Sexual Behaviour and Factors Affecting Female Reproduction in House and Field Crickets

by

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ABSTRACT

The objective of this investigation was to clarify the adaptive significance of female sexual behaviours in the house cricket, Acheta domesticus, and the Texas field cricket, Gryllus integer. Experiments were focussed primarily on: nutritional factors affecting female reproductive success; the ontogeny of female sexual behaviours; female mating frequency and progeny production; and the pattern of sperm competition.

Reproduction of singly mated female A. domesticus assigned to 3 nutritional regimes was compared. Females fed a vitamin and protein-enriched mouse chow, cannibalistic females, and starved females produced on the average, 513, 200 and 68 offspring, respectively. Cannibals probably could not obtain the same amounts of essential nutrients as females fed mouse chow. Reabsorption of oocytes was likely the major factor contributing to the decreased reproduction of starved females. In addition, female A. domesticus fed mouse chow, but allowed constant access to males produced 11 times as many offspring than did females fed corn meal. Females fed corn meal probably could not absorb or synthesize enough dietary lipids, thus resulting in poor ovariole growth.

Female A. domesticus first mate at an average adult age of 7 days, closely corresponding to when they first exhibit positive phonotaxis. Females mate repeatedly and often consume

the externally attached spermatophore. In A. domesticus, females allowed constant access to males produced significantly more offspring than did single maters. Similarly, doubly mated G. integer females produced more offspring than did single maters. This difference resulted largely from the failure of many single maters to reproduce. Remating by female crickets partly functions in offsetting the possibility of a failed initial mating. Nymph production increased significantly with the time the spermatophore was attached in singly mated A. domesticus. Spermatophore consumption by the female was not affected by male guarding behaviour, and the interval between mating and eating of the spermatophore may often be shorter than the time required for maximum insemination. Some degree of sperm depletion in singly mated A. domesticus and G. integer may have occurred. The patterns of daily offspring production of singly and multiply-mated females suggests that a factor provided by a male during mating stimulates female oviposition and/or egg production. Female crickets also might acquire nutrition from spermatophore consumption, a benefit that is augmented by female multiple mating. The electrophoretic examination of various allozymes in G. integer did not permit determination of a pattern of sperm competition. However, the possibility of last male sperm predominance is related to male guarding behaviour.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Female insect reproduction has long been of interest. Recently, several studies have focussed on social factors that affect female insect reproductive success. These include male-female interactions, mating frequency, and mate preference (Engelmann 1970; Thornhill 1976a, 1979). Also, there are important non-social factors that influence female insect reproductive success. These include nutrition, temperature, humidity, and photoperiod, of which nutrition is probably the single most important factor in the majority of insect species (Engelmann 1970).

Sakaluk (1978) previously studied female reproductive behaviour in crickets and specifically examined the role of mating frequency in Acheta domesticus (Orthoptera; Gryllidae). Doubly mated A. domesticus produced significantly more offspring than did single maters, and nymph production increased significantly with the time the spermatophore remained attached in singly mated females. These results indicated that female house crickets remate and thus correct for insufficient first matings. In many cricket species, females multiply mate and recently, a high degree of multiple mating was demonstrated in the Texas field cricket, Gryllus integer (Sakaluk and Cade 1980).

In addition, Sakaluk (1978) observed a high degree of cannibalism between A. domesticus individuals in a mating chamber. This coupled with the knowledge that females of various

cricket species often consume the externally attached spermatophore (Alexander and Otte 1967), suggests that the acquisition of nutrition might be an important facet of the reproductive behaviour of female crickets.

The initial direction of the research reported here centred on two principle areas: the influence of social factors such as mating frequency, spermatophore consumption, and cannibalism on the reproductive success of female crickets; and the influence of varying nutrition on female cricket reproduction. To this end, the ontogeny of female mating behaviour in A. domesticus, and the ontogeny of female phonotaxis to male calling song were studied. The relationship between mating frequency and daily progeny production was examined, and the influence of the duration of spermatophore attachment on both reproductive components was assessed. Similar parameters also were studied in G. integer, and an electrophoretic analysis was used to investigate the pattern of sperm competition in this species. The effects of varying nutrition were examined only in A. domesticus, and the importance of spermatophore consumption to progeny production was assessed.

Results from these experiments were interpreted in terms of the evolution of female cricket reproductive behaviours. Sexually dimorphic behaviours probably are best examined in the context of sexual selection theory. Darwin (1871) first recognized two types of selective forces operating simultaneously on the sexes during reproduction. Citing several insect examples,

he noted that in many species males compete for females, whereas females exercise a choice of mates. Competition between individuals of one sex for individuals of the other is intrasexual selection, and choice of one sex for certain individuals of the other is intersexual selection.

Why, in most species, males compete for females and females choose males has only recently been made clear. Trivers (1972) argued that selective forces act differently on the sexes during reproduction because of a difference in parental investment by the sexes. He defined parental investment as "any investment by the parent in an individual offspring that increases the offspring's chance of survival (and hence reproductive success) at the cost of the parent's ability to invest in other offspring." This was a refinement of Bateman's (1948) observation that male Drosophila provide little energy investment in their gametes, whereas females invest a considerable amount. Therefore males are limited only by their ability to fertilize females and females by their ability to produce eggs. Females thus are a limiting resource for males for which the males compete. Females presumably are selected to choose those mates that bestow the greatest fitness on the females's offspring.

In most insects, there is no investment by males beyond the energy invested in spermatozoa, and intense levels of male-male competition have been documented in various species. By contrast, intersexual selection is poorly understood (Thornhill 1979), and an understanding of female insect reproductive

behaviours appears to lag far behind that of males. Much of the data on female cricket reproduction presented here is novel therefore, and should promote a greater appreciation of female insect reproduction in general.

LITERATURE REVIEW

The literature which is relevant to this thesis is organized into four sections. I first review the sexual behaviour of crickets with specific reference to A. domesticus and G. integer. Cricket reproductive behaviour is discussed in the context of sexual selection. Also, the evolution of female multiple mating in insects in examined, as is evidence for sperm competition and it's mechanisms. Lastly, non-social factors affecting female cricket reproduction are considered.

1. Reproduction in crickets: Pair formation, mating behaviour, and sexual selection

In crickets, formation of sexual pairs normally is facilitated either by male calling or accidental encounters (Alexander and Otte 1967). Both males and females have one pair of auditory receptors or tympana on the inside of each of the tibiae of the forelegs. Only males are able to produce songs, a process termed stridulation. As the forewings of a male are rubbed together, a "scraper" on one strokes over a "file" on the ridge of the other producing a single pulse of sound. A cricket chirp consists of many pulses produced together. In detailing the acoustical component of reproduction in crickets, Alexander (1962) described various types of songs. These include: 1) a calling song which functions to attract conspecific females; 2) a courtship song which induces females to mount males; 3) an

aggressive song produced in the context of intense male-male aggression; 4) a copulatory song; 5) a post-copulatory song; and 6) a courtship-interruption song produced when contact is broken between a courting male and female. A copulatory song and a post-copulatory song are not known in either Acheta domesticus or Gryllus integer (Alexander 1962).

The calling song generally is a loud, rhythmic, intense song which is produced continually over long periods of time. The courtship song also is rhythmic, but is much softer and almost inaudible. The aggressive song is a loud but brief, sharp signal elicited when conspecific males come in close proximity. The courtship-interruption song is similar, and often identical to the aggressive song.

Additionally, olfaction may function in sex and/or species recognition in A. domesticus and G. integer (Otte and Cade 1976), and Teleogryllus commodus (Rence and Loher 1977). Otte and Cade's experimental apparatus consisted of five chambers through which air was forced and included two chambers that contained the odours to be tested, two in which the odours were released, and one in which the test animal was placed. Individuals of both A. domesticus and G. integer exhibited a significant attraction to chambers containing conspecific odours of the opposite sex, as opposed to those containing no odours. Males of both species preferred chambers conditioned by conspecific females over those conditioned by conspecific males. These results demonstrated the existence of volatile sex and species-specific odours in

these two species. In Rence and Loher's (1977) study, sexually receptive male T. commodus were touched on their antennae with freshly severed antennae of male and female conspecifics. When touched with male antennae, males produced the aggressive song, but when touched with female antennae, they produced the courtship song. Rence and Loher repeated Otte and Cade's (1976) experiment, but T. commodus individuals exhibited a random choice of chambers. Their results indicated the presence of a contact (non-volatile) pheromone in this species.

In an extensive review, Alexander and Otte (1967) described the mating behaviour of many cricket species and other Orthoptera. Their treatment included a description of the mating behaviour of both A. domesticus and G. integer, which is summarized here. After a male A. domesticus encounters a female, his mode of chirping changes from the calling song to the courtship song. If the female remains stationary, the male continues to produce the courtship song while gently rocking from side to side and backing towards her. During this courtship "dance", the pronotum and head of the male point downward and the posterior end of his abdomen almost touches the substrate. If the female antennates and palpitates the male, he flattens his body and extends it under her. The female may then mount the male. Once in position, the male inserts his epiphallus between the base of her ovipositer and subgenital plate. In response, the female everts papilla surrounding the genital orifice which the male grasps with his phallic complex. The tube of the male's extruded

spermatophore is threaded into the bursa copulatrix of the female with the aid of his guiding rod or virga (Alexander and Otte 1967). The spermatophore is emptied of sperm through the development of internal osmotic pressures (Khalifa 1949). Male A. domesticus apparently attempt to stay in contact with the female by watching and continually antennating her. Male production of spermatophores varies from 15 to 65 minutes in A. domesticus (Khalifa 1950), so male guarding behaviour may allow a male to inseminate a female repeatedly. In G. integer, the mating behaviour is nearly identical to that of A. domesticus, although there are slight morphological differences in the size and shape of the genitalia (Alexander and Otte 1967).

The externally attached spermatophore is composed almost entirely of protein (Khalifa 1950). It is removed by the female some time after copulation by rubbing her lower abdomen against the substrate, or by bending her abdomen sharply and consuming it (Alexander and Otte 1967; Khalifa 1950). Females consume spermatophores in other cricket species as well. In Nemobius sylvestris for example, a mating routinely involves the attachment of two spermatophores. The female quickly removes and consumes the first spermatophore. The male then transfers a second spermatophore, approximately three times as large as the first, which remains attached for a lengthy period.

As in crickets, spermatozoa are transmitted to the female in a spermatophore in most insect species. Sperm are then transferred within the female to the sperm storage organ or

spermatheca. The mode of sperm transference in the female may be due to pumping movements in the female's reproductive system. Alternatively, sperm may migrate to the spermatheca in response to unknown chemical stimuli (Wigglesworth 1965). In some insect species, sperm are deposited directly into the female's spermatheca through the male's penis or aedeagus (Bonhag and Wick 1953; Thornhill 1979). Fertilization of eggs occurs some time after copulation, usually just before oviposition. An egg moves down the female's oviducts by peristalsis to the spermathecal duct pore, where sperm are released and the egg is fertilized (Elzinga 1978).

Female crickets deposit fertilized eggs into moist soil by means of a long, thin ovipositer. Eggs do not hatch until most of the individuals from the previous generation have died. When hatched, an immature cricket or nymph is approximately the same size as an egg. It reaches maturity by undergoing a series of molts, and the period between two molts is termed an instar. A female A. domesticus can oviposit as many as 2600 eggs during her lifetime (Thompson 1977).

Different forms of male-male competition have been intensively studied in field crickets. Alexander (1975) and Otte (1977) described the various modes of male-male competition in acoustically signalling insects and these include: moving into a new area if a conspecific male is in the vicinity; attacking signalling competitors; outsignalling competitors; silently intruding into the vicinity of other calling males and

intercepting responsive females; seeking out and attacking courting males and attempting to steal their mates; reducing signal intensity when females are nearby, thus reducing the probability of cuckoldry; and producing a more directional signal when females are in close proximity.

Intrasexual selection in field crickets may be mediated directly through aggressive encounters between males that include head butting, grappling with mandibles and biting. A struggle of this nature usually is terminated by the retreat of one individual. After a high intensity encounter, the "winning" male produces aggressive stridulation, whereas the retreating male rarely chirps. In small experimental arenas, dominance hierarchies are formed through aggression between males (Alexander 1961).

A similar scenario has been described by Burk (1979) for the cricket, Teleogryllus oceanicus. In this species, males also interact aggressively through fighting, and successful fighters stridulate more often than losers. Virgin female T. oceanicus mate only with males that produce the courting song, and Burk showed a positive, significant correlation between male fighting success and being the first male to court a female. However, no correlation was found between fighting success and the probability of a male encountering a female. Additionally, if a losing male did court a female, it was no less likely to lead to a copulation than if a successful fighter courted her.

Male-male competition also has been extensively detailed in Gryllus integer by Cade (1979a). In this species, competition between males is expressed primarily in acoustical signalling behaviour which functions to attract females. However, males may also obtain females by silently searching for them. Cade showed that males compete by adopting any one of several behaviours. Some males call constantly at high intensities which enable them to maintain territories. When previously recorded conspecific song was broadcast to calling males, some increased their song intensity and often attacked male conspecifics tethered close to the loudspeader. Regularly calling males probably communicate a threat to other males, thus excluding males in the immediate vicinity from obtaining mates. However, regularly calling males do incur two potential costs, the attraction of other males which can either attack or steal mates, and the attraction of parasites. Cade (1975) demonstrated that calling males are parasitized significantly more often by an acoustically orienting parasitoid, Euphasiopteryx ochracea, than are non-callers. Parasitized crickets die about a week later. Overall, calling males probably have a reduced survivorship when compared to males that don't call and thus remain free of fly parasites.

To a lesser extent, sexual selection has been studied in A. domesticus. Heiligenberg (1966) showed that male A. domesticus increase their song rate in response to other singing males. He reasoned that since responsive females are attracted to calling

males, the most actively singing males should be the most reproductively successful. Thus, a male that increases its song rate when it hears another male, may attract a female that might otherwise go to a rival.

Crankshaw (1979) examined female choice in A. domesticus. Four males at a time were placed in a large container until a dominance hierarchy was established. Songs of the most dominant males were recorded, as were those of the least dominant. Songs of dominant and subordinant males were played at the same intensity, from separate speakers at the end of a Y maze in which individual females were tested. Females displayed a positive phonotactic response to dominant song 60% of the time, as opposed to 40% for subordinate song. Also, when the tegmina were removed from four males, no matings were recorded when each male was placed with a female. However, when courtship song was played, these males mated 5 times out of 8 opportunities to do so. Thus, it appears the production of the courtship song is necessary to stimulate females to mount males.

2. Multiple matings in insects: benefits and costs

One form of female preference is the number of times a female mates (Walker 1980). The evolution of female multiple mating in crickets was studied here and females of many insect species often mate repeatedly. Repeated matings occur even though females store sperm internally and in many insect species, a

single insemination is sufficient to maintain normal fertility and oviposition rates for the entire reproductive life of the female (Wigglesworth 1965). Daly (1978) reviewed the costs of female matings in a variety of animals and these include time and energy expenditures, risks of predation and the possibility of injury. These costs are increased for females that mate repeatedly. Indeed, unless females benefit from multiple matings, the time and energy wasted in remating should select against additional matings (Parker 1970a, 1979).

However, benefits also are attributed to female multiple matings. Kirkendall (1977) extensively reviewed female multiple mating behaviour in insects. He identified the following advantages for females that remate: correction for insufficient first matings; production of genetic variability in offspring; acquisition of male-controlled resources such as oviposition sites; and in species where males feed the females, the acquisition of nutrition. Other benefits include obtaining an adequate and functional supply of sperm, and avoiding the physiological costs of storing and maintaining sperm (Alcock et al. 1978). Remating by female insects should only occur if the benefits outweigh the costs such that increased reproductive success is likely. I here review the evidence pertinent to this prediction.

Fecundity and/or fertility increase

Several studies have demonstrated the importance of multiple mating in increasing fecundity and/or fertility in

female insects. In a mating study of the olive fruit fly, Dacus oleae (Diptera; Tephritidae), Economopoulous et al. (1976) allowed females to mate with a sterilized male and then with an untreated male. These females produced an average of 2.6 more eggs than singly mated females. A similar result was obtained for the cricket Plebeiogryllus guttiventris (Orthoptera; Gryllidae). Virgin females produced 125.1 $\pm$ 20.5 eggs, whereas females mated with castrated males left 214.7 $\pm$ 40.6 eggs (Bentur and Mathad 1975).

Gordon and Bandal (1967) showed that virgin female Oncopeltus fasciatus (Hemiptera; Lygaeidae) lay infertile eggs in clusters at intervals of one week. The presence of one or two males tripled female egg cluster size and also the frequency of oviposition. These effects continued for up to 3 weeks after males were removed. Gordon and Loher (1978) found that egg production is enhanced by frequent matings in O. fasciatus, and that not enough sperm is transferred in one mating to fertilize all of a female's eggs.

In the spruce budworm, Choristoneura fumiferana (Lepidoptera; Tortricidae), females rarely mate more than once over 24 hours in the field. Sexual activity apparently is governed by the photoperiod. Copulation lasts 3 to 7 hours so that by the end of a copulation, individuals are at the non-responsive end of the activity cycle. However females can mate more than once over several days (Sanders 1975). Outram (1971) showed that as the frequency of mating increases for male C. fumiferana, their

spermatophore size becomes progressively smaller. Also the presence of a spermatophore in a female's bursa did not always indicate successful insemination, as some singly mated females did not lay any fertile eggs. By remating, females may be ensuring an adequate sperm supply.

Replenishing spent spermatozoa

In some insect species, females remate and replenish spermatozoa spent in previous ovipositions. This was clearly demonstrated by Pyle and Gromko (1978) for Drosophila melanogaster (Diptera; Drosophilidae). Singly mated females produced an average of 528 progeny compared to 1053 for remated females. Fecundity and fertility in singly mated females decreased drastically after 7 days, but remained constant in remated females. Also, remating was correlated with the rate of progeny production and sperm utilization. Pyle and Gromko concluded that females remated and replenished spent sperm when female productivity began to decrease. In the Mediterranean fruit fly, Ceratitis capitata (Diptera; Tephritidae), singly matedfemales don't remate for several weeks. Females become receptive when the sperm in the spermatheca is depleted. The quantity of sperm in a female's spermatheca may be detected by stretch receptors and transferred to the insect's brain (Cunningham et al. 1971).

In the apple maggot, Rhagoletis pomonella (Diptera; Tephritidae), females who had males present only occasionally

averaged 4 matings, a production of 360 eggs, and a 46% hatch. Females with a male always present averaged 30 matings, a production of 395 eggs, and a 95% hatch. Since during the first 2 weeks of egg laying females of both groups had a similar percent hatch, remating probably functions to replenish spent spermatozoa (Neilson and McAllan 1965). Similarly in the red bollworm, Diparopsis castanea (Lepidoptera;Noctuidae), doubly mated females averaged 97.4 ± 0.3% fertility of eggs laid over a 10 day oviposition period. Females mated once averaged a 98.3 ± 0.6% fertility rate over days 1 to 5, but this dropped to 73.8 ± 5.2% during days 6 to 10. Fecundity did not differ between the 2 groups (Marks 1976). In this species multiple mating also appears to have a sperm replenishing function.

Correction for infertile matings

The probability of an infertile mating also may explain why multiple mating has evolved in some female insects. In Atteva punctella (Lepidoptera; Yponomeutidae), females involved in infertile copulations had a significantly higher probability of remating than females from fertile matings. However, fertility was based on eggs darkening and no attempt was made to distinguish fertile, but inviable eggs in early embryogenesis. Forty percent (N=55) of fertile females mated a second time, whereas 68% (N=28) of infertile females remated (Taylor 1967). The contributing factor to female infertility apparently was either male inability to transfer a spermatophore, or an inbreeding

effect. However Taylor dissected infertile females and found in some, a quantity of dead sperm in the seminal ducts indicating blockage within the female had occurred.

Mishra and Krishna (1979) studied reproduction in the rice moth, Corcyra cephalonica (Lepidoptera; Galleridae). Newly emerged females mated with males 0, 3 and 6 days old, left an average of 142.4, 49.0 and 7.0 fertile eggs respectively. Older males are probably unable to inseminate females fully. Multiple mating females of the tsetse fly, Glossina pallidipes (Diptera; Glossinidae), have a higher fecundity and a lower percentage of infertility than singly mated females. By remating, females reduce the probability of undergoing two sterile copulations Jaenson 1979).

Acquisition of nutrition

Nutrition provided by males to females is an integral part of the reproductive behaviour of many insect species. Females which mate repeatedly increase this benefit. Nutrition provided by males comes in 3 types: glandular secretions; nuptial prey items ; and the male's body. In many cases, this has evolved in the context of female preference (intersexual selection) for increased male parental investment (Thornhill 1976a).

In the scorpion fly, Bittacus apicalis (Mecoptera; Bittacidae), males provide females with nuptial prey items and females mate repeatedly. The duration of copulation and the number of sperm transferred (a measure of male reproductive

success) show a significant positive correlation. The size of the prey item provided by the male and duration of copulation also is positively correlated for prey sizes of 3 to 19 mm^2. When prey items are less than 18 mm^2, copulations are terminated by the female. At prey sizes of 18 to 55 mm^2, copulations last from 20 to 31 minutes and are terminated by the male. Since at 21 minutes the correlation between duration of copulation and sperm transfer breaks down, males receive no fitness gains by copulating any longer.

By terminating the copulation soon after maximal insemination, a male can provide what is left of the nuptial prey to another female. Male-male competition is exhibited then, in the ability to catch or steal prey items of adequate size. Females are maximally inseminated only by those males providing adequate-sized nuptial prey and by so choosing, increase oviposition, enhance egg production, and conserve time and energy which otherwise would have been utilized in hunting (Thornhill 1976b).

A similar mating arrangement occurs in species of the genus Rhamphomyia (Diptera; Empididae). In R. nigrita, males catch their prey items in male swarms of mosquitoes and carry them back to a mating site identified by a visual marker. If a male approaches another male with a prey item, the other male moves away. However, if the conspecific approached is a female, pairing takes place and the prey is transferred to the female who feeds on it while copulating. Females obtain food in no other way. Prey items are usually small, and appear necessary

for ovarian maturation. Remating is more frequent than necessitated by adequate insemination (Downes 1970).

Boggs and Gilbert (1979) demonstrated that nutrients from spermatophores are incorporated into the eggs of the tropical butterflies Danaus plexippus, Heliconius erato, and H. hecale (Lepidoptera; Nymphalidae). Females of all three species multiply mate and in these as in other Lepidoptera, spermatophores are inserted internally. Boggs and Gilbert used radioactive tracers to label males. The first eggs laid by females contained the label indicating rapid incorporation of male-provided nutrition. Unfertilized eggs were as radioactive as fertilized ones, indicating that eggs were not radioactive simply because of labelled spermatozoa.

Interestingly, in a study of the pink bollworm, Pectinophora gossypiella (Lepidoptera; Gelechiidae), Henneberry and Leal (1979) showed that female multiple mating increases neither fecundity or fertility. Singly mated females laid 208 ± 20 eggs and multiply mated females laid 217 ± 18 eggs. The average hatch for single matings was 69 ± 3% and for multiple matings, 59 ± 6%. This contrasts with the suggestion that additional spermatophores increase fecundity (Gilbert 1976), and the nutritional role attributed to spermatophores (Boggs and Gilbert 1979).

Access to resources

In some insects, males control resources required by

females such as food and oviposition sites. The acquisition of these resources appears to be more readily facilitated by females that mate repeatedly. This situation was studied in the dung fly, Scatophaga stercoraria (Diptera; Scatophagidae), by Parker (1970b,c). Females of this species lay up to 4 batches of eggs without any drop in fertility after a single mating. Females deposit eggs in fresh cattle dung (the freshest dung being most advantageous for larval development). A female invariably mates every time she lays a batch. Females usually fly over a dung pile to an upwind position, where male density is lowest. While walking to the dung, a female is usually mated by a single male. However, if a female approaches from the downwind side where male density is highest, she may be grasped simultaneously by several males. This can involve a large cost, as it takes some time for one male of a group to dominate and achieve a copulation. Females leave the dung immediately after ovipositing.

Similarly, males of the leafcutting bee, Anthidium maculosum (Hymenoptera; Megachilidae), establish territories at patches of flowering Monarda sp., a food resource for both sexes. Females multiply mate although they receive enough sperm from one mating to fertilize the few eggs they produce. However, since the average copulation lasts only 27 seconds, females save time and energy and reduce foraging interruptions by copulating directly, rather than trying to evade or resist the larger males (Alcock et al. 1977).

In an unusual example of male resource control, male giant water bugs, Abedus herberti (Hemiptera; Belostomatidae), brood eggs on their back. Male brooding is necessary for offspring development and survival. Mating with additional males by females with remaining eggs, frequently occurs. Females should mate with any male when male back space is in short supply. This is of course, a somewhat unique circumstance in the insects as male A. herberti parental investment approaches, and possibly equals that of females (Smith 1979a).

Genetic benefits

Potential genetic benefits have been assigned to some species in which females multiply mate. Pease (1968) reasoned that multiple mating in some Lepidoptera functions to increase interpopulation hybridization so as to reduce the competitive advantage of an invading population. For example, female Hypaurotis crysalus (Lepidoptera;Lycaenidae), usually mate once, but sometimes twice and rarely, three times (Scott 1974). Scott implied that the reason females of this species are willing to remate, whereas females of other butterflies mate only once, is to lessen inbreeding in the population. Similarly, Dunbar (1972) found that female Geocoris punctipes (Hemiptera; Lygaeidae) can mate up to 10 times, although singly mated females produce viable eggs over a mean of 30 days, and exhibit an average hatch of 73%. He reasoned that multiple mating was necessary to maintain genetic variability in the population.

However, Boorman and Parker (1976) correctly pointed out that these types of explanations rely on interpopulation or group selection. The importance of population selection as a major evolutionary force is at best, tenuous, and restricted to only a very unique set of circumstances (Lewontin 1970). The observations of Pease (1968), Dunbar (1972), and others are best explained in terms of individual selection. Hybridization of offspring might be advantageous to the individual female in the case where females lay eggs continuously throughout life (Boorman and Parker 1976).

Richmond and Ehrman (1974) demonstrated that female Drosophila paulistorum (Diptera; Drosophilidae) mate repeatedly under experimental conditions, and that females remate before their sperm supply is exhausted. They suggested that competition between larvae is more likely to be reduced if they differ in genotype if the female multiply mates.

Byers (1978) studied multiple mating in 13 species of the genus Euxoa (Lepidoptera; Noctuidae) and found the average number of matings in these ranged from 1.65 to 10.86. He suggested that females of species mating at a low frequency do so to ensure adequate insemination. In those species with a high level of multiple mating, the acquisition of nutrition and genetic benefits were considered of greater importance.

Costs of multiple mating

Some striking examples of the costs of mating to female

insects have been reported. In the lovebug, Plecia nearctica (Diptera; Bibionidae), females copulate once or occasionally twice, oviposit, and then die. Copulations last on the average, an astounding 51 hours. Females live on the average only 86 hours after their first copulation (Thornhill 1976c).

Risk of physical damage is another mating cost. Labine (1964) proposed that the risk of physical damage selected against multiple inseminations in Euphydryas editha (Lepidoptera; Nymphalidae). After mating, a female's bursa is greatly distended by a single spermatophore. Severing innervation to the bursae of recently mated females significantly increased the probability of their remating.

Nilakhe (1977) varied male:female ratios of the boll weevil, Anthonomous grandis (Coleoptera; Curculionidae), and kept them together for the entire duration of the female's life. When male: female ratios were 1:1, 10:1, and 20:1, the average longevity of females was 63.3 $\pm$ 1.3 days, 38.3 $\pm$ 7.3 days, and 16.8 $\pm$ 1.3 days, respectively. The fecundity of females was 387.5 $\pm$ 69.1 eggs, 238.0 $\pm$ 155.3 eggs, and 87.8 $\pm$ 23.5 eggs, respectively. The bursae of females were greatly distended after several matings. Fecundity was affected because ovarioles were probably compressed, thus preventing descent of eggs.

In Macrocentrus ancylivorus (Hymenoptera; Braconidae), an insect parasitic on the potato tuber worm, Gnorimoschema operculella (Lepidoptera; Gelechiidae), 15% of singly mated females were not impregnated, whereas 45% of multiply mated

females were not impregnated. Dissections of multiply mated females showed that crowding of spermatophores in the vagina may have prevented spermatophores from connecting with the sperm ducts (Flanders 1945).

In the dung fly, Scatophaga stercoraria (Diptera;Scatophagidae), females are sometimes injured by the simultaneous grasping of several males. Occasionally, females are dragged down in the dung and drown (Hammer 1941; cited in Parker 1970c).

Although reproductive behaviour might result in increased susceptibility to predation, no documented cases of this are known for females. Bell (1979) showed that little blue herons, Florida coerulea, are attracted to tape recorded Anurogryllus celerinictus (Orthoptera; Gryllidae) songs. Herons were observed to stalk, catch, and eat crickets at night. Walker (1964) demonstrated that cats can acoustically locate singing Orthopteran prey. Cade (1975) documented the acoustical orientation of a parasitoid, Euphasiopteryx ochracea (Diptera; Tachinidae), to cricket calling songs. Although most female Orthoptera do not call, their association with calling males may render them more susceptible to acoustically orienting predators.

3. Sperm competition in the insects

Female multiple mating is an important precondition for the occurrence of a high level of sperm competition in an animal

group. Clearly this applies to many insect species. Sperm competition is competition between the sperm of two or more males for the fertilization of eggs of a single female (Parker 1970a). Competition also can occur among the sperm of a single male, but this is outside the scope of this review. Natural selection should favour adaptations in males which ensure that their sperm, in competition with the sperm of other males, will fertilize most of a female's eggs (Boorman and Parker 1976). These adaptations can be either behavioural, or manifested in the sperm themselves. This selection necessarily leads to two opposing evolutionary forces: males that mate with non-virgin females should be selected to displace previous sperm or somehow ensure that their sperm will contribute maximally to subsequent fertilization; and males that mate with virgin females should be selected to reduce competition from the sperm of other males in some way (Parker 1970a).

Parker (1970a) listed 4 preadaptations which if fulfilled, probably would lead to the occurrence of a high level of sperm competition in an animal group. These are: females mate repeatedly before all their eggs are fertilized; females can store sperm; sperm live for the entire life of the female; and sperm utilization at fertilization is highly efficient, thus increasing the probability of the overlapping of various ejaculates. Many insect species satisfy these prerequisites to at least some degree, so sperm competition is probably a widespread phenomenon in this class. However, serious investigations of this phenomenon

have been undertaken only within approximately the last 10 years, and the mechanisms of sperm competition are poorly understood. In this section I describe methods common to the study of sperm competition, survey the evidence, and review the current understanding of possible mechanisms underlying sperm competition.

Methods for the determination of sperm competition

Basically, three methods have been utilized in determining how sperm from different matings are used in the fertilization of a female's eggs. These will be described only generally, as deviations from them will become apparent when individual experiments are described.

One common procedure to study sperm competition consists of mating females with a recessive character to both normal and recessive males. Hence, progeny exhibiting normal characteristics can be attributed to the normal male and those possessing the recessive properties, to the recessive male. However, this method requires the existence of different and readily identifiable phenotypes whose genetic basis is known. It also assumes that the sperm from normal and mutant males are equally viable.

Another common method consists of labelling sperm by inducing sterility in one of the males, usually either by irradiation or chemosterilization. Sperm from irradiated males are able to fertilize eggs, but induce lethal abnormalties during the development of zygotes. Hence, eggs that hatch can be

attributed to normal males, and those that don't to irradiated males. Since sperm from irradiated males may not be as competitive as that from normal males, it is necessary to reverse the order of mating of the two types of males as a control. If the sperm from both treated and untreated males are equally competitive, then the total offspring of each from the two types of matings should be equal (Parker 1970a).

For the remainder of this section, the following conventions will be adopted. An NR mating will denote a normal female mated first with a normal and then with an irradiated male. An RN mating will denote a normal female mated first with an irradiated, and then with a normal male. When chemosterilization, a genetic marker, or hybridization is utilized to label males, R will be replaced by C, G, or H, respectively.

More recently, gel electrophoresis has been used to investigate sperm competition. Briefly, strains of different genotypes established previously by electrophoresis are crossed. The enzyme under study is examined by electrophoresing parents and their resultant offspring. Paternity of offspring is ascertained by comparison of their enzyme separation patterns with those of their parents. Multiple allelic loci are particularly sensitive indicators of multi-paternity because of the large number of potential mating types (Sassaman 1978).

Evidence for sperm competition and its mechanisms

a) Coleoptera

Schlager (1960) utilized a black mutant to study sperm competition in the flour beetle, Tribolium castaneum (Tenebrionidae). Initially, 100% of the offspring produced subsequent to a second mating were fathered by the second male to mate. However, this percentage dropped off with time. Schlager described this as a "first sperm in, last sperm out" phenomenon. Apparently, sperm from the first male was displaced from the vagina into the spermatheca by the second male's sperm. As more of the second male's sperm was utilized, the first male's sperm began to be employed.

The predominance of the second male's sperm in fertilizing subsequent eggs was also shown in the plum curculio, Conotrachelus nenuphar (Curculionidae) by Huettel et al. (1976). Various allozymes of this species were assayed in a horizontal starch gel electrophoresis. Two strains, having different frequencies of an isocitrate dehydrogenase allele, were crossed and parents/offspring examined. Mean percent progeny produced by the second male to mate was 85.8%.

Several investigators have examined sperm competition in the boll weevil, Anthonomus grandis (Curculionidae). Gilliland and Davich (1966) and Lindquist and House (1967) mated females to normal and apholate-sterilized males alternately, and estimated second male predominance at approximately 67% and 80% respectively. Klassen and Earle (1970) used bulsulfan as a chemosterilant and arranged only NC matings. Females were mated once with a normal male, and then with a chemosterilized male

which previously had mated once or several times. The percentage hatch of females when the chemosterilized male mated only once was 12%, whereas it was 35% when the chemosterilized male previously had mated 5 times. Their results indicated that the proportion of subsequent offspring fathered by the second male ranges from 65% to 85%, depending on how many times the male had mated previously.

Nilakhe and Villavaso (1979) surgically removed spermathecae from A. grandis females so that sperm would enter directly into the copulatory pouch. Females were mated alternately to irradiated and normal males. The NR group of females produced 11.2% less offspring than expected if no sperm precedence had taken place. Similarly, RN females produced 11.8% more offspring than expected. Nilakhe and Villavaso suggested that direct sperm competition within the copulatory pouch was more important than sperm displacement within the spermatheca, since 61% of the subsequent offspring were fertilized by the last male to mate, even though the spermatheca had been removed. They attributed this predominance to the occurrence of clotting and binding of sperm with male accessory gland material. Sperm from first matings are subjected twice to this process.

b) Diptera

Although females of the mosquito, Aedes aegypti (Culicidae), have frequent copulations, experiments with genetic markers showed they are inseminated only once (Craig 1967). When the

male accessory gland was implanted in the thorax of virgin females, they often would copulate but were never inseminated. Craig suggested that the active agent stimulates females to hold the vaginal lips together. Bryan (1968) examined sperm competition in another mosquito species, Anopheles gambiae (Culicidae). She produced sterile hybrid males by crossing A. gambiae with A. melas. In 75 NH matings most eggs hatched, whereas in 75 HN matings none of the eggs hatched. This indicated that the first male to mate fertilized all subsequent eggs.

Boorman and Parker (1976) mated normal female Drosophila melanogaster (Drosophilidae) to irradiated and normal males. They found that the proportion of offspring fathered by the second male was dependent on the interval between first and second matings. If a female was remated within a day, the proportion of offspring fathered by the second male was 0.83. However, if the interval was 14 days, second males fertilized all subsequent offspring. These results are supported further by the irradiation experiments of Hennebery et al. (1967) who reported an 85%, second male predominance. Lefevre and Jonsson (1962) utilized a sex-linked character and only examined daughter offspring of GN and NG matings. In GN matings, the second male to mate was predominant in fertilizing 75.3% of the subsequent offspring, whereas in NG matings it was 76.6%.

Boorman and Parker (1976) developed an excellent method for calculating the proportion of eggs fertilized by R and N males. This method incorporates the proportion of eggs which

fail to hatch due to natural infertility and the expected proportion of hatching eggs which are products of R sperm. If P_r is the proportion of eggs fertilized by an R male, x is the proportion of eggs hatching after a double mating, z is the proportion of eggs hatched produced from R sperm as indicated by single matings involving R males (ideally, should be 0%), and p is the proportion of eggs produced from N sperm as indicated by single matings involving normal males (ideally, should be 100%), then:

$$P_r = (1 - x/p) + ((z/p)(1 - (x/p)/1 - (z/p)).$$

A numerical example is included in Appendix 1.

Olivieri et al. (1970) mated D. melanogaster YXY/O males with Oregon R females. This type of mating produced only XO males and XXY females. A sex ratio (proportion of males) higher than 0.5 would indicate that a higher number of O sperm than YXY sperm are utilized in fertilization. Olivieri et al. found a sex ratio significantly less than 0.5, indicating a greater utilization of YXY sperm. Johnsen and Zarrow (1971) also showed differential utilization of sperm in D. melanogaster, and their results supported the observations of Olivieri et al. (1970). Both studies indicate that the sperm of certain genotypes are more efficient in fertilizing eggs than the sperm of other genotypes.

Gromko and Pyle (1978) found that for D. melanogaster females mated twice, 72% of the subsequent offspring were

produced by the second male to mate. However, the net effect of the female's remating on the first male's productivity was a loss of only 16% of the progeny he would have produced had the female not remated. This indicated that the amount of sperm displaced by the second male to mate was substantially less than suggested earlier. Gromko and Pyle reasoned that a large proportion of subsequent progeny was sired by the second male because females did not remate until most of their sperm was spent. Lefevre and Johnson (1962) reported a high degree of sperm displacement for D. melanogaster, but confined their males and females for up to 48 hours which resulted in females remating before sperm depletion.

Parker's (1970b) study of the dung fly, Scatophaga stercoraria (Scatophagidae), is one of the very few detailing a complete pattern of sperm competition in a species. Using irradiation as a sterilant, Parker found that second males in RN matings fertilized 92.12% of the subsequent offspring, whereas second males in NR matings only exhibited a 70.7% predominance. This indicated a lower number of viable irradiated sperm, or a reduction in the total number of irradiated sperm. Parker also mated females to many different males. By labelling matings at various positions (ie. N male at 1st mating, 2nd mating, 3rd etc.), he ascertained that the last male to mate before a female oviposits fertilizes 80% of the subsequent offspring regardless of the number of previous matings. Parker constructed a mathematical model which assumed that sperm from matings previous to the last one, would compete for the remaining 20% of the batch

in the same proportionate relationship as they did for the previous batch. If E is the number of eggs in a given batch, p is the proportion of eggs in the present batch to be fertilized by the last male to mate, n is the batch number of the female, and ΣE is the total number of eggs fertilized by a given male then, if a male mates with a virgin female:

$$\Sigma E = E_1 + E_2p(1-p) + E_3p(1-p)^2 \ldots + E_np(1-p)^{n-1}$$

and if a male mates with a non-virgin female:

$$\Sigma E = E_1p + E_2p(1-p) + E_3p(1-p)^2 \ldots + E_n(1-p)^{n-1}.$$

Using these equations, Parker was able to predict the total number of eggs a male would fertilize over all a female's egg batches. His predictions, with one exception, did not differ significantly from the observed hatch. A numerical example is included in Appendix 2.

Curtis (1968) utilized irradiation to investigate sperm competition in the tsetse fly, Glossina austeni (Muscidae). In this species, the first male to mate fertilized 71%[1] of the subsequent offspring. The proportion of subsequent offspring fathered by first males in NR matings was 0.84%[1] and in RN matings involving the apple maggot, Rhagoletis pomonella (Tephritidae), the second male fertilized 77.1%[2] of the subsequent eggs. In 20 NR matings, the second male fertilized 74.1%[2] of the

1 calculated from Curtis' (1968) raw data using Boorman and Parker's (1976) equations

2 calculated from Myer's et al. (1976) raw data using Boorman and Parker's (1976) equations

eggs (Myers et al. 1976).

Two Diptera exhibited a relatively lower level of sperm competition. The proportion of subsequent offspring fathered by the second male to mate in the olive fruit fly, Dacus oleae (Tephritidae), and Mediterranean fruit fly, Ceratitis capitata (Tephritidae), was approximately 50% to 60% (Cavallora and Debrio 1974; Katiyar and Ramirez 1970).

c) Hemiptera

In a study of the large milkweed bug, Oncopeltus fasciatus (Lygaeidae), Riemann (1974) found that in RN matings, second males fertilized 83.8% of the subsequent offspring, whereas in NR matings they fertilized only 28.7%. Based on these ambiguous results, Riemann hypothesized that sperm from the first male was displaced by the sperm of the second male to mate. Walker (1979) demonstrated a photoperiod effect on sperm competition in this species. Using a genetic marker, he found that second matings displaced 60% of the previous sperm when they occurred in early photophase, whereas if second matings occurred in late photophase they displaced 97%. Copulations were significantly greater in duration in late photophase.

In the giant water bug, Abedus herberti (Belostomatidae), males brood eggs and always copulate with females before receiving their eggs. Sperm precedence is almost complete. Experiments using a genetic marker in double matings showed than an average of 99% of subsequent offspring were fathered by the second male

to mate. By copulating with a female repeatedly before receiving her eggs, a male assures his paternity of the eggs he broods (Smith 1979a,b).

d)Hymenoptera

Holmes (1974) used eye colour as a genetic marker to investigate sperm competition in the parasitic wasp, Nasonia vitripennis (Pteromalidae). In double matings involving two virgin males, the first male to mate fertilized 98% of the subsequent offspring. However, when the first male or both were non-virgins, the data indicated random use of sperm, or occasionally a second-male predominance. Holmes suggested that mating with a virgin male resulted in the spermatheca of the female being filled, preventing the entry of any additional sperm. If a virgin female mates with a non-virgin male her spermatheca is probably not filled, allowing the inclusion of another male's sperm and sperm mixing.

e) Lepidoptera

Retnakaran (1971) used ^{3}H or ^{14}C- leucine labelled sperm to investigate the female multiple mating system of the spruce budworm, Choristoneura fumiferana (Tortricidae). By assaying the female abdomen for radioactivity, he was able to determine whether a double mating had occurred. By coupling this with sterilization techniques, he showed that most of the female's eggs were fertilized by the first male to mate.

However, more recently, Retnakaran (1974) demonstrated that the first male to mate was not always predominant in fertilizing subsequent eggs, and that this depended on the interval between matings. Of 15 doubly mated females, 6 produced offspring fathered solely by the first male, 6 produced offspring solely by the second male, and 3 produced a mixture of both. Male C. fumiferana transfer a spermatophore into the bursa copulatrix of a female during copulation. Initially this spermatophore is soft, but after approximately 30 minutes it becomes rigid. If a remating occurs before the spermatophore hardens, sperm of a second male displaces some of the first male's sperm. After some time, however, the female's sperm storage organs will be filled leaving no room for additional sperm from other males.

Labine (1967) found that in 6 of 9 double matings involving Euphydryas editha (Nymphalidae) females, the second male to mate fathered 100% of the offspring. In 3 other double matings, the first male was 100% predominant in fertilizing subsequent offspring. Similar results were obtained for Heliothis virescens (Noctuidae) by Flint and Kressin (1968). This probably indicates an "all or nothing" displacement of sperm by the second male to mate, depending on whether the remating occurs before or after the mating plug hardens.

Brower (1975) utilized black wings as a genetic marker to study sperm competition in the Indian meal moth, Plodia interpunctella (Pyralidae). In 30 crosses, he found that 77% to 98% of the subsequent offspring were fathered by the second

male to mate. He hypothesized a packing effect whereby older sperm are displaced upwards by the sperm of the last male to mate.

Etman and Hooper (1979) studied radiation induced sterility in Spodoptera litura (Noctuidae), and mated females to RN and NR combinations of males. In both instances, sperm was found in the spermatheca immediately after the second mating, at 30-45 minutes no sperm was found in the spermatheca, and one hour after the second mating, the spermatheca again contained sperm. Since it takes about an hour for sperm from a mating to reach the spermatheca, Etman and Hooper suggested that a second mating triggers a female physiological response resulting in expulsion of sperm of the first mating from the spermatheca.

Two Lepidoptera exhibited a relatively moderate level of sperm competition (predominance of second male=50% to 65%). These were the codling moth, Carpocapsa pomonella (Olethreutidae), and the fall armyworm, Spodoptera frugiperda (Noctuidae) (Proverbs and Newton 1962 and Snow et al. 1970). Sims (1979) mated only one female Papilio zelicaon (Papilionidae) with a normal and genetically marked male. Sperm from the second male was gradually favoured as the elapsed time from both matings increased.

f) Odonata

In the only Odonate studied, Waage (1979a,b) clearly delineated the mechanism of sperm competition in the damselfly, Calopteryx macubata (Calopterygidae). He found that singly and

doubly mated females did not differ with respect to the amount of sperm contained in their spermathecae. Field collected females (presumably non-virgin), interrupted in copula, had little or no sperm in their bursae. However, perching females, females flying in tandem with males, and recently mated females had significantly greater amounts of sperm in their bursae. These results, along with evidence from scanning electron microscopy studies, indicated that males, during an undulatory phase of copulation, use their penis to remove sperm deposited in a female's bursa copulatrix and spermatheca. Immediately thereafter they deposit their own sperm.

g) Orthoptera

Hunter-Jones (1960) utilized albinoism as a genetic marker to investigate sperm competition in the desert locust, Schistocera gregaria (Acrididae). In 4 GN matings, all subsequent offspring were normal in appearance and hence fathered by the second male. In 4 NG matings, all subsequent offspring were albinos.

In Locusta migratoria migratorioides (Acrididae), the last male to mate fertilized about 90% of the subsequent eggs in a one mating/one oviposition cycle. However, if a female mates with two males before a given oviposition, the second male fertilizes only 40% of the subsequent eggs. This probably occurs because the second male has difficulty everting a second spermatophore tube past the one left by the first male in the female's spermatheca (Parker and Smith 1975).

In the German cockroach, Blattella germanica (Blattidae), only 2% of mated females remate. In cases where females do remate, the first male almost always fathers all of the subsequent offspring. Cochran (1979) examined 50 double matings in which mutant females were mated first to mutant males, and then remated to normal males. Of the 49 egg cases produced, 48 left only mutant progeny, whereas only 1 produced both mutant and normal progeny.

Mechanisms by which males reduce sperm competition

In many of the insect species reviewed here, the last male to mate fertilized the majority of the subsequent offspring. Therefore, selection strongly favours mechanisms by which males reduce competition from the sperm of other males. Mechanisms by which males reduce sperm competition basically are of 4 types: mating plugs, prolonged copulation, passive phases, and mate guarding (Parker 1970a). Many examples of these already have been cited.

Mating plugs are secretions of the male accessory gland which block the genital orifices of females. They are common in many insect groups but most notably, the Diptera and Lepidoptera (Parker 1970a). However, in some cases, the last male to mate fertilizes the majority of the subsequent progeny if a remating occurs before the mating plug hardens (Labine 1967; Flint and Kressin 1968). In some species such as the spruce budworm, Choristoneura fumiferana (Lepidoptera; Tortricidae), the

spermatophore, after it hardens, functions as a mating plug (Retnakaran 1974).

A striking example of prolonged copulation which possibly serves as a mechanism to reduce sperm competition occurs in the lovebug, Plecia nearctica (Diptera; Bibionidae). In this species, copulations last on the average 51 hours, although maximal sperm transfer appears to require only 12.5 hours. Although these lengthy copulations are costly to males in terms of reduced feeding times, fewer copulations, and energy expenditures, these costs are likely offset by the benefits of a reduction in sperm competiton (Thornhill 1976c).

A passive phase is similar to prolonged copulation except that while the male remains attached to the female, there is no genital contact (Parker 1970a). This behaviour occurs in the dung fly, Scatophaga stercoraria (Diptera; Scatophagidae). After mating, a male terminates genital contact and raises his abdomen, allowing the female to oviposit. A male displays specialized reaction responses if the pair are approached by other males (Parker 1970c).

Mate guarding is a behaviour in which a male remains close, but not in contact with a female with whom he has recently mated (Parker 1970a). Mate guarding occurs in several species of crickets including Acheta domesticus (Orthoptera; Gryllidae) and Gryllus integer (Orthoptera; Gryllidae; Alexander and Otte 1967). Males attempt to stay in contact with females by watching and continually antennating them after mating.

4. Non-social factors affecting growth, development, and reproduction in crickets

The final section of this review details non-social factors affecting cricket growth, development, and reproduction. I first will describe the role of nutrition in cricket biology from the macro-nutrient to the micro-nutrient level, and then describe patterns of food utilization. This will comprise the majority of the discussion as nutrition has received the most attention of the non-social factors. Following this, other factors including population density and group effects, temperature, and ionizing radiation will be considered.

Gross nutritional effects

McFarlane *et al.* (1959) compared the growth of house crickets, *A. domesticus*, reared on 10 different artificial diets with the growth of those reared on commercially available rabbit pellets. For the artificial diets, they varied combinations and amounts of dextrose, cellulose powder, cholesterol, casein, and various vitamins. Cricket growth on rabbit pellets was greater than on any diet using purified chemicals. The average weight of nymphs for those fed rabbit pellets was 202 mg. For crickets reared on a chemical diet, 153 mg was the largest mean weight obtained and 11.5 mg, the smallest. The nymphal stage was of shortest duration for crickets reared on the pellets, an average of about 39 days. Of the 10 chemical and 1 commercial diets, crickets reared on rabbit pellets exhibited a 73% survivorship

to the adult stage, a 75% survivorship was recorded for the best chemical diet and 0% recorded for the worst.

Patton (1967) reared A. domesticus on different diets, varying protein, carbohydrate, and fat levels by mixing commercially available feeds. The greatest cricket growth and development was achieved when protein ranged from 20% to 30%, carbohydrate from 32% to 47%, fats (including sterol) from 3.2% to 5.2%, plus a normal complement of vitamins and minerals. Interestingly, McFarlane (1964) showed that growth of A. domesticus was not significantly altered when protein in the diet ranged from 10% to 50%. Patton's (1967) optimum diet consisted of 30% soybean meal, 25% standard wheat middlings, 15% powdered milk, 10% corn meal, 10% powdered brewers yeast, and 10% powdered animal liver.

Patton (1978) compared the growth of house crickets reared on his optimum diet with that of those reared on different commercial animal feeds. Growth was greater on the optimum diet than when crickets were fed chick mash, dog food, or rabbit pellets. After 6 weeks, nymphs reared on the optimum diet weighed, on average, 500 mg whereas on dog food, the worst commercial diet, they averaged 280 mg. Each female on the optimum diet laid from 1200 to 1500 eggs. The best commercial diet was chick mash with an average adult cricket weighing from 300 to 350 mg.

Richot and McFarlane (1962) examined the role of lipid in the growth, development, and reproduction of A. domesticus. They used a basic diet previously shown to be adequate for cricket

growth and development. Crickets were presented with 3 experimental diets: the basic diet; the basic diet plus 20 mg/g of wheat germ oil; and the basic diet plus 5 mg/g of linoleic acid. More rapid cricket growth was achieved on the wheat germ oil than either the basic or linoleic acid diet. Adult weight of crickets was larger for those reared on the wheat germ oil and linoleic acid diets than on the basic diet. The average fecundity of females maintained on the basic diet was 586 eggs with a 0% hatch. Average fecundity and percent hatch of females reared on the linoleic acid and wheat germ oil diets was 950 eggs with a 1.1% hatch, and 1319 eggs with a 53.6% hatch, respectively. These results indicated that dietary lipid was essential for cricket reproduction. Richot and McFarlane suggested that linoleic acid may have met the cricket reproductive requirements if it had been presented in greater amounts.

Meikle and McFarlane (1965) tested the constituents of wheat germ oil by single omission to determine those which were required for reproduction. Females reared on the basic diet plus 2% wheat germ oil exhibited a 55% egg hatch and those reared on the basic diet plus vitamin E (a constituent of wheat germ oil), a 57% hatch. When vitamin E was present with other constituents including linoleic acid, linoleic acid and oleic acid, females yielded approximately a 45% egg hatch. The spermathecae of females, mated to males that had been reared on diets containing no vitamin E, were examined and they contained negligible amounts of sperm. Females, mated with males

that had been reared on diets containing vatamin E, had full spermathecae. Meikle and McFarlane concluded that vitamin E was required for spermatogenesis in the male.

Additionally, when lipid starved females were mated with lipid starved males, the average female preoviposition period (period between adult emergence and first record of oviposition) was 26.4 days and the average number of eggs laid per day per female was 11.4. In comparison, when vitamin E was present in the diet of males and females, the average preoviposition period and mean number of eggs laid were 11.8 days and 31.5 eggs, respectively. Meikle and McFarlane suggested that this enhanced egg production may have been due to sperm or seminal fluid in the spermathecae, mediated directly by a chemical substance in the semen or by the mechanical effect of pressure in the spermatheca.

Dakshayani and Mathad (1973a) studied the effects of different commercial and artificial diets on growth, development, and survival in Plebeiogryllus guttiventris. The optimal diet was concentrated poultry feed (Royal Products®). Crickets reared on this diet weighed on average 101.9 ± 43 mg, and 83% survived to adulthood. Crickets reared on the worst diet, dog biscuits (Royal Products®), exhibited a mean weight of 29.0 ± 1.9 mg and a 27% survival. The Nutritional Index (average adult weight (mg) + % survival/ duration of nymphal stage (days) X 10) of the poultry feed was 73, and of the dog food, 27.

Tennis et al. (1979) examined the effect of food size on

the fitness of 2 size strains of A. domesticus. These strains were developed using an artificial mass selection technique. The smallest 5% of crickets and largest 5% were selected for 6 generations. The 2 strains were subjected to 3 different food sizes, small, large, and mixed food particles. For 0 to 14 days of the life cycle, both strains exhibited the least growth on the large food size and greatest growth on the small food size. Food size had no effect on the survivorship of the large strain. However, survivorship of the small strain was inversely related to the size of food.

Asay et al. (1975) used A. domesticus in a bioassay of forage quality in tall fescue. Twelve diverse genotypes of tall fescue were presented as cricket diets. Survival and weight of crickets were measured after 21 days. Survival on the different fescues ranged from 0% to 95% and weight, from 16 mg to 239 mg.

Micro-nutritional effects

Richot and McFarlane (1961) reared A. domesticus on the same basic diet previously shown to be adequate for cricket growth and development (McFarlane et al. 1959), minus 10 B vitamins. Each B vitamin was tested by single omission. When thiamine, pyridoxine, nicotinic acid, pantothenic acid, choline, and biotin were omitted, crickets exhibited poor growth and negligible survival to adulthood. When riboflavin, inositol and folic acid were omitted, crickets exhibited poor growth but fairly high survival. Only the omission of p-aminobenzoic acid

had no effect on cricket growth or survival.

After Meikle and McFarlane (1965) demonstrated the importance of vitamin E for spermatogenesis in male A. domesticus, McFarlane (1972a,b,c,d) further studied other nutritional effects of this vitamin. McFarlane (1972a) showed a pronounced effect of vitamin E on A. domesticus reproduction. Only 0.3% of eggs laid by females reared on a basic diet, developed, whereas 72.9% developed when females were reared on the basic diet plus 86 ug/g vitamin E. Generally, production of eggs was lower on the basic diet. The threshold for vitamin E activity on reproduction was approximately 17.2 ug/g, that is, the vitamin had no effect below this amount. However at 17.2 ug/g of vitamin E in the diet, McFarlane (1972b) found that 31% of adult males obtained were albinos. He suggested that vitamin E also may inhibit the phenolase system producing melanin. This suggestion further was supported when methyl laurate, which induces melanization or blackening of eggs, was applied to the surface of cricket eggs. When 5% vitamin E was added to the application, melanization was inhibited.

McFarlane (1974) investigated the copper requirements of house crickets and the metabolic relationship between copper and vitamin E. Copper alone increased the growth of crickets and optimal concentrations for males and females were 10 ug/g and 2 ug/g, respectively. When copper was tested in the presence of vitamin E, the average weight of adults obtained increased significantly. The sex difference with respect to copper

requirements primarily was due to the accumulation of copper in male testes. Vitamin E appeared to increase the male testes requirement for copper.

McFarlane (1976a) studied the effect of zinc and copper on house cricket growth with threshold amounts of vitamin E in the diet. Copper alone increased growth in males, but zinc had no effect. The presence of both copper and zinc in the diet resulted in the highest increase in male growth. This same trend was exhibited in females except that the beneficial effect of zinc was not as pronounced. McFarlane suggested that the higher male requirements of copper might demand a higher requirement of zinc, resulting in the observed sex difference. There appeared to be no clear connection of copper to reproduction except that when no dietary copper was presented, reproduction varied greatly from negligible to normal.

McFarlane (1976b) examined vitamin K as a natural dietary factor which might replace vitamin E in it's effect on A. domesticus growth and reproduction. McFarlane noted that vitamin K is abundant in many potential plant food sources. Cricket growth was greater with 18.7 ug/g of vitamin K in the diet than with the same amount of vitamin E. However, vitamin K had no effect on reproduction. The growth effect of vitamin K was further enhanced with the addition of optimal concentrations of copper and zinc (McFarlane 1978a).

Food utilization

Woodring et al. (1977) related feeding, growth and

metabolism to age in nymphal A. domesticus. Maximum growth occurred in the first half of the seventh and eighth instars, a period corresponding to maximal food and water consumption. The metabolic and locomotory rates in the first 2 to 3 days of each instar were twice that of the last 2 to 3 days. Percent total protein of crickets remained constant over each instar. Percent total lipids increased in the first half of each instar and then remained constant. However, total carbohydrates quadrupled during the first half of each instar and was then approximately halved towards the last day of each instar. Woodring et al. suggested that carbohydrates are utilized for maintenance energy when feeding declines, whereas lipids remain untouched.

Woodring et al. (1979) also examined food utilization in both female nymphal and adult house crickets. Growth during the last instar was entirely somatic whereas during the first 10 days of adulthood, virgin female growth was entirely gonadol. Almost all carbohydrates were used in energy production in both nymphs and adults. Proteins were utilized for somatic growth in nymphs, but in adults, contributed to both ovariole and somatic growth, and some energy production.

Population density and group effects

Tennis et al. (1977) separated the effects of population density and food surface area on body weight in A. domesticus. When food surface area was kept constant and population density

varied from 50, 200 and 400 crickets per microcosm, average adult weights obtained were 330 mg, 260 mg, and 225 mg respectively. Food surface area was tested at a population density of 200 crickets. When crickets were reared with the availability of 1, 2 or 3 petri dishes (petri dish diam.=9.0 cm), average cricket weights were 225 mg, 250 mg, and 290 mg, respectively.

McFarlane (1978b) studied the growth of nymphal A. domesticus as affected by the excretory products of nymphs. Jars were conditioned with nymphs for 12 to 14 days. Nymphs were kept singly or in groups of 10 and their growth compared with that of nymphs kept in clean jars. Singly and grouped nymphs in clean jars exhibited significantly greater growth at 9 days than their counterparts in conditioned jars. However, for both clean and conditioned jars, grouped nymphs exhibited greater growth than singly kept nymphs. This group effect, whereby individuals reared in small groups exhibit accelerated growth compared to that of those singly reared, was also demonstrated by Dakshayani and Mathad (1973b) for Plebeiogryllus guttiventris. Crickets were reared in 30 oz. jars in densities of 1, 5, 10, 15, and 20 individuals. The optimal density was 10 individuals with the shortest nymphal stage, 36.7 days, greatest average weight, 218.5 mg, and the highest survival, 80%. Singly reared individuals had an average nymphal stage of 45.9 days, weighed on average, 150 mg, and exhibited a 43.3% survival. Crickets reared in groups of 20 yielded results similar to that of singly reared individuals.

McFarlane (1966a) attempted to determine the type of mechanism through which the group effect might be mediated in A. domesticus. Methyl linolenate, a substance attractive to nymphs, and methyl linoleate, a substance chemically similar to methyl linolenate, were absorbed on filter paper and placed in jars containing single nymphs or groups of 10 nymphs. Methyl linolenate significantly shortened the nymphal development time of singly reared individuals, but reduced the survivorship of group reared nymphs. Although vitamin E normally increases cricket growth, when fed to crickets exposed to methyl linolenate conditioned paper, it's effect was negated. However, when vitamin E was not present in the diet, the average weight of adults obtained was significantly greater when nymphs were reared in groups of 5, as opposed to being singly reared.

Because methyl linolenate was shown, to some extent, to mimic the group effect, McFarlane (1966b) suggested that the group effect might be mediated by a chemical substance, namely a pheromone . The possibility that a fatty acid and/or fatty acid methyl ester was involved first was indicated by McFarlane and Henneberry's (1965) study of growth inhibition of Gryllodes sigillatus by representatives of this chemical group. Various fatty acids and their methyl esters were absorbed on filter paper and placed in jars each containing 10 newly hatched individuals. Of the methyl esters tested, methyl palmitate and methyl oleate significantly inhibited growth and caused reduced survivorship, while methyl stearate and methyl myristate caused

some growth inhibition. A few methyl esters prolonged the female nymphal period. Of the fatty acids, only myristic acid proved growth inhibitory, while other fatty acids tended to lengthen the nymphal development period. These substances probably entered nymphs through the cuticle of the tarsi, a suggestion subsequently confirmed (McFarlane 1972d). McFarlane and Henneberry (1965) suggested that fatty acids and their methyl esters serve as an energy reservoir, and also might function in the control of growth and development.

McFarlane (1966b) repeated similar experiments with methyl laurate. The interaction of the temperature at which A. domesticus nymphs were reared and the concentration of this methyl ester modified the intensity of the group effect. The group effect was more promounced at 29°C than at 35°C. At 29°C, a concentration of 0.040M methyl laurate increased the weight of singly reared nymphs. At a concentration of 0.053M, methyl laurate increased the weight of singly reared males, but decreased the weight of singly reared females. McFarlane (1966c) showed that when methyl palmitate was added to rearing jars, survival of group reared nymphs was reduced, but not that of singly reared nymphs. McFarlane considered this further evidence that fatty acids and/or their methyl esters were involved in the action of some developmental pheromone, since the group effect tended to magnify the toxicity of methyl palmitate.

McFarlane (1968), in an extensive review, summarized the importance of fatty acids and their methyl esters to insect

growth and development. He reiterated that because some methyl esters increase growth in singly reared but not group reared crickets, this indicated that the group effect was chemically mediated. He also noted that in some insects, free fatty acids and their methyl esters are present on the surface of the cuticle.

Recently, Watler (1979) noted a group effect on A. domesticus female reproduction. Grouped females began laying eggs significantly earlier than females that had been isolated during development. Grouped females began ovipositing 4.80 ± 0.83 days after adult emergence, whereas isolated females began ovipositing 5.59 ± 1.39 days after adult emergence. Watler suggested that vitallogenesis proceeded faster in grouped females.

Temperature

Ghouri and McFarlane (1958) investigated the effects of temperature on A. domesticus longevity, fecundity, and survival. Females reared at 28°C had an average longevity of 64 days and produced, on average, 728 eggs. Females reared at 35°C lived on average 57 days and produced a mean of 1060 eggs. Crickets reared at 23°C, 33°C, and 41°C exhibited 42%, 88%, and 10% survival to adulthood, respectively.

Bate (1972) collected A. domesticus in January and September. In each rearing jar were placed one male and female, and crickets were kept at either 26.5°C or 35°C. Temperature had no

significant effect on fecundity for both seasonal collections. However, females collected in January produced significantly more eggs than those collected in September, at both temperatures.

Hoffmann (1974) found that for Gryllus bimaculatus, the greatest longevity was attained when crickets were kept at 20°C. However, maximum egg production (1000 eggs/female) occurred at 34°C with a 53% hatching success.

Ionizing radiation

Hunter and Krithayakiern (1971) examined possible injuries or benefits of ionizing radiation using various doses of gamma irradiation to observe effects on longevity and reproduction in house crickets. Adult crickets were irradiated at 3 days of age. The control crickets lived, on average, 37.8 days. However, females exposed to 500R, 1000R, and 2000R had average longevities of 59.1 days, 56.4 days, and 48.7 days, respectively. Male longevity was unaffected in this range of irradiation. Cricket longevity dropped to 9.5 days at 4000R. Fecundity was reduced in the 500R to 4000R range, dropping from an average of 304 eggs for the control group to 44.9 eggs for crickets exposed to 500R. Crickets exposed to doses greater than 4000R laid no eggs. The percent of eggs hatching was reduced immediately at 500R, and no eggs hatched when crickets were exposed to 1000R or greater.

METHODS OF STUDY

Immature A. domesticus, purchased from Armstrong's Cricket Farm, Monroe, La., were housed in a fibreglass chamber containing ample food (Purina® Mouse Chow), test tubes containing water and plugged with cotton, and layered egg cartons for cover. Late instar females were removed and held separately thus ensuring virginity upon the final molt. Adult females were considered 1 day old on the day they were found to have molted. Adult and late instar G. integer were collected at the Brackenridge Field Laboratory of The University of Texas at Austin in August, 1978. They were kept in individual containers (0.58 l), brought to Canada and treated identically to A. domesticus. First generation G. integer of the initial stock also were used. Nymphs were obtained by allowing adult females to oviposit in metal trays which contained a layer (5 cm) of moistened fine vermiculite. Nymphs were provided with ample food, water and cover. All crickets were maintained at 26-31°C with a 12 hour light: 12 hour dark photoperiod.

Ontogeny of female sexual behaviour

To determine the age at which females first mate, one day old virgin A. domesticus were marked distinctively by coating the pronotum of each individual with Liquid Paper® and writing a number thereon. Females were observed for 15 consecutive days in a plastic mating arena (78 x 49 x 10 cm). A jar lid which

contained crushed mouse chow, another which contained moistened vermiculite for oviposition, and 2 test tubes of water plugged with cotton were placed in the arena. On a given day, the number of females in the arena ranged from 15-20 depending on the molting frequency and the death of some females. The same number of sexually mature males were placed in the arena each day and observed for 2 hours under red lights, 3 hours into the dark portion of the photoperiod. Several replicates of this procedure were performed. The number of matings over 15 days was recorded for each female. Females often mounted males without transfer of the spermatophore. These were designated abortive matings. A mating was considered successful, however, only if the spermatophore was clearly inserted into the bursa copulatrix of the female. The time the spermatophore remained attached was determined by repeatedly locating (approximately every 2 min.) recently mated females. Additionally, the frequency of abortive matings and spermatophore consumption, and all deaths occurring in the mating chamber were recorded.

To determine the onset of positive phonotactic response to male calling song in female A. domesticus, 28 females were tested using tape-recorded A. domesticus song. One day old virgin females were housed in a separate terrarium without males. Trials were conducted one hour into the dark portion of the photoperiod, in a circular arena (105 cm(diam) x 15 cm (ht)) constructed of expanded polystyrene (Styrofoam®). The arena contained two Phillips AD 0160/T8 loudspeakers embedded in the

wall of the arena at a height of 3 cm and two red lights at 90° to the speakers. A Sanyo M2211 cassette tape recorder broadcasted previously recorded A. domesticus calling song through one loudspeaker at 65-75 dB (A scale) as measured with a General Radio Sound Level Meter - 1565B held 10 cm in front of the loudspeaker. This sound intensity was consistent with previous measurements of A. domesticus males calling in the laboratory. Recordings were made with a Uher 240 CR stereo cassette tape recorder and a Uher M640 microphone on a BASF 60 magnetic cassette. In each trial, a single female was placed under an inverted vial in the centre of the arena. After a 5 min period in which the female was allowed to become quiescent, the beaker was removed and broadcasting of taped song commenced. A trial lasted until a positive response was recorded, until 5 minutes had elapsed, or until the female left the arena. A positive response involved a female remaining in a marked area (15 cm x 11 cm) under the speaker and/or on the speaker for a total duration of 1 min of the 5 min trial. All females were tested every day until a positive response was recorded, and 9 of the 28 females were tested for 15 consecutive days regardless of the age at which they first showed positive phonotaxis.

Female isolation and spermatophore attachment

To test the effects of male presence on female retention of the spermatophore, 30 female A. domesticus from another group of crickets were removed from the observation arena immediately

after mating and isolated from males. Their spermatophore attachment times were compared with 30 females which remained with males in the arena after mating. Females of both groups were 5 day old virgins before mating.

Nutrition and progeny production in house crickets

To determine the effects of varying nutrition on relative progeny production in female A. domesticus, virgin females were randomly assigned to the following 3 nutritional classes: 30 females fed on Purina® mouse chow, a high protein diet; 30 females allowed to cannibalize female conspecifics; and 30 starved females. At five days of age, virgin females were placed in the mating chamber with sexually mature males and allowed to mate only once. To test the effect of the consumption of one spermatophore on progeny production within each nutritional category, approximately half of the females within each class were allowed to consume the attached spermatophore, whereas the remaining females had their spermatophores removed with forceps. Spermatophore attachment times between groups were equalized by removing spermatophores at times approximating those of females allowed to consume their attached spermatophores.

Mated females were housed in individual, plastic-coated cardboard containers (0.58 l) and placed on their respective diets. Each container had a layer (2 cm) of moistened fine vermiculite for oviposition and cotton-plugged test tubes containing water. Females fed mouse chow had their food contained

in bottle caps that were replaced every 3 days. Female cannibals were each presented with a female conspecific every 3 days. The legs of crickets fed to females were removed to facilitate more readily cannibalistic activity on the part of the experimental female. In each container, fungal growth on both food and faecal matter was removed when necessary by gently scraping the surface of the vermiculite, so as not to disturb any deposited eggs.

In all categories, nymphs were first seen in the containers at about 3 weeks. Nymphs were mouth aspirated and counted daily. To test whether females adjust the sex ratio of their offspring under severe nutritional stress, approximately 200 offspring of starved females were cultured to the third instar and their sex ascertained with the use of a Lietz dissecting microscope. As a control, some of the offspring of females fed mouse chow were also reared and their sex determined. The date of death for all experimental females was recorded to observe the effects of varying nutrition on female longevity.

Constant access to male and female reproduction in house crickets

To test the effects of allowing females constant access to males on relative progeny production, 2 groups of females provided with mouse chow or corn meal, were established. In one group, 30 newly molted, adult females each were placed in individual containers with one adult male and provided with mouse chow as a food substrate. Each female was kept with the same male until the male died, in which case, he was replaced. On the death

of the female, the male was removed. In the other group, 30 females, provided with corn meal, were treated identically to those fed mouse chow with the single exception that males were replaced in the containers every three days.

All containers were maintained in the same manner as those of the previous experiment. Nymphs were mouth aspirated and counted daily. Dates of death were recorded for all females. Comparisons of female progeny production between singly mated females of the previous experiment and those here, were made.

Mating frequency and progeny production in field crickets

To determine the relative progeny production of females mated 1 or 2 times, virgin female G. integer were randomly assigned to classes for which 1 or 2 matings were planned. This was arranged for 2 groups of females, a small group maintained on a corn meal substrate and a large group fed high protein mouse chow.

In the group maintained on corn meal, females were housed individually and each presented with a sexually mature, adult male the day they molted. Spermatophore attachment times were equalized by removing the spermatophore with forceps, approximately 30 min after a female mated. Females of the double mating group were each presented with a male one day after their first mating and each subsequent day until they remated.

Essentially the same procedure was followed with G. integer females fed mouse chow with the following exceptions. Three days

after their first mating, females of the double mating group were transferred to new oviposition containers and then each presented with a second male. These females were also used in an investigation of sperm competition (see next section). Additionally, females of the double mating group that did not remate within periods of observation were permitted constant access to a male for 24 hours. The progeny produced by these females were treated for purposes of comparison as a distinct experimental group.

In both nutritional classes, nymphs first appeared in their containers at about 3 weeks. Nymphs were mouth aspirated and counted daily.

Sperm competition in field crickets

To determine the pattern of sperm utilization in multiply mated female G. integer, virgin females were each mated to 2 different males. Females were transferred to new oviposition containers 3 days after their first mating, so that any subsequent offspring appearing in the first container could be attributed to the first male to mate. This permitted the detection of infertile first matings which if unnoticed, possibly might have obscured later interpretation. All males and females were marked clearly, and a record of mating combinations and sequences was kept (see Appendix 3). Every day, offspring were collected from oviposition containers and placed in separate

plastic vials, marked so as to identify their maternity, date of emergence, and oviposition container from which they originated (1st or 2nd). They immediately were stored in an ultra-freeze at -76°C until further analysis. Similarly, all experimental adults were frozen after they died.

Acrylamide vertical slab-gel electrophoresis (Davis 1964; Ormstein 1964) was used to survey genetically polymorphic enzymes in the parental G. integer. Specifically, an attempt was made to detect mating combinations in which the 2 males were of different genotypes. Only matings of this type would allow the paternity of the offspring to be established.

Gels were prepared using 60 ml of 10% W/V Cyanogum (Sigma Chemical Co) in 0.1M Tris Borate buffer and 60 ml of 0.1M Tris Borate EDTA (gel buffer). Added to this mixture were the polymerization catalysts TMEDA (0.5 ml), and ammonium persulfate (1.5 ml). Nymphs were individually homogenized in gel buffer containing 5% sucrose. For adult G. integer, body parts including the head, thorax and legs, and whole individuals were homogenized. Homogenates were injected directly into pockets of the gel, which allowed placement of 24 samples.

Samples generally were electrophoresed for 30 minutes at 50V, and then for 3½ hours at 280V. The following enzymes were examined: gross protein, malic dehydrogenase (MDH), alcohol dehydrogenase (ADH), lactic dehydrogenase (LDH), various esterases, and amylase. Only 1 or 2 gels for each of the first five enzymes were utilized, for reasons described in the results section. For

specific details on their electrophoretic times and staining procedures, see Appendix 4. However, approximately 50 gels (1200 samples) were run for esterase and amylase enzymes. For the esterase assay, the gel was incubated in 0.5M Boric acid for 20 min and then rinsed with water. It then was stained for approximately 16 hours in a solution consisting of 40 mg of β Napthyl acetate in 1 ml of 50% acetone, and 100 mg of fast blue TRN in 100 ml of 0.1M phosphate buffer (pH 6.5), mixed together. The procedure for amylase consisted of incubating the gel in a solution consisting of 135 ml of H_2O, 15 ml of 1.0 Tris Borate buffer (pH 7.5), and 3 g of starch for 1½ hours. After rinsing, the gel was stained with a solution of 8 ml of 0.1M IKI and 150 ml of H_2O, for about 5 min (until light bands appeared). All gels were fixed after appropriate staining in a 5:5:1 mixture of methanol: water: acetic acid (glacial).

RESULTS

Parameters of female sexual behaviour

Female A. domesticus mated for the first time at an average age of 6.9 days (Fig. 1). They mated an average of 2.8 times over the 15 day observation period (Fig. 2) and after reaching an age of 5 days, the percentage of females mating at any given age remained constant (Fig. 3). One female that did not have any successful matings in 15 days had 8 abortive matings, 2 of these occurring in the same observation period. This was also the case with another female for which 5 abortive matings were recorded. Of 364 instances in which females were observed to mount males, 127 or 34.9% resulted in abortive matings. Abortive matings occurred at more or less the same frequency for females of different ages.

The average interval between first and second matings for female A. domesticus was 3.0 days (Fig. 4). In 11 instances, females had both an abortive mating and a successful mating in the same observation period. In 8 of these, the abortive mating occurred before the successful mating. On one occasion, a female successfully mated twice in the same observation period and following this had an abortive mating.

The average duration of spermatophore attachment for female A. domesticus that mated during the 15 day observation period and lived for the entire duration was 29.3 min (N=189;

Figure 1. Frequency distribution of the age at which female *A. domesticus* first mate.

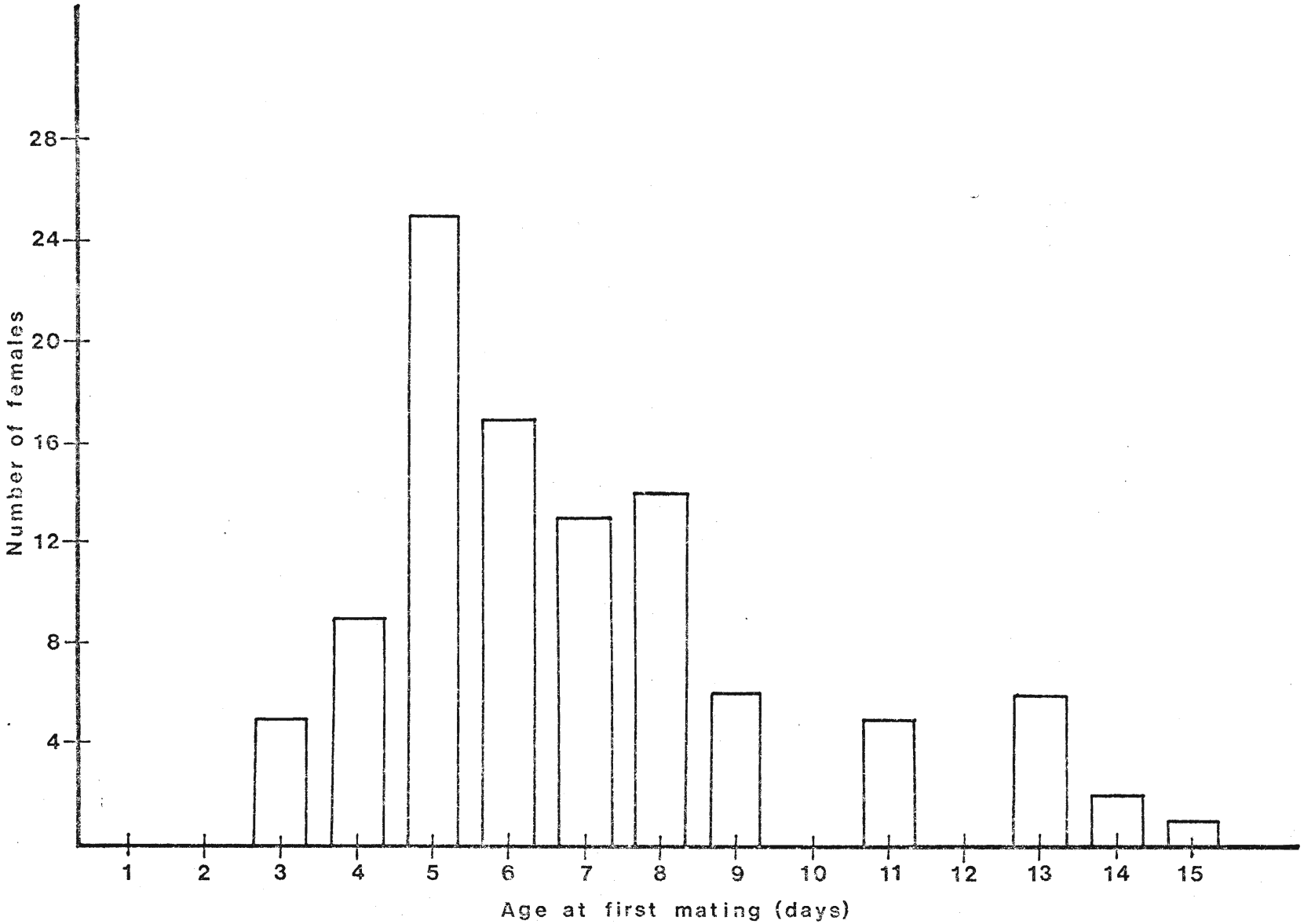

Number of females
28
24
20
16
12
8
4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Age at first mating (days)

Figure 2. Female mating frequency distribution for A. domesticus observed 2 h for 15 nights.

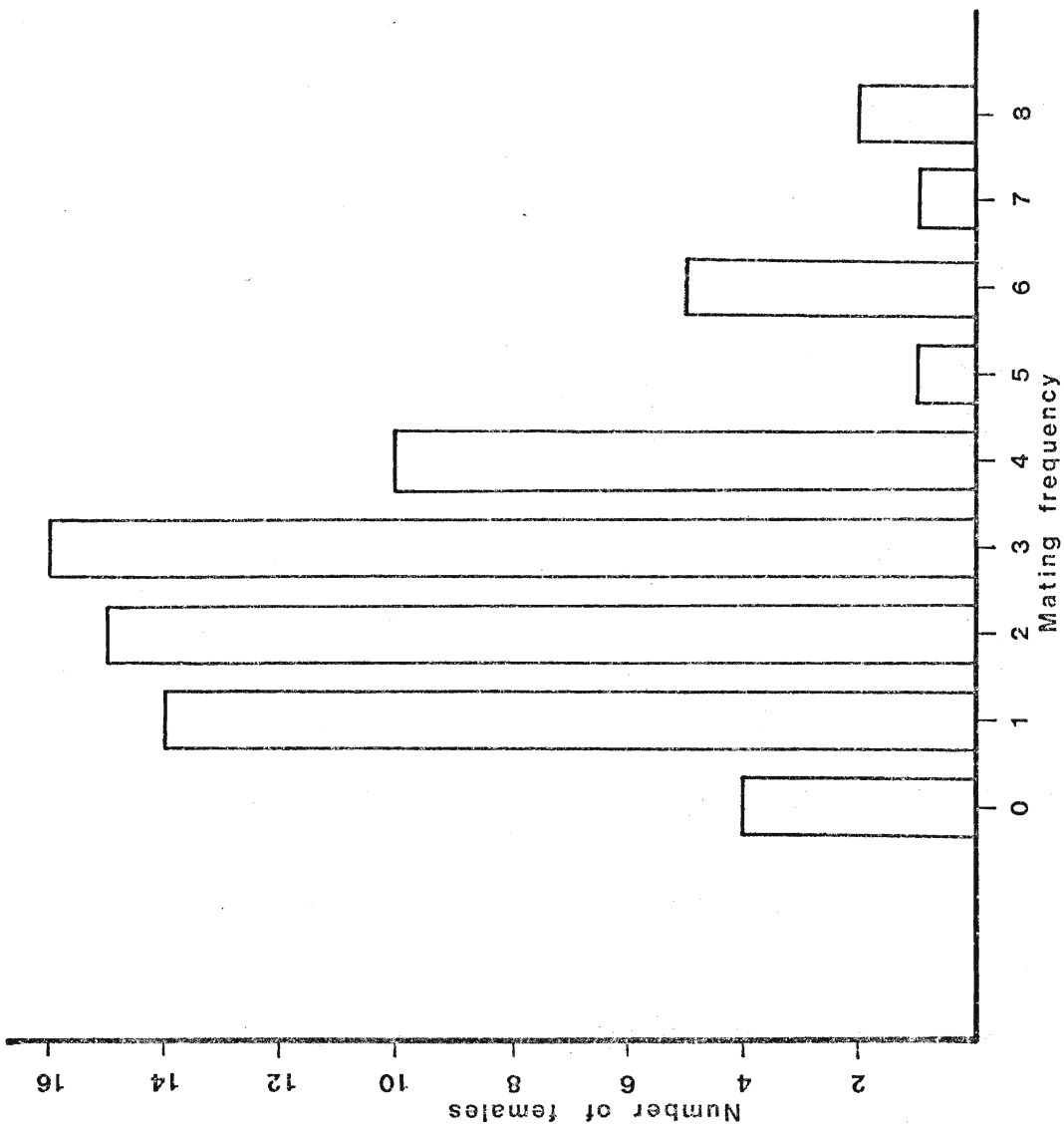

Number of females
2
4
6
8
10
12
14
16
Mating frequency
0
1
2
3
4
5
6
7
8

Figure 3. Frequency distribution of the percent of females mating at any given age for A. domesticus observed 2 h for 15 nights.

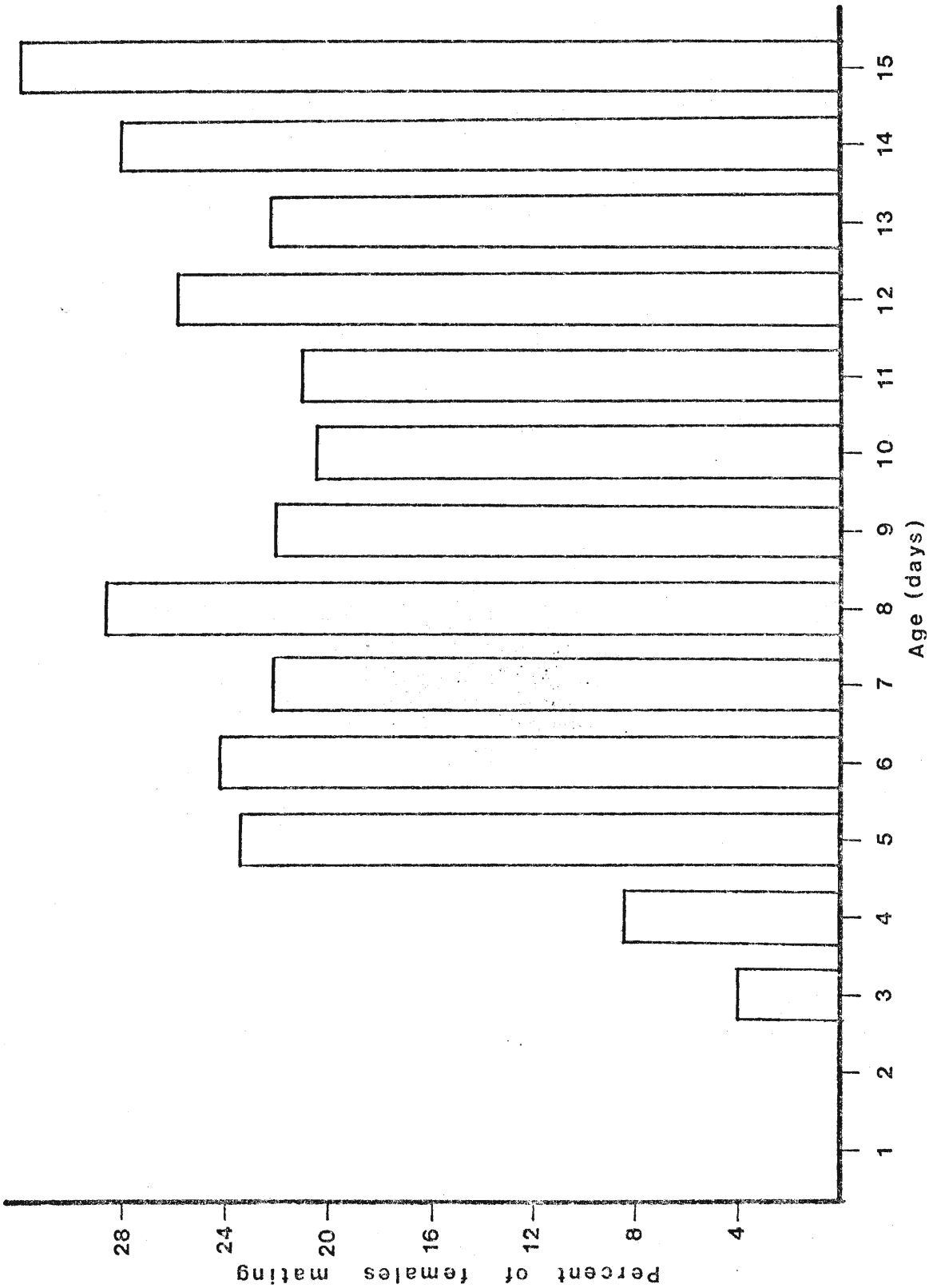
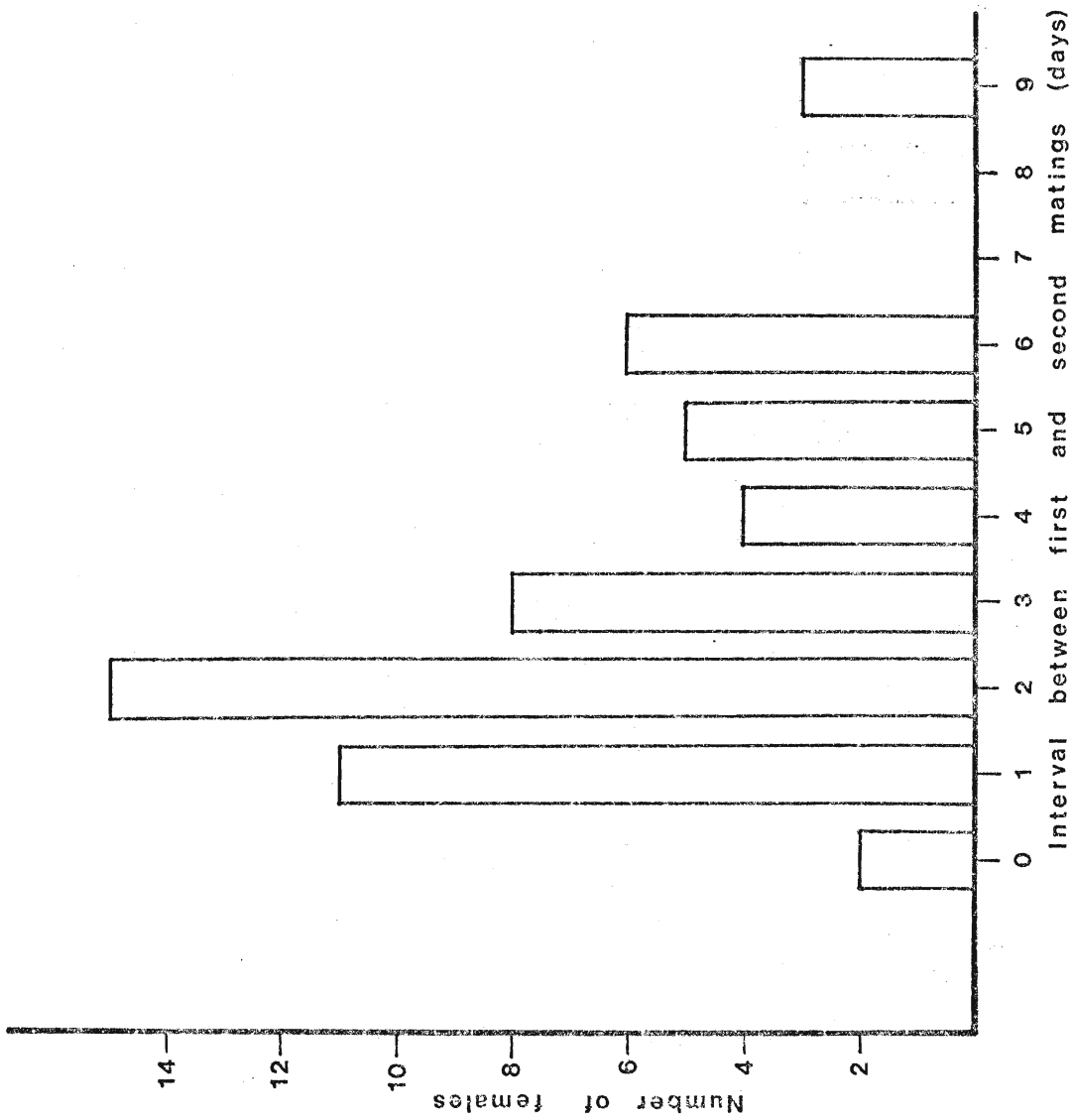

Percent of females mating
28
24
20
16
12
8
4
Age (days)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figure 4. Frequency distribution of the interval between first and second matings for female A. domesticus observed 2 h for 15 nights.


Number of females
2
4
6
8
10
12
14
0
1
2
3
4
5
6
7
8
9
Interval between first and second matings (days)

range=1-116; SD=22.2). Females ate their spermatophores after 178 matings, but in 35 other cases the spermatophore was dislodged against the substrate. (These data include some females that were in the arena less than 15 days and that are not represented in Fig. 2.). In 2 matings a female other than the one copulating was observed chewing on the male's extruded spermatophore. Data on the time the spermatophore was attached after the first mating and the age at which females first mated are summarized in Table 1. Although the average duration of spermatophore attachment increased with the age at which females first mated, this trend was not significant (one way ANOVA; $F=0.90$; $p > 0.05$). Additionally, the average durations of spermatophore attachment for first, second, third, and fourth through eighth matings were compared (Table 2), but no significant trend was observed (one way ANOVA; $F=1.46$; $p > 0.05$). Data on the time the spermatophore was attached after the first mating for females and the elapsed time until they remated are shown in Table 3. Females whose second mating was on the same or following day as their first mating had much lower spermatophore attachment times than did those females that remated on the second, third or fourth, and fifth through ninth day after the initial mating (one way ANOVA; $F=8.26$; $p < 0.001$).

Although many successfully mated female A. domesticus were observed ovipositing over the 15 day observation period, some were not. On several occasions, females were observed ovipositing subsequent to mating in the same observation period. No females

Table 1 The relationship between the age at which females first mate and the time the spermatophore was attached after mating for female A. domesticus.

Age at which females first mate (days)	N	Spermatophore attachment time (min)		
		$\bar{X}^*$	SD	Range
3-5	31	29.19	23.38	3-79
6-8	40	31.60	26.79	1-116
> 9	19	41.68	29.63	3-99

*ANOVA $F = 0.90$; $p > 0.05$

Table 2 The duration of spermatophore attachment for first, second, third, and fourth through eighth matings for female A. domesticus.

nth mating	N	Spermatophore attachment time (min)		
		$\bar{X}^*$	SD	Range
first	91	32.86	26.13	1-116
second	46	28.48	18.53	2-104
third	27	20.96	14.03	2-48
fourth-eighth	34	27.41	18.13	1-74

*ANOVA $F = 1.46$; $p > 0.05$

Table 3 The relationship between the number of days between the first and second matings for female *A. domesticus* and the time the spermatophore was attached after the first mating.

Nights between 1st and 2nd matings	N	Initial Spermatophore Attachment Time (min)		
		$\bar{X}$*	SD	Range
0-1	13	14.07	11.72	1-30
2	15	47.60	35.61	11-116
3-4	12	49.50	28.85	16-99
5-9	14	34.14	30.37	7-99

*ANOVA $F=8.26$; $p<0.001$

that did not mate successfully at least once over the 15 day observation period were observed ovipositing.

Thirty-four female deaths were recorded in the mating chamber and many males also died. Several deaths resulted from cannibalism by conspecifics of both sexes. Prior to the initiation of a new observation period, partially eaten or whole dead crickets often were found.

The results of the experiment to determine the onset of phonotactic response to male calling song in female A. domesticus are detailed in Fig. 5. Virgin females first became acoustically receptive at an average age of 5.4 days. The average number of positive phonotactic responses exhibited by the 9 females that were tested over 15 consecutive days was 7.78 (SD=2.22; range= 5-11).

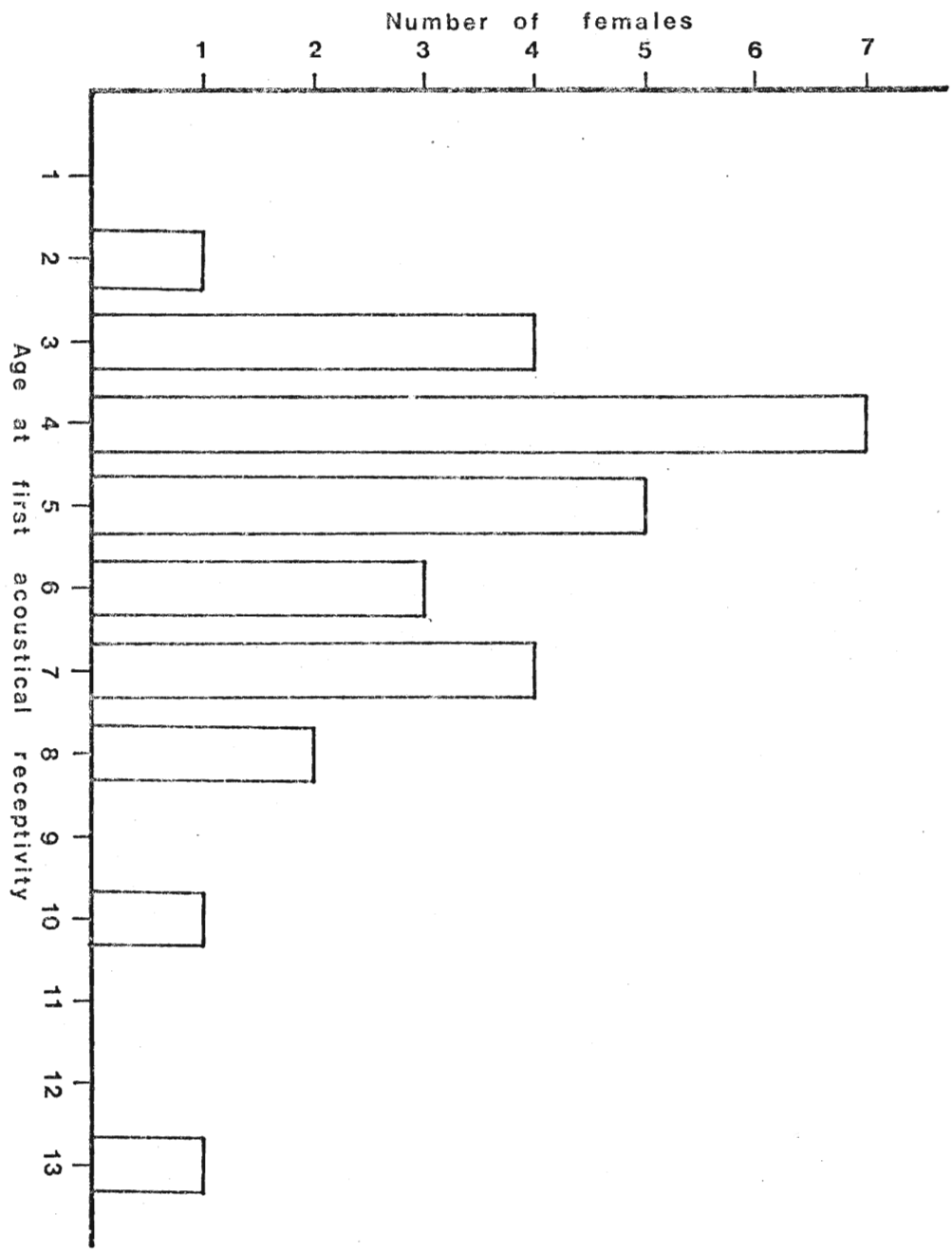
Female isolation and spermatophore attachment

For the 60 five-day-old virgin female A. domesticus, those isolated from males immediately after mating had an average spermatophore attachment time of 38.3 min (SD=30.3; range=10-107). Females that remained with males did not differ significantly with respect to spermatophore attachment time ($\bar{X}$=36.0 min; SD= 25.4; range=3-99).

Nutrition, constant male access, and offspring production in female A. domesticus

Results comparing the reproduction of female A. domesticus

Figure 5. Frequency distribution of the age at which female *A. domesticus* first show a positive phonotactic response.


Number of females
1
2
3
4
5
6
7
Age at first acoustical receptivity
1
2
3
4
5
6
7
8
9
10
11
12
13

maintained on different diets, and either mated once or allowed constant access to a male, are summarized in Table 4. Comparisons of experimental groups are shown which both include and exclude reproductive failures (females leaving no progeny). The statistical analysis was applied to groups excluding the reproductive failures because: 1) it was not known whether reproductive failures of females allowed constant access to males had mated; and 2) it could not be determined whether reproductive failures of singly mated females had been inseminated. Females allowed constant access to a male and maintained on mouse chow produced significantly more offspring than singly mated females fed mouse chow ($t=2.09$; $p< 0.025$ on log transformed to normal data).Considering singly mated females only, nymph production of those fed mouse chow was significantly greater than that of cannibalistic or starved females ($t=2.61$; $p< 0.01$ and $t=5.74$; $p< 0.001$). Cannibals left significantly more nymphs than starved females ($t=3.04$; $p< 0.005$). Singly mated females maintained on mouse chow and cannibals left significantly more offspring than females fed corn meal even though females fed corn meal were allowed constant access to males ($t=5.62$; $p< 0.001$ and $t=3.65$; $p < 0.005$).

Of the 215 offspring of starved females that were cultured to an instar at which their sex could be ascertained, 115 males and 100 females were obtained. This result did not differ significantly from a 50:50 sex ratio ($X^2=0.536$; $p< 0.05$). Offspring of females maintained on mouse chow also were examined and of 32

Table 4A The number of early instar nymphs produced per female A. domesticus : 1) maintained on different diets; and 2) either mated once or allowed constant access to a male.

[a]Nutritional and mating status	N	Number of progeny including reproductive failures			% reproducing
		$\bar{X}$	SD	Range	
A. fed mouse chow; 1 mating	30	444.53	348.71	0-989	86.67
B. cannibals; 1 mating	30	180.03	148.79	0-533	90.0
C. starved; 1 mating	30	56.40	41.64	0-130	83.33
D. fed mouse chow; constant access	30	571.40	489.98	0-2164	76.67
E. fed corn meal; constant access	30	52.83	64.20	0-206	80.0

[a] see Appendix 6

Table 4B The number of early instar nymphs produced per female A. domesticus: 1) maintained on different diets; and 2) either mated once or allowed constant access to a male.

Nutritional and mating status	N	Number of progeny excluding reproductive failures		
		$\bar{X}$	SD	Range
A. fed mouse chow; 1 mating	26	512.92[a,b]	323.38	5-989
B. cannibals; 1 mating	27	200.04[c]	143.31	5-533
C. starved; 1 mating	25	67.68	36.05	3-130
D. fed mouse chow; constant access	23	745.30[d,e]	425.40	81-2164
E. fed corn meal; constant access	24	68.91	65.37	1-206

[a] significantly greater than B at $p < 0.01$
[b] significantly greater than C, E at $p < 0.001$
[c] significantly greater than C, E at $p < 0.005$
[d] significantly greater than A at $p < 0.025$
[e] significantly greater than B, C, E at $p < 0.001$

that were reared, 19 males and 13 females were obtained. This result also did not differ significantly from unity ($X^2=1.25$; $p > 0.05$).

Data regarding the effect of the consumption of one spermatophore on progeny production within each nutritional category are presented in Table 5. For all three nutritional classes, singly mated females allowed to consume the externally attached spermatophore did not differ significantly in offspring production from those whose spermatophores were removed.

Results comparing the time after mating at which offspring first appeared in the oviposition containers of singly mated females of different nutritional categories are shown in Table 6. The time at which offspring of singly mated females first appeared did not differ significantly between the three nutritional categories and on average, offspring first emerged 22.63 days after a mating. For females allowed constant access to males, the time at which offspring first appeared in the containers after females were enclosed, also is shown. The offspring of females maintained on corn meal and allowed constant access to a male appeared significantly later in the containers than did the offspring of females fed mouse chow ($t=8.63$; $p< 0.001$ on log transformed to normal data).

Results detailing the number of days over which offspring hatched for female A. domesticus of different nutritional and mating categories are shown in Table 7. There was no significant difference in the duration over which offspring hatched for

Table 5 The number of early instar nymphs produced per female *A. domesticus* either allowed to consume the attached spermatophore or whose spermatophore was removed. Three nutritional categories are presented.

Nutritional Category	N	Number of Offspring $\bar{X}$	SD	Range	p
Cannibals					
ate spermatophore	8	270.50	166.18	88-533	>0.05
spermatophore removed	19	170.37	131.63	3-395	
Reared on Mouse Chow					
ate spermatophore	13	459.69	301.48	54-972	>0.05
spermatophore removed	13	566.15	363.06	5-989	
Starved					
ate spermatophore	12	70.08	37.85	18-151	>0.05
spermatophore removed	13	65.46	35.70	3-130	

Table 6 The time after a mating at which nymphs first appeared in the oviposition container for female A. domesticus: 1) maintained on different diets; and 2) either mated once or allowed constant access to a male.

Nutritional and mating status	N	Days post-mate when offspring first emerge		
		$\bar{X}$	SD	Range
fed mouse chow; 1 mating	26	22.08	3.22	19-32
cannibals; 1 mating	27	23.00	3.67	17-28
starved; 1 mating	25	22.80	3.53	18-31

Nutritional and mating status	N	Days after females enclosed in containers when offspring first appear		
		$\bar{X}$	SD	Range
fed mouse chow; constant access	23	23.70	4.94	19-38
fed corn meal; constant access	24	34.54*	3.75	26-40

* $p < 0.001$

Table 7 The number of days over which offspring hatched for female *A. domesticus*: 1) maintained on different diets; and 2) either mated once or allowed constant access to a male.

Nutritional and mating status	N	Duration of nymph production (days) $\bar{X}$	SD	Range
A. fed mouse chow; 1 mating	26	22.11[a,b,c]	12.18	3-46
B. cannibals; 1 mating	27	13.89[d]	6.97	4-26
C. starved; 1 mating	25	6.88	2.64	4-14
D. fed mouse chow; constant access	23	25.70[e]	8.61	6-44
E. fed corn meal; constant access	24	11.96	7.57	1-24

[a] significantly greater than B at $p < 0.025$
[b] significantly greater than C at $p < 0.001$
[c] significantly greater than E at $p < 0.005$
[d] significantly greater than C at $p < 0.001$
[e] significantly greater then B, C, E at $p < 0.001$

singly mated females fed mouse chow and females fed mouse chow and allowed constant access to a male ($t=1.62$; $p > 0.05$ on log transformed to normal data). However, the duration over which offspring hatched for singly mated females fed mouse chow was significantly greater than that of cannibalistic and starved females ($t=2.21$; $p < 0.025$ and $t=6.00$; $p < 0.001$). The duration of offspring production for cannibalistic females was significantly greater than that of starved females ($t=5.04$; $p < 0.001$). Additionally, singly mated females fed mouse chow left offspring over a significantly longer period than females fed corn meal, even though the latter group of females was allowed constant access to males ($t=3.05$; $p < 0.005$).

Patterns of daily offspring production of females of different nutritional and mating categories are shown in Figures 6-10. Day 1 of average offspring production represents the first day that offspring emerged in the oviposition containers. Although the duration over which offspring hatched did not differ significantly between singly mated females fed mouse chow and females allowed constant access to males and fed mouse chow (Table 7), on a daily basis, females of the latter group (Fig. 6) left consistently more offspring than singly mated females (Fig. 7). The day of progeny production at which the average number of offspring produced was greatest, occurred at 2 days for both experimental groups. Considering singly mated females only, the average number of offspring produced each day by females fed mouse chow (Fig. 7) consistently was greater than that of

Figure 6. The average number of offspring produced per day by female A. domesticus maintained on mouse chow and having constant access to a male. Closed bars represent 95% confidence intervals. Days 0-3 (1 day post-peak offspring production) are described by: $Y=51.20 + 7.88 \ln(x)$ ($n=69$; $r=0.115$; $p > 0.1$). Days 3-26 are described by: $Y=54.65 - 1.89x$ ($n=474$; $r=-0.478$; $p < 0.001$).

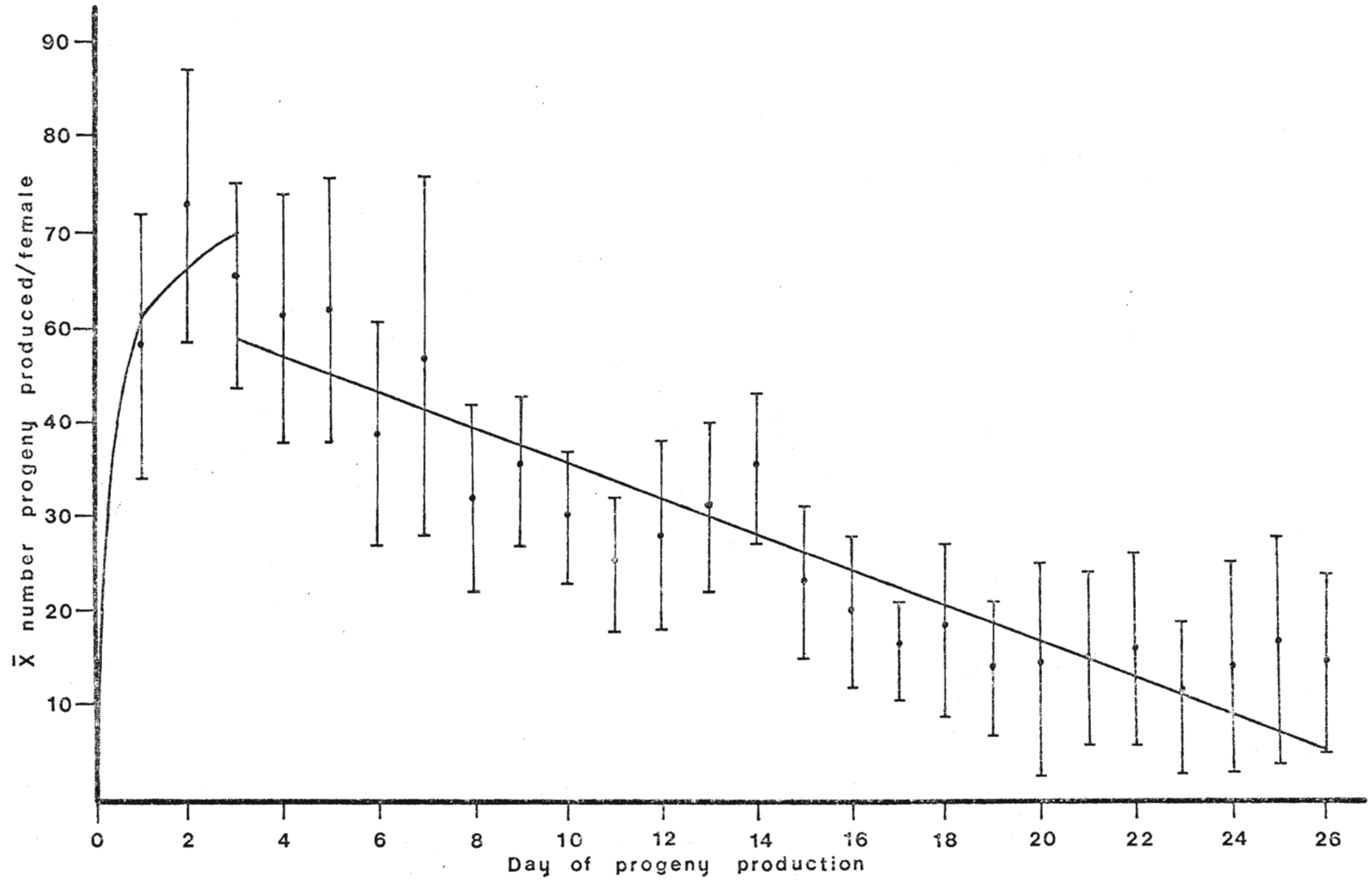

90
80
70
60
40
30
20
10
$\bar{X}$ number progeny produced/female
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Day of progeny production

Figure 7. The average number of offspring produced each day by singly mated A. domesticus, maintained on mouse chow. Closed bars represent 95% confidence intervals. Days 0-3 (1 day post-peak offspring production) are described by: $Y=20.70 + 18.12 \ln(x)$ ($n=77$; $r=0.339$; $p < 0.01$). Days 3-25 are described by: $Y=37.53 - 0.955x$ ($n=424$; $r=0.335$; $p < 0.001$).

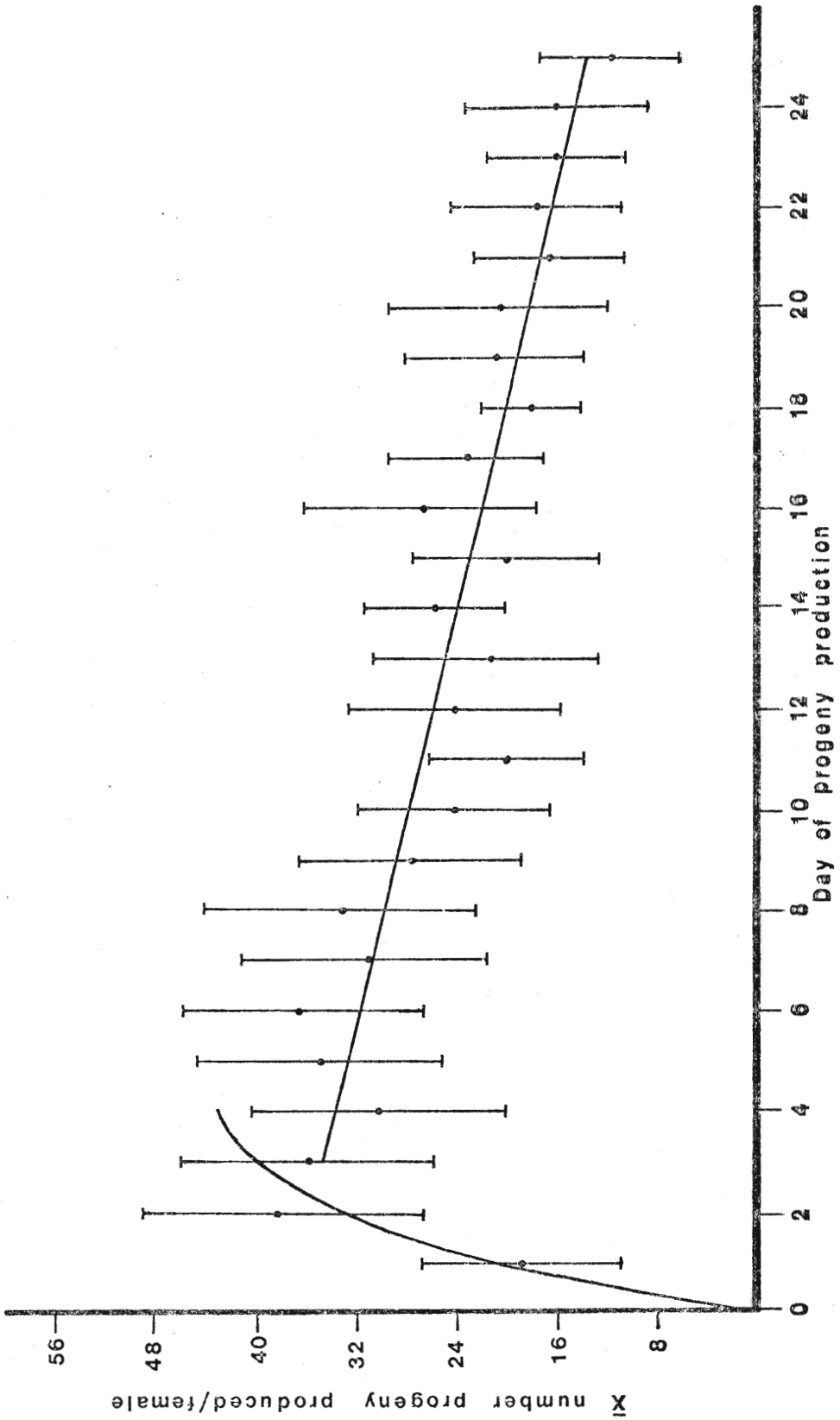

X̄ number progeny produced/female
56
48
40
32
24
16
8
0
2
4
6
8
10
12
14
16
18
20
22
24
Day of progeny production

Figure 8. The average number of offspring produced each day by cannibalistic female A. domesticus, mated once. Closed bars represent 95% confidence intervals. Days 0-3 (1 day post-peak offspring production) are described by: $Y=18.42 + 4.27 \ln(x)$ ($n=81$; $r=0.093$; $p > 0.1$). Days 3-16 are described by: $Y=27.32 - 1.56x$ ($n=255$; $r=-0.331$; $p < 0.001$).

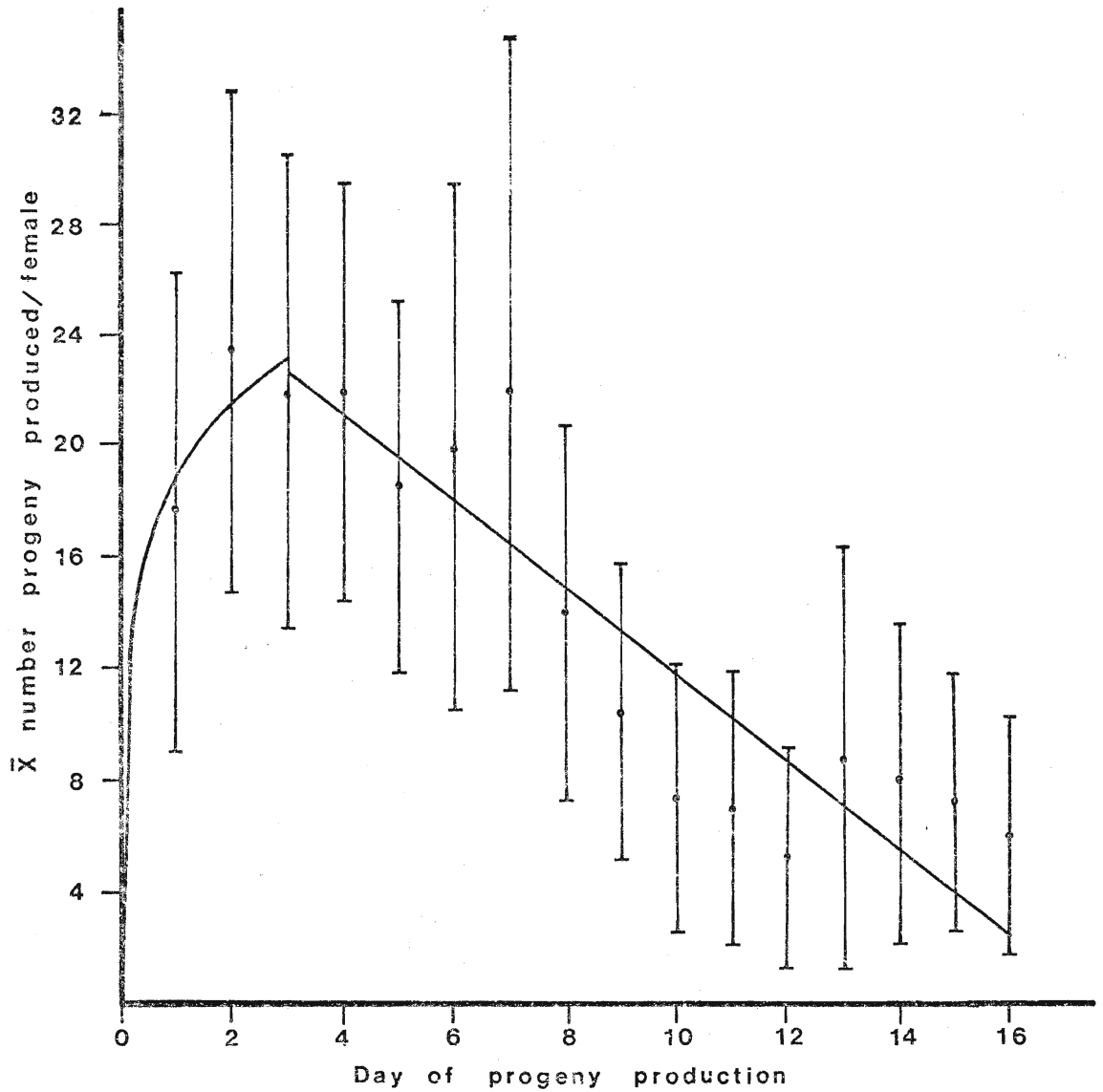

$\bar{X}$ number progeny produced/female
32
28
24
20
16
12
8
4
0
2
4
6
8
10
12
14
16
Day of progeny production

Figure 9. The average number of offspring produced each day by starved female A. domesticus, mated once. Closed bars represent 95% confidence intervals. Days 0-3 (1 day post-peak offspring production) are described by: $Y=12.14 + 3.37 \ln(x)$ ($n=75$; $r=0.119$; $p > 0.1$). Days 3-7 are described by: $Y=20.76 - 2.53x$ ($n=100$; $r=-0.395$; $p < 0.001$).

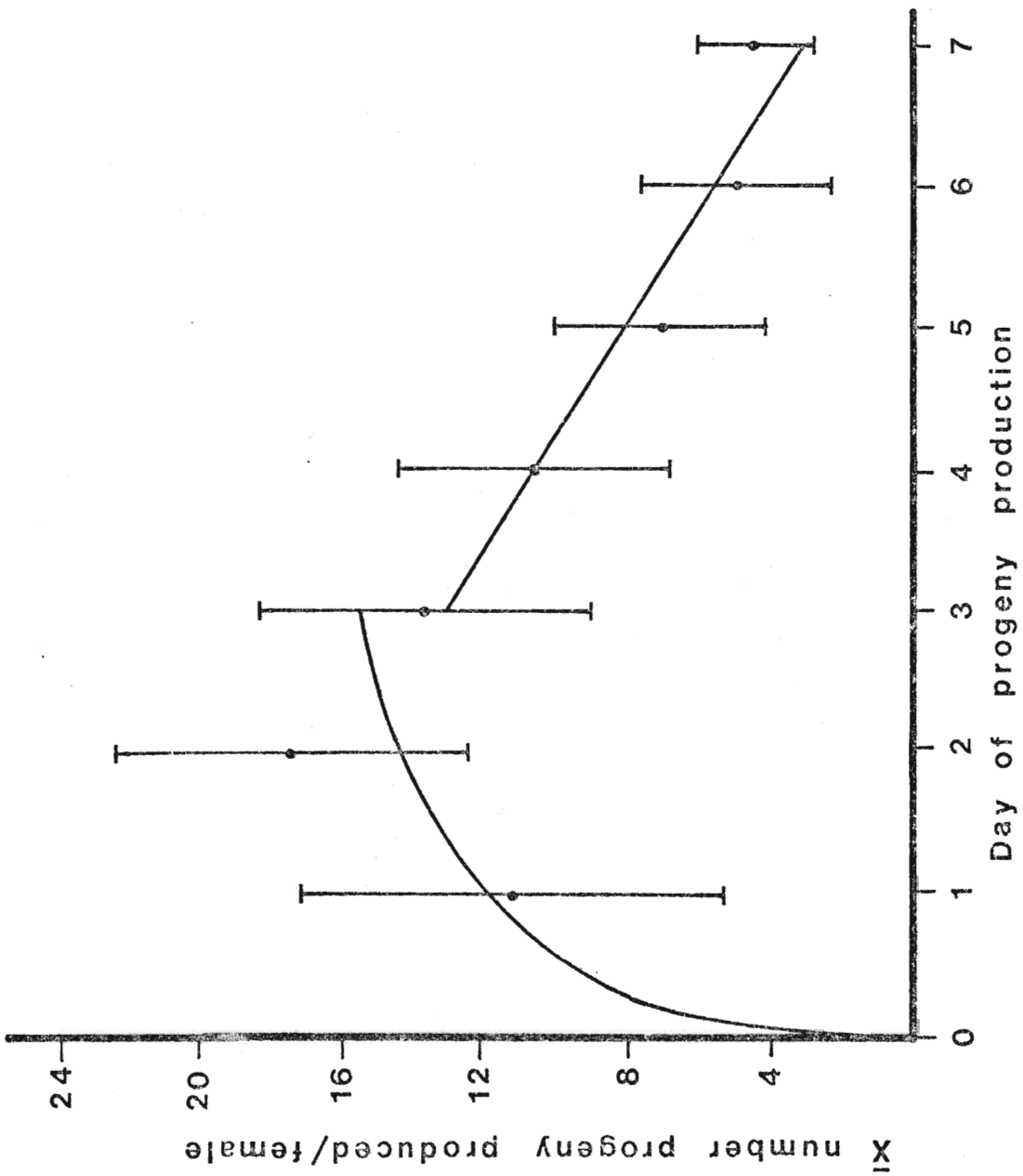
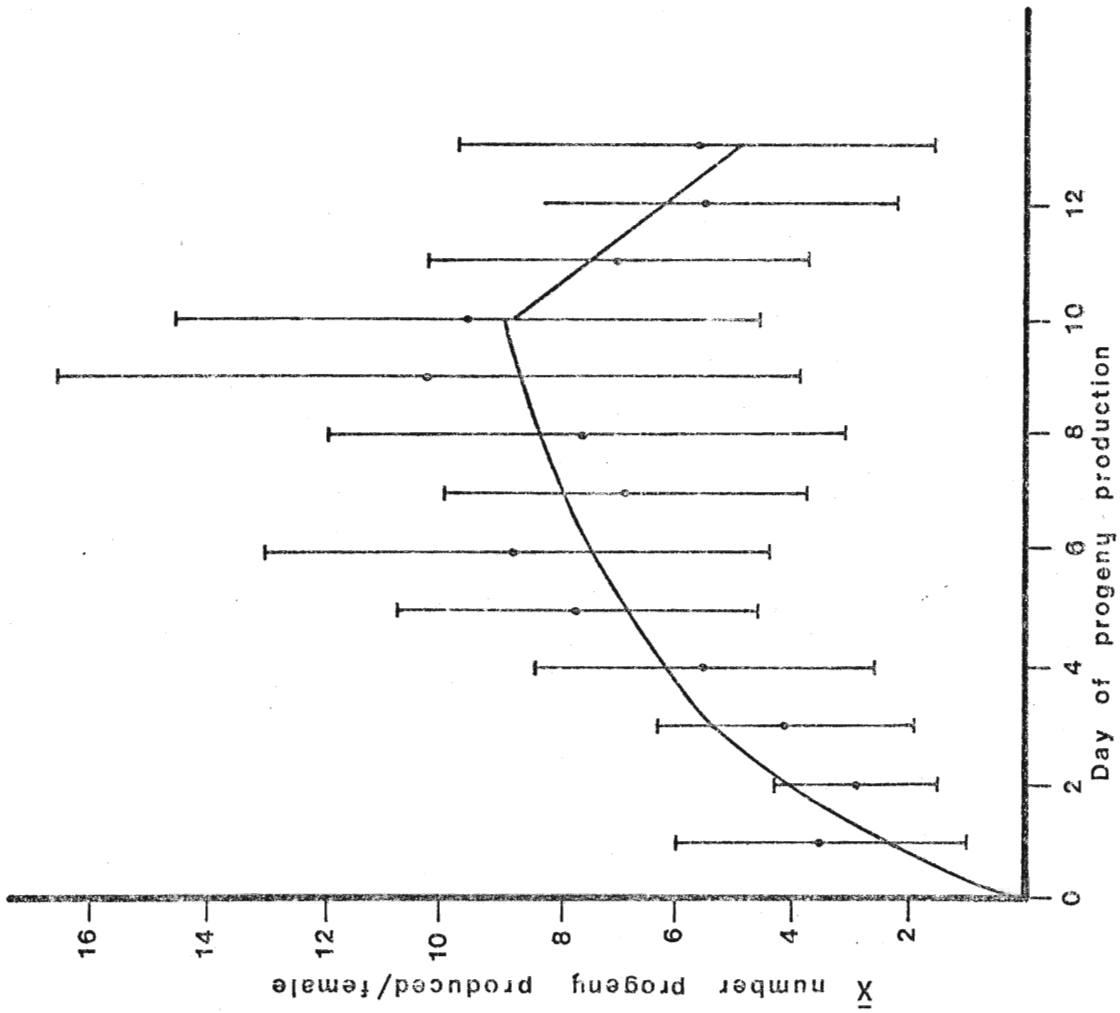

$\bar{X}$ number progeny produced/female
24
20
16
12
8
4
0
1
2
3
4
5
6
7
Day of progeny production

Figure 10. The average number of offspring produced each day by female A. domesticus, maintained on corn meal and having constant access to a male. Closed bars represent 95% confidence intervals. Days 0-10 (1 day post-peak offspring production) are described by: $Y=2.06 + 3.01 \ln(x)$ ($n=179$; $r=0.317$; $p < 0.001$). Days 10-13 are described by: $Y=22.16 - 1.32x$ ($n=50$; $r=-0.243$; $0.05 < p < 0.1$).


$\bar{X}$ number progeny produced/female
16
14
12
10
8
6
4
2
0
2
4
6
8
10
12
Day of progeny production

cannibals (Fig. 8) and starved females (Fig. 9). Also, daily offspring production of cannibals consistently was greater than that of starved females. The day of progeny production at which the average number of offspring produced was greatest, occurred at 2 days for both cannibals and starved females. The average number of offspring produced each day by females fed corn meal and allowed constant access to a male (Fig. 10) was consistently lower than that of the other experimental groups. Additionally, the peak of daily offspring production of females fed corn meal and allowed constant access to a male occurred at 9 days post nymph emergence as opposed to 2 days for the other experimental groups.

Data on the time the spermatophore was attached after a female mated and subsequent offspring production for singly mated females are in Fig. 11-13. For females fed mouse chow (Fig. 11), a logarithmic regression analysis produced the highest correlation coefficient of 4 regression analyses including linear, exponential and power regressions. Offspring production showed a significant positive correlation with the time the spermatophore was attached ($r=0.413$; $p < 0.05$). A linear regression analysis produced the highest correlation coefficient for cannibalistic females (Fig 12; $r=0.262$) although the correlation between offspring production and spermatophore attachment time was not statistically significant ($p > 0.1$). Such was the case for starved females (Fig. 13) for which an exponential regression provided the highest correlation between offspring production

Figure 11. The relationship between the average number of offspring produced per day (total number of offspring/longevity of the adult female (days)) by singly mated female A. domesticus maintained on mouse chow and the time the spermatophore was attached after mating. The curve shown is described by: $Y=8.74\ \ln(x) - 6.60$ ($n=25$; $r=0.413$; $p< 0.05$).

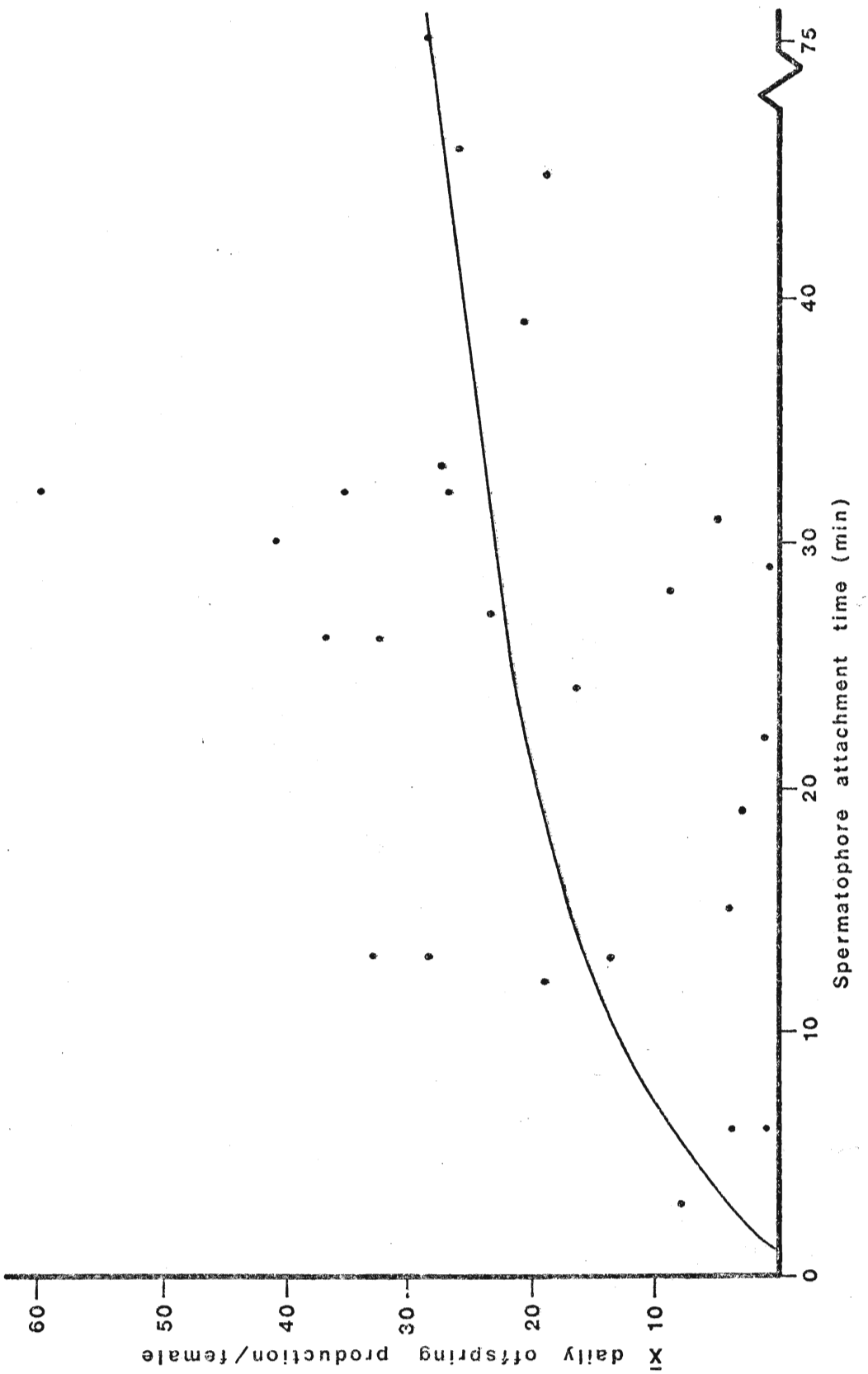

$\bar{x}$ daily offspring production/female
60
50
40
30
20
10
0
10
20
30
40
75
Spermatophore attachment time (min)

Figure 12. The relationship between the average number of offspring produced per day (total number of offspring/longevity of the adult female (days)) by cannibalistic, singly mated female A. domesticus and the time the spermatophore was attached after mating. The curve shown is described by: $Y=12.03 + 0.160x$ ($n=26$; $r=0.262$; $p > 0.1$).

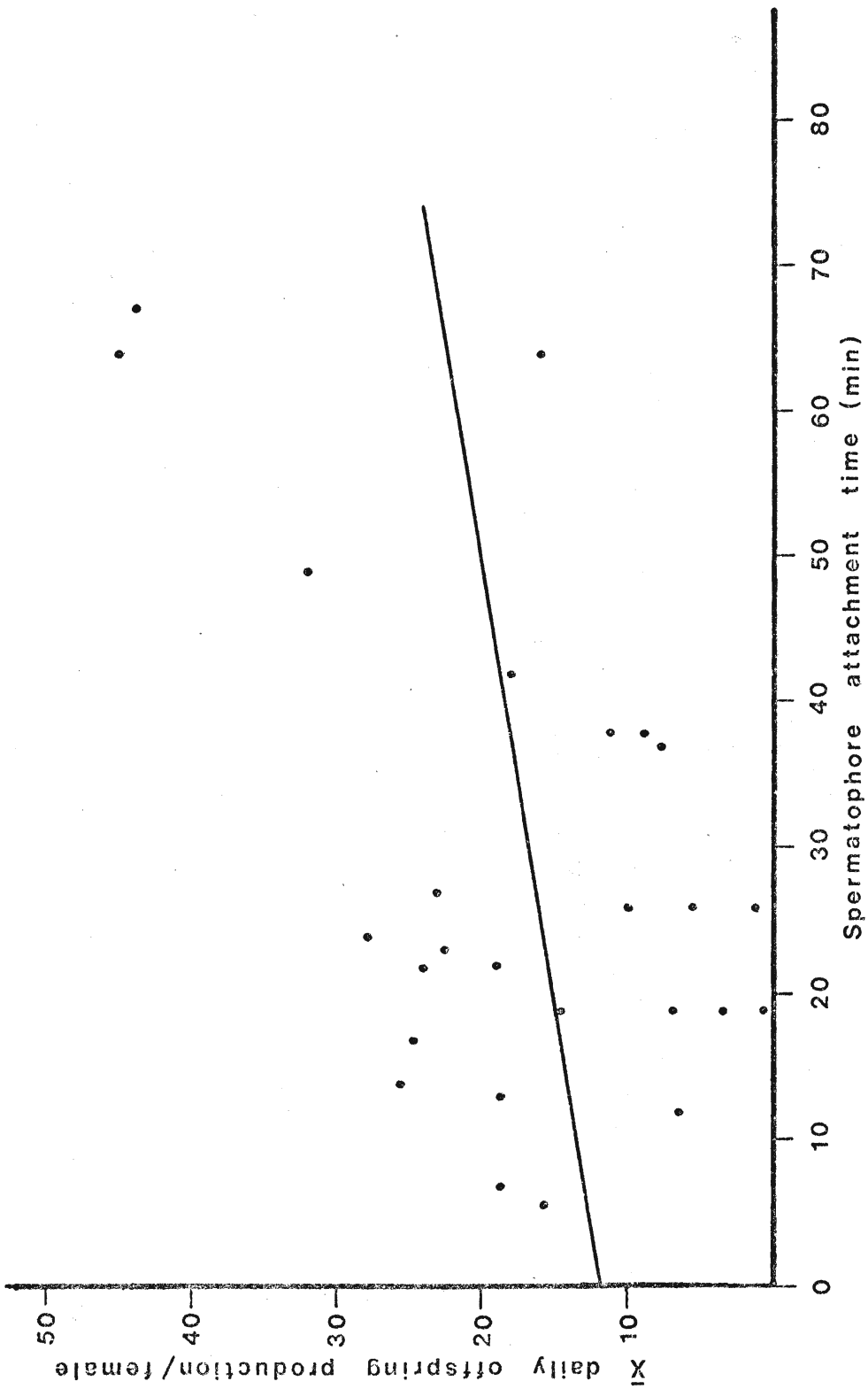
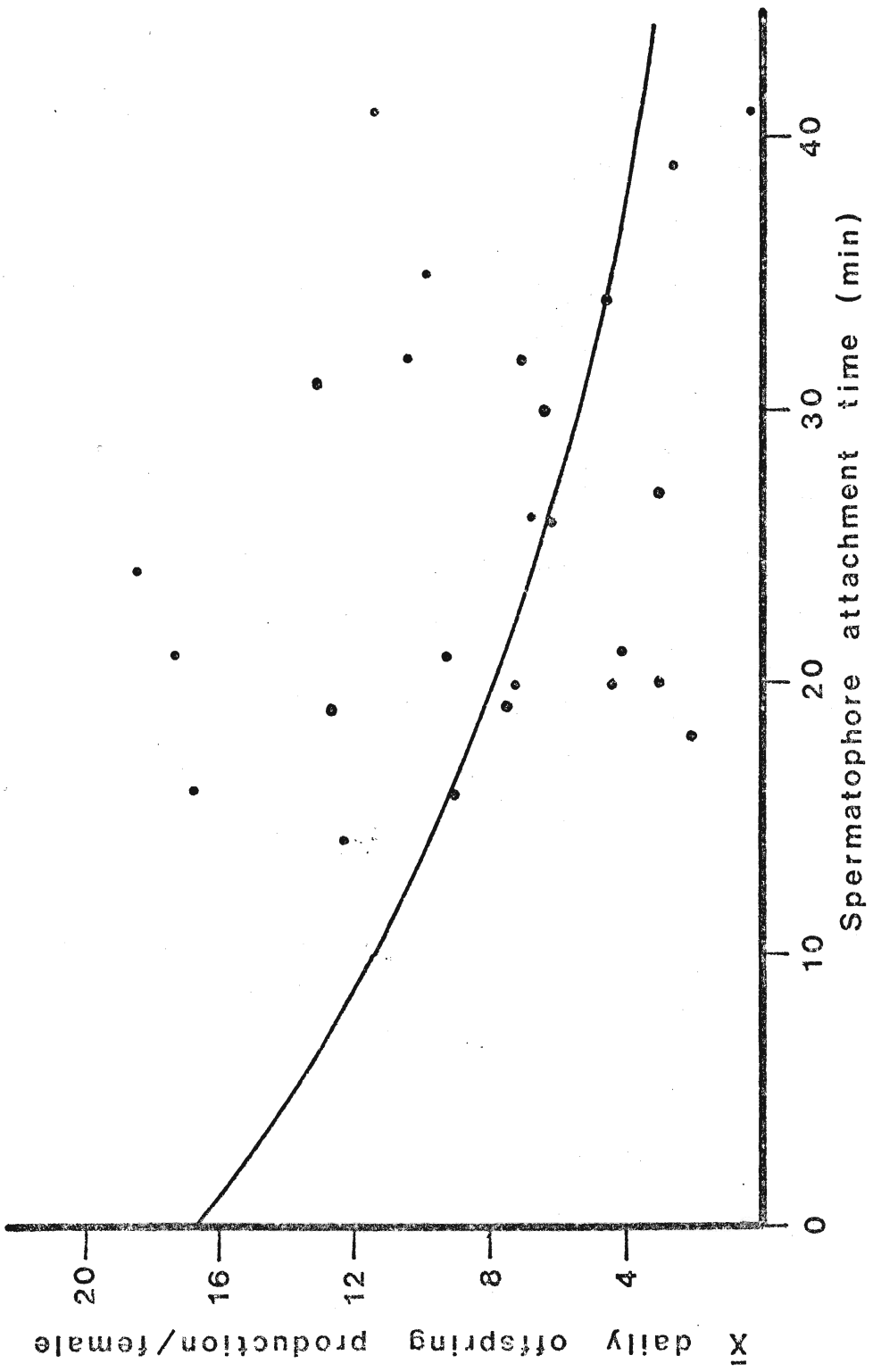

X̄ daily offspring production/female
50
40
30
20
10
0
10
20
30
40
50
60
70
80
Spermatophore attachment time (min)

Figure 13. The relationship between the average number of offspring produced per day (total number of offspring/longevity of the adult female (days)) by starved, singly mated female A. domesticus and the time the spermatophore was attached after mating. The curve shown is described by: $Y = 16.80 x^{-0.038}$ ($n=24$; $r=-0.354$; $p > 0.1$).


X daily offspring production/female
20
16
12
8
4
0
10
20
30
40
Spermatophore attachment time (min)

and spermatophore attachment time ($r=-0.354$; $p > 0.1$).

Results detailing the adult longevity of females of different nutritional and mating categories are shown in Table 8. Singly mated females maintained on mouse chow lived significantly longer than females fed mouse chow and allowed constant access to a male ($t=3.65$; $p < 0.001$ on log transformed to normal data). Regarding singly mated females only, those fed mouse chow lived significantly longer than both cannibals and starved females ($t=7.31$; $p < 0.001$ and $t=13.84$; $p < 0.001$). Also, cannibalistic females exhibited a significantly greater longevity than starved females ($t=3.54$; $p < 0.001$). Although females fed mouse chow and allowed constant access to males left 11 times as many offspring as those maintained on corn meal (Table 4), females fed corn meal lived significantly longer ($t=1.91$; $p < 0.05$). Additionally, females fed corn meal and allowed constant access to males lived significantly longer than cannibals and starved females ($t=4.75$; $p < 0.001$ and $t=8.49$; $p < 0.001$).

Mating frequency and progeny production in G. integer

Data on female mating frequency and subsequent reproduction in Gryllus integer maintained on corn meal are summarized in Table 9. Although doubly mated females produced more offspring on the average than did those mated once, this difference was not significant. Four of 8 single maters produced no offspring, whereas only one of 8 double maters failed to reproduce.

Results on female mating frequency and subsequent

Table 8 Adult longevity of female A. domesticus: 1) maintained on different diets; and 2) either mated once or allowed constant access to a male.

Nutritional and mating status	N	Average longevity (days) $\bar{X}$	SD	Range
A. fed mouse chow; 1 mating	30	33.80[a]	9.28	17-64
B. cannibals; 1 mating	30	18.83[b]	7.25	9-38
C. starved; 1 mating	30	13.57	3.00	7-20
D. fed mouse chow; constant access	30	25.17[c,d]	6.64	5-36
E. fed corn meal; constant access	30	32.00[e,f]	11.49	8-56

[a] significantly greater than B, C, D at $p < 0.001$
[b] significantly greater than C at $p < 0.001$
[c] significantly greater than B at $p < 0.005$
[d] significantly greater than C at $p < 0.001$
[e] significantly greater than B, C, at $p < 0.001$
[f] significantly greater than D at $p < 0.05$

Table 9 The number of early instar nymphs produced per female G. integer, allowed to mate once or twice, and maintained on corn meal.

Number of Matings	N	Number of Progeny			% reproducing
		$\bar{X}$	SD	Range	
1	8	121.0*	136.8	0-338	50.0
2	8	172.5	107.5	0-315	87.5

* $p > 0.05$

reproduction in G. integer maintained on mouse chow are summarized in Table 10. Doubly mated females produced significantly more offspring than did those mated once ($t=4.30$; $p< 0.001$ on log transformed to normal data). Additionally, females allowed constant access to a male for 24-48 hours left significantly more offspring than single maters ($t=2.36$; $p< 0.025$). Seven singly mated females (24%) did not produce offspring, whereas only 1 doubly mated female (2%) did not leave any nymphs. Excluding reproductive failures, doubly mated females still produced significantly more offspring than did reproductively successful single maters ($t=2.75$; $p< 0.001$).

Data comparing the time after mating at which offspring first appeared in the oviposition containers of female G. integer are shown in Table 11. The offspring of females maintained on corn meal emerged significantly later than the offspring of females maintained on mouse chow including single maters, double maters, and females allowed constant access to a male for 24-48 hours ($t=7.15$; $p< 0.01$, $t=5.48$; $p< 0.001$ and $t=2.51$; $p< 0.025$, respectively on log transformed to normal data). Considering females maintained on mouse chow, the offspring of double maters and females allowed constant access to a male for 24-48 hours emerged significantly later than the offspring of singly mated females ($t=5.18$; $p< 0.001$ and $t=3.43$; $p< 0.005$).

Results detailing the number of days over which offspring hatched for female G. integer are shown in Table 12. Females fed corn meal left offspring over a significantly longer period

Table 10 The number of early instar nymphs produced per female by allowing virgin *G. integer*, fed on mouse chow, to mate once, mate twice, or remain constantly with a male for 24-48 hours.

Mating Status	N	Number of progeny including reproductive failures			% reproducing	N	Number of progeny excluding reproductive failures		
		$\bar{X}$	SD	Range			$\bar{X}$	SD	Range
A. mated once	29	368.09	372.31	0-1062	75.9	22	485.18	354.82	1-1062
B. mated twice	45	815.64[a]	524.28	0-2410	97.8	44	834.18[c]	515.42	30-2410
C. with male 24-48 hours	8	1015.50[b]	661.43	159-2018	100	8	1015.50	661.43	159-2018

[a] significantly greater than A at $p < 0.001$
[b] significantly greater than A at $p < 0.05$
[c] significantly greater than A at $p < 0.001$

Table 11 The time after a first mating when nymphs first appeared in the oviposition containers for female G. integer maintained on either corn meal or mouse chow and allowed to mate once, mate twice, or remain with a male for 24-48 hours.

Nutritional and Mating Status	N	Day post-mate (first) when offspring first emerge		
		$\bar{X}$	SD	Range
A. fed corn meal; singly & doubly mated	11	27.18[a,b]	5.01	19-39
B. fed mouse chow; mated once	22	17.86	2.73	15-28
C. fed mouse chow; mated twice	44	21.07[c]	2.75	18-35
D. fed mouse chow; with male 24-48 hours	8	22.00[d]	3.87	19-32

[a] significantly greater than B,C at $p < 0.001$
[b] significantly greater than D at $p < 0.025$
[c] significantly greater than B at $p < 0.001$
[d] significantly greater than B at $p < 0.005$

Table 12 The number of days over which offspring hatched for female G. integer maintained on either corn meal or mouse chow and allowed to mate once, mate twice, or remain with a male for 24-48 hours.

Nutritional and Mating Status	N	Duration of offspring production(days) $\bar{X}$	SD	Range
A. fed corn meal; singly & doubly mated	11	36.64[a,b]	14.47	13-71
B. fed mouse chow; mated once	22	16.82	9.43	1-38
C. fed mouse chow; mated twice	44	21.86[c]	8.78	4-47
D. fed mouse chow; with male 24-48 hours	8	23.12	8.89	14-40

[a] significantly greater than B, C at $p < 0.005$
[b] significantly greater than D at $p < 0.025$
[c] significantly greater than B at $p < 0.025$

than females maintained on mouse chow including single and double maters, and females allowed constant access to males for 24-48 hours. For females maintained on mouse chow, double maters left offspring over a significantly longer period than single maters ($t=2.36$; $p<0.025$ on log transformed to normal data).

Patterns of daily offspring production of singly and doubly mated G. integer fed mouse chow are in Figures 14 and 15. As well as leaving offspring over a significantly longer period (Table 12), doubly mated females consistently produced, on the average, more offspring each day than did single maters. The day at which offspring production peaked was 5 days for singly mated females and 7 days for double maters.

Data describing the adult longevity of female G. integer are shown in Table 13. Longevity of females fed corn meal did not differ significantly with that of females maintained on mouse chow. Interestingly, doubly mated females fed mouse chow lived significantly longer than single maters ($t=2.20$; $p<0.025$).

Sperm competition in G. integer

Two representatives of acrylamide vertical slab-gel electrophoreses performed in this study are shown in Figures 16 and 17. Of the enzymes surveyed, only 2 were examined in detail and these were esterase (stain: β-napthyl acetate, fast blue TRN; Sigma Chemical Co.), illustrated in Plate 1 and amylase, shown in Plate 2. Other enzyme examinations were discarded after 1 or 2 gels when: 1) little to no variation

Figure 14. The average number of offspring produced each day by female G. integer, maintained on mouse chow and singly mated. Closed bars represent 95% confidence intervals. Days 0-5 (1 day post-peak offspring production) are described by: Y=19.70 + 16.23 ln (x) (n=104; r=0.335; $p < 0.001$). Days 5-17 are described by: Y=41.40 - 0.846x (n=204; r=-0.122; $0.05 < p < 0.1$).

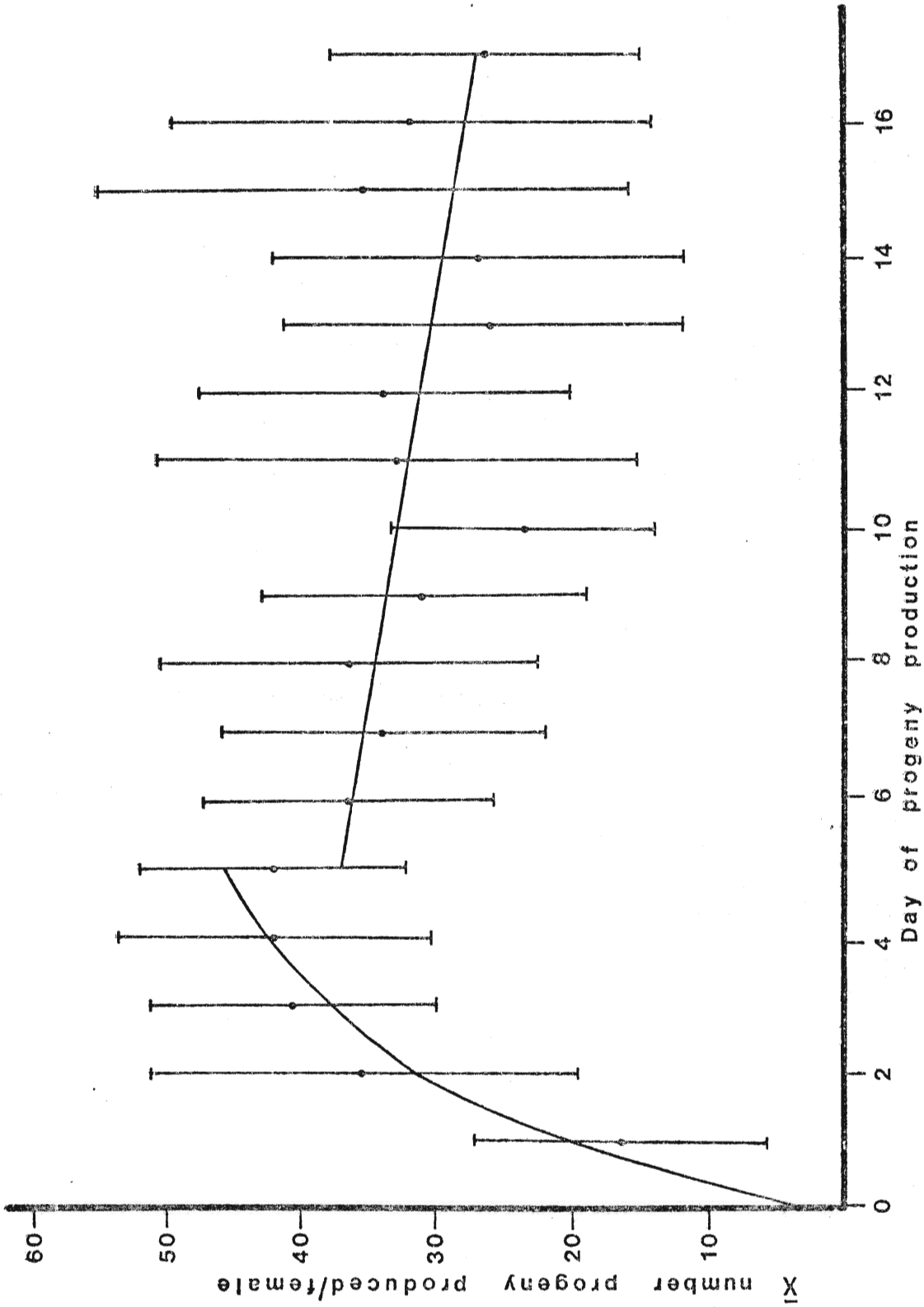

X̄ number progeny produced/female
10
20
30
40
50
60
0
2
4
6
8
10
12
14
16
Day of progeny production

Figure 15. The average number of offspring produced each day by doubly mated G. integer, maintained on mouse chow. Closed bars represent 95% confidence intervals. Days 0-7 (1 day post-peak offspring production) are described by: $Y=38.11 + 8.46 \ln(x)$ ($n=295$; $r=0.168$; $p < 0.01$). Days 7-24 are described by: $Y= 73.95 - 2.52x$ ($n=581$; $r=-0.414$; $p < 0.001$).

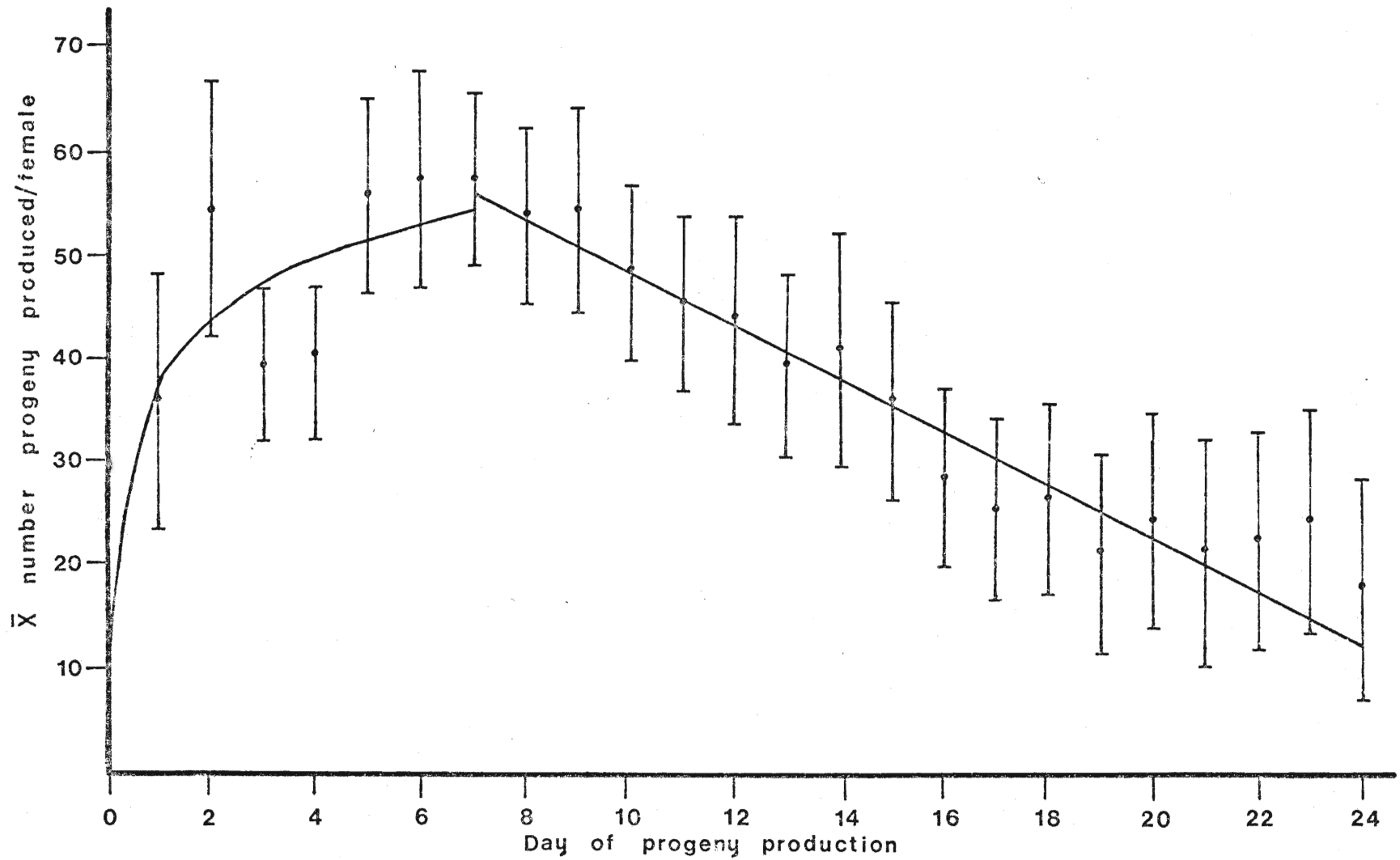

70
60
50
40
30
20
10
$\bar{X}$ number progeny produced/female
0
2
4
6
8
10
12
14
16
18
20
22
24
Day of progeny production

Table 13 Adult longevity of femal G. integer maintained on either corn meal or mouse chow and allowed to mate once, mate twice or remain with a male for 24-48 hours.

Nutritional and mating status	N	Average longevity (days)		
		$\bar{X}$	SD	Range
A. fed corn meal; singly & doubly mated	12	45.17	18.22	13-77
B. fed mouse chow; mated once	29	41.52	10.56	19-55
C. fed mouse chow; mated twice	45	49.33[a]	12.30	10-71
D. fed mouse chow; with male 24-48 hours	8	46.25	10.47	28-60

[a] significantly greater than B at $p < 0.025$

Figure 16. Allozyme phenotypes of esterase in body extracts of G. integer. At least 3 esterase systems (1, 2 and 3) are present in this sample of 24 individuals. In esterase system 2, "b" and "c" represent 2 individuals for which there appears to be no overlap in the allozyme bands. However, the number of allozymes for which this system is polymorphic was not clear. The mushroom shape of "a" indicates that protein denaturation of this sample has occurred (D. Hickey, pers. comm.).

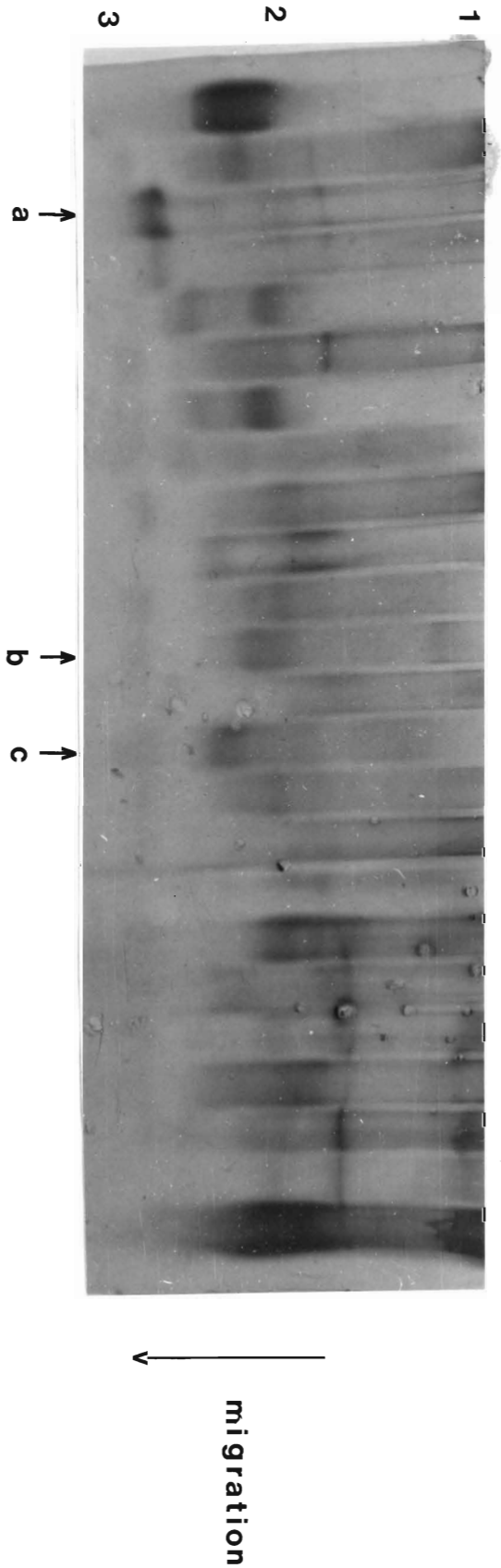
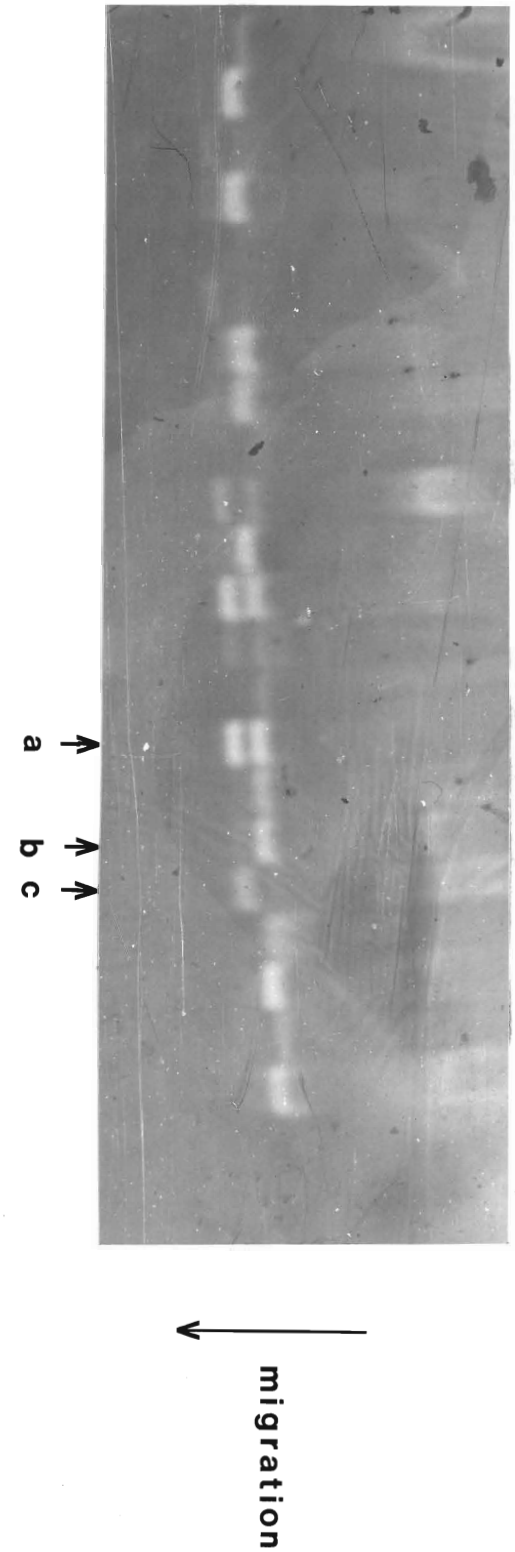

1
2
3
a
b
c
migration

Figure 17. Allozyme phenotypes of amylase in body extracts of *G. integer*. In this sample of 24 individuals, the amylase system is polymorphic for fast- and slow-migrating allozymes (F and S respectively). Phenotypes (and presumed genotypes) of individuals a, b, and c are FS (heterozygote, 2 bands), SS (homozygote, 1 slow band), and FF (homozygote, 1 fast band), respectively.


a
b
c
migration

was found; 2) low enzyme activity occurred; or 3) band resolution was extremely poor.

For the esterase system shown in Figure 16, individuals "b" and "c" represented potentially useful genotypes for determining the paternity of offspring. For example, if a female of "c" genotype first was mated to a male of the same genotype and remated to a male of the "b" genotype then, because there is no overlap in the allozyme bands, resultant offspring of genotype "c" could be ascribed to the first male to mate and those of a genotype intermediate to "b" and "c", attributed to the second male to mate. However, no mating combinations of this type were found. Paternity of offspring could not be established and hence the pattern of sperm competition could not be determined.

The amylase system shown in Figure 17 accorded greater resolution of potentially useful genotypes for determining the paternity of offspring. For example, if an SS female (one slow band) twice was mated with an SS male and FF male (1 fast band), then FS progeny (2 bands, heterozygote) could be attributed to the FF male and SS progeny, ascribed to the SS male. However, as with the esterase allozymes, appropriate mating combinations of this type were not found. Therefore, the pattern of sperm competition in G. integer was not determined in this electrophoretic study for either esterase or amylase allozymes.

DISCUSSION

This investigation focussed primarily on social and non-social factors affecting female cricket reproductive success. Results will be discussed in four sub-sections. I consider the onset of female sexual behaviour, the role of nutrition in the reproductive success of females, the adaptive significance of female multiple mating in A. domesticus and G. integer, and sperm competition and mate guarding in crickets.

Onset of female sexual behaviour in house crickets

Female A. domesticus first mate at an average adult age of 6.9 days, an age very close to when they first become positively phonotactic. The frequency distribution of the age at which females first mated was virtually duplicated by the frequency distribution of the age at which females first became positively phonotactic. This provides the first direct evidence for a positive relationship between copulatory readiness and phonotaxis in female crickets. Additionally, Shuvalov and Popov (1971) showed that only sexually mature female A. domesticus exhibit a positive phonotaxis (cited in Stout et al. 1976). Female sexual responsiveness in A. domesticus probably is mediated by the corpora allata. When the corpora allata of sexually responsive females (females that mounted a male within 30 min of confinement) were transplanted to non-responsive females, the latter became sexually responsive within 24 hours

(Stout et al. 1976).

The age at which females first mate probably corresponds to when mature eggs are available for fertilization. No ovariole growth occurs in the last nymphal stadium, but begins to increase drastically at an adult age of 3 days in A. domesticus (Woodring et al. 1977 and 1979). Therefore, adult females that mated too soon after the imaginal molt (ie. before the availability of mature eggs), would encounter reduced fitnesses due to time and energy expenditures. Also, adult crickets are soft-bodied for approximately 3 days after eclosing and females might risk physical damage if they mated before the exoskeleton hardened.

Some females began ovipositing after mating in the same observation period. This indicates that soon after insemination, fertilization of the egg occurs. Indeed, some mechanical or chemical stimulus from mating probably triggers oviposition in females , as females that did not mate never were observed ovipositing. The presence of males alone, without the occurrence of mating, does not appear to be sufficient stimulus for oviposition. Woodring et al. (1979) also found that virgin females not permitted to mate, never oviposited and retained eggs in a viable state throughout their entire life. It is not surprising that some successfully mated females were not observed ovipositing. Females had constant access to an oviposition substrate and it is likely that these females oviposited during the time no observations were made.

Nutrition and reproduction in female house crickets

Data on the reproductive success of singly mated female A. domesticus allows comparison of the effects of an enriched diet (mouse chow), cannibalism, and starvation on female reproduction. Females fed mouse chow were the most successful reproducers, leaving many more offspring than both cannibals and starved females, over a significantly longer period of time. Additionally, cannibalistic females left more offspring than starved females and over a longer duration.

Direct comparison of my results on female reproduction in A. domesticus with other published reports is not possible due to differences in a wide variety of variables including nutrient composition, temperature, rearing and maintenance conditions, and mating frequency. However, reproduction of female A. domesticus fed mouse chow, the optimal diet in this study, was comparable to maximum reproductive rates reported by Ghouri and McFarlane (1958), Richot and McFarlane(1962), Patton (1967), and Woodring et al. (1979).

Patton (1967,1978) determined that A. domesticus optimal growth and reproduction occurred when protein ranged from 20% to 30%, carbohydrate from 32% to 47%, and fats from 3.2% to 5.2%. Protein, carbohydrate and fat levels in Purina® mouse chow were comparable to these optimal amounts (see Appendix 5 for approximate chemical composition of Purina® mouse chow 5020). In addition, the mouse chow used in this study contained vitamins

and minerals experimentally determined necessary for A. domesticus growth and/or reproduction. These included B vitamins (Richot and McFarlane 1961), vitamin E or alpha-tocopherol (Meikle and McFarlane 1965; McFarlane 1972a), copper (McFarlane 1974), zinc (McFarlane 1976a), and vitamin K (McFarlane 1976b).

Given the above considerations, the relatively high reproduction exhibited by female A. domesticus maintained on mouse chow is not strikingly peculiar. It may not be readily apparent why cannibals left less than half as many offspring as females fed mouse chow. However, it seems likely that cannibals simply could not obtain the same amounts of essential nutrients as females maintained on mouse chow. Although female crickets incorporate various dietary nutrients in different body organs and tissues (Woodring et al. 1979), cannibalized crickets were never more than partially eaten. Therefore, cannibals were deficient in specific nutrients. Although cannibalistic females experienced decreased reproduction compared to females maintained on mouse chow, the observed intensity of cricket cannibalism indicates that this trait is a normal facet of A. domesticus social behaviour. It may be that under field conditons, cannibalism furnishes individuals with important nutritional supplements.

Not surprisingly, reproduction of starved female A. domesticus was the lowest of all experimental groups. Lack of adequate nutrition in insects generally results in the reabsorption of oocytes by females, before yolk is deposited (Wigglesworth 1965). Recently, egg reabsorption has been linked

to a decrease in juvenile hormone synthesis by the corpora allata in locusts, Schistocerca americana gregaria, subjected to starvation (Tobe and Chapman 1979). It seems likely that reabsorption of eggs was a major factor contributing to the decreased reproduction of starved female A. domesticus.

The effects of varying nutrition on female adult longevity probably affected the relative reproduction of singly mated females. Females fed mouse chow lived significantly longer than cannibals, and cannibals lived much longer than starved females. It seems reasonable that the longevity of an adult female limits the duration over which offspring can be produced. The period of time over which females of different nutritional categories left progeny corresponded to their average longevities. This is not to imply that the indirect effects of longevity on relative female reproduction take precedence over the direct effects of varying nutrition. Even when the relative difference in the time over which females left offspring are ignored, females fed mouse chow consistently produced more offspring per day than cannibals. Similarly, cannibals produced more offspring daily than starved females.

An important aspect of the reproduction of singly mated females which appeared to be unaffected by varying nutrition was the pattern of daily progeny production. For the 3 experimental groups, offspring production began at about the same time ($\bar{X}$=22.63 days post-mate). Initially, daily offspring production increased abruptly, peaking at the second day of offspring

production and then gradually tapered off. In that this pattern of offspring production was exhibited by females regardless of the nutrition they received, it possibly is an evolved characteristic, optimizing individual female reproductive success. Although this possibility requires further investigation, nonetheless these data represent, to the best of my knowledge, the first time that the daily offspring production of female crickets has been documented.

The sex ratio of offspring produced by starved females also was examined. Theoretical considerations suggest that environmentally stressed females may alter the sex ratio of their progeny. Trivers and Willard (1973) proposed that for species exhibiting low parental investment, starved females should produce more female than male progeny, since unhealthy male offspring would be more unsuccessful in mating than poor quality female progeny. Alternatively, females might be selected to produce more offspring of the sex requiring the least maternal investment (Myers 1978). In this study, neither starved or well-fed females produced an offspring sex ratio differing significantly from unity. This may indicate that female crickets are not capable of altering their primary sex ratio under severe environmental stress.

The effects of the consumption of one spermatophore also was examined in singly mated females. Females allowed to consume the attached spermatophore did not exhibit greater reproduction than females whose spermatophores were artificially removed.

However, females ate spermatophores in 90% of their matings, and because many females mate frequently a typical female probably consumes several spermatophores during her lifetime. Females also might obtain and consume spermatophores from other copulating pairs, a behaviour observed once in this study. It may be that a slight nutritional benefit accrued from the consumption of only one spermatophore could not be discerned in this study.

Examination of the reproduction of female A. domesticus allowed constant access to males permits, as well, comparison of mouse chow and corn meal diets. Females fed mouse chow produced significantly more offspring over a longer period of time than females maintained on corn meal. Also, singly and doubly mated G. integer fed corn meal exhibited significantly reduced reproduction compared to those fed mouse chow. The decreased reproduction of females fed corn meal possibly was due to an inadequate supply of dietary lipid. Commercial corn meal consists of 78.9% carbohydrates, 7.6% protein, and 0.9% fats (D. Goslin, Quality Control Manager, Quaker Oats of Canada Ltd., personal communication). Patton (1967) estimated that a range of 3.2% to 5.2% dietary lipids are optimal for A. domesticus growth and reproduction. Although house crickets can synthesize lipids from dietary carbohydrate (Woodring et al. 1979), Richot and McFarlane (1962) found that lipid starved females leave no viable progeny. Additionally, lipid starved females exhibit decreased oviposition (Meikle and McFarlane 1965). During the

first 10 days of adult life, almost all absorbed and synthesized lipids contribute to ovariole growth in female A. domesticus (Woodring et al. 1979). Thus it may be that females fed corn meal could not absorb or synthesize enough dietary lipids, resulting in poor ovariole growth. A longevity effect could not have contributed to these relative differences in reproduction, because A. domesticus females fed corn meal lived significantly longer than females fed mouse chow. In G. integer, there was no difference in longevity.

Females fed mouse chow and allowed constant access to males exhibited the same pattern of daily offspring production as singly mated females. Daily progeny production increased drastically, peaking at the second day and thereafter, gradually tapered off. However, females fed corn meal exhibited a somewhat irregular pattern of offspring production. Offspring production of females fed corn meal began significantly later than females fed mouse chow in A. domesticus and G. integer. Also, offspring production did not peak until 9 days after mating in A. domesticus. Reproduction in females fed corn meal probably was delayed because more time was required to incorporate enough dietary lipid to permit adequate ovariole growth and egg production.

In summary, female cricket reproduction varied widely under different nutritional conditions. Indeed, the acquisition of adequate nutrition may have contributed to the evolution of female multiple mating and spermatophore consumption, one

hypothesis developed in the following analysis of female mating behaviour.

The adaptive significance of female cricket multiple mating

In most cricket species, the parental investment of females far exceeds that of males (Alexander 1975). Various forms of male-male competition and female choice should therefore predominate (Trivers 1972). Different modes of male-male competition in crickets have been documented (Alexander 1975; Cade 1979a), but the role of female choice is poorly understood. However, the frequency at which females mate can be considered one form of female preference (Walker 1978). In this study, female A. domesticus mated on the average 2.8 times. This observed mating frequency likely underestimates the number of times females mate. Females were exposed to males for only 2 hours each day, whereas continued access to males probably would have increased their mating frequency. Females also were observed for only the first 15 days of adult life, although they live approximately 30 days in the laboratory. The frequency distribution of the percent of females mating at any given age strongly suggests that females continue to mate after the adult age of 15 days. Female G. integer also mate repeatedly and 2 females each were observed to mate 10 times in an 18 hour observation period (Sakaluk and Cade 1980). These observations suggest that female crickets mate several times in the field. Indeed, female field crickets

become increasingly phonotactic to the male calling song when they are isolated from males for only a few days (Cade 1979b). Similarly, in this study female A. domesticus that were constantly isolated from males were tested for positive phonotaxis through the age of 1 to 15 days. They exhibited, on the average, 7.78 positive phonotactic responses (n=9; SD=2.22; range=5-11).

The evolution of a high degree of female multiple mating in crickets probably is due to many selective factors. In G. integer fed mouse chow, doubly mated famales, and females confined with males for 24 to 48 hours left significantly more offspring than single maters. This difference in reproduction resulted largely from the failure of 24% of singly mated females to reproduce. Similarly, in G. integer females fed corn meal, double maters left more offspring than single maters. Although this difference was not significant, 4 of 8 single maters did not reproduce. The number of offspring produced by singly and doubly mated female A. domesticus was previously compared (Sakaluk and Cade 1980). This study produced results comparable to those found for G. integer. Doubly mated females produced more offspring than single maters and 12.5% of singly mated females did not leave any offspring. Interestingly, Bentur et al. (1977) found no difference in the number of eggs produced by singly and doubly mated female G. bimaculatus. However, they did demonstrate that additional matings were required to stimulate higher levels of oviposition. Some of the reproductive failures that occurred in singly and doubly mated female A. domesticus

and G. integer may have been due to female physiological defects. This possibility is supported indirectly by 2 female A. domesticus that had many abortive matings over 15 days, but no successful matings. However, many more singly mated females than doubly mated A. domesticus and G. integer failed reproductively, suggesting that female physiological defects are not usually involved. Instead, spermatozoa and/or egg development stimulants may not be successfully transferred from the male spermatophore (Bentur et al. 1977; Loher and Edson 1973). Females that remate thus correct for the possibility of an infertile mating.

Male inability to thread the thin tube of the spermatophore into the female may contribute to the failure of matings (Loher and Rence 1978). In support of this possibility, abortive matings occurred in 34.9% of the instances in which female A. domesticus mounted males. Additionally, the probability of an abortive mating was not reduced by the increased mating experience of females. However, natural selection should favour male ability to efficiently transfer spermatophores. Indeed, male crickets often remove old spermatophores which presumably contain inviable sperm (Alexander and Otte 1967).

The occurrence of reproductive failures does not sufficiently account for the difference in reproduction between singly and doubly mated G. integer. Doubly mated G. integer fed mouse chow produced significantly more offspring than single maters, even when reproductive failures were excluded from the analysis. This was also the case for singly and doubly mated A. domesticus

(Sakaluk and Cade 1980). Early spermatophore removal resulting in the incomplete transfer of spermatozoa or egg production stimulants probably accounts for some of the difference. Indeed, the duration of spermatophore attachment was positively correlated with offspring production in singly mated female A. domesticus fed mouse chow. Not unexpectedly, these variables exhibited a logarithmic relationship. At some critical time, the spermatozoa presumably would be emptied of sperm or alternatively, a female would have received sufficient sperm to fertilize all of her eggs. A female that removed her spermatophore before this time would be expected to encounter reduced reproduction. A female that left her spermatophore attached for any time after this critical period, would encounter neither decreased or increased reproduction. The average duration of spermatophore attachment in 189 matings for A. domesticus females was 29.3 minutes, a time corresponding to when daily offspring production appears to level off (Fig. 11). Cannibalistic and starved singly mated females did not exhibit a significant relationship between spermatophore attachment and offspring production. It may be that the reproductive constraints imposed by inadequate nutrition prevents any expression of the potential reproductive benefits of increased spermatophore attachment time. However, a significantly positive relationship was shown for singly mated A. domesticus fed corn meal (Sakaluk and Cade 1980), even though results presented here demonstrate that corn meal is an inadequate diet.

It often has been suggested that male cricket post-copulatory behaviour functions, in part, to prevent the early removal of spermatophores by females, thus ensuring that females are maximally inseminated (Alexander 1961,1962; Alexander and Otte 1967; Loher and Rence 1978). Khalifa (1950) observed that guarding behaviour in A. domesticus consisted of males standing close to mates and apparently watching them. Males attempt to prevent females from removing spermatophores by continuous antennation (Khalifa 1950; Alexander and Otte 1967). However, results comparing the spermatophore attachment times of females isolated from males and females remaining with males after mating, indicates that the post-copulatory behaviour of male A. domesticus generally is unsuccessful in preventing premature removal of spermatophores. Khalifa's (1950) data also show no such effect on spermatophore attachment. Similar behaviours by males occur in other crickets. In tree crickets (Grillidae; Oecanthinae), females feed at specialized male glands, which may distract them from prematurely removing spermatophores (Alexander and Otte 1967; Bell 1979; Walker 1978). Indeed, Bell (1979) showed that when the metanotal glands of males were waxed over, females ate spermatophores approximately 15 minutes after mating, whereas spermatophore consumption normally occurs 27 minutes after a mating. Loher and Rence (1978) demonstrated that female Teleogryllus commodus retained their spermatophores longer when males were guarding them. They suggested that mate guarding time should be influenced by the time required for

complete insemination. Compete sperm transfer in T. commodus requires 68 minutes, whereas the average male guarding time is 83 minutes.

Many singly mated female A. domesticus fed mouse chow did not reproduce or did so at very low levels, despite lengthy spermatophore attachment times. This result was shown previously by Sakaluk and Cade (1980) who suggested that blockage of the narrow spermatophore duct might prevent the flow of spermatozoa. If blockage of this duct occurred shortly after the spermatophore was attached, any additional spermatophore time would not benefit the female in terms of increased offspring production.

Additionally, multiple mating might serve to replenish spent spermatozoa. Such is the case in Drosophila melanogaster, where female remating corresponds to the depletion of sperm in the bursa copulatrix. When the productivity of D. melanogaster singly and multiply mated females was compared, the daily offspring production of singly mated females mimicked that of multiple maters for about 5 days after the first mating. After the fifth day, offspring production of singly mated females dropped abruptly, whereas multiply mated females continued to produce offspring at approximately a constant rate (Pyle and Gromko 1978). Some degree of sperm depletion may have been responsible for the difference in reproduction between singly and doubly mated G. integer and A. domesticus (Sakaluk and Cade 1980). The average interval between first and second matings for female A. domesticus was 3.0 days. Females with a short

interval (0 to 1 day) between the initial and second mating had shorter durations of spermatophore attachment than other females. Newly mated females with insufficient periods of spermatophore attachment should remate more rapidly if additional sperm are required for optimal reproduction. This result suggests that females are capable of assessing the degree of sperm transfer or the duration of spermatophore attachment. Taylor (1967) showed that female Atteva punctella (Lepidoptera; Yponomeutidae) involved in infertile matings had a significantly higher probability of remating than females from fertile matings. Additionally, Labine (1964) showed that severing innervation to the bursae of recently mated female Euphydryas editha (Lepidoptera; Nymphalidae), increased the chances of their remating.

The potential for a similar relationship between the age at which females first mate and spermatophore attachment also was examined in A. domesticus. Sakaluk (1978) found that females, singly mated at an age of 5 to 6 days, produced more offspring than females that were singly mated at an age of 11 to 12 days. Females that mated for the first time at an older age might be selected to compensate for this decreased reproduction by increasing the time at which they consumed spermatophores. No such effect was found.

The importance of female cricket multiple mating in replenishing spent spermatozoa, however, must remain speculative. If a high degree of sperm depletion was responsible for the decreased reproduction in singly mated female A. domesticus fed

mouse chow, then their daily offspring production would have been similar, at least for some time, to the daily offspring production of females confined constantly with males. Also, the duration over which offspring hatched for singly mated females would have been much shorter than that of females confined constantly with males. Instead of exhibiting this characteristic pattern of sperm depletion (see Gromko and Pyle 1978), female A. domesticus exhibited a much different trend. There was no significant difference in the duration over which offspring hatched for singly mated females fed mouse chow and females fed mouse chow and allowed constant access to a male. Also, the daily offspring production of singly mated females did not equal that of females allowed constant access to males for the initial period of offspring production, nor did it abruptly decrease. Instead, the pattern of daily offspring production of singly mated females reflected that of females allowed constant access to males over the entire duration of offspring production. However, singly mated females consistently left fewer offspring daily. To a reduced extent, these trends also were found in singly and doubly mated G. integer. Singly mated females consistently left fewer offspring daily than doubly mated females. However, the duration over which offspring hatched for doubly mated females was significantly greater than that of singly mated females. These results may indicate that a factor(s) provided by a male during mating stimulates female oviposition and/or egg production. Repeatedly mating females would increase this

benefit and exhibit increased levels of offspring production. The existence of a male mating factor that stimulates egg production and oviposition in females was documented in Teleogryllus commodus (Loher and Edson 1973). Apparently, this factor arises in the testes and is transferred to the female via the spermatophore, although it is not known whether the sperm or some other substance causes the effect. Recently, however, prostoglandin synthetase has been linked to increased oviposition in A. domesticus. This enzyme was found in the testes, seminal vesicles, and spermatophores of male A. domesticus. It also was detected in the reproductive organs of mated females, but not virgin female house crickets. Additionally, an inhibitor of this enzyme suppressed oviposition in mated females (Destephano and Brady 1977).

Female crickets might acquire nutrition from spermatophore consumption. This benefit also would be augmented by female multiple mating. Although the consumption of one spermatophore did not increase reproduction in singly mated female A. domesticus, it may be that a slight nutritional benefit could not be discerned. Spermatophore feeding and possibly, egg production stimulants might be considered forms of parental investment if the male's contribution resulted in the increased survival of his offspring at the expense of his ability to invest in other offspring (Boggs and Gilbert 1979; Thornhill 1976; Trivers 1972). Sakaluk and Cade (1980) suggested that spermatophore feeding in crickets should be considered a form of mating effort (Alexander and Borgia 1979)

and not parental investment. This was largely based on two observations. First, male A. domesticus and G. integer, selected to invest parentally, should produce the large spermatophores characteristic of katydids. In these species, the large spermatophylax coupled with the loss of female sexual receptivity after mating, strongly indicates male parental investment (Morris 1979). Secondly, the apparent inability of male field crickets in preventing females from departing after mating (Cade 1979a) such that females likely mate with many different males, reduces the possibility that the nutrition provided by males is incorporated into the eggs he fertilizes.

However, the distinction between mating effort, a necessary condition or unavoidable consequence of males acquring mates, and male parental investment may still appear to be somewhat ambiguous. The reason for this is due largely to the lack of data regarding the incorporation of nutrition contained in male spermatophores into a female's eggs. In house and field crickets, a clear distinction might possibly require a better understanding of how often nutrition provided by males is incorporated into eggs he fertilizes, the functional significance of mate guarding, the costs of spermatophore production to males, and the pattern of sperm competition. This further will be considered in the section discussion sperm competition and mate guarding in crickets.

Female multiple mating in G. integer and A. domesticus produced additional effects on female reproduction and longevity.

The offspring of doubly mated female G. integer and females allowed constant access to males for 24 to 48 hours, emerged later than those of single maters. This difference was significant but small, and may not have any biological significance. Also, doubly mated female G. integer lived significantly longer than single maters (Table 13). Interestingly, starved virgin female Plebeiogryllus guttiventris that had spermatophores artificially inserted into their genitalia, lived significantly longer than starved virgin females that did not have artificially inserted spermatophores (Bentur and Mathad 1975). However, singly mated female A. domesticus fed mouse chow lived significantly longer than females confined constantly with males. Constant confinement with males would result in continual male-female behavioural interactions. This might restrict the time in which females could acquire nutrition and/or conserve energy, resulting in reduced survivorship.

Sperm competition and mate guarding

The electrophoretic examination of various allozymes in G. integer did not allow determination of a pattern of sperm competition. Although 56 females each were mated to 2 different males, the number of different males involved in matings totalled only 16. This small number of males severely limited the probability of the occurrence of requisite mating combinations. Compounding the problem was the denaturation of protein in 4

of the male samples (eg. "a" in Fig. 16), further limiting potentially favorable mating combinations. This was, however, an exploratory study and one which previously had not been attempted in crickets. The obvious benefit of this study was that it established a potential protocol for determining the pattern of sperm competition in G. integer. Such a protocol would incorporate the following necessary changes. Amylase allozymes solely would be utilized since of all the enzymes examined, they provided the clearest system for the establishment of the paternity of offspring. Many more males would be used, to ensure that the necessary matching of genotypes would be assured. One hind leg of each male first would be electrophoresed, as hind legs contain sufficient enzyme to be detected (pers. observation). Males are able to mate even after the removal of a hind leg. This would save a great deal of time because genotypes of parents would be established before matings, and the requisite genotype pairings could be arranged. This procedure clearly would allow paternity of offspring to be established. The pattern of sperm competition could be accurately determined for each day of offspring production.

It is tempting to speculate that the last male to mate fertilizes most of the subsequent offspring in A. domesticus and G. integer, as is the case in other Orthoptera (Hunter-Jones 1960; Parker and Smith 1975). Certainly the mate guarding exhibited by male crickets does, in other insect species, function to prevent last male sperm predominance (Parker 1970a).

Mate guarding in crickets studied here did not appear successful in preventing females from prematurely removing spermatophores. Indeed, any delay in the removal of the spermatophore is probably only an incidental function of post-copulatory behaviour (Alexander 1961). Instead, the post-copulatory behaviour of crickets probably functions to keep males and females together and promote repeated copulation (Alexander 1961; Alexander and Meral 1967; Loher and Rence 1978). However, in 12 field matings observed in G. integer, males were unsuccessful in preventing females from departing shortly after mating (Cade 1979a). Also, inseminated females disperse by flying in the night and probably encounter other males (Cade 1979c). Given these observations, the temporary monopolization of females by males for more than a short time is doubtful, especially in high density populations. Regardless of how long males are able to monopolize, even short-term holding of the female could reduce last-male sperm predominance. In this case, subsequent spermatophores consumed by the female might well contribute to the offspring of the monopolizing male, and must therefore be viewed as parental investment. Indeed, given the assumption of last-male predominance and the fact that a female must ultimately have a last mating, then for each female there may be at least one male that contributes parentally to his offspring. However, parental investment of females far exceeds that of males. Female crickets mate many times over their lifetime and probably, with several different males. Therefore, for the majority of males, spermatophore feeding is mating

effort. Females probably consume many spermatophores whose sperm never fertilize eggs.

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Appendices

Appendix 1. Method for Calculating the Proportion of Subsequent Eggs Fertilized by the Second Male to Mate (Boorman and Parker, 1976)

$Pr = (1 - x/p) + (z/p \times (x/p) / 1 - (z/p))$

Pr= proportion of eggs fertilized by R male

x = proportion of eggs hatching after double mating

z = proportion of eggs hatched produced from R sperm, as indicated by singly mating females, to N males (ideally, should be 0%)

eg. for NR mating

$x = 0.1314$; $z = 0.049$; $p = 0.945$

$Pr = 0.861 + 0.045 = 0.906$.

Second male fertilized 90.6% of the offspring.

eg. for RN mating

$x = 0.8751$; $z = 0.049$; $p = 0.945$

$Pr = 0.074 + 0.004 = 0.078$

First male (R) fertilized 7.8% of the offspring, thus the second male fertilized 92.2%

Mean progeny fertilized by second male = 92.2 + 90.6 / 2

= 91.4%

Note: When I used this equation I did not incorporate the second half of it because in both instances, z was less than 1%

Appendix 2. Method for Determining the Total Number of Eggs a Male *S. stercoraria* Fertilizes over All a Female's Egg Batches (Parker, 1970)

mates with virgin female

$$\Sigma E = E_1 + E_2(1-p) + E_3(1-p)^2 .... + E_n(1-p)^{n-1}$$

mates with non-virgin

$$\Sigma E = E_1 p + E_2 p(1-p) + E_3 p(1-p)^2 .... + E_n p(1-p)^{n-1}$$

ΣE = total number of eggs fertilized by the male

E = number of eggs in a given batch

p = proportion of eggs in the present batch to be fertilized by last male to mate

n = batch number

eg. A male mates with a virgin female, which, over the course of her lifetime, mates with three more males.

$E = 60$ for all batches

$p = 0.8141$

$n = 4$

$$\Sigma E = 61 + 11.15 + 2.07 + 0.39 = 74.24$$

The male fertilized approximately 74 of the female's total number of eggs.

Appendix 3. Electrophoretic examination of G. integer: Mating combination.

Female #	Age at 1st mate (days)	Date of 1st mate	♂ #a	Date of 2nd mate	♂ #b	Date of Death
1	3-10	2/2/79	1	5/2/79	2	8/4/79
2	3-10	2/2/79	2	5/2/79	1	5/4/79
3	3-14	9/2/79	1	13/2/79	2	2/4/79
4	6	4/3/79	3	7/3/79	4	12/4/79
5	6	4/3/79	4	7/3/79	3	16/4/79
6	9	7/3/79	5	10/3/79	4	28/3/79
7	9	7/3/79	6	10/3/79	4	29/4/79
8	9	7/3/79	7	10/3/79	5	2/4/79
9	-	----	-	----	-	2/3/79
10	6	4/3/79	3	7/3/79	4	17/4/79
11	6	4/3/79	4	7/3/79	3	15/4/79
12	9	7/3/79	8	10/3/79	6	27/4/79
13	6	5/3/79	3	8/3/79	6	25/3/79
14	6	5/3/79	4	8/3/79	8	24/4/79
15	7	6/3/79	3	9/3/79	4	30/4/79
16	7	6/3/79	4	9/3/79	6	14/4/79
17	8	7/3/79	8	11/3/79	4	19/4/79
18	7	7/3/79	8	11/3/79	8	19/4/79
19	7	7/3/79	4	13/3/79	7	12/4/79
20	7	7/3/79	5	10/3/79	7	12/4/79
21	7	8/3/79	4	22/3/79	9	12/4/79
22	7	8/3/79	4	12/3/79	5	9/5/79
23	-	----	-	-----	-	10/3/79
24	7	9/3/79	6	13/3/79	4	18/4/79
25	7	9/3/79	7	14/3/79	4	6/5/79
26	10	12/3/79	4	15/3/79	8	7/5/79
27	6	8/3/79	4	13/3/79	5	15/4/79
28	6	9/3/79	4	13/3/79	7	30/4/79
29	10	14/3/79	7	19/3/79	5	7/5/79
30	5	16/3/79	7	19/3/79	8	3/5/79
31	7	19/3/79	8	23/3/79	9	29/4/79
32	13	25/3/79	9	28/3/79	10	11/5/79
33	10	23/3/79	9	28/3/79	11	10/5/79
34	6	21/3/79	LF	25/3/79	10	3/5/79
35	10	26/3/79	9	30/3/79	10	5/5/79
36	6	22/3/79	10	26/3/79	11	18/5/79
37	6	23/3/79	10	27/3/79	9	28/3/79
38	6	23/3/79	9	4/4/79	10	3/5/79
39	10	27/3/79	10	30/3/79	9	23/4/79
40	10	28/3/79	9	6/4/79	12	9/5/79
41	10	28/3/79	10	2/4/79	9	29/4/79
42	7	25/3/79	12	28/3/79	10	3/5/79

Female #	Age at 1st mate	Date of 1st mate	♂#a	Date of 2nd mate	♂#b	Date of Death
43	6	25/3/79	9	28/3/79	12	10/5/79
44	7	26/3/79	9	------	-	28/3/79
45	6	27/3/79	9	11/4/79	10	19/4/79
46	6	27/3/79	10	30/3/79	9	21/4/79
47	7	28/3/79	9	4/4/79	BF	19/4/79
48	6	28/3/79	9	6/4/79	11	30/4/79
49	6	28/3/79	12	2/4/79	9	21/5/79
50	6	2/4/79	9	18/4/79	101	13/5/79
51	6	2/4/79	9	6/4/79	11	8/5/79
52	9	7/4/79	9	11/4/79	1 dot	15/5/79
53	6	7/4/79	9	11/4/79	109	23/5/79
54	9	11/4/79	9	15/4/79	BF	17/5/79
55	5	7/4/79	BF	18/4/79	102	23/5/79
56	9	11/4/79	BF	18/4/79	103	2/6/79

Appendix 4. Staining Solutions and Electrophoresis Times

Protein Gel

1. 5% gel, using 0.1M Tris Borate EDTA as the buffer solution.

2. Electrophoresis. 350V and less than 100 mA. Run for one hour. Change to 410V for 40 mins.

3. Staining.
   Use 1 cc of 1% Coomassie Blue in methanol, H_2O and Acetic acid mixture per 100 cc of the prepared Methanol, H_2) and CH_3COOH solution. Put the gel in this mixture until deeply stained bands appear. De-stained afterwards in the same mixture, but without the dye.

Esterase

Electrophoresis. Run for 1 hr and 45 mins. at 350 V

Staining: a. Inculate gel in 0.5M Boric acid in the refrigerator for 1 hr.

b. Discard Boric acid after inculation and rinse gel with H_2O

c. 1. Dissolve 20 mg of α Nepthyl acetate in 1 cc of 50% acetone.
   2. Dissolve 50 mg fast red TRN in 100 cc of 0.1M phosphate buffer pH 6.5.
   3. Mix solutions 1 and 2.
   4. Stain gel with this solution and leave on shaker until red bands appear.

Amylase

Electrophoresis. Start at 350 V, 100 mA for 1 hr.
Raise to 450 V for 1.5 hr.

Staining.
1% solution of soluble starch in 0.1M Tris HCl. pH 7.5 and $CaCl_2$. Solution make by boiling. Pour on gel. Leave on shaker for one hour. Wash off starch solution. Pour on I_2 - KI - H_2O solution. Amylase bands appear as clear spots.

Malic Dehydrogenase

Electrophoresis. 400 V and less than 100 mA for 1 hr and 50 mins.

Staining.
10 ml 1M Sodium Malate
20 mg NAD
30 mg NBT
100 ml 0.1M Tris - HCl pH 7.5
Inculate for 1 hr, then add 1 ml of PMS solution

Alcohol Dehydrogenase

Electrophoresis. 400 - 500 V for 6 hrs,
80 - 100 mA.

Staining.
same as MDH execpt use Iso-propanol in place of Sodium Malate.

Lactic Dehydrogenase

Electrophoresis. 400 - 450 V for 3 hrs.

Staining.
same as MDH except use sodium lactate in place of Sodium Malate

Appendix 5. Chemical Compostition of Purina® Mouse Chow

Nutrients	% of ration
PROTEIN	20.6
Arginine	1.03
Cystine	.30
Glycine	.90
Histidine	.45
Isoleucine	1.01
Leucine	1.48
Lysine	1.02
Methionine	.46
Phenylalanin	.88
Threenine	.71
Tryptophan	.23
Valine	.99
FAT	9.0
FIBER	2.5
TDN	84.1
NFE(by difference)	54.8
Gross Energy, KCal/gm	4.3
ASH	4.8
Calcium	.81
Phosphorus	.60
Potassium	.64
Magnesium	.17
Sodium	.30
Chlorine	.40
Fluorine, ppm	6.4
Iron, ppm	97.7
Zinc, ppm	40.7
Manganese, ppm	51.1
Copper, ppm	10.0
Cobalt, ppm	.04
Iodine, ppm	.36
VITAMINS	
Carotene, ppm	--
Manadione (added, ppm	8.8
Thiamin, ppm	8.0
Riboflavin, ppm	8.0
Niacia, ppm	54.8
Pantothenic Acid, ppm	21.0
Choline, ppm x 100	22.0
Folic Acid, ppm	2.1
Pyridoxine, ppm	5.0
Biotin, ppm	.2
B-12 mcg/lb.	6.0
Vitamin A, IU/gm	30.0
Vitamin D, IU/gm	3.3
Alpha-tocopherol, IU/lb.	30.0
Ascorbic Acid, mg/gm	---

Appendix 6. Clarification of *A. domesticus* experimental groups.

Tables comparing the parameters of reproduction in *A. domesticus* females record five experimental subgroups. Three consist of singly mated females while two consist of females allowed constant access to males. However, these two groups represent two separate experiments conducted at different times and probably under slightly different conditions (see Methods). Comparisons between singly mated females of different nutritional regimes and females allowed constant access to males therefore, must be made with caution. This is true particularly for comparisons of females fed corn meal and allowed constant access to males and singly mated females of the other three nutritional regimes. Females fed corn meal were placed on their diet at an adult age of one day. However, singly mated females of all three nutritional regimes were fed high protein mouse chow up until five days of age, at which time they were mated and placed on their respective experimental diets. Additionally, slight differences in temperature and/or season may or may not affect *A. domesticus* female reproduction (Ghouri and McFarlane 1958; Bate 1972). Of the two experimental groups, perhaps the only relevant comparison is the reproduction of females fed mouse

chow that were either singly mated or allowed constant access to males. Females of these two sub-groups were fed the same diet as nymphs and throughout adult life. For this reason, only this last comparison is stressed in the discussion.