Effect of Age On Female Choice

in the Field Cricket,

Gryllus integer

by

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Submitted to the Department of Biological Sciences

in partial fulfillment of the requirements

for the degree of

Master of Science

July 1994

Brock University

St. Catharines, Ontario

ABSTRACT

Female choice is an important element of sexual selection that may vary among females of the same species. Few researchers have investigated the causes of variation in selectivity with respect to potential mates and overall level of motivation toward a stimulus source representative of a mate. This study demonstrates that female age may be one cause of variation in female choice. Females of different ages may have different mate preferences. As females age, they have less time left to reproduce, and their residual reproductive value decreases. This should correspond to a higher reproductive effort which may be represented as increased motivation and/or decreased selectivity.

The effect of age on mate choice in *Gryllus integer* was investigated by using a non-compensating treadmill, called the Kugel, to measure female phonotaxis. Artificially generated male calling songs of varying pulse rates were broadcast in either a single-stimulus or a three-stimulus experimental design. The pulse rates used in the calling song stimuli were 70, 64, 76, 55 and 85 pulses per second. These corresponded to the documented mean pulse rate for the species at the experimental temperature, one standard deviation below and above the mean, and 2.5 standard deviations below and above the mean, respectively. Test females were either 11-14 days or 25-28 days post-ecdysis. Trials usually were conducted two to seven hours into the scotophase.

In the single-stimulus experiment, females were presented with stimuli with only one pulse rate. Older females achieved higher vector scores than younger females, indicating that older females are

more motivated to mate. Both groups showed little phonotactic response towards 55 or 85 p/s, both of which lie outside the natural range of *G. integer* calling song at the experimental temperature. Neither group discriminated among the three pulse rates that fell within the natural range of calling song.

In the three-stimulus experiment, females were presented with stimuli with one of three pulse rates, 64, 70 or 76 p/s, in alternation. Both age groups had reduced responsiveness in this experiment, perhaps due to an increase in perceived male density. Additionally, younger females responded significantly more to 64 and 70 p/s than to the higher pulse rate, indicating that they are selective with respect to mate choice. Older females did not discriminate among the three pulse rates. Therefore, it was concluded that selectivity decreases with age.

A further study was conducted to determine that these effects were due to age and not due to the differing periods without a mating between the two age groups. Again, stimuli were presented in a three-stimulus experimental design. Age was held constant at 28 days and time since last mating varied from 11 to 25 days. Females varying in time since last mating did not differ in their responses to the calling song pulse rates. This indicated that the increased motivation and decreased selectivity exhibited in the initial experiments were due to age and not to time without a mating. Neither time of trial nor female weight had an effect upon female phonotaxis. Data are discussed in terms of mate choice, residual reproductive value, and costs of choice.

ACKNOWLEDGEMENTS

I would like to extend my thanks to my supervisor, Dr. W.H. Cade for his tremendous guidance and support. I would also like to thank Dr. Anne-Marie Murray and Dr. William Wagner Jr. for their advice on the project and invaluable help with statistics. I would like to thank the members of my supervisory committee, Dr. J. Mercier and Dr. C. Lewis for their advice on many aspects of my project and academics throughout my stay at Brock. Special thanks to Tom MacDonald and crew for assisting me to learn the use of the machinery. Finally, I would like to thank my lab mates, Leslie Proctor, Allen MacDougall, and Dayle Belme for making my time at Brock enjoyable and memorable.

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INTRODUCTION

Darwin (1872) noted certain traits which increase the reproductive success of the holder, and suggested that members of the opposite sex may have preferences for these traits. Mate preferences have been documented in a number of taxa but the causes of variation in these preferences are still under investigation. It has been demonstrated that female mate choice increases the reproductive success of the female in a variety of taxa (Crocker and Day, 1987; Reynolds and Gross, 1992).

One cause of variation in mating preferences may be the costs associated with female choice in terms of time and energy spent searching for a mate. It is predicted that these costs increase as females age (Caswell, 1982). An older female, with a shorter reproductive future may have fewer mating opportunities. As animals age, their residual reproductive value decreases and therefore their investment in reproductive effort should increase (Stearns, 1992). Older females should have a broader range of mate acceptance as was demonstrated in *Acheta domesticus* (Walikonis *et al.*, 1991).

Crickets are useful subjects for studying mate choice. Pair formation is elicited by females phonotactically orienting to species-specific calling song (Loher and Dambach, 1989). Crickets can be studied in the field or in the laboratory. Females orient well to naturally calling males (French and Cade, 1987), taped recordings of naturally calling males (Schmitz *et al.*, 1982), and artificially generated calling songs (Loher *et al.*, 1992). Female phonotaxis is relatively easy to measure, either by observation in the field or in

laboratory, or by quantitative computer analysis in conjunction with a locomotory treadmill.

Often it is questioned whether orientation towards a sound stimulus adequately represents motivation to mate. A female is unable to mate with a male until she orients to his calling song and frequently, mating follows orientation. In a study by Cade (1979), female *Gryllus integer* that had been deprived of males for three days exhibited higher levels of phonotaxis, indicating that phonotaxis and mating may be linked. Additionally, the onset of phonotaxis occurs only slightly before the onset of mating (Sakaluk, 1982). Phonotaxis is used as a measure of mate choice in many animals (Morris *et al.*, 1977; Doherty and Gerhardt, 1984).

The purpose of this study was to determine whether female age affects mate choice. Females of the Texas field cricket, *G. integer* were divided into distinct young and old groups. A non-compensating treadmill was used to quantify phonotaxis toward different variations of artificially generated calling song. It was predicted that older females should show higher reproductive effort, in the form of increased levels of phonotaxis compared to younger females. Older females also were predicted to exhibit decreased selectivity with respect to mate choice.

LITERATURE REVIEW

In this section, literature that pertains to this thesis is reviewed. The three main topics covered are Sexual Selection and Female Choice, Mating Behaviour in Gryllidae, and Phonotaxis and Mate Choice.

Sexual Selection and Female Choice

Sexual selection refers to the differential reproductive success of conspecific individuals of the same sex (Darwin, 1872; Lande, 1981; Arnold, 1983). Darwin proposed two mechanisms which operate together to cause these differences. The first is intrasexual competition, which typically involves males competing for the opportunity to mate with females. The second of these mechanisms is mate choice exerted by the sex undergoing less competition, which in most cases is the female. Intrasexual selection and mate choice and which sex exhibits each can be predicted by the relative costs involved if a low quality mating or a heterospecific mating were to occur. In most polygynous animals, males invest very little in their offspring. Sperm are small, numerous and easy to produce. Therefore, the reproductive success of males should be limited by the number of eggs they can fertilize, rather than the number of sperm they can produce. Eggs are fewer, larger, and require nutritional investment for development. It is much more costly for a female to mate with a heterospecific or a low quality conspecific male and consequently, to lose an entire batch of eggs, than it is for the male to make this same error. For this reason, females are most often the more discriminatory of the two sexes, whereas males

actively compete for the opportunity to mate with as many females as possible (Trivers, 1972).

In species in which much more parental investment is required from each parent, such as in monogamous birds, this dichotomy is not as distinct. It becomes more costly to the male to raise offspring that are unhealthy or infertile or to pair with a female that is not able to provide the young with the necessary care. Males in these systems also are seen to be discriminatory with respect to mate selection (Trivers, 1972).

In some species, the females compete for the males, and the males are the more selective sex. In these systems, either the males provide an important resource for the females such as food, territory, or parental care, or the chances of encountering a high-quality, healthy male are slim. The discriminating males usually choose their mates on the basis of fecundity. Often weight is an indicator of fecundity because heavier females are likely to produce more eggs. Males of some species thus select females on the basis of weight. Males that discriminate with respect to female weight will produce more offspring and therefore achieve a higher reproductive success (Bradbury and Davies, 1987).

Models of Intraspecific Female Choice

Models of female choice often are divided into two categories on the basis of whether the female gains resources directly from the male such as attaining nutrition or an oviposition site, or gains indirectly by producing offspring that possess heritable

characteristics that may increase their viability or mating success (Maynard-Smith, 1985).

Resource-Based Choice

Resource-based mate choice involves female preference for males that offer superior resources, such as food, territories and oviposition sites. In this system, the female derives direct benefits from the male. Males of the hangingfly, *Hylobittacus apicalis* (Thornhill, 1980), the scorpionfly, *Harpobittacus similis* (Gwynne, 1984), and the Japanese scorpionfly, *Panorpa japonica* (Thornhill, 1992) attract females by everting pheromonal glands. During courtship, the male presents an arthropod prey item to the female. Females of each of these three species appear to accept or reject males on the basis of size of the prey offering. The arthropod provides the female with the required nourishment for egg development. By accepting these nuptial gifts, the female can produce more offspring, hence her fitness is increased. Females also benefit from not having to hunt, which reduces their risk of predation. The female feeds on the arthropod during copulation. A female receiving a large food item copulates longer and accepts more sperm from the male. Larger sperm loads increase the male's probability of successful fertilization. Therefore, a male presenting a large nuptial gift will copulate longer, and thereby transfer more sperm to the female, which increases his inclusive fitness.

Another example of resource-based selection is in the bullfrog, *Rana catesbeiana*. Early embryo mortality, which is caused by developmental abnormalities, depends on the site of oviposition. Temperatures above 32°C promote developmental abnormalities and

therefore sites that reach this temperature should be avoided. Females ovipositing at sites with low temperature greatly enhance their reproductive success. Oviposition sites are controlled by territorial males. Larger and older males control the most ideal territories. Females choosing to mate with larger or older males improve their chances of acquiring ideal oviposition sites (Howard, 1978a,b).

Non Resource-Based Female Choice

Fisher's Runaway Selection

Fisher (1958) proposed that females may choose mates on the basis of a trait that may reduce viability, but is not correlated directly with fitness. In many secondary sexual characteristics, there is a lot of variation among the males in the population. If these characteristics are heritable, they will be passed on to the offspring. Females may vary in their preferences for these characteristics. If the majority of females prefer males with a certain trait, these males may incur an increased mating success, are likely to produce more offspring and may pass this characteristic to their offspring. In some males, the trait may be exaggerated, and this may be more attractive to many of the females. The exaggerated trait may then be passed on to the offspring and increase in the population. The male trait and female choice for the trait can proliferate in this manner at an exponential rate. This runaway process will continue until enhancement of the character begins to inhibit the survival potential of the individual (Fisher, 1958).

Fisher's hypothesis is very difficult to prove in the field because it is not easy to determine that over time the male trait became enhanced due to the female preference for a superstimulus. Some evidence has come from work on the widowbird, *Euplectes progne* in which females mated preferentially with males possessing long tails. Andersson (1982) demonstrated that males with artificially lengthened tails achieved a higher mating success than controls or groups with shortened tails. However, long-tailed males may have had more difficulty in flight, and in obtaining prey and maintaining a territory, thereby decreasing their fitness. They also may have been more conspicuous to predators. For this reason, the length of the tail is unlikely to change further from its current length (Andersson, 1982).

"Good Genes" Hypothesis

It also has been suggested that females are capable of choosing mates on the basis of "good genes". Good genes refers to those genes coding for heritable traits that increase the viability or reproductive success of the individual. Females can increase their own inclusive fitness by mating with a male that possesses superior survival capabilities compared to other males. These qualities might include large body size, health and vigour, and if heritable, will be passed on to their offspring.

Size is easily assessed by females. Female finches, *Geospiza fortis*, appear to choose larger males (Price, 1984). Other aspects of male quality may not be as assessable. Often, mate quality is

correlated with another trait which may or may not contribute to a reduction in fitness. This correlated trait is referred to as a handicap.

There are three types of handicaps. A Zahavi's handicap is one in which the males possessing the trait must be of high viability to survive the handicap (Zahavi, 1975; Pomiankowski, 1988). Females can assess the fitness of males by the presence or absence of the trait. The trait is such a burden that only males of high quality would possess it.

The second type is the Condition-dependent handicap in which the gene is expressed only under certain environmental conditions (Andersson, 1986). Males of the guppy, *Poecilia reticulata*, vary in colour pattern and females are attracted to more brightly coloured males. Male brightness depends on the amount of carotenoids consumed. Therefore, in environments when food is scarce, even males possessing the genes for bright colouration may be dull in colour (Endler, 1980).

The third type of handicap is the revealing handicap. In this model, the ornament or display accurately reflects the viability of the male. Hamilton and Zuk have applied this theory to parasitized males. They believe that females can assess parasite load through the condition of the ornament. Males with no parasites are expected to have brighter or more elaborate ornaments. Empirical evidence in various species of birds supports this view. Both bright plumage (Hamilton and Zuk, 1982; Borgia and Collis, 1989) and complex song (Read and Weary, 1990) in birds are indicative of healthy, parasite-free males. In parasitized birds, much of the energy required for bright colour or elaborate song is diverted to the hematozoa.

Females discriminate on the basis of male brightness, and complexity of song (Hamilton and Zuk, 1982; Borgia and Collis, 1989).

The preceding examples deal with females choosing the most fit males on the basis of certain quality indicators. Females also may gain indirectly by mating with males possessing characteristics that typically are attractive to females, but uncorrelated to fitness, if these traits are heritable. The female then may pass the trait to her offspring whom are thus likely to achieve a higher mating success (Maynard Smith, 1987). This often is referred to as the sexy son hypothesis (Weatherhead and Robertson, 1979). Some genetic lines of female ladybirds of the species *Adalia bipunctata* have shown a distinct preference for the melanic form of males. Melanism does not appear to be directly correlated with fitness, therefore females do not benefit from mating with a male possessing this trait by passing fitness genes to her offspring. The offspring will be likely to receive traits that will be more attractive to other females, and therefore will attain a higher reproductive success. By mating with these preferred males, the offspring of the female may acquire the genes for the desired characteristic (Majerus *et al.*, 1986).

Few studies have examined whether sexual characteristics are heritable or whether females benefit from choosing. Reynolds and Gross (1992) found that female mate preference resulted in increased offspring growth and reproduction in a fish, *Poecilia reticulata*. Females preferred large males, and size was heritable to both sons and daughters. Large sons achieved higher mating success due to their attractiveness, and large daughters achieved higher reproductive success due to their high fecundity. Choice also

increases reproductive fitness in the seaweed fly, *Coelopa frigida*. Females that are allowed to choose their mates produce more offspring. There is a particular inversion system on chromosome I and heterokaryotypes have better survival rates. Females chose mates with the opposite karyotype to their own (Crocker and Day, 1987).

Sensory Exploitation

Sometimes, males may develop a secondary sexual characteristic corresponding to an existing female preference of non-sexual context (Kirkpatrick, 1987). This phenomenon is termed sensory exploitation. Water mites locate their copepod prey by sensing the movement of the legs of the copepods during swimming. Over time, males have evolved a courtship ritual that begins with vibrating their legs at the same frequency common in swimming copepods. The remainder of the mating ritual continues once the female has oriented to and clutched the male (Proctor, 1992).

Mating Behaviour in Gryllidae
Pair Formation

Mating involves close contact between the male and the female and this can be accomplished through the use of several sensory pathways (Paul, 1976; Otte, 1977; Atkins *et al.*, 1987; Stiedl and Kalmring, 1989).

Close range orientation can be accomplished by vibrational stimuli. Females of the bushcricket, *Ephippiger ephippiger*, were presented with calling song and tremulations conducted through the plant in the laboratory. After a certain distance had been reached by

the female, the auditory stimulus was switched off. Vibrotaxis was apparent only when the female was a relatively short distance, approximately 58 cm, from the source. This suggests that vibration is significant only in the localization of nearby males (Stiedl and Kalmring, 1989).

Visual cues may also assist in mate localization at close range. Adult *Acheta domesticus* females were attracted to dark objects for which the horizontal visual angle was greater than or equal to 30°, and the horizontal measurements exceeded the vertical dimensions. Complex shapes such as two or more superimposed rectangles caused significantly lower scototactic responses (Atkins *et al.*, 1987).

In laboratory experiments, several species of nemobines were presented with paper from the cages of female crickets. They responded with antennation and production of calling song (Paul, 1976). Similarly, Otte and Cade (1976) demonstrated that in the presence of female odours, male crickets of the genera *Acheta* and *Gryllus* groomed more and became more aggressive. This suggests that some acoustical insects may use pheromonal cues in mate recognition.

The best studied and most characteristic means of pair formation involves female orientation to male calling song. Such acoustic communication has several advantages over other modes. Although visual and vibrational cues may be utilized at close range, auditory stimuli are the most effective over long distances. Acoustic and visual signals may be turned off and on rapidly whereas chemical signals are persistent and thus cannot be turned off if danger approaches. Visual signals are relatively ineffective at night.

Crickets are nocturnal and therefore can make little use of visual stimuli, except at close range. Due to limitations in distance vision in the insect eye, vision plays no role in orienting to mates over vast distances. A further advantage of acoustic stimuli is that they can be transmitted over relatively greater distances. (Otte, 1977).

Most male crickets possess the ability to produce a species-specific calling song by a process termed stridulation which is the rubbing together of body parts to produce a sound (Alexander, 1966; Toms, 1986). Among species there is a lot of variation in the male song and mating behaviour. In the Gryllidae, interspecific variation in song typically is with respect to temporal properties of the song. Female crickets discriminate between heterospecific and conspecific males (Moiseff *et al.*, 1978; Pollack and Hoy, 1979; Pollack, 1986; Doherty and Callos, 1991; Doherty and Storz, 1992). There is also a great deal of variation in the songs and behaviour of males within each species (Souroukis *et al*, 1992).

Calling

The most conspicuous sound produced by Orthopterans is the calling song, which typically is a loud, long-lasting, rhythmical sound emitted by the male (Alexander, 1957; 1962). The sounds are generally in the range of 2 to 10 kHz (Bennet-Clark, 1989) and are some of the loudest sounds produced by animals. Orthopterans are the only animals known to produce a pure frequency by stridulation (Alexander, 1966).

The cubital fields are specialized veins of the modified forewings or tegmina that are responsible for sound production. The

specialized region on the upper posterior surface of one of the wings, called the plectrum, acts as a scraper, and is used as a file on the undersurface of the other wing (Bennet-Clark, 1989). As the plectrum and teeth of the file are engaged, the wings close, causing the plectrum to distort until it slips to the adjacent tooth (Elliot and Koch, 1985; Bennet-Clark, 1989). Sound is emitted only during the closing stroke of the wings, and each closure represents a pulse of sound. The rate at which the plectrum activates the teeth residing on the file is determined by the resonant properties of the wings. The teeth are dropped at the rate of one tooth per cycle of the sound. The carrier frequency of the song is determined by the size of the file or the spacing between the teeth (Bennet-Clark, 1989). The pure frequency of the song depends on the number of teeth (Alexander, 1962). The intensity of the call depends on the resonance properties and the angle of the tegmina during calling (Loher and Dambach, 1989). Angles range from 45° in *Acheta domesticus* (Khalifa, 1950) to 65° in *Teleogryllus oceanicus* (Loher and Dambach, 1989). In most species of crickets, the females do not possess any of the components of the stridulatory apparatus (Bennet-Clark, 1989).

The song can be perceived over long distances (Cade, 1985). Females exhibit positive phonotaxis toward the male calling song, thus attracting females appears to be one of the primary functions of the calling song. Additionally, the calling song elicits calling and/or aggressive behaviour in other males and appears to maintain spacing between males (Alexander, 1962; 1966). *Teleogryllus commodus* (Campbell and Shipp, 1979) and *G. integer* (Cade, 1981) males were found to remain no less than one meter apart, and can be up to 8 or

9 meters apart in low density habitats (Campbell and Shipp, 1979; French *et al.*, 1986). Males of several burrow-inhabiting species of gryllids call from the entrance of the burrow, thereby amplifying the intensity of the call (Loher and Dambach, 1989). Males not in possession of burrows may call in the field and change position frequently and thus maximize their broadcast distance (Loher and Dambach, 1989).

Diel Patterns of Calling

Males do not call persistently throughout a 24 hour period. Most crickets are nocturnal, and therefore call by night. However, if the nightly temperatures are excessively low, female movement is restricted. In this instance, males will begin to call by day instead (Loher and Dambach, 1989). Calling males typically sing for part of the night only. In *Anurogryllus muticus* and *G. rubens*, song bouts lasted two to three hours per night (Loher and Dambach, 1989; Cade, 1991). Some individual *G. integer* males called 6.2 hours per 24 hour period, on average (Cade, 1991). Peak male calling usually begins within a few hours of sunrise and lasts until a few hours after sunrise in *G. veletis, G. pennsylvanicus* and *G. integer* (French and Cade, 1987).

Cade (1975) observed that in populations of *G. integer*, some males do not call. Instead, they orient to the song of calling males, and remain near the calling male. As females orient towards the calling male, the silent, satellite males intercept the oncoming female.

The presence of this alternative reproductive strategy suggests that there is a cost involved in calling (Cade, 1981).

Any form of signalling not only attracts the attention of the intended recipient of the message, but also possible predators or parasitoids. Several studies have indicated that predators such as cats (Walker, 1964), bats (Belwood and Morris, 1987) and geckos (Sakaluk and Belwood, 1984) orient towards the calling songs of male crickets. Cade and Rice (1980) found cricket fragments in droppings of the toad, *Bufo marinus* indicating that it, too, is a predator.

Cade (1975) discovered an acoustically orienting parasitoid that uses *G. integer* as a host. The female fly, *Ormia ochracea*, deposits its larvae on or near a calling male cricket. Males that sing are more at risk of being parasitized by these flies. Therefore there would be an obvious cost to calling. The benefit of achieving a mating may be outweighed by the cost of predation risk. Satellite males that intercept phonotactic females, still achieve matings, but with less risk of predation or parasitism. Although flies sometimes lay their eggs in the vicinity of a calling male, satellite males often intercept females at a distance from the calling male. Additionally, production of song is energetically costly, and satellite males avoid this cost as well (Cade, 1984). The fly has been noted also to attack *G. rubens* (Walker, 1986) and *T. oceanicus* (Zuk *et al.*, 1993). It was demonstrated that a population of *T. oceanicus* that is attacked by the fly had shorter pulse durations, trill durations, inter-chirp intervals and inter-song intervals than unparasitized populations, suggesting that the population that is vulnerable to parasitism is

selected to reduce song parameters to avoid parasitism (Zuk *et al.*, 1993).

Mechanism of Hearing

Pair formation requires that the female receives the acoustic message from the male, and orients towards the source (Murphey and Zaretsky, 1972). Each of the auditory organs of crickets resides in one of the foreleg tibiae and is thought to receive auditory input from the tympana and spiracles of the ipsilateral and contralateral sides of the animal. The tympana consist of several auditory sensilla that are surrounded by a fluid that contacts both the tympanal membrane and the acoustic tracheae. The tracheae are sensitive to high intensity sound (Ball *et al.*, 1989).

It is thought that the binaural senses of the cricket and therefore, the power to localize sounds are due to the possession of one tympanal organ in each foreleg. This was confirmed by work involving destruction to both tympanal organs in the cricket, *Scapsipedus marginatus*, which resulted in no behavioural response to the calling song stimulus. Destruction to one ear only meant that the cricket was unable to locate the sound source. Its movements were directed towards the side of the intact ear (Murphey and Zaretsky, 1972).

The field cricket, *G. campestris*, was studied in order to determine the importance of posterior tympana and prothoracic spiracles, another source of auditory input, in locating a sound source. Experiments involving occlusion of the auditory channels with wax and testing phonotaxis upon a locomotion compensator

gave rise to the following results. Occlusion of both spiracles resulted in a higher intensity threshold for accurate orientation to begin. Blocked tympana necessitated an even higher intensity threshold and the highest was seen when all spiracles and tympana were occluded. The occlusion of one spiracle and one tympanum at the same time resulted in an increased threshold and course deviations of 49° towards the side of the intact tympanum. These results suggest that while both types of sound structures are important in orienting to sound, the effect of an impaired tympanum overrides that of an occluded spiracle (Schmitz, 1985).

Information flows from the auditory organs to the brain and subsequently to the muscles via the nervous system. The auditory organ resides in the foreleg tibiae and possesses 60-70 auditory receptors. These receptors are arranged in a row and lie in close contact with the acoustic trachea system which is responsible for respiration (Huber, 1984). The axons of the receptors make up the auditory nerve which terminates in an area of the prothoracic ganglion called the acoustic neuropile (Huber, 1983). Some of the receptors are sharply tuned to 4-5 kHz, and there are bursts of nerve impulses that are associated with each syllable of the calling song. In this sense the temporal pattern of the calling song is copied by the receptors. Also, they are sensitive to sound intensity which may assist in determining the direction of the stimulus (Huber, 1984).

Local interneurons in the prothoracic ganglion, Omega neurons, consist of two types. Type-I Omega-neurons are tuned to the calling song carrier frequency, and copy the temporal pattern of the calling song at all intensities (Janiszewski and Otto, 1989). Information is

attained from both ears with reciprocal inhibition, which is believed to assist in directional hearing. Type-II Omega-neurons receive information from both ears and do not copy the temporal pattern of the calling song. The information then is conveyed to the interneurons, AN1 and AN2, which in turn, are connected to the cephalic ganglia. AN1 neurons are very sensitive to the calling song carrier frequency, copy the syllable length and rate of the calling song and receive excitatory input from the ipsilateral ear only. AN2 neurons are tuned both to the calling song carrier frequency and frequencies greater than 10 kHz. Activity in these neurons reflects the chirp length and rate, and the neurons acquire excitatory and inhibitory information from both ears (Huber, 1983). The brain interprets the information and relays messages to the motorneurons which deliver motor output to the muscles. Several of the neurons mentioned copy the temporal pattern of the calling song within a particular range. This range is correlated with the range to which females have oriented in phonotaxis studies (Walikonis *et al.*, 1991). The frequencies to which the various neurons are sensitive are also correlated with the behavioural responses of females (Huber, 1983).

Courtship, Mating and Events After Mating

It is thought that the first cricket sounds were of low intensity and detected only at close range. Courtship song was likely the first acoustical stimulus to arise in Orthopterans and initially, females may not have possessed auditory receptors (Alexander, 1962; Toms, 1986). The raised forewings may have provided visual cues to the female (Alexander, 1966). Courtship also may have been

effective due to the vibrations of the wings. Calling song provided greater vibration and thus may have been preferred by females. Males exhibiting calling may have had increased mating success and therefore this trait was carried on through subsequent generations. Over time the female ear may have evolved in conjunction with the evolution of calling. Calling and courtship are physiologically linked. A calling male often is primed to initiate courtship. If courtship is interrupted, calling sometimes will resume (Alexander, 1962).

Males begin to court females when antennation of the female by the male results in passivity by the female rather than the aggression typical of male behaviour (Alexander, 1961). Courtship may also be initiated by a male upon cercal contact with an object during calling. The wings are held in a roof-like position (Alexander, 1957), and produce a soft, chatter-like and quite rhythmical sound. It is usually accompanied by a distinctive courtship dance, in which the male rocks back and forth in time with the motion of the wings (Alexander, 1962). The courtship song acts to stimulate the female to assume the proper mating position (Alexander, 1962; 1966).

If, during courtship, the male loses contact with the female, he may immediately antennate and palpate the area behind him, while producing a few pulses which resemble the aggressive sounds. These sounds in this context are often termed courtship interruption sounds. They likely function in reattracting the departing female. If unsuccessful, he may revert back to the calling song after a short period of time (Alexander, 1957; 1962).

Courtship may eventually lead to mating. All gryllids assume the female-above mating posture. A male, when primed to mate,

will produce a spermatophore, which consists of a sperm-filled ampulla, an anchor plate and a tube. During copulation, the male attaches the spermatophore to the female externally. The tube is threaded through the genital chamber and passes into the aperture of the spermathecal duct. The ampulla portion remains outside of the body of the female. The entire spermatophore complex is held in place by the attachment plate which is slipped into the female above the subgenital plate. Two fluids inside the ampulla differ in osmotic pressure such that when the tip of the spermatophore is dissolved, the evacuation fluid flows into the pressure bodies. The pressure bodies then swell, causing the contents of the spermatophore to be forced from the ampulla into the tube. The male epiphallus is three-pronged and the spermatophore has hook-like projections. Mating takes approximately 30 seconds and can be repeated as soon as every 15 minutes in some crickets (Loher and Dambach, 1989) including those of the genus *Gryllus* (Alexander and Otte, 1967).

Sperm storage organs called spermathecae, store enough sperm from one mating to fertilize all of the eggs of the female. However, females mate multiply, even though there should be a cost involved in doing so. Sakaluk and Cade (1983) showed that females that were allowed to mate a second time, produced more offspring than those that were not. Burpee and Sakaluk (1993) demonstrated that females of *G. veletis* and *Gryllodes similis* that had limited opportunities to mate suffered a reduction in survival. Females that were given unlimited mating opportunities lived longer and produced significantly more offspring than females that were restricted with respect to their access to males. This suggests that

there is a benefit to multiple mating that offsets the cost of reproduction. The benefit may be achieved in the form of nutrient acquisition from the spermatophore.

After the spermatophore has been attached, most male crickets perform post-copulatory guarding of the female which prevents her from prematurely removing the spermatophore. Guarding also prevents other males from inseminating the same female, thereby displacing the first male's sperm prior to fertilization. Another function of guarding may be to monopolize the female for further matings. If the female makes any attempt to leave or remove the spermatophore, the male becomes very aggressive towards her. The most intense activity occurs within the first 10-20 minutes following copulation. This time corresponds to that of maximum sperm transfer. Each egg travels past the aperture of the spermathecal duct on its way to the genital chamber. Sperm is released from the spermatheca. A maximum of one spermatozoon can fertilize each egg by entering through one of three micropyles on the surface of the egg. The ovipositor of the female is long and slender and is composed of two valvulae. Scales along the inner side of the valvulae grip the egg and move it along the ovipositor. When a moist oviposition site is found, the female inserts her ovipositor into the substrate. Prostaglandin in the spermatophore stimulates the female to release her eggs (Loher and Dambach, 1989).

Phonotaxis and Mate Choice

Most studies involving mate attraction in acoustical insects have dealt with orientation with respect to sound. Phonotaxis is

defined as movement in response to a sound. Positive phonotaxis refers to net movement toward the source, whereas net locomotion away from the stimulus is termed negative phonotaxis. Negative phonotaxis may be important as a means of avoiding predators such as bats (Moiseff *et al.*, 1978). Phonotaxis plays a significant role in pair coupling in crickets and other acoustical insects, with the females exhibiting positive phonotaxis toward the calling song of conspecific males. Females have been shown to exhibit positive phonotaxis to conspecific song much more often than to heterospecific song (Pollack and Hoy, 1979; Pollack, 1986; Doherty and Callos, 1991; Doherty and Storz, 1992). Females also appear to possess preferences for certain males within their species. In many studies, researchers have attempted to determine the characteristics that females prefer. Phonotaxis thus can serve as a means of studying mate choice.

Females generally are most receptive to male calling song within the first few hours after sunset. The songs of conspecific males, as well as artificial conspecific song broadcast from speakers, attracted the greatest number of flying *G. integer* males and females from two to six hours past sunset in a natural arena study. Significantly more females than males were attracted (Cade, 1989).

Methods of Measuring Phonotaxis

Researchers of cricket phonotaxis have used a variety of techniques for measuring phonotaxis. Arenas have been commonly used to measure phonotaxis. Phonotaxis quantification sometimes has been facilitated by dividing the arena into quadrants and

recording which quadrant the cricket is in at certain intervals of time (Stout and McGhee, 1988). Arenas often were lined with fibreglass (2-16 cm thick) along the sides (Zaretsky, 1972; Stout and McGhee, 1988) and sand on the bottom (Zaretsky, 1972; Stout and McGhee, 1988; Murphey and Zaretsky, 1972; Simmons, 1988) to reduce echoes of the broadcast sound and the shape of the arena was usually rectangular or circular. Arenas typically were set up with one to four loudspeakers that broadcast calling song. In contrast to some studies in which previously taped calling song was broadcast, later researchers produced computer generated song in order to standardize the stimulus presented to all of the females (Zaretsky, 1972; Oldfield, 1980; Stout and McGhee, 1988). Prior to the initiation of the sound broadcast, the container with the female cricket was placed in the centre of the arena. Since the response of a cricket cannot be determined uness the cricket orients to the sound source, researchers usually discounted any crickets that did not leave the centre region in a specified amount of time (5-10 minutes), or did not reach a certain distance from the active loudspeaker in a specified amount of time (usually 5 minutes). Trials were discontinued if the female walked along the edge of the arena, rather than approaching the loudspeaker (Murphey and Zaretsky, 1972; Zaretsky, 1972; Hoy et al., 1977; Cade, 1979; Sakaluk, 1982). Observations were conducted with the aid of red light (Hoy et al., 1977; Sakaluk, 1982). The advantage of arena studies is that they are very natural and provide little stress to the individual. The disadvantage is that the researcher must be relatively subjective in deciding when movements are considered sufficient to be counted

and documented. Additionally, random movement may cause the female to approach the speaker. She then may be attracted by the higher intensity of the song, rather than the properties of the song. Another disadvantage is that subsequent crickets may detect pheromonal trails of previously tested females.

In forced choice systems, the females walk up the base arm of a T or Y-shaped enclosure. At the fork, they must choose one of the two arms of the container, on the basis of the stimuli at the ends of the arms. In phonotaxis studies, a speaker broadcasting male calling song is placed at the end of each of the arms of the tube (Popov and Shuvalov, 1977; Crankshaw, 1982). In some studies, only one speaker at a time is active (Crankshaw, 1982). In others, both are played simultaneously (Popov and Shuvalov, 1977; Crankshaw, 1982). Quantification of a positive response is different for individual researchers. Some researchers count only those females that actually reach and contact the speaker (Popov and Shuvalov, 1977), whereas others consider a positive response to be one where the female has moved two thirds of the way up one of the arms (Crankshaw, 1982). The advantages of using Y-mazes are that they provide less stress to the individual than some of the techniques that involve tethering the subject. Also, because the female must choose only one of two paths, it is easy to document her choice. They are easy to clean between successive trials and therefore females cannot be influenced by pheromonal trails of previous subjects. One disadvantage of using Y-mazes is that females are restricted in potential directions of movement compared to in an arena.

Another method for testing phonotaxis is to use flight assays. In these assays, the crickets are mounted and suspended in a windstream with a loudspeaker on either side of them. As sound is broadcast through the speakers, either singly or simultaneously, the cricket will make bending movements of the abdomen towards or away from the sound. These movements have been interpreted as an attempt to steer (Moiseff *et al.*, 1978). The advantage of flight assays is that they appear more sensitive in determining female preferences. They also allow simultaneous neurological measurements. The disadvantage is that they provide a great deal of stress to the organism.

Treadmills have also been used for the study of phonotaxis. Paired-wheel treadmills are the least common. The apparatus consists of two disc-shaped wheels that rotate independently, side by side. The cricket is tethered to a balance and therefore is not able to change its position upon the apparatus. It runs with its left and right legs on each of the two wheels. The different degrees of rotation of the two wheels serves as an indicator of the turning tendency of the cricket in response to a stimulus (Stabel *et al.*, 1989).

Locomotion compensatory treadmills originated in Germany and are generally called Kramer treadmills, after the scientist who first used them. In this system, the cricket sits untethered on top of a large polystyrene sphere, 33-50 cm in diameter (Schmitz *et al.*, 1982; Loher *et al.*, 1992). Speakers surrounding the sphere alternately play a variety of stimuli. The cricket bears a small piece of reflecting foil on her pronotum. As she moves from the centre of the sphere, infra-red beams from above detect the deviation of the

foil from the centre of the scanning field. Some of the apparati are accompanied by a computer in which the tracks from each individual are digitized. The mean response of all of the females responding to one stimulus often is represented by the construction of a polar orientation diagram.

A variation of this design is the non-compensatory treadmill, sometimes referred to as the Kugel which is German for the word sphere. In this system, the cricket is tethered and kept in a central position on a light plastic sphere supported by a column of air. As with the compensatory treadmill, surrounding speakers broadcast various acoustic stimuli. The tethering system allows the female to rotate 360° and make walking and jumping movements, but is not able to leave the center of the sphere. Instead, as she travels in one direction, the power of her locomotory movements propels the sphere in the opposite direction. Two detectors, similar to those found in a computer mouse, contact the sphere, detect its motion and relay this information to an adjacent computer. The detectors sample the movement of the sphere every second that the stimulus is played and the computer subsequently generates a vector score for each stimulus (Doherty and Pires, 1987).

The disadvantage of any of the treadmills is the possible effect of stress on the female thus affecting her response. An advantage is that with the adjacent computer detecting all motion, there is no longer any need to rely on the subjectivity of the researcher to quantify female movement.

Ontogeny of Phonotaxis

The average age for the initiation of phonotaxis to male calling song in *Acheta domesticus* was 5.4 days after the final molt even though, on average, they did not mate until 6.9 days following ecdysis. This premature phonotaxis increases the probability that there will be mates available as soon as the female becomes sexually receptive (Sakaluk, 1982). Similarly, other studies involving walking assays demonstrated that onset of phonotaxis in female crickets does not occur until 4-8 days following the final molt (Walikonis *et al.*, 1991; Loher *et al.*, 1992). However, in flight assays, female *Gryllus bimaculatus* initiated positive phonotaxis at one day following the imaginal moult (Sergeyeva and Popov, 1990).

Negative Phonotaxis

Tethered flight assays indicated that adult female *Teleogryllus oceanicus* are attracted to conspecific calling song. They responded by bending their abdomens towards the sound source, probably in an attempt to steer towards the attractive stimulus. The crickets turned toward the stimuli within the frequency range of 3 to 9 kHz if they possessed the correct species specific temporal pattern. However, when this temporal pattern was played at carrier frequencies from 30 to 70 kHz, the females attempted to steer away from the speaker (Moiseff *et al.*, 1978).

It has been suggested that crickets are capable of bat evasion by means of acoustical recognition of ultrasonic stimuli. Echolocating bats emit ultrasonic pulses that reflect off insects and relay positional information back to the bats. Some crickets fly by night

and therefore are prone to predation by bats. Bats have been located in regions that are dense with crickets and have been noted to eat crickets and other Orthopterans (Belwood and Morris, 1987). Other insects, such as moths and green lacewings, are also capable of bat avoidance by responding to the ultrasonic cries of bats. These organisms have no known use for acoustics other than predator avoidance and therefore their auditory system is highly specialized for predator avoidance. In contrast, crickets require the use of their auditory structures to assist in conspecific communication, and the organs are specialized for this purpose. However, the auditory structures still are able to function in predator avoidance (Pollack and Hoy, 1989).

Researchers have investigated whether separate mechanisms were responsible for positive and negative phonotaxis. They argue that the negative phonotactic response of the females could result in their inability to adequately locate the sound. Oldfield (1980) showed that the turning angle of females toward the conspecific calling song temporal pattern at various carrier frequencies was greatest at carrier frequencies closest to the natural calling song. It was thought that negative phonotaxis occurs because of an inability of the female to locate the source if played at high frequencies. Pollack *et al.* (1984) showed that location error is not the cause of different responses to high and low frequency sounds, by studying the responses of one-eared crickets. When stimulated by a 5kHz song model, one-eared crickets always turned towards the intact side, whereas they turned away from the intact side when presented with the song pattern at high frequency (33 kHz). This illustrated

that positive and negative phonotaxis follow different rules and thus are discrete behaviours. Further support for the possibility that positive and negative responses are physiologically independent is that negative phonotaxis occurs regardless of the temporal pattern (Nolen and Hoy, 1986). Therefore, negative phonotaxis cannot be linked to an orientation error while attempting to locate the sound source as first was suggested.

Crickets habituate to high frequencies, even though high frequencies are representative of sounds produced by predatory bats. *Teleogryllus oceanicus* females responded less to repeated stimuli, and this response shows stimulus generalization. The habituation is more pronounced if the intensity is relatively low. A novel stimulus destroys the habituation, but itself becomes habituated upon repeated trials (May and Hoy, 1991). Unattractive stimuli not in the range of bat sonar do not always result in negative phonotaxis. More often, there is no net movement in any direction (Atkins *et al.*, 1987).

Mate Recognition and Discrimination
Interspecific Discrimination

It is essential for a female to recognize correctly signals from conspecific males and thus avoid mating with members of other species. Heterospecific matings decrease the reproductive fitness of females through loss of batches of eggs, or production of offspring of low viability or fertility as demonstrated in *G. rubens* and two different populations of *G. integer* that are believed to be different

species, one of which likely has an incorrect designation (Smith and Cade, 1987; Cade and Tyshenko, 1990).

Genetic Coupling Versus Genetic Correlation

There is great controversy regarding how the communication link between sender and receiver of a signal has been established. One train of thought, called genetic coupling, is that sender and receiver characteristics have common genetic basis, and the outcome depends on the sex of the individual. On the other hand, it has been suggested that the production of a particular signal and the reception of that signal have become part of a mate recognition system through coevolution. Females with a preference for a particular signal will mate with males possessing that signal. This assortative mating eventually is likely to lead to linkage disequilibrium of these alleles. Often, this is termed genetic correlation. Empirical evidence suggested for genetic coupling came from work on hybridization studies in two species of *Teleogryllus*. F1 hybrid females of *T. oceanicus* and *T. commodus* were tested for their preference for hybrid brothers, reciprocal hybrids, or the parental species. The females of each of the two hybrid groups chose the songs of their hybrid brothers significantly more often than the songs of any of the other groups. It was thus concluded that there is genetic coupling between sender and receiver in auditory communication in these species. Males and females each must receive the same gene for acoustic communication but females lack the stridulatory apparatus and therefore, do not call (Hoy *et al.*, 1977). However, this work has been criticized. F1 hybrids obviously

will show intermediate traits compared to their parents. Only in subsequent F2 generations or through backcrosses can linked traits possibly be identified. Additionally, it is unlikely that a physiological link exists between sender and receiver in this system. The production of the male calling song is likely due to a neural oscillator. Through work by Pollack and Hoy (1979), it has been demonstrated that females recognize shuffled song and therefore a common oscillator is not possible. (Butlin and Ritchie, 1989; Boake, 1991).

Additional support for genetic coupling comes from work on temperature coupling (Walker, 1962; Doherty, 1985a). It was noted that temperature affects the temporal pattern of the male calling song. In *Oecanthus quadripunctatus* and *O. nigricornis* the pulse repetition rate increased linearly with temperature, and females preferred the pulse rate that corresponded to the temperature that she was exposed to. The fact that females responded to those syllable and chirp rates that corresponded to the male at that temperature, implies that the same genes that are responsible for the male singing at a particular rate are inherent in the female and cause her to respond to the "correct" rate for that temperature. This same phenomenon was observed in *G. bimaculatus*. There must be genetic coupling between males and females for the female to orient to the pulse rate that corresponded with the ambient temperature (Doherty, 1985a). The support of genetic coupling with this evidence has been criticized as well. Bauer and von Helversen (1987) were able to control the head and thoracic temperatures of grasshoppers, *Chorthippus spp.* independently. It was demonstrated that the thoracic temperature affects the production of song, and the head

temperature was responsible for changes in female response. This indicates that common neural pathways could not affect both production and reception and therefore, genetic coupling is an unlikely explanation for the coevolution of signal production and reception in these animals.
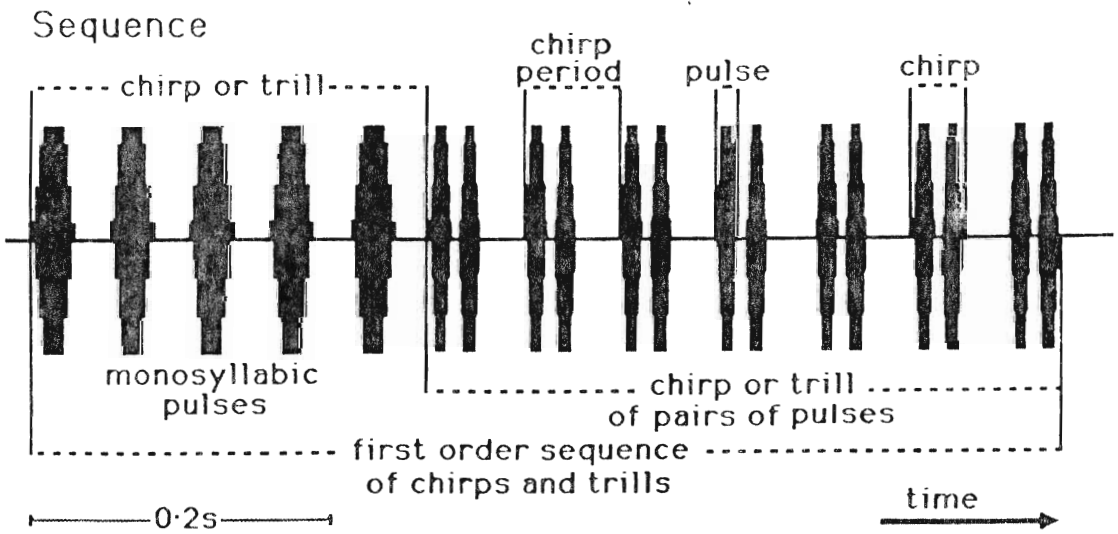
Recognition of Conspecific Song

There is additional debate as to how females recognize calling song. Some researchers believe that females compare incoming stimuli to an internal template. In birds, it is thought that young songbirds compare the sounds that they produce to an internal template to construct the entire song. Evidence supporting this view has been provided for several species (Konishi and Nottebohm, 1969). This does not appear to be the case in Orthoptera. Several studies have indicated that several song characteristics can be altered without affecting phonotaxis (Zaretsky, 1972; Doherty, 1985b). In addition, if females were to match stimuli to an internal template, the sequence of the song components would be important fdor song recognition. Hoy *et al.* (1977) demonstrated that this is not the case for *T. oceanicus*. *Teleogryllus oceanicus* males produce a calling song composed of three different classes of intertrill interval arranged in fixed order. If females matched input to a distinct template, they would not be attracted to a song in which the three types of intertrill interval were shuffled. Therefore, neurons sensitive to particular song parameters, feature detectors, may be important, rather than matching input strictly to an internal template.

Females may choose males on the basis of song parameters that exceed a particular threshold value (Lande, 1981). Moore and Moore (1988) examined female mating preferences in the cockroach, *Nauphoeta cinerea* to determine whether females compared males to each other or to an internal template. Females were presented with either a dominant or subordinate male. It was discovered that females approached, and responded to dominant males much more quickly than to subordinate males. This indicates that female *N. cinerea* compare incoming stimuli to an internal template, rather than to the stimuli arising from other males. In acoustical insects the song parameter of interest to the female may be different among species.

If several males within the range of a female exceed the threshold value, the female may sample several males, compare them and choose the best one. This is referred to as the Best-Of-N sampling method (Maynard Smith, 1987). In *Drosophila melanogaster*, females are usually courted by no more than two males, and they typically mate with the male with the longer wings. In this situation, the female appears to be making a Best-Of-2 comparison in that she compares only two males and mates with the best one (Wilkinson, 1987).

Many researchers have attempted to determine the precise component of the calling song by which a female cricket recognizes a conspecific male. Crickets can be classified as trillers or chirpers, on the basis of mode of syllable repetition in the song, and syllable rate varies from species to species (Pringle, 1957). The different parameters of cricket song are illustrated in Figure 1. Thorson *et al.*

Figure 1.   Representative diagram of cricket calling song
(from Bennet-Clark, 1989).

Pulses

syllable or pulse duration · · · · inter-pulse interval · · carrier frequency · · build up · · · decay

10 ms · · · · · · pulse period · · · · · · · · · number of cycles in pulse

Chirp

· · · · · · · number of pulses in chirp · · · · · · · · ·

50 ms · · · · · · · · · chirp duration · · · · · · · · ·

Sequence

· · · · chirp or trill · · · · · · chirp period · pulse · chirp

monosyllabic pulses · · · · · · · · · chirp or trill of pairs of pulses

· · · · · · · · · · first order sequence of chirps and trills

0·2s · · · · · · · · · · · · · time
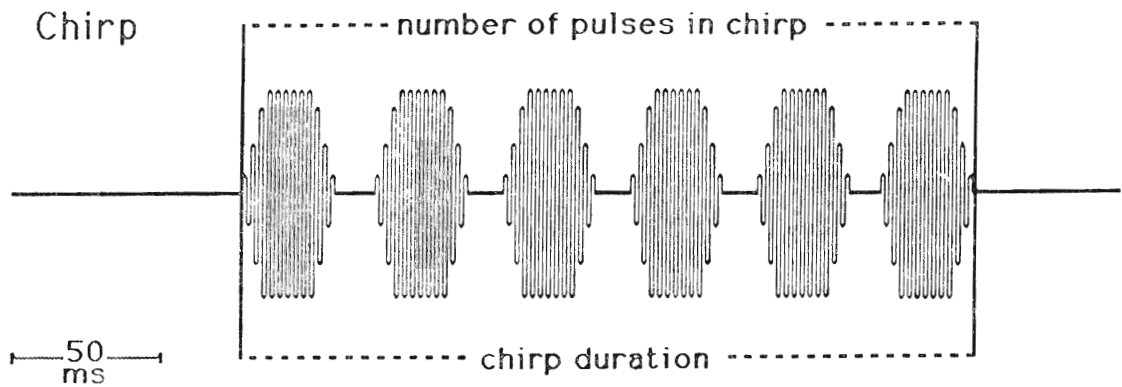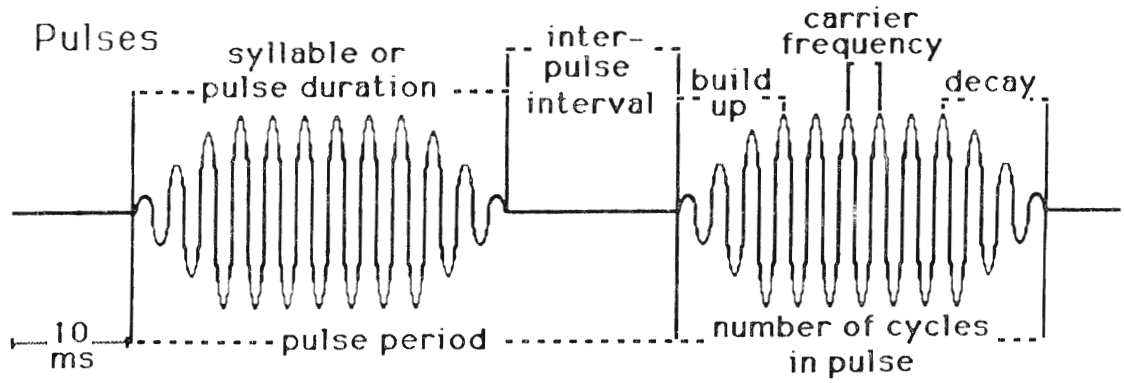
(1982) proposed that a syllable rate near 30 Hz in natural or artificial song is both necessary and sufficient for positive phonotaxis to occur. They found that in *G. campestris*, a species in which the males produce a chirping song, females responded to a song containing pure trill. In addition, the duty cycle, the ratio of syllable duration to syllable period, could be varied greatly and still would be attractive to females, as long as the syllable rate was maintained at 30 Hz. In other species of crickets syllable rate is not the important component.

Scapsipedus marginatus females preferred the pulse interval pattern characteristic of the males of their species. Chirp interval, fundamental frequency and the number of pulses per chirp all can drastically be altered without affecting phonotaxis (Zaretsky, 1972).

In some species of crickets, the male song consists of a chirp component and a trill component. *Teleogryllus oceanicus* females preferred the chirp component to the trill portion. The song containing all chirp was preferred to the model of conspecific song which contained 16% chirp. Song consisting of only trill was not preferred in any trial (Pollack and Hoy, 1981). In some species of crickets, the chirp portion of song is not the attractive component. Thorson *et al.* (1982) found that *G. campestris* females preferred songs containing all trill to the conspecific song and to songs containing all chirp. Doherty and Callos (1991) had identical findings for *G. rubens*. In some species, the frequency of the call is important. *Teleogryllus oceanicus* females responded more consistently to songs in which the carrier frequency was in the range of 3.5 to 4.5 kHz, which is similar to the frequency of the normal conspecific calling

song. They did not respond at all to 2.5 kHz or 12.5 kHz (Oldfield, 1980). Latimer and Lewis (1986) found that carrier frequency was not the only important aspect of *T. oceanicus* calling song but that harmonics were important as well. In a two-choice Y-maze experiment, females preferred a song that was rich in harmonics to one that contained no harmonics.

The important characteristic in *G. firmus* (Doherty and Storz, 1992), *G. campestris*, *Melanogryllus desertus* (Popov and Shuvalov, 1977) and *G. bimaculatus* (Popov and Shuvalov, 1977; Simmons, 1988) is the pulse rate. Females prefer the pulse rate indicative of their conspecific males to either faster or slower pulse rates. This preference persists even when the conspecific pulse rate is presented at a 12 dB lower intensity (Doherty and Storz, 1992).

In other cases, the preference for the temporal components of the calling song is intensity dependent. As the stimulus intensity was increased, the selectivity of *T. oceanicus* females towards particular temporal parameters also increased. However, if intensities were increased to 100 dB, a negative phonotactic response was elicited, even if previously attractive temporal pattern was maintained (Doolan and Pollack, 1985).

In addition to pulse rate, *G. campestris* and *G. bimaculatus* discriminate with respect to the chirp duration (Popov and Shuvalov, 1977; Simmons, 1988), whereas *Melanogryllus desertus* requires an increase in amplitude of pulses within the chirps (Popov and Shuvalov, 1977). *Acheta domesticus* females recognize conspecific song by chirp rate, but however also exhibit phonotaxis to songs of varying chirp rates if the intensity is raised. Syllable period

had no effect on its own, but appeared to be a very important parameter when combined with intensity and chirp rate (Stout and McGhee, 1988).

Trade-Off

Sometimes there are several components to the song that the females find attractive. Often the apparent importance of one parameter will be reduced if another song component is within the attractive range. This is referred to as a trade-off of one song parameter for another. In *G. bimaculatus*, the syllable period is an important component of the song for species recognition. Females will not track a song with a syllable period that is outside the natural range of calling song for that species. However, if a chirp component is added, the extreme song becomes more attractive to the females (Doherty, 1985b).

The temporal components of male song vary with temperature. Since temporal components are often important cues for females to recognize their conspecific males, this could have a severe effect upon the pairing process. Fortunately, it appears that male signalling and female reception are matched by a phenomenon referred to as temperature coupling. Females orient to songs whose temporal properties are "correct" for conspecifics at that temperature.

The trade-off phenomenon exists in the context of temperature coupling as well (Walker, 1962). The range of syllable periods tracked by females was broad if the chirp period was within the natural range for that temperature, and much narrower if the chirp period was outside or at the margins of the range. Chirp duration

had little effect upon phonotaxis if the syllable period was within the natural range at that temperature. However, if syllable period was at the margins, particularly at the lower margin, females would track longer chirp durations (Doherty, 1985b).

Intraspecific Discrimination

A female further may increase her reproductive success if she can discriminate among males of her species, and selectively mate with stronger or healthier partners. Females may be able to assess the dominance status of potential mates. In a study by Crankshaw (1982), female *A. domesticus* crickets chose the song from the dominant male significantly more often than that of the subordinate male. It was noted that the song from the dominant males was sharper and lower in pulse rate than the song from the subordinate males. Any of the song characteristics may be the criterion utilized for mate discrimination and a great deal of attention has been focused upon determining which of these song characteristics are most important. In *G. bimaculatus*, the intensity and pulse rate of the call are positively correlated with the body size of the male. In playback experiments that controlled for intensity, females were attracted preferentially to the songs from large males. Large body size was shown to be heritable (Simmons, 1988) and may lead to increased fighting success (Dixon and Cade, 1986).

The most essential characteristic for *Gryllodinus kerkennensis* females is the continuity of the calling song. Unlike other trilling species, pulse rate does not seem to be important. Broadcast stimuli with interrupted trills result in reduced phonotactic response (Popov

and Shuvalov, 1977). Singing duration is also important for *Gryllus integer* females from California populations. Hedrick (1986) demonstrated that females are preferentially attracted to certain conspecific males, on the basis of calling song. Calling bout length was the factor of discrimination, with females responding best to songs of males with long bout lengths. Perhaps this preference for longer calling bouts represents non-random mating in the direction of fit males.

Discrimination and Mate Availability

Population density and sex ratio are likely to affect the pairing of individuals, especially in terms of mate discrimination. If there are more members of the opposite sex with whom to mate, it is to be expected that animals will be more discriminatory when selecting a potential partner. Additionally, mating effort need not be great. If potential partners are scarce, choosiness is expected to decrease. Individuals also may have to put out a greater mating effort in order to secure a mating (Shelly and Bailey, 1992).

Population density affects male reproductive behaviour (Alexander, 1961). At low population density, female encounter rates are low. Therefore, a larger proportion of *G. campestris* males call than in high density situations, when a male is likely to encounter a female just by walking through the population (Hissmann, 1990). Similar results were seen in *G. integer* males. Males in low density called for a longer duration, walked less, and mated more frequently than did those males in moderate density populations in *G. integer* (Cade and Cade, 1992) and *G. veletis* (French

and Cade, 1987). None of these differences were apparent in *G. veletis* in a study by Ciceran (1992).

Male katydids exert mate choice when having been exposed to high density field situations or high female contact in laboratory environments. Typically, if males have had contact with females, they tend to discriminate with respect to with whom they mate, and reject lighter females (Shelly and Bailey, 1992).

Density affects females as well. If females have been isolated from males for a period of time, the sex ratio would not seem to be favouring males. It thus would be expected that they would be more receptive (ie., more phonotactic toward male song), and less selective with respect to song characteristics. In a laboratory study by Cade (1979), *G. integer* and *G. veletis* females were isolated from males for several days to determine whether male deprivation enhances the phonotactic response. After three days of male deprivation, females exhibited a significant increase in positive phonotaxis to broadcast tape-recorded male calling song.

Mate Discrimination and Female Age

As females age, they have less time remaining for reproduction, and their residual reproductive value decreases. Residual reproductive value can be defined as the number of offspring that an individual can be expected to have in the future. As residual reproductive value decreases, females should be selected to undergo increased reproductive effort (Stearns, 1992), and possibly an increase in the range of stimuli that they will find attractive.

Empirical evidence with *Acheta domesticus* supports the theory that discrimination is reduced as females age. Positive phonotaxis to correct syllable periods at 65 dB is apparent after day three post-ecdysis in *A. domesticus* (Walikonis *et al.*, 1991). As females aged, they progressively became more attracted to a broader range of syllable periods. The initiation of Juvenile Hormone III, which is produced by the corpora allata, and is responsible for vitellogenesis and ovocyte maturation (Renucci and Strambi, 1983) peaks at day three, at the same time as the females are very selective. As females age, the hormone concentration remains fairly constant, but at lower levels than at day three. This corresponds with a tendency to become unselective (Walikonis *et al.*, 1991).

Topical application of JHIII to older, unselective females, causes these individuals to become selective within four days of the procedure (Stout *et al.*, 1991). The L3 auditory interneuron in the prothoracic ganglion has a specific decrease in response in young females. In older females, the response decrement is not as pronounced. Upon JHIII application to old females, their L3 interneurons exhibit a response decrement similar to those of young females (Henley *et al.*, 1992).

In *A. domesticus*, females that were classified as non-responsive by their refusal to mount courting males, became the recipients of the corpora allata from responsive females. Within one day, the originally unresponsive females with the corpora allata transplant became responsive to calling and courting males (Stout *et al.*, 1976). Researchers then wished to examine the effect of JHIII upon phonotaxis. The phonotactic threshold is very high prior to 3

days post-imaginal molt. After 3-5 days in *A. domesticus*, it gradually lowers. The application of JHIII to one day old females causes the threshold to decrease in these individuals. The application of transcription or translation blockers prevented the drop in the threshold. Each drop in the behavioural threshold was closely matched with a drop in the threshold of the L1 auditory neuron. Loher *et al.* (1992) studied four species of crickets after allatectomy, *G. campestris, G. bimaculatus, T. commodus* and *A. domesticus*, and found that those females that had their corpora allata removed in adulthood continued to exhibit phonotaxis. Surprisingly, the females that were deprived of their corpora allata in the last nymphal stage also developed phonotaxis and maintained it. After the tests were run, it was noted that all females that had their corpora allata surgically removed were completely lacking in JHIII (Loher *et al.*,1992).

Juvenile Hormone is important in the reproductive development of insects. The above experiments indicate that Juvenile Hormone III is a possible proximate mechanism whereby the response of a female toward sound stimuli changes with age. As levels of Juvenile Hormone III change in the hemolymph of the female, the range of song parameters that are attractive is broadened.

MATERIALS AND METHODS

All experiments were conducted at Brock University, St. Catharines, Ontario, Canada, between March 1993 and March 1994. The subjects used, *G. integer* females, were obtained from cultures maintained in the laboratory and were descendants of individuals collected as last instar nymphs and adults at the Brackenridge Field Laboratory, University of Texas at Austin in 1992.

Maintenance of Cricket Cultures

The cultures were maintained in plastic containers (17cm X 32.5cm X 37.5cm) at 29 ± 2°C and approximately 50% relative humidity in a reversed light cycle of 12hL:12hD. Crickets were supplied with Purina® Cat Chow and water-filled vials plugged with cotton wool *ad libitum*. Cultures were inspected daily and newly moulted adults removed to ensure the virginity of all test subjects. Test females were placed in individual plexiglass containers (9.1cm X 9.2cm X 16.1cm) and kept in acoustical isolation in a separate room from conspecific males at 21 ± 2°C, the same temperature at which the phonotactic trials were conducted.

The Kugel

The Kugel, a non-compensating treadmill, was used to measure phonotaxis of *G. integer* females to variations in artificially produced calling song (Figure 2). The circular anechoic chamber is constructed of wood, stands 47.4 cm tall and has a diameter of 101.9 cm. In the centre of the enclosure, a hollow plastic sphere, with a circumference of 51 cm and weight of 34.4g is supported by a cushion of air (pressure = 120 P.S.I.) in order to minimize friction. Surrounding the

Figure 2.   Representative diagram of the Kugel (from Doherty and Pires, 1987).
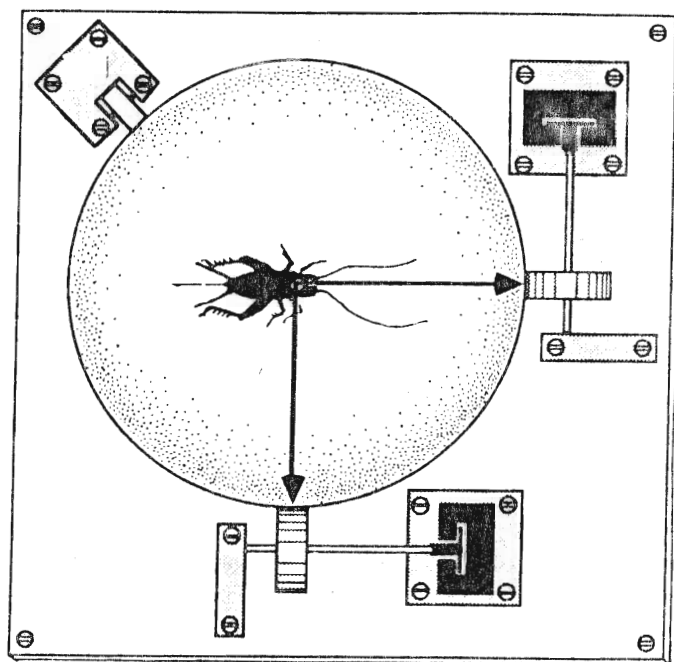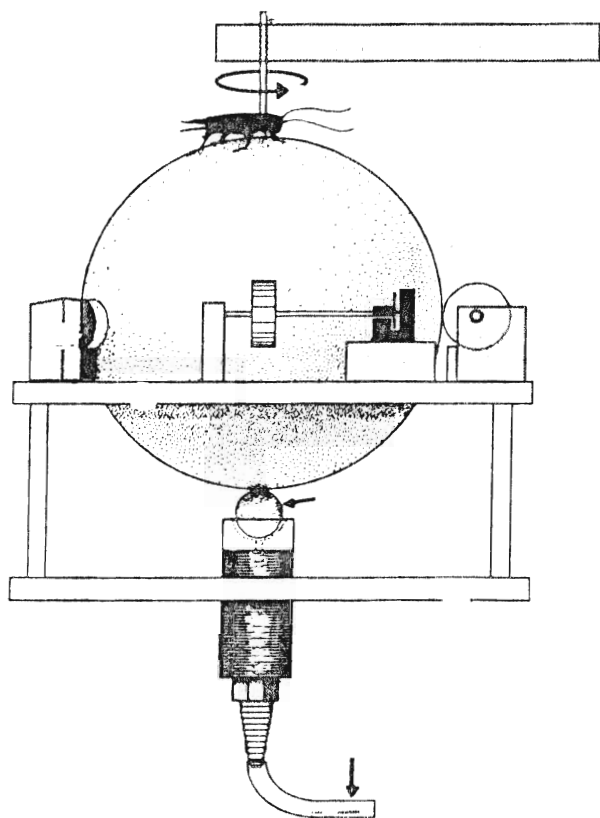
sphere, at 90° intervals, are four speakers (Linear, B-4-5 JO full-range frequency) through which songs are broadcast. Each speaker is 35.4 cm from the centre of the top of the sphere.

For each of the experiments, a tiny block of wood (4 mm X 2 mm X 2 mm) was attached to the pronotum of the female by melted beeswax. A thin wire (0.01 mm diameter X 5 cm length) attached vertically to the block, was passed through a small hole at the end of a horizontal metal arm that positioned the cricket over the centre of the sphere (Figure 2). This tether maintained the female cricket on the sphere but allowed her to rotate about her vertical axis and allowed limited vertical movement. As the cricket moved in one direction, the power of her step caused the sphere to rotate in the opposite direction. Two "mouse" detectors contacted the sphere and collected information regarding the direction and speed of motion of the sphere and hence of the female. The entire apparatus was attached to an adjacent personal computer (Goldstar 1460 plus) to which the information was relayed. A computer program calculated speed and direction of motion for each second of stimulus presentation. The total vector score for one female for each stimulus was calculated as in Doherty and Pires (1987):

$$\text{vector score} = \sum \cos (\text{vector angle}) * \text{vector length}$$

The speaker from which sound was broadcast was always considered to be position 0°, and the direction of locomotion was assessed with respect to the active speaker.

Experimental Design

Artificial Song

The calling song model used in this study was devised by taking one pulse from a recording of a naturally calling *G. integer* male and constructing a computer model representing the average calling song of the species. The various song parameters were based on song data collected in previous studies (Souroukis *et al.*, 1992) and were chosen to match natural songs recorded at temperatures of 20-22°C, the temperature at which the Kugel trials were conducted. The intertrill interval was 267 ms, there were 38 pulses per trill, and the percentage of missing pulses was 5.36. Pulse rate was the only song component that was varied in this study. The pulse rates used were the mean pulse rate for the species at this temperature (70 pulses per second), values one SD below and above (64 and 76 pulses per second, respectively), and values approximately 2.5 SD below and above the mean (55 and 85 pulses per second, respectively). Other song characteristics remained constant. The intensity of the broadcasts was approximately 83 dB at the position of the cricket, which is well within the range of intensities of calling males in the field and measurements taken in the laboratory (Cade, 1976).

Acclimation Protocol

Prior to exposure to the trial stimuli, the female remained on the sphere in complete silence to become acclimated to the surroundings. All test females were allowed 5 min on the sphere before any acoustic stimuli were presented. Females were then presented with a standard array of acoustic stimuli. Each stimulus

lasted 40s and successive stimuli were separated by silent intervals lasting 10s. Each of three stimuli was presented twice randomly. If the female responded to two of the stimulus presentations consecutively, the actual trial was initiated. This pre-trial standard presentation was used to control for the latency period that often was observed before females began to respond to test stimuli.

Age and Phonotaxis: Single-Stimulus Presentation

The two age groups used in this study were 11-14 days old and 25-28 days old, and were called young and old subjects, respectively. Ages were with respect to the imaginal moult. Each individual cricket was presented with an artificially generated calling song of one of the five pulse rates. The song stimulus was presented fifteen times within a trial. Each of the fifteen broadcasts lasted 60s, and successive broadcasts were separated by a 15s period of silence. The active speaker during each broadcast was chosen at random to control for speaker bias. Vector scores for all of the 15 broadcasts were summed to give a total score for each female for a specific pulse rate. Trials were conducted from 1.5 to 11.5 hours following the initiation of the scotophase. A single stimulus presentation design tests for female choice of the type involving comparisons with a template rather than comparisons between several potential mates.

Age and Phonotaxis: Three-Stimulus Presentation

The two age groups for this experiment were identical to those in the single-stimulus experiment, but in this study, each individual cricket was presented with three song types. This modification to the experimental design was deemed necessary to compare relative

responses of the females to different stimuli. This design tests for female choice involving comparisons among stimuli, rather than to an internal template. The three pulse rates were 64, 70, and 76 pulses per second. All other song variables were held constant as before. Each of the three songs was presented in random order four times throughout a trial, which gave a total of 12 broadcasts heard by each female. Each broadcast was 100 s in duration and successive broadcasts were separated by a 25 s silence. Each female therefore achieved three vector scores, each one a total for each of the three pulse rates presented. Trials were conducted from 2.5 to 6.5 hours into the dark period of the light cycle.

Mating and Phonotaxis: Three-Stimulus Presentation

In this study, the females first were mated, in order to separate the effect of time without a mating from the effect of age. All crickets were tested at 28 days of age. The first experimental group consisted of females that had mated at three days of age and therefore had no mating experience for 25 days prior to the trial. The second group had mated at 17 days of age and had been without a mating for 11 days. These mating times were chosen to be equivalent to time without a mate in the first experiments, in which the ages of the virgin females, and thus, time without a mate, were 11-14 days, or 25-28 days. Female *Gryllus integer* have been shown to first mate at 3.6 days of age, on average (Solymar and Cade, 1990). Mating trials were conducted just before and after dawn, a time corresponding to peak mating time in the field (French and Cade, 1987). Females were introduced into a small plastic tub containing a

randomly chosen conspecific male. Males were from 7-28 days old, and prior mating experience was not known. Mating was considered to have occurred if a spermatophore was attached to the genital plate of the female. The spermatophore was allowed to remain attached for 45-60 minutes, and the male remained present during this time in order to prevent the female from prematurely removing the spermatophore. If the spermatophore did not remain attached for the desired period of time, the female was discarded. If a mating did not occur, the pair was separated. Three day old females that did not mate were tried again at 17 days of age. All test females were allowed to mate only once. If the male initiated courtship once again, the pair was separated. Similar to the protocol for the three-choice experiment involving virgin females, the mated females were presented with 12 broadcasts, four of each of three pulse rates, 64, 70 and 76 pulses per second. Each broadcast was played for 100 s, with 25 s silent intervals. Again, the vector scores for each pulse rate were tallied to give three overall vector scores, one for each pulse rate. Trials were conducted from 2.5 to 6.5 hours into the scotophase.

Statistical Analysis

Frequency distributions of vector scores were not normally distributed, and variances were not homogenous (Kolmogornov - Smirnov, Lillefors test; Bartlett's test). Several transformations were applied to the data but they still did not conform to a normal distribution. Therefore, non-parametric statistics were used to analyze the results. Ranked two-way ANOVAs were used with

female age and pulse rate as the two factors. Significant differences were compared across groups separately using Kruskal-Wallis one-way ANOVAs followed by non-parametric Tukey-type multiple comparison tests on ranked data (Zar, 1984) All statistics were carried out using the SYSTAT statistical package (Wilkinson, 1989).

A Kruskal Wallis test was applied to determine whether there was any significant difference in vector scores achieved at various trial times.

Spearman rank correlations were used to determine whether there was any significant correlation between weight and either vector score or absolute vector score.

RESULTS

Age and Phonotaxis: Single-Stimulus Presentation

Frequency distributions for vector scores generated by young *G. integer* females in the single stimulus presentation study, are presented in Figure 3. The mean vector scores were -1691 ± 12174, 682 ± 2403, 2539 ± 7876, 846 ± 2980, and -101 ± 2018 for 55, 64, 70, 76 and 85 pulses per second, respectively. Distributions of vector scores for the old females, are in Figure 4. Mean scores were 249 ± 2048, 7892 ± 14981, 3574 ± 9370, 4695 ± 13273, and -125 ± 2085 for 55, 64, 70, 76, and 85 p/s, respectively.

Old females achieved significantly higher vector scores than did young females (ANOVA: F=9.15, p=0.003, df=1, 506) irrespective of pulse rate, indicating a higher motivation level. Females, irrespective of age, varied in their response to the three stimuli (ANOVA: F=5.4, p<0.0001, df=4, 506). Multiple comparison tests revealed that females achieved higher vector scores for 64 p/s over either 55 or 85 p/s, but did not discriminate among 64, 70 and 76 p/s. There was no interaction between age and pulse rate (ANOVA: F=0.931, p=0.45, df=4, 506).

Age and Phonotaxis: Three-Stimulus Presentation

Figure 5 shows frequency distributions for the young females in the three-stimulus experiment. The mean vector scores for each pulse rate were 3054 ± 6707, 2707 ± 4087, and 2023 ± 5400 for 64, 70 and 76 p/s, respectively. Figure 6 displays frequency histograms for old females in the three-stimulus experiment. The

Figure 3.   Frequency distributions of the vector scores
            for young *Gryllus integer* females for each of
            the five pulse rates in the single-stimulus
            experiment

NUMBER OF FEMALES

55 p/s

64 p/s
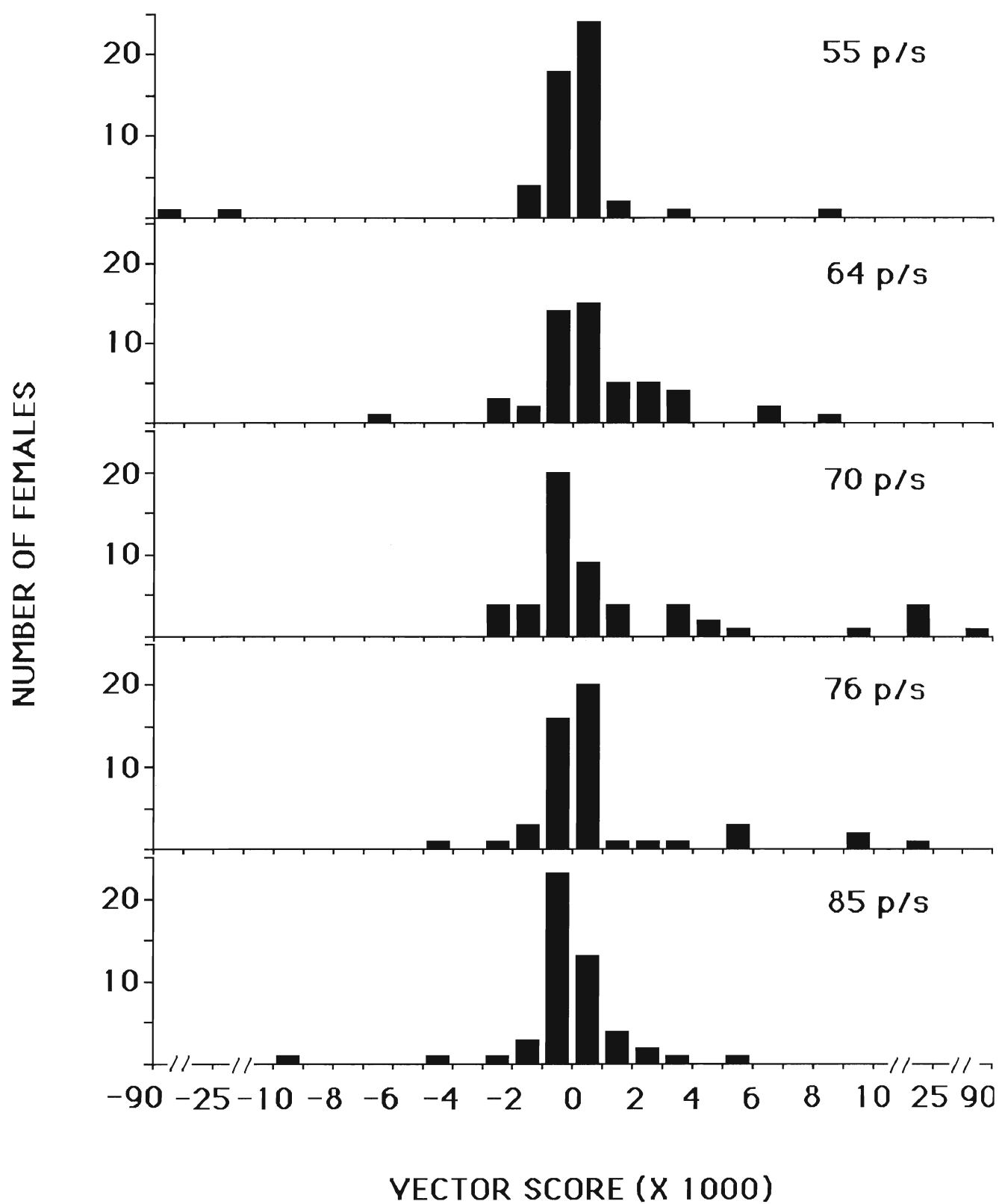
70 p/s

76 p/s

85 p/s

VECTOR SCORE (X 1000)

Figure 4.   Frequency distributions of the vector scores for old *Gryllus integer* females for each of the five pulse rates in the single-stimulus experiment
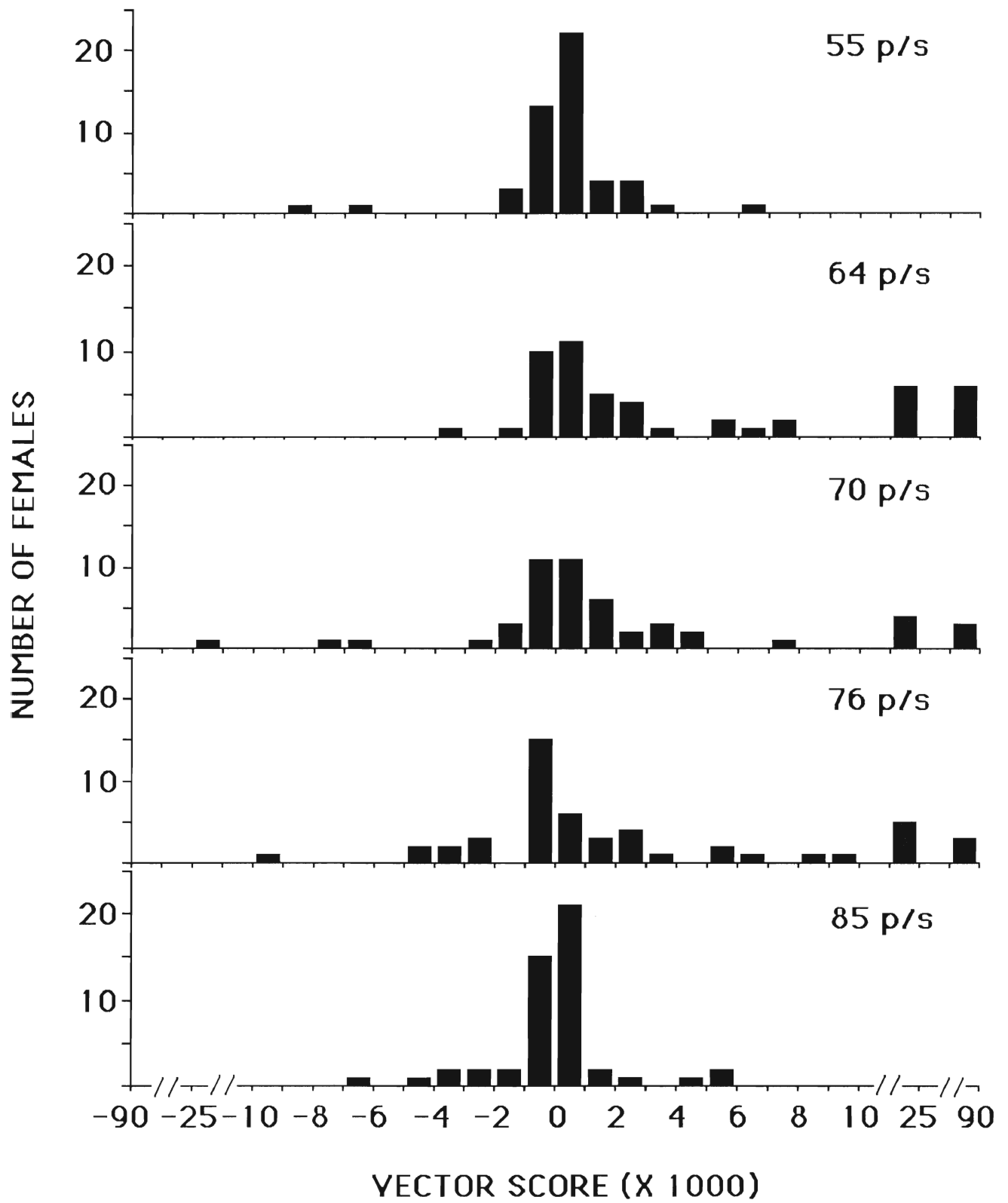
Figure 5.   Frequency distributions of the vector scores for young virgin *Gryllus integer* females for each of the three pulse rates in the three-stimulus experiment

NUMBER OF FEMALES

64 p/s

70 p/s

76 p/s

-50 -25 -10 -8 -6 -4 -2 0 2 4 6 8 10 25 50
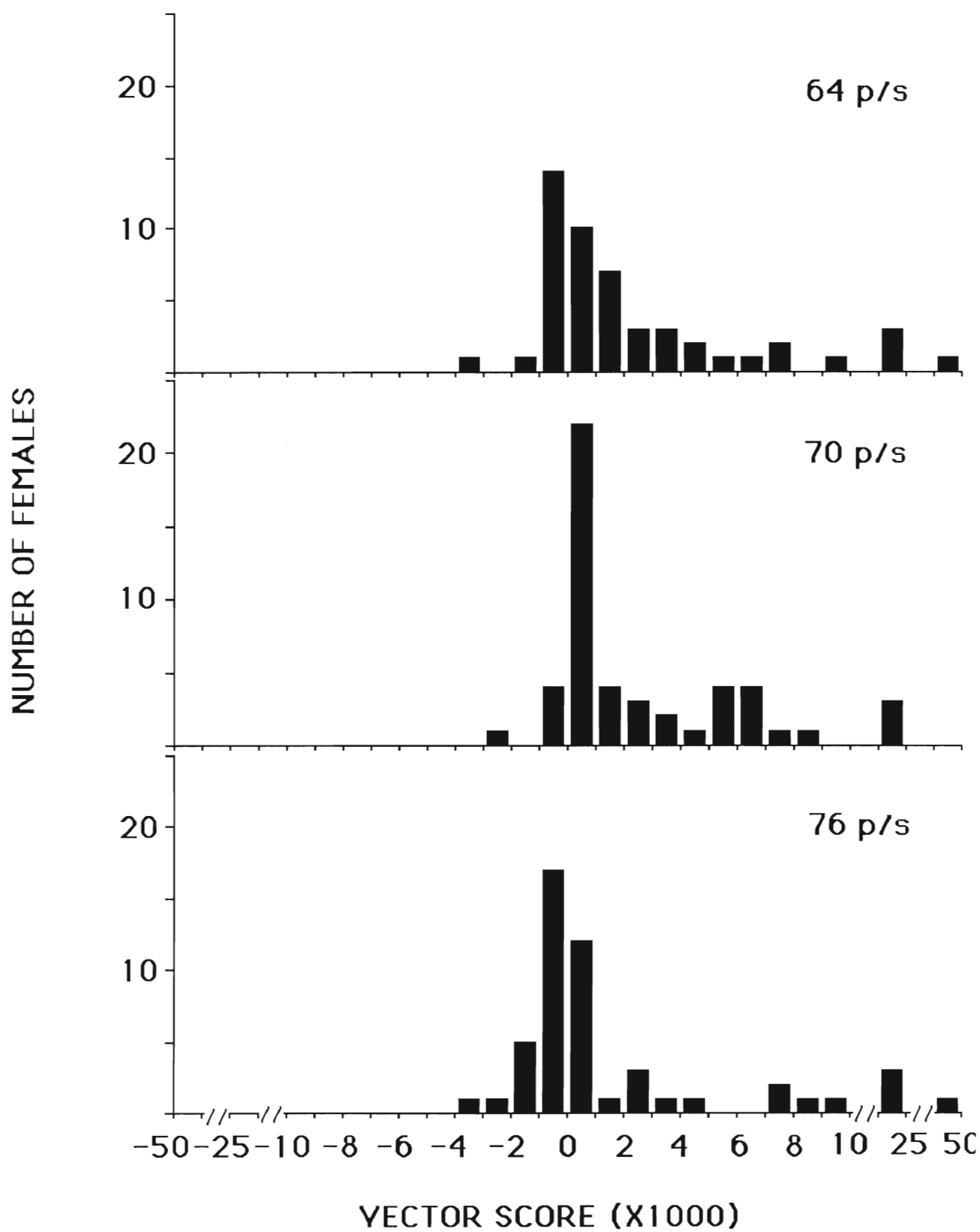
VECTOR SCORE (X1000)

Figure 6.   Frequency distributions of the vector scores for old virgin *Gryllus integer* females for each of the three pulse rates in the three-stimulus experiment
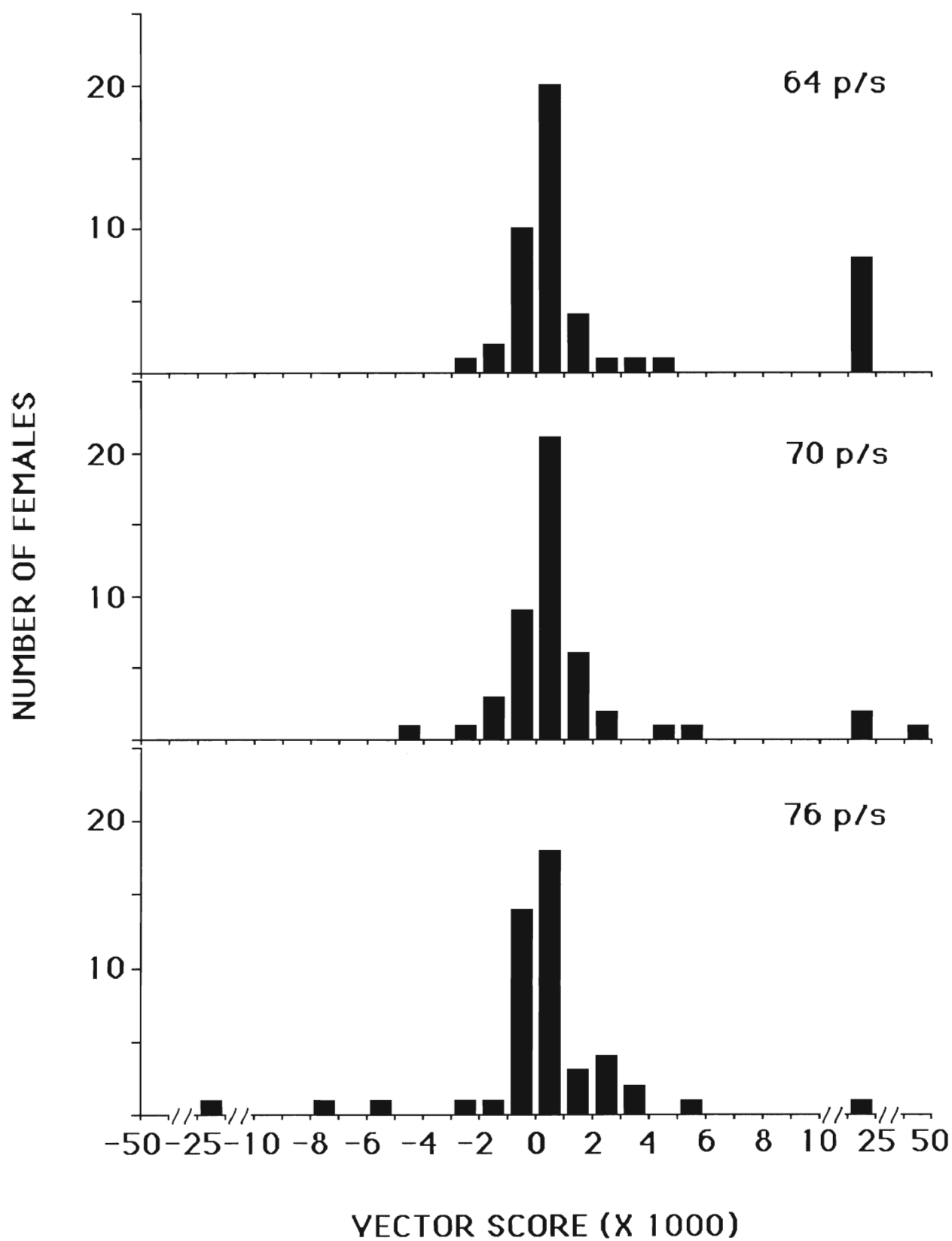
VECTOR SCORE (X 1000)

Figure 7.   Frequency distributions of the vector scores
for *Gryllus integer* females mated at three days
for each of the three pulse rates in the three-
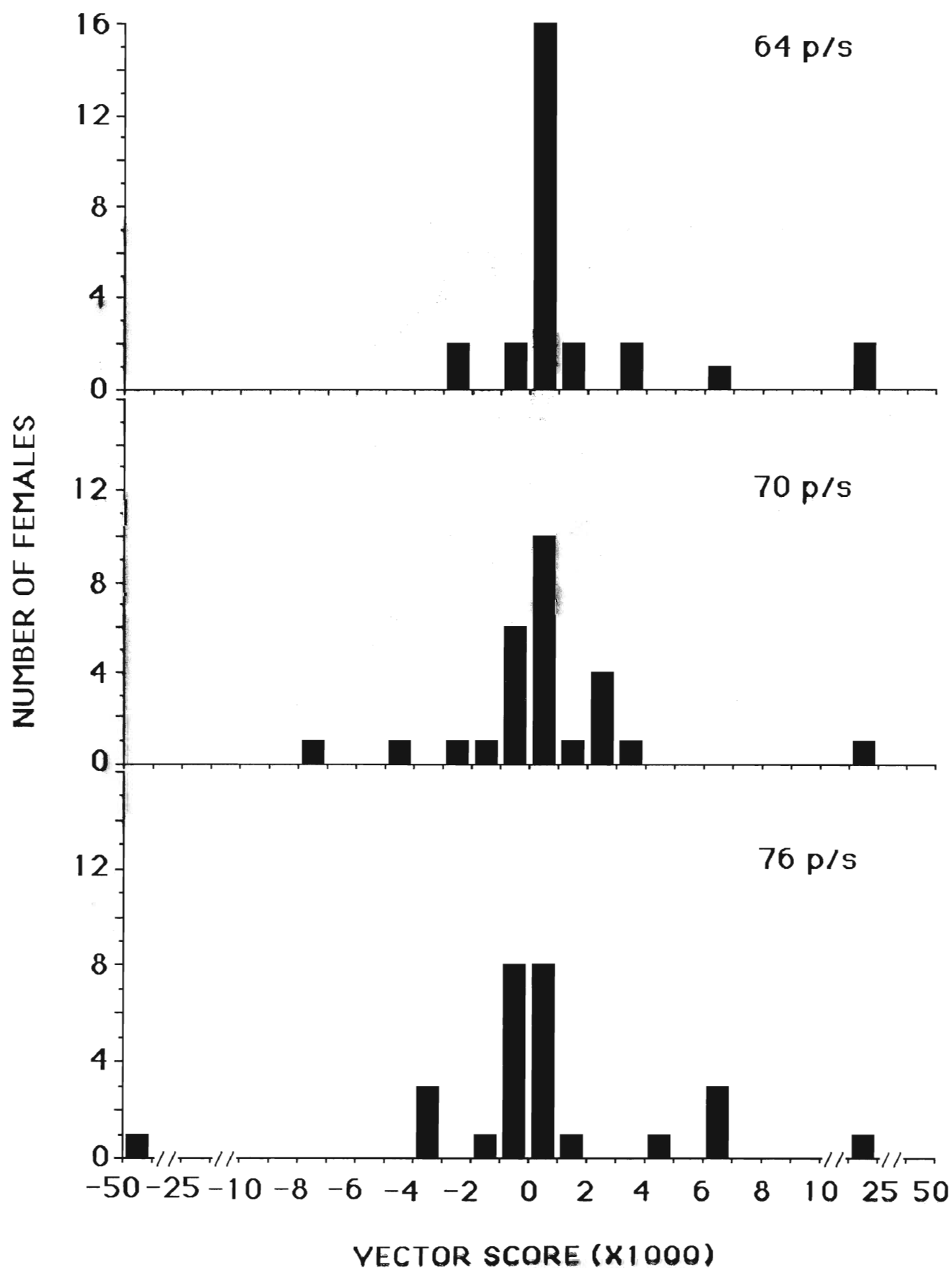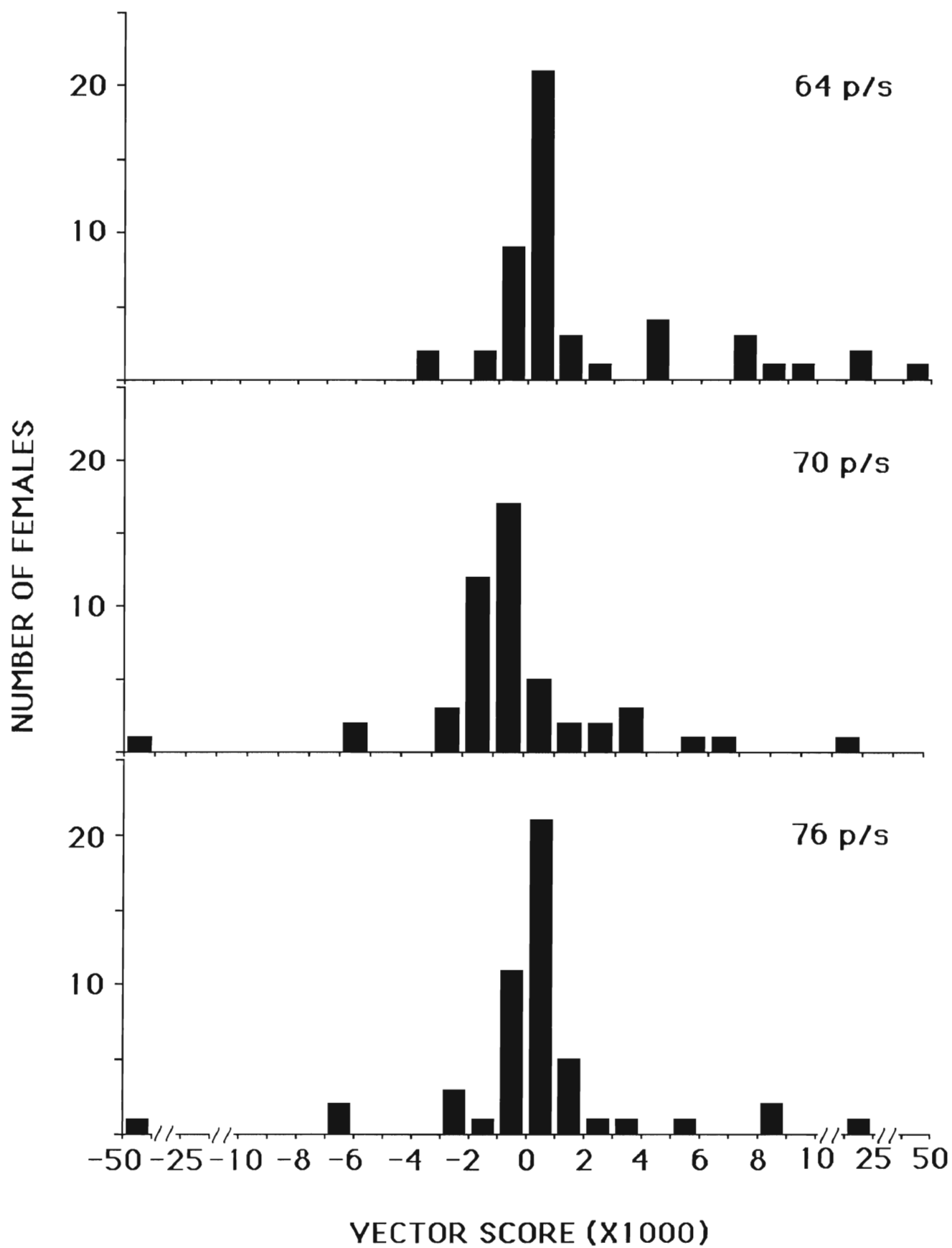stimulus experiment

VECTOR SCORE (X1000)

Figure 8.   Frequency distributions of the vector scores
for *Gryllus integer* females mated at 25 days
for each of the three pulse rates in the three-
stimulus experiment

was also no significant difference in the responses of females in general, irrespective of age at mating, across the three pulse rates (ANOVA: F=2.66, p=0.07, df=2, 150). There was also no significant interaction between age at mating and pulse rate (2-way ANOVA: F=0.02, p=0.99, df=2, 150).

Other Factors - Time of Trial and Weight

Figures 9, 10 and 11 illustrate the relationships between mean vector score and time of trial, for each of the three experiments. Trial times were grouped into 0.5 hour blocks for the purpose of analysis. Time of day did not influence the vector scores in the single-stimulus study (Kruskal-Wallis: H=8.71, p=0.99, df=20, 494), the three-stimulus experiment involving virgin females (Kruskal-Wallis: H=11.70, p=0.17, df=8, 294), or the three-stimulus study involving mated females (Kruskal-Wallis: H=11.72, p=0.16, df=8, 210).

Figures 12, 13 and 14 present scatter diagrams of weight and vector score for the three experiments. Weight did not influence vector score in the single-stimulus study (r=0.019, p=0.70, N=443), in the three-stimulus study involving virgin females (r=-0.015, p=0.81, N=273), or in the three-stimulus experiment involving mated females (r=0.085, p=0.23, N=198). However, the correlations between weight and absolute vector scores (Figures 15, 16 and 17) gave rise to somewhat different results. In the single-stimulus design, there was a significant correlation between weight and absolute vector score such that heavier females generated significantly higher values (r=0.233, p=0.0001, N=443). A similar

trend was found for the three-stimulus experiment involving mated females (r=0.245, p=0.0006, N=198), but not in the data from the three-stimulus experiment involving virgin females (r=-0.436, p=0.66, N=270).

Figure 9.   Relationship between time of trial and vector
             score for *Gryllus integer* females in the single-
             stimulus experiment.   The standard deviation is
             represented by error bars.   Sample sizes are
             indicated by the numbers above the error bars.

Figure 10. Relationship between time of trial and vector score for virgin *Gryllus integer* females in the three-stimulus experiment. Standard deviation is represented by error bars. Sample sizes are indicated by the numbers above the error bars.

Figure 11. Relationship between time of trial and vector
score for mated *Gryllus integer* females in the
three-stimulus experiment. The standard deviation
is represented by error bars. Sample sizes are
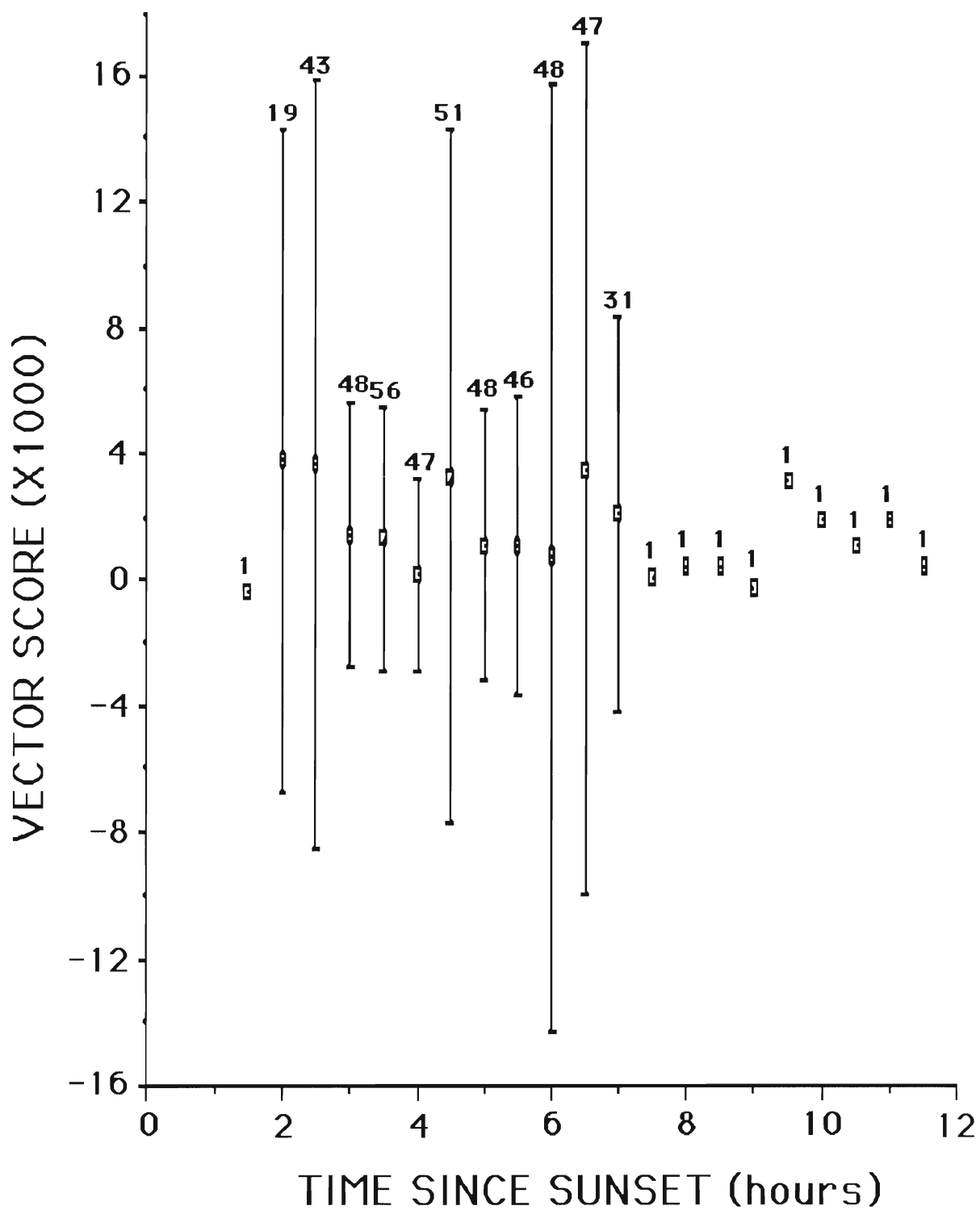indicated by the numbers above the error bars.

Figure 12.   Correlation between female weight and vector
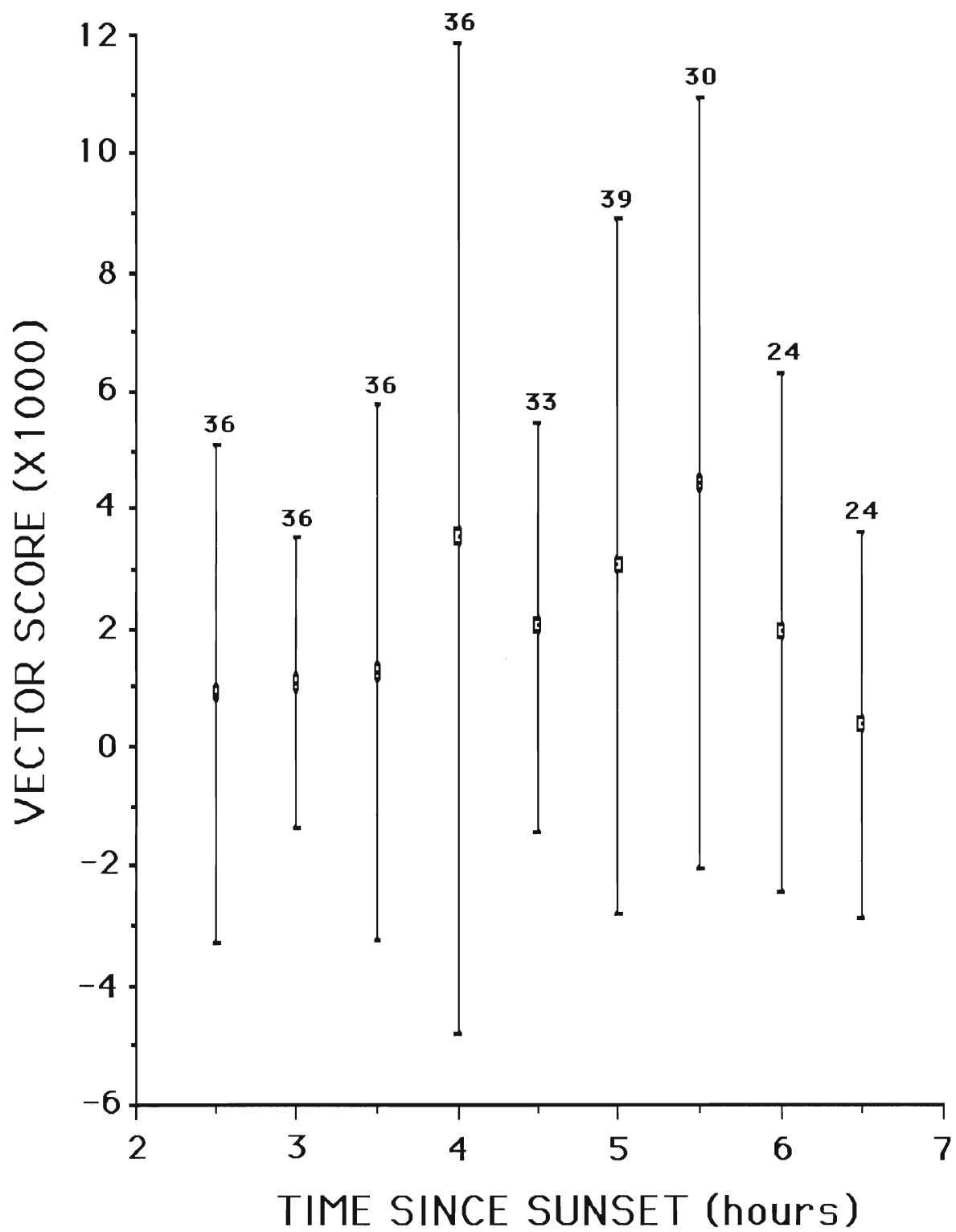score for *Gryllus integer* females in the
single-stimulus experiment.   The dotted line
represents the zero axis.   Each dot represents the
vector score achieved by one individual.

VECTOR SCORE (X1000)

WEIGHT (grams)

Figure 13.   Correlation between female weight and vector
score for virgin *Gryllus integer* females in the
three-stimulus experiment.   The dotted line
represents the zero axis.   Each dot represents
the vector score achieved by one individual for
one of the three stimuli.

Figure 14. Correlation between female weight and vector score for mated *Gryllus integer* females in the three-stimulus experiment. The dotted line represents the zero axis. Each dot represents the vector score achieved by one individual for one of the three stimuli.
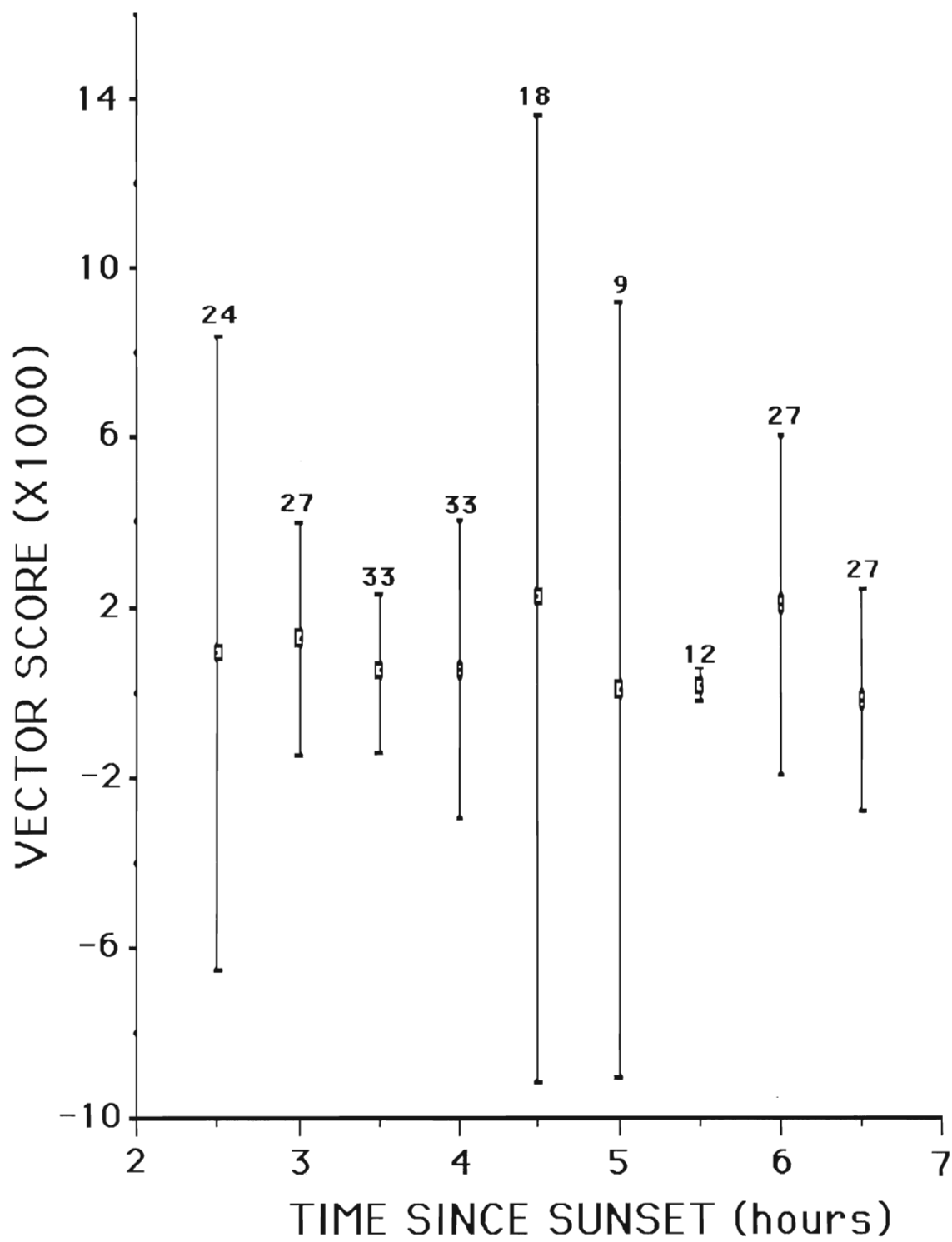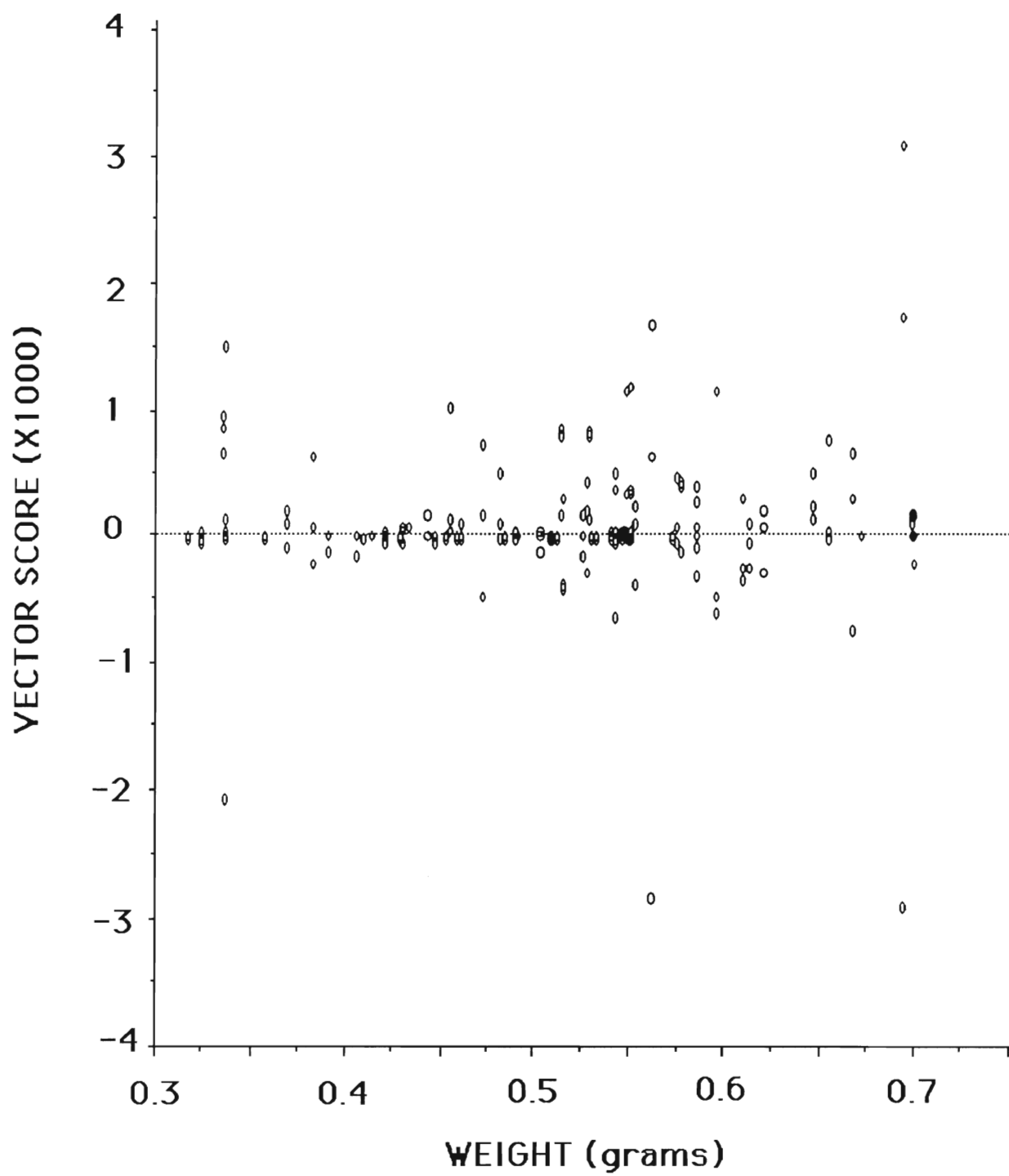
Figure 15. Correlation between female weight and absolute vector score for *Gryllus integer* females in the single stimulus experiment. The dotted line represents the zero axis. Each dot represents the vector score achieved by one individual.

Figure 16. Correlation between female weight and absolute vector score for virgin *Gryllus integer* females in the three-stimulus experiment. The dotted line represents the zero axis. Each dot represents the vector score achieved by one individual for one of the three stimuli.
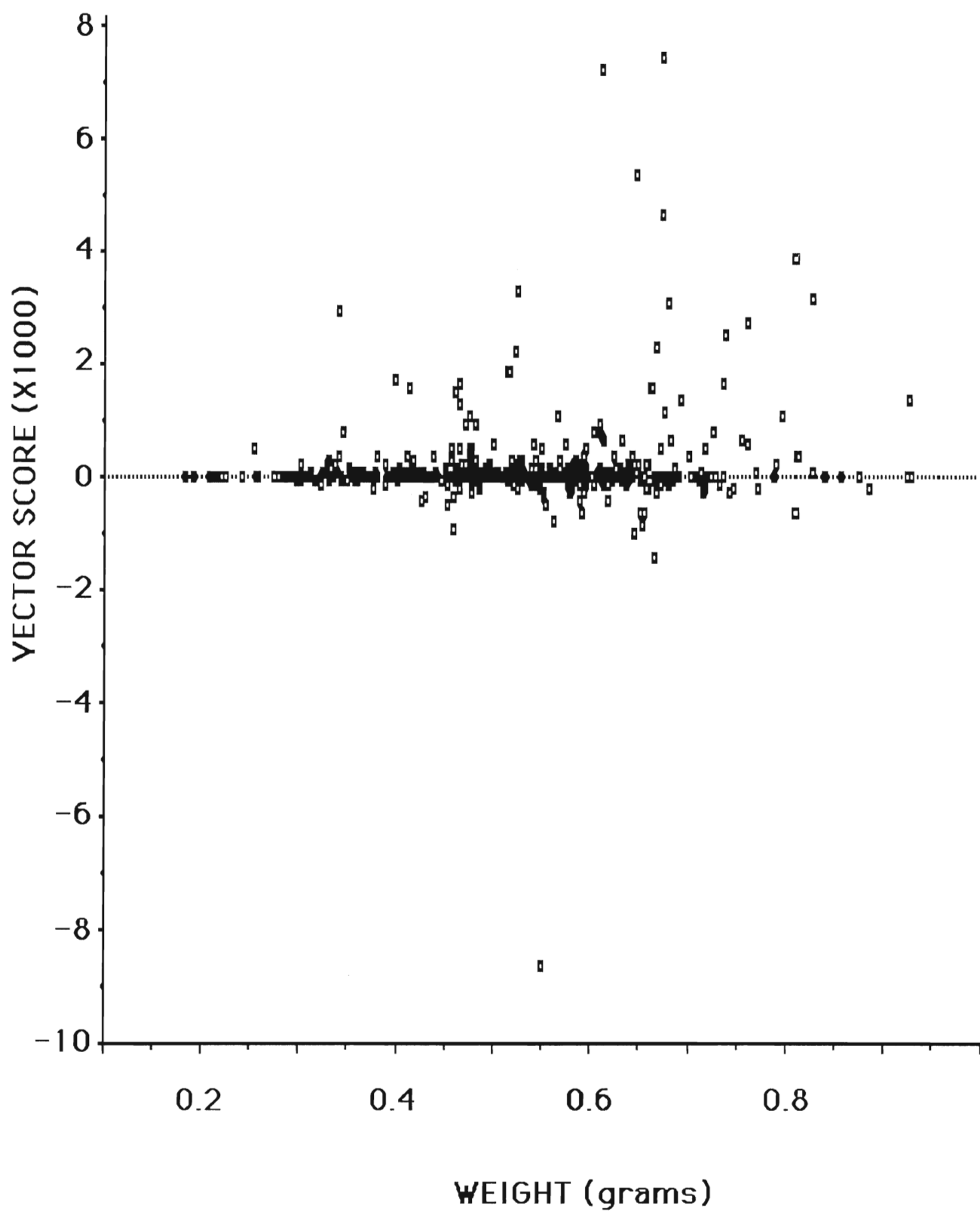
Figure 17. Correlation between female weight and
absolute vector score for mated *Gryllus integer*
females in the three-stimulus experiment. The
dotted line represents the zero axis. Each dot
represents the vector score achieved by one
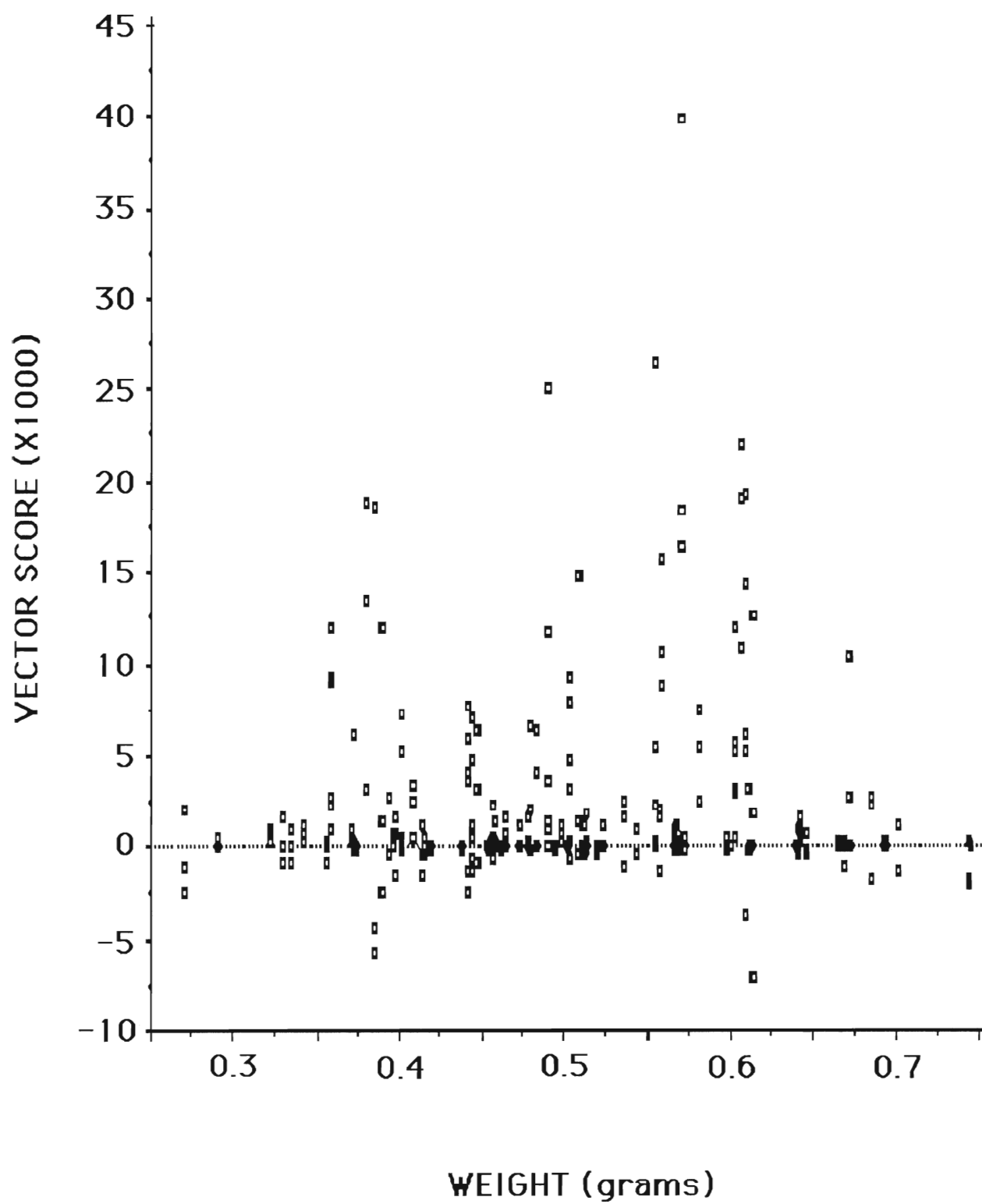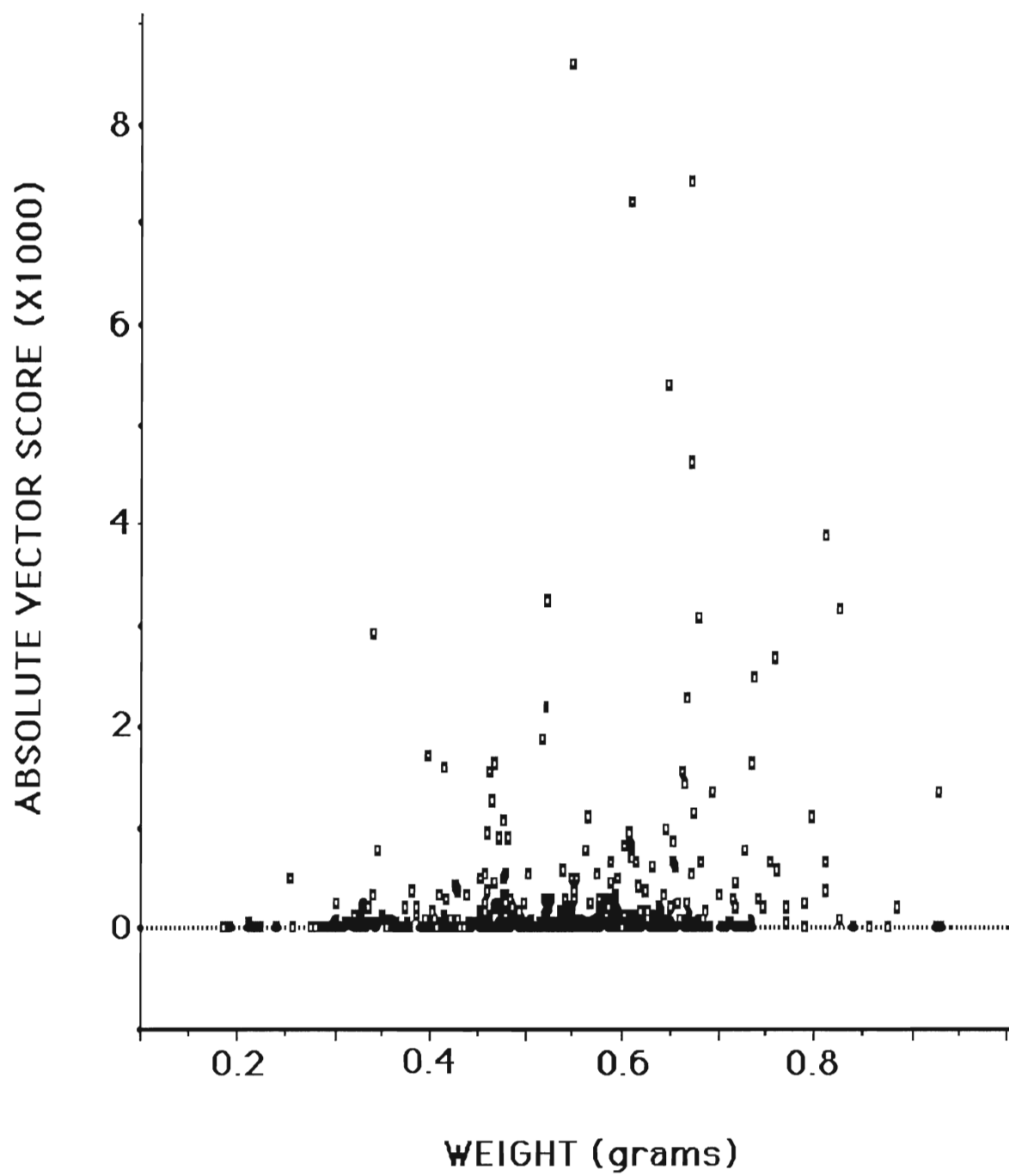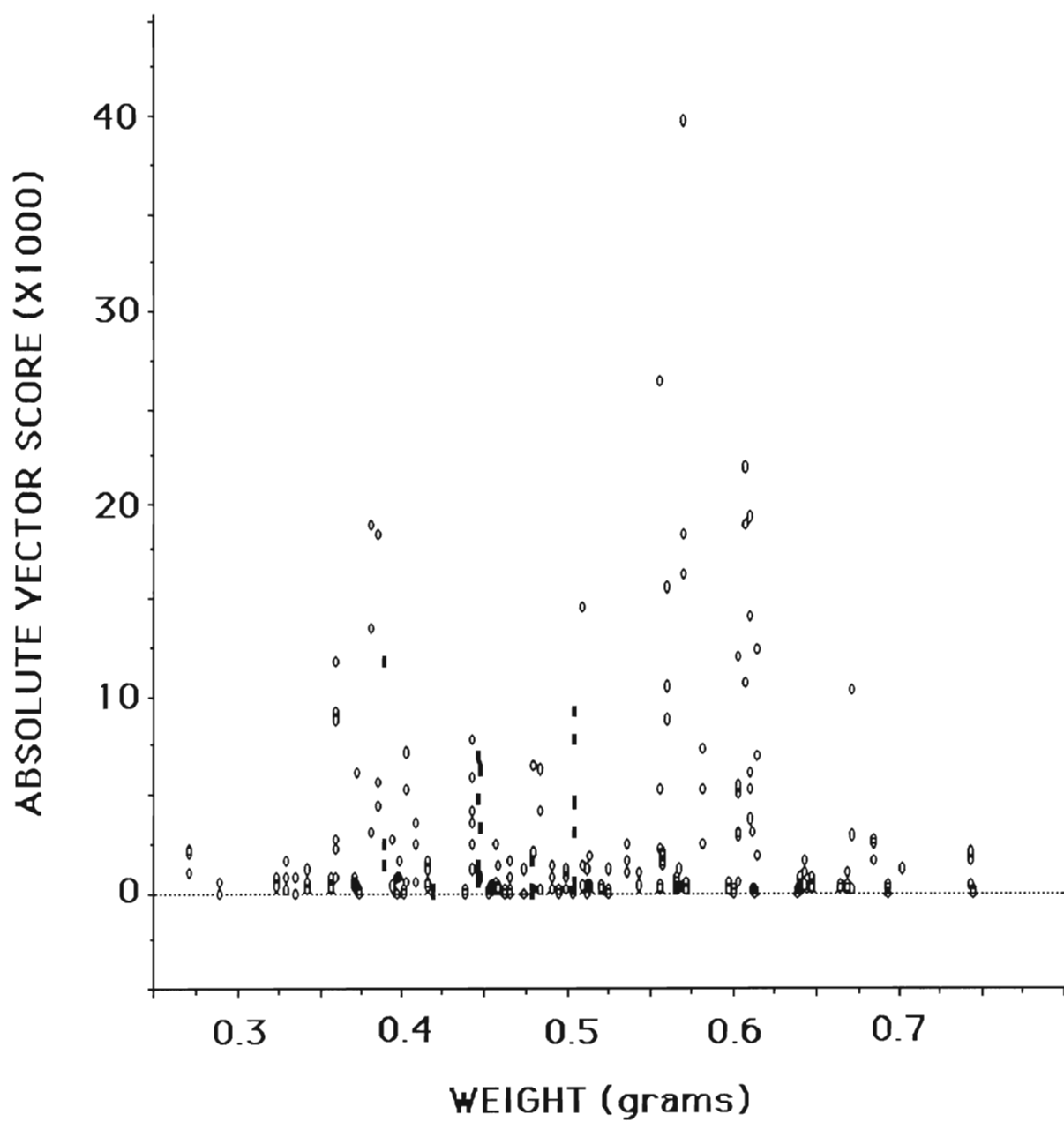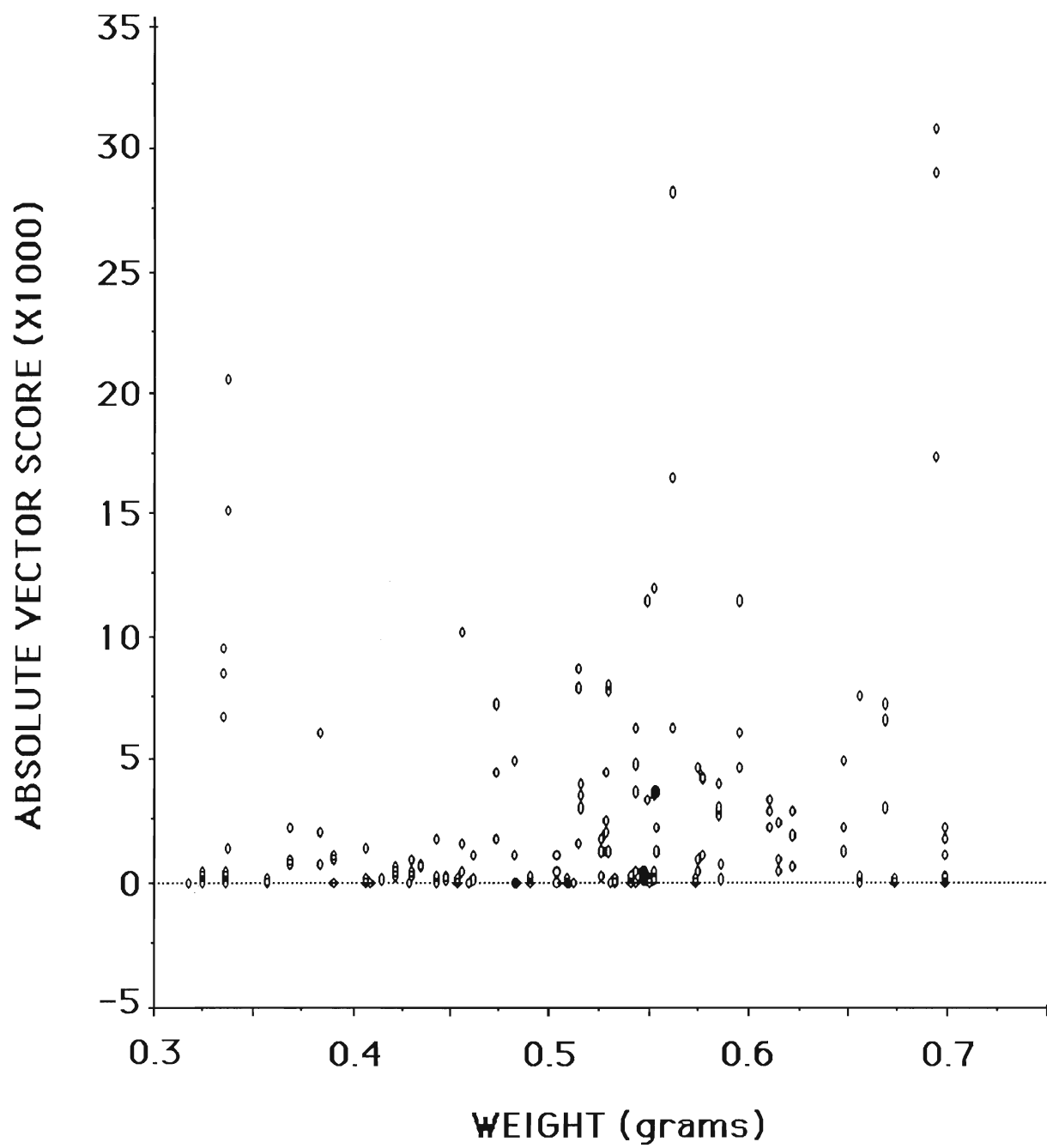individual for one of the three stimuli.

DISCUSSION

Female choice occurs both at the interspecific and intraspecific level (Otte, 1972; Trivers, 1972). Females may benefit from interspecific choice by avoiding heterospecific matings which typically result in reduced offspring viability (Smith and Cade, 1987; Cade and Tyshenko, 1990). There also may be an advantage for females to mate preferentially with certain males within the species. Although it would be advantageous for all females to be discriminatory with respect to intraspecific mate choice, females vary in their responses to conspecific male calling song. Some females may demonstrate a higher degree of motivation or responsiveness, which is represented by increased rate of movement towards the male. Some females may be less selective or discriminatory, and respond equally well to all conspecifics. Few researchers have dealt with the factors contributing to variation in motivation or selectivity in intraspecific mate choice. This study investigated female age as a potential factor contributing to this variation. It was demonstrated that both motivation and discrimination are influenced by female age, with older females becoming more motivated to mate and less discriminatory with respect to mate choice. The results from phonotaxis experiments are discussed in ultimate terms of motivation, cost of selectivity and residual reproductive value.

Age and Phonotaxis: Single-Stimulus Presentation

The results from the single stimulus experiment indicated that older *G. integer* females had significantly higher vector scores than

younger females. This is in agreement with predictions based on residual reproductive value. As females age, their residual reproductive value decreases. With less time left to mate and develop and lay eggs, females should maximize reproductive effort in present mating opportunities. This should correspond to higher vector scores, indicating a higher motivation level in older females as was seen in this study.

Female *G. integer* discriminated with respect to pulse rate. In this study, the stimulus presentations 64, 70 and 76 p/s were within the normal range for that experimental temperature and were preferred over 55 or 85 p/s, stimuli outside the natural range for that temperature. This suggests that females are selected to avoid orienting to males singing at pulse rates outside the natural range for the ambient temperature. This increases the probability that they will respond to conspecific songs. The eastern periphery of the range of *Gryllus integer* is shared with a congeneric species, *Gryllus rubens*, whose pulse rate is approximately 62 pulses per second at $25 \pm 1\,^{\circ}\mathrm{C}$ (Smith and Cade, 1987). It is important that females recognize the songs of males of their species. Heterospecific matings often result in decreased survivorship of offspring and hence a reduction in reproductive fitness of both the male and the female (Smith and Cade, 1987; Cade and Tyshenko, 1990).

It is essential that the song recognition system in the female is coupled with the song emission mechanism of the male so that females are able to recognize conspecific males under all conditions. Since certain parameters of male calling song are temperature-dependent, female response must be temperature-coupled in a

parallel fashion to the male. The phenomenon of temperature coupling has also been studied in other species (Prestwich and Walker, 1981; Doherty, 1991; Souroukis *et al.*, 1992). Prestwich and Walker (1981) discovered that in three trilling species of gryllids, *Anurogryllus arboreus, Oecanthus quadripunctatus,* and *O. celerinictus*, wing stroke rate, which corresponds to pulse rate, increased linearly with temperature. Doherty (1991) studied the effect of temperature on pulse period in *Gryllus rubens*. Pulse period is the time from one pulse to the next, and the reciprocal of pulse rate. It was found that as temperature increases, the pulse period decreases. Similarly, in *G. integer*, pulse rate increases with temperature (Souroukis *et al.*, 1992). Females respond to male calling song with temporal properties that are characteristic of the song that is produced at that temperature (Walker, 1962; Doherty, 1985a).

Females did not discriminate among 64, 70 and 76 p/s, indicating that in this experiment, females, in general, were not selective intraspecifically. Young and old females did not differ significantly in their responses across the five pulse rates. This is not in agreement with initial predictions. It was expected that younger females would discriminate with respect to pulse rate and older females would not. Perhaps this experimental design did not allow differences to be found. Females were presented with only one stimulus, and thus were unable to compare among different songs. Discrimination may be more apparent when there are several songs for the females to choose from. When females were presented with

multiple stimuli rather than a single stimulus, the results were quite different.

Age and Phonotaxis: Three-Stimulus Presentation

In the three-stimulus experiment, each individual female was presented with three pulse rates in alternation. There was no significant difference in overall response between young and old groups. This finding is quite different from the prediction that older females should show increased motivation as was seen in the single-stimulus experiment. Doherty (1985c) had similar findings in *G. bimaculatus*. The difference between the two experiments may be explained by the method by which females may recognize conspecific song. In a single-stimulus design, the female is able to compare incoming stimuli to a template only. In three-stimulus presentations, the females may compare stimuli to an internal template and/or compare various stimuli to each other. For this reason, there may be a relatively higher perceived mate availability.

Mate availability has been shown to influence female mating behaviour. Souroukis and Murray (in press) found that in female-biased *G. integer* populations, females accepted more courting males than in male-biased or populations of equal sex ratios. In this study, all test females were acoustically isolated upon eclosion. A sexually naive old female may be selected to undergo increased reproductive effort upon hearing the single broadcast of calling song and the single-stimulus may elicit a stronger response from the female. The single-stimulus presentation may be representative of a low density situation in the field. It would be too costly for this female to forego

mating in case the opportunity to mate did not occur again. In contrast, a young female has a higher residual reproductive value and should have a greater probability of encountering another mate in the future. She may benefit from being selective and not mating at random. If mate availability seems high, such as in the three-stimulus experimental designs, females irrespecitve of age may benefit from being selective. Such reduced selectivity in multiple stimulus presentations has been recorded for *G. bimaculatus* and *A. domesticus* (Popov and Shuvalov, 1977; Stout and McGhee, 1988). In this study, selectivity resulted in a lower magnitude of vector scores, indicating lower motivation levels. Charalambous *et al.* (1994) also found a negative correlation between responsiveness and choosiness in the grasshopper, *C. brunneus*. Souroukis and Murray (in press) found that in female-biased *G. integer* populations, females accepted more courting males than in male-biased or populations of equal sex ratios. This indicates that the density of potential mates affects both motivation and selectivity.

There was a significant difference in vector scores generated across the three pulse rates, irrespective of age. Females preferred 64 or 70 to 76 p/s, but did not discriminate between the lower two pulse rates. It had been expected that females would orient preferentially to 70 p/s. This was reported to be the mean pulse rate of the species in the laboratory at 18-23°C, similar temperatures to those under which the present phonotaxis tests were conducted (Souroukis *et al.*, 1992). However, females responded equally well to 64 p/s. Males from the same laboratory population as those used in these experiments exhibited a mean pulse rate of about 62 ± 7 p/s

(A.M. Murray, unpublished data; L. Proctor, unpublished data). It appears that there is a lot of variation with respect to pulse rate means across different populations of field crickets. The preference for lower pulse rates noted in this study corresponded with the variation in pulse rate recorded for the males of the same population. Therefore, females orienting to stimuli with 64-70 p/s were responding to pulse rates indicative of conspecific song. A similar preference for modal rather than extreme values was documented for *A. domesticus* (Stout and McGhee, 1988). Females showed greater discrimination for songs with modal values of syllable period (Stout *et al.*, 1983; Stout and McGhee, 1988).

Multicomparison tests indicated that there was an interaction between age and pulse rate. There was a difference in the responses across the three pulse rates when comparing vector scores of young and old females. Old females did not vary significantly across the test stimuli. In contrast, young females differed in their responses across the three pulse rates. Young females preferred the 70 over the 76 but did not discriminate 64 from either 70 or 76. Often, multi-comparison tests lead to such ambiguous results due to lack of power in analysis (Zar, 1984). However, the important finding is that there was a difference in response across pulse rates between young and old females, and that the young females were more discriminatory. This is what was predicted initially because older females have less time left in which to mate, compared to young females.

Similar predictions regarding age and selectivity have been tested in other species. Charalambous (1990) suggested that in the

grasshopper, *Chorthippus brunneus*, females would be most selective at maturity and would become less selective as they neared oviposition, but this was not supported by subsequent work (as cited in Charalambous *et al.*, 1994). However, it is possible that there was not enough of a range in age, since only females aged 4-9 days were studied. Walikonis *et al.*, (1991) found that female *A. domesticus* over 12 days of age were less selective than at 3-5 days after the imaginal moult. Older females demonstrated positive phonotaxis to a broader range of syllable periods. It was discovered that this behavioural pattern corresponded to a decrease in Juvenile Hormone III levels.

Young and old females may differ in the mode by which they assess conspecific song. Young females may compare stimuli to a fixed internal template to recognize songs produced by conspecifics, and use a Best-Of-N comparison method to discriminate further among conspecifics. As they age, they may compare stimuli to a less rigid template, and thus be less discriminatory in mate choice. It is also clear from this study that prior mating experience may affect motivation and selectivity.

Mating and Phonotaxis: Three-Stimulus Presentation

There was no significant difference in overall response between females mated at three days and those mated at 17 days. Therefore, time since last mating did not affect motivation as seen in the magnitudes of vector scores. There also was no difference in scores across the three pulse rates, irrespective of mating group. Therefore, mated females did not exhibit selectivity with respect to

pulse rate. Additionally, there was no interaction between age at mating and pulse rate, indicating no difference in discrimination between the mating groups. It is possible that the effect seen in the three-choice study was not due to age *per se*, but due to differing periods of isolation from males. However, the results obtained from the three choice experiment involving mated females indicated that differences in vector scores in the two age groups in the three-choice experiment with virgin females were due to age and not due to time since last mating.

It was noted that the magnitude of vector scores for mated females was lower than in either study involving virgin females. This was surprising considering female *G. integer* can benefit from mating multiply. A study of multiple mating in *G. integer* and *A. domesticus* revealed that doubly mated females produced significantly more offspring than those that mated only once (Sakaluk and Cade, 1983). Similarly, in *G. veletis*, females given unlimited mating opportunities produced more offspring (Burpee and Sakaluk, 1993). Females in this study were isolated from males until testing on the Kugel. Male deprivation is known to result in increased phonotaxis. In an arena study, female *G. integer* and *G. veletis* that had been deprived of males for three days were significantly more phonotactic than those that had been kept with males (Cade, 1979). In the grasshopper, *Orchelium gladiator*, the phonotactic response to broadcast tape-recorded conspecific calling song decreased after mating (Morris *et al.*, 1975). In this study, mated females appeared to have decreased motivation levels. However, seven days after mating, only 20% of females were

attracted to conspecific song. Females may show reduced motivation immediately after mating because at this time they may be selected to search for oviposition sites, rather than orienting to other males. The smallest interval between mating and testing in this study was eleven days. Therefore, it was expected that females would be responsive to calling song stimuli after this period of isolation.

Other Factors - Time Of Trial and Weight

It has been demonstrated that *G. integer* females tend to be most active between two and nine hours after sunset (French and Cade, 1987). It may be that females are more motivated at different periods of the night. In this study, however, phonotaxis was not affected by the time of the trial.

It is possible that weight could have an influence on vector score. Heavier females may carry more eggs and therefore be more motivated to respond to a calling male. In this study, it was determined that weight did not affect vector score. However, weight had an effect on absolute vector score in two of the three experiments. This indicated that weight affects the magnitude to which a female is able to rotate the sphere, but not the direction that she will travel in. It is likely that heavier females have more muscle mass and therefore are better able to move the sphere. Weight did not have an effect on absolute vector score in the three-stimulus experiment involving virgin females. Females in this experiment had a similar range in weight comparable to the other experiments and so variation in weight between experiments cannot explain this result. It cannot be explained why weight affected the absolute vector score in only two of the three experiments. In the single-stimulus experiment, the weights of the females covered a broader range then in either of the three-stimulus experiments. Therefore, it is not surprising that there was a stronger correlation between weight and absolute vector score in this study than in the three-stimulus presentation involving virgin females. However, this cannot

explain the fact that there was a correlation in the three-stimulus experiment involving mated females, but not in that involving virgin females. In these two studies there was little difference in the ranges of weight.

The fact that in two of the experiments there was a correlation between weight and the magnitude of the vector score may be one of the limitations of the apparatus. The Kugel relies on the female to propel the sphere and heavier females may be able to exert more force to do this. In other systems such as arenas, Y mazes, flight assays, or the locomotion compensator females are not required to move part of the apparatus. However, heavier females may walk faster and therefore weight is a factor that should be considered in these assays as well. This is something that should be considered when choosing the apparatus to be used in an experiment.

On the other hand, there are several advantages to using the Kugel. It is an easily manipulated system that allows several stimuli to be broadcast in alternation. Due to the digitized quantification of movement of the subject, the researcher remains objective. Successive studies can be repeated and replicated with ease. In contrast to arena studies, the female is neither exposed to increased intensity upon approach to the loudspeaker, nor to pheromonal cues from previously tested subjects. Although stress may be a factor in the responsiveness of the female, the crickets tested in this study seemed to quiesce rather quickly. Therefore, the Kugel is an effective and efficient apparatus that can be used to explore factors that affect phonotaxis and female choice.

It is beneficial for females to first recognize the males of their species and then to also exert choosiness intraspecifically to mate with the male of highest quality. Phonotaxis is an excellent measure of mate choice in acoustical animals because females orient to the species-specific calling song prior to mating. There appears to be a great deal of variation in female phonotaxis and at least part of this variation may be caused by female age.

CONCLUSIONS

It is important for females to be choosy and avoid heterospecific males because of costs in terms of reduced reproductive success. Results from the single-stimulus presentation in this study supported this as females did not orient to very high or very low pulse rates but chose modal values. Pulse rate may be an important parameter for species recognition as males of different species often produce different pulse rates (Smith and Cade, 1987; Cade and Tyshenko, 1990).

Females also exhibited intraspecific mate choice. In the three-stimulus experiment using virgin females, females preferred 70 or 64 to 76 p/s. Females may use variation in calling song to assess males. Several song parameters are known to vary in their attractiveness to females (Wagner *et al.*, in press). In the three-stimulus experiment in this study, young females discriminated with respect to pulse rates within the natural range of calling song at that experimental temperature.

Mate choice is a very complicated phenomenon in which several factors may play a role. Researchers have commented on the variability of phonotaxis among females (Doherty and Storz, 1992; Wagner *et al.*, in press), but few studies have focussed on the possible causes of this variation. This study demonstrated that female age may contribute to variation in mate choice. Older females were more motivated to mate and less discriminatory with respect to mate choice. This is what was expected from initial predictions regarding decline in residual reproductive value. Female choice also

may be affected by environmental factors such as sex ratio and population density as well as by prior mating experience.

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