Behavior and evolution of periodical cicadas (*Magicicada* spp.)

by

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PREFACE

This dissertation is the partial product of five years of intensive study of periodical cicada biology, much of which was conducted in collaboration with John R. Cooley. Although we began with general questions we hoped the cicadas might be uniquely qualified to answer, such as the nature of female choice in insect leks, we have allowed the cicadas to guide our research, believing that the best questions are often revealed only after one begins to know an organism in detail. The brilliant may occasionally manage to anticipate significant discoveries while wading through the confusion of published information, to guess correctly where and when to look and what to measure, but for the rest of us the best investment is simply time spent in the field testing the best questions we have at the moment, watching and listening to the organisms, and waiting for the surprises. This fitful but fun approach has been productive during the past five years -- we have stumbled upon, among other interesting finds, a female signal that unravels the *Magicicada* communicative sexual sequence in all seven species, a new species of 13-year periodical cicada, and a case of reproductive character displacement. These findings have solved problems, raised questions, and opened doors for future research. To a certain extent, it is only now, after five seasons of watching the cicadas and thinking about sexual selection, lek evolution, signal evolution, and speciation, that I am beginning to realize (at least little I hope) what I should have been measuring all along, what the really key questions are. Fortunately there will be periodical cicadas emerging in seven of the next nine years!

The first two chapters of the dissertation discuss studies of *Magicicada* mating behavior and communication. Chapter 1 describes a female "wing-flick" signal and its

implications for understanding (1) pair-formation strategies of males and females, (2) the adaptive significance of calling song structure in *Magicicada* -decim species, and (3) the nature of male-male acoustic interference competition. Chapter 2 considers the origin of *Magicicada* leks and the nature of sexual selection within them, using experimental data on courtship behavior, female remating tendencies, and male mating success.

The focus on *Magicicada* -decim song structure that developed during the research in Chapters 1 and 2 facilitated the discovery of 13-year *Magicicada neotredecim* and the pattern of reproductive character displacement that occurs where the new species overlaps its closest 13-year relative (*M. tredecim*), as discussed in Chapter 3. Playback techniques developed in the prior behavioral studies became essential in testing the reproductive isolation of these species in sympatry. In Chapter 4, morphological analysis is used to further test the hypothesis of interspecific hybridization between *M. neotredecim* and *M. tredecim*, as well as to test its hypothesized origin from Midwestern populations of a 17-year species, *M. septendecim*.

In Chapter 5, problems of signal evolution and species interactions are unified in an analysis of female "preference curves" for male song pitch in 17-year *Magicicada septendecim*. These data afford an estimate of the risk of wasted interspecific mating effort that faced female *M. neotredecim* upon first contact with *M. tredecim*. In addition, playback techniques are used to determine if acoustic background interference alone could drive song divergence in sympatry.

Chapter 6 concludes by moving to an analysis of the historical record of *Magicicada* emergences in the Midwest. These records have been used by earlier investigators to develop and test hypotheses of periodical cicada biogeography that suggest massive brood range shifts occurring in the past 150 years. Reanalysis of these records indicates that *Magicicada* broods have remained stable in distribution, with the appearance of range shifts having been created in part by occasional delayed emergences. Populations of *Magicicada neotredecim* appear to have contributed a large number of these straggling emergences,

perhaps reflecting the recent allochronic derivation of this species from 17-year *M. septendecim*.

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CHAPTER 1

SEXUAL SIGNALING IN PERIODICAL CICADAS, *MAGICICADA* SPP.

Abstract

We describe a previously unknown mating receptivity signal in female *Magicicada* and its role in periodical cicada sexual pair formation. Receptive female *Magicicada* flick their wings with a quick motion in timed response to an individual chorusing male; this female response is hereafter referred to as a "wing flick" signal. We document the nature, timing, and species-specificity of this signal as well as the unresponsiveness of immature or mated females. We also document changes in male chorusing and searching behavior in response to this signal, and male responses to visual and acoustical components of the signal. We present and test the hypothesis that female sexual responsiveness has shaped calling song evolution in *M. septendecim* by favoring males whose calls are more readily distinguished from a background chorus. Within mating aggregations, male *Magicicada* engage in acoustic interference competition by acoustically obscuring the terminal downslurs of calls of potential interlopers with a timed "interference buzz", reducing the likelihood of a female response. We suggest that the traits characterizing periodical cicada sexual pair formation have resulted from selection for efficient mate-location in dense

aggregations and that intense male-male competition for limited mating opportunities is primarily responsible for complex sexual behaviors in *Magicicada*.

Introduction

Periodical cicada (*Magicicada* spp.) adult behavior is characterized by an extraordinary combination of attributes, some rare or unknown in other acoustic insects. Males form extremely dense, multispecies[1] mating aggregations (Alexander & Moore 1958, 1962, Alexander 1975), perform conspicuous searching behavior by alternating short (ca. 3-15 s) calling bouts with short flights (Alexander & Moore 1962), and engage in complex courtship (Alexander and Moore 1962, Alexander 1968, Dunning et al. 1979) which in most species includes distinctive behaviors and at least two distinctive acoustic signals in addition to the calling song (Table 1, Figure 1). Courtship may be followed quickly by mating or may last many hours (Alexander 1968, Dunning et al. 1979), and copulation is lengthy, typically lasting 4.5 h (Cooley 1999).

Attempts to understand the evolutionary significance of *Magicicada* behavioral strategies of both sexes (Alexander 1968, 1975; Dunning et al. 1979), and to place them into a general comparative model of cicada pair-forming systems, have achieved limited success because of incomplete knowledge of intersexual communication during pair formation. The cues causing males to switch from chorusing (not directed at a specific individual) to courtship (directed at an individual female), or from extended courtship to copulation, have until now remained unknown (Alexander 1968). Here we demonstrate that *Magicicada* pair-formation fits a pattern observed in some other Cicadidae: A receptive female perceiving a nearby conspecific male responds to each of the male's calls with a wing flick signal performed with species-specific timing; this repeated signal-response

[1] Because each 13-year *Magicicada* species has a 17-year counterpart with similar ecology, morphology, and behavior (see Table 1 of Chapter 3 for distinguishing traits), we refer to the cognate groups as –decim (17-year *M. septendecim*, 13-year *M. neotredecim*, and 13-year *M. tredecim*), –cassini (17-year *M. cassini*, 13-year *M. tredecassini*), and –decula (17-year *M. septendecim*, 13-year *M. tredecula*).

"duet" leads to localized searching by the male and then to later stages of courtship. We demonstrate that the female signal contains both visual and acoustic components and that males respond to these stimuli by localizing their mate search efforts and beginning late stages of courtship. These discoveries clarify the adaptive strategies of both males and females and lead to new findings involving the evolution of male -decim calling song structure. The results consistently show that periodical cicada sexual behavior and communication has been potently influenced by the extraordinary density of their mating aggregations. We focus in additional detail in this paper on two ways in which selection deriving from high intraspecific population density has influenced the evolution of the *Magicicada* sexual sequence.

Male -decim calling song structure

Males of five of the seven *Magicicada* species produce bouts of short (ca. 1.5-4s) calls each containing two primary components, a constant-pitch "main element" followed by a terminal "frequency downslur;" the downslur is most prominent in the -decim species. As activity levels increase in dense *Magicicada* choruses, the nearly pure-tone main elements of individual *M. tredecim, M. neotredeci*m, or *M. septendecim* calls blend to produce a uniform chorus drone, and only the downslurs of nearby males stand out (to a human ear) from the background chorus. Because female responses to male calls are timed in a species-specific manner, we suggest that the terminal downslur of the male call has evolved secondarily to improve the likelihood that a nearby female will accurately perceive the end of the male's call in dense aggregations. This hypothesis leads to several predictions. First, increasing background chorus intensity should decrease response rates of females to playbacks of whole male calls and of call fragments lacking the downslur. Second, downslurs alone should not be sufficient to elicit wing-flick signals, unless the background chorus is loud enough to take the place of the main element in stimulating the

female. Third, neither the downslur nor the rest of the call alone should be as effective as the intact call in eliciting the female response at any background chorus intensity.

Male-male acoustic interference competition -- The structure of male calling song and female responses to it creates opportunities for males to compete among themselves acoustically for mates, especially given that males and females are often in close proximity in *Magicicada* aggregations. In 1996 we first observed a previously undescribed male sound in *M. septendecim and M. cassini*; this sound is composed of a short (≈ 0.25 s) "buzz" with frequency content roughly comparable to that of the calling song main element. Males always produce this sound precisely during the downslur of another male's call (see Fig. 2) and always when recently or currently engaged in a courtship interaction, strongly suggesting that the buzzes are not explainable simply as aborted calls. In part because the downslur is important in eliciting female wing-flick signals (see below), we suspected that a male engaged in close-range courtship uses this "buzz" sound to interfere acoustically with downslurs of calls produced by individual chorusing males who land nearby. Such males are potential interlopers, and the buzz sound may reduce the likelihood that the courted female will reveal her presence with a wing-flick signal before the chorusing male completes his short calling bout and departs. This hypothesis predicts that buzzes should be elicited only in circumstances surrounding the arrival of a competitor male during a courtship interaction, and not during chorusing behavior in general. In an alternative hypothesis, males might use this sound to signal their sex to other males; males sometimes mistakenly court other males, so the sound may discourage misdirected sexual attention. A similar explanation has been proposed for a "flick-tick" signal produced by *M. cassini* males (Dunning et al. 1979). This hypothesis predicts that the signal should be observed most often when males are crowded and encounter one another commonly in the chorus.

Materials and Methods

General methods

We observed male-female interactions and conducted experiments on sexual signaling from 1995-1999 (Table 2) using both 13-and 17-year cicadas. We concentrated on the -decim species but included others when sufficient numbers were available. We used only unmated females in our study, except when experimental designs required otherwise. Although mated females commonly have a hardened white seminal plug in the genital opening (White 1973), this plug is occasionally absent or difficult to detect in mated females and is therefore an imperfect indicator of mating status. Periodical cicadas remain teneral for several days after their final moult while the exoskeleton hardens and other maturation processes occur (Maier 1982; see also Karban 1981, Young and Josephson 1983); during this period they are generally inactive, do not mate, and are identifiable by their dull color and soft bodies. Thus, by collecting teneral females early each morning and caging them away from males, we could be certain that we were studying unmated females.

We used a Macintosh computer and Canary software (Cornell Bioacoustics Laboratory) for acoustical analyses, and SoundEdit software (MacroMedia, San Francisco, CA) for model song synthesis. Playback equipment consisted of a Sony WM-D6C cassette player or a Macintosh computer connected to a Radio Shack SA-10 amplifier driving a 3" midrange speaker for -decim calls or a tweeter for -cassini calls. We maintained playback call intensity at natural levels (≈ 72 dB at 20 cm from the sound source as measured by a Radio Shack 33-2050 sound level meter set to "A" weighting). In all years, we kept cicadas in captivity, but within their natural environment, by placing them in ca. 200 liter cages made by wrapping living vegetation with black fiberglass screen or white nylon tulle. Some observations were completed in small 22 x 24 x 22 cm screened test chambers or in larger ca. 3 x 3 x 2.5 m "flight cages" placed over living woody vegetation. For all parts of

our analysis, we used simple, nonparametric statistics on untransformed data; all statistical analyses were conducted with Systat Version 5.0 (MacIntosh).

Documenting the nature, timing, and species-specificity of the female signal

In 1995-1998, we observed sexual interactions between male and female *Magicicada*. We noted whether females produced wing-flick signals and how the responses were timed in relation to male calling and courtship songs (details of these signals are in Table 1) using sonograms generated from audiotape and videotape to measure timing. We also recorded female –decim and -cassini responses to playbacks of male calling song and how males responded to natural and simulated wing-flick signals.

To demonstrate the species-specific nature of the wing-flick response, in 1996 we played recorded *M. septendecim* calling phrases alternating with *M. cassini* calling phrases to 25 caged, unmated *M. septendecim* females and noted their responses. We also included three unmated *M. cassini* females as controls. We tested the *M. septendecim* females in groups of five, playing a series of 15 alternating *M. septendecim* and *M. cassini* calls (30 calls total) and recording female responses.

Documenting the relationship of female signals to sexual receptivity

To demonstrate that immature females are not sexually receptive and do not signal, in 1998 (in a 13-year cicada emergence) we made six daily collections of approximately 25 newly-emerged females each. Each day, we played recordings of *M. septendecim* male song to each of the cohorts and watched for wing-flick signals. We at first presumed that the cicadas were *M. tredecim*, which at that time was believed not to differ from *M. septendecim* in any behavioral attributes. However, we later discovered that two 13-year -

decim species were present, one previously undescribed and both distinguishable by calling song pitch (see Chapter 3 and discussion). Because both 13-year species differ from *M. septendecim* in calling song pitch in the study location, the recorded *M. septendecim* calls were probably not as stimulating as conspecific calls for females of either species (see Chapter 5).

To determine whether mated females are sexually unreceptive and do not wing flick, we allowed 22 individually-marked (Cooley et al. 1998) female *M. septendecim* to mate once and divided them between two cages along with 22 marked, unmated females of the same age. In each of four consecutive days we played recorded *M. septendecim* calling songs to the females for two minutes and observed the number of mated and unmated females responding.

Assessing the effects of female signals on male chorusing behavior

In 1996 and 1997, we examined how chorusing male *M. septendecim* respond to female signals by producing sounds and movements imitating female wing-flicks. Before we identified a suitable device for producing simulated wing-flicks, we used a strip of paper that we flicked with our fingers. Later, we discovered that toggling an ordinary household electric light switch was more convenient for the experimenter. Because male responses to both artificial stimuli appear indistinguishable, we combined trials with both methods in our analyses.

To document the importance of correct timing in eliciting male *M. septendecim* courtship, we produced simulated wing-flicks in response to the calls of males that had landed and begun calling on nearby vegetation, placing the flicking device within 25 cm of the male along the branch on which he had just landed and timing our signals either (1) during the main element, (2) during the downslur, or (3) after the downslur. In control trials, the experimenter did everything but produce simulated wing-flicks. We scored a

male as responding positively if he moved toward the stimulus and began late-stage courtship behaviors such as CII or CIII calling, foreleg vibration, or attempts to mount the object used to make the stimulus. Each trial ended when the male flew or walked away from the stimulus, or when the male remained motionless longer than 20 seconds.

The above experiment simulated a scenario in which a male alights near a signaling female. To examine male responses in a scenario involving weaker and/or less consistent female responses, we conducted trials in which we presented individual chorusing males that had just landed with a single nearby (25 cm) or distant (1.3 m) simulated wing-flick response, with the timing appropriate for the species (see Table 1 and Fig. 3). We included control trials in which the experimenter approached in the same manner but did not produce a signal. In each trial we recorded the number of calls the male made in his current bout (call number), the nature of his next action (sit, walk, fly), and the direction and distance of movement. We stopped monitoring males that paused for longer than 20 seconds.

Comparing male responses to visual and acoustical components of the female signal

Female wing-flick signals contain visual and broad spectrum acoustical (Fig. 3) stimuli. It is possible that different *Magicicada* species perceive different aspects of female signals; although high-frequency sounds are within the range of maximal hearing sensitivity of the –cassini species, much of the acoustical content of wing-flick signals is above the range of maximal hearing sensitivity for male –decim (see Simmons et al. 1971, Huber et al. 1980).

To examine the effects of timed flick sounds alone on male chorusing behavior, in 1997 we constructed a clicking device by attaching a 12 volt relay to the end of a 1-meter wooden pole, covering all wires and dark (cicada-colored) parts of the relay with white masking tape; this device produced a sharp, broad-frequency click. In test trials, we

identified a chorusing male *M. septendecim* or *M. cassini* that had just landed, placed the relay 25 cm from him along the same branch, and clicked the relay in response to each call in his next two calling bouts with timing appropriate for the species (see Table 1 and Fig. 3). We avoided making any timed movements observable by the male; our control was to place the device in the same manner without clicking the relay. For each male, we measured the number of calls in each of two calling bouts and the distance moved between the two calling bouts.

In 1998, to investigate the effects of visual signals (both timed movements and color) without sound, we approached individual chorusing male –decim that had just landed with a model consisting of a white plastic ballpoint pen with a black cap or with an identical cap painted white, holding the pen 25 cm away so that the male faced the cap. In response to the male's calls, and without making sound, we twitched the model once rapidly back and forth about 2 cm with the appropriate timing (see Table 1 and Fig. 3), or we held it still. In controls we approached with the model but did not move it. We recorded all courtship behaviors directed toward these models, discontinuing if the male moved away, climbed onto the model, or remained still longer than 20 seconds. For *M. tredecassini*, we were able to obtain enough males to conduct only anecdotal observations.

To determine whether combined visual and acoustical stimuli are more potent than either alone, in 1997 we captured single males of *M. septendecim* or *M. cassini* from the surrounding chorus and placed each in a 22 x 24 x 22 cm test chamber. Two opposite walls of the chamber consisted of three layers of dark, opaque cloth to allow sound but not light to penetrate; the remaining walls consisted of fiberglass screen. In the first treatment, we suspended a motionless model cicada constructed of a thimble covered with black cloth inside the test chamber and responded with appropriate timing to the male's calls with light-switch clicks produced behind the opaque chamber sides. In the second treatment, the experimenter held the model inside the chamber and responded to the calling male by moving the model slightly with the appropriate timing, without making clicks. In the third

treatment, we responded by simultaneously producing clicks and moving the model. After the start of each trial, we recorded the male's behaviors for the next two minutes. Male *M. septendecim* and *M. cassini* were scored as responding positively to the model if they exhibited any of the following behaviors during the 5 minute trial: CII or CIII courtship, extrusion of genitalia, mounting attempts, foreleg vibration, or copulation attempts.

Evaluating the functions of -decim call components

In 1997, to test our hypothesis that the downslur of the –decim call functions to distinguish the call from the background chorus, we noted the wing-flick responses of female *M. septendecim* to playbacks of pure-tone model calling phrases, pure-tone main elements, and pure-tone downslurs. We played each of these call types five times at an intensity of 72-79 dB (call intensity varied within the test chamber) against each of four pure-tone background choruses differing in intensity (0 dB, 58-62 dB, 63-77 dB, 65-80 dB). We tested 19 groups of five females each. In previous experiments, we found that females respond similarly to playbacks of recorded and pure-tone model calls. The experiment was carried out in a field where the natural *Magicicada* chorus was faint.

Documenting male-male acoustical interference behavior

We conducted three experiments in 1997 to evaluate the hypotheses for the function of –decim buzzing behavior. In the first experiment, designed to determine if crowded chorus conditions alone can induce buzzes, we placed 30 chorusing males together in a 1 x 1 x 1 m screen cage placed over a stump sprout and listened for the male buzzes. After 20 minutes, we engaged one or more males on one side of the cage with artificial wing-flick signals for one minute. We then listened for male buzzes for another five minutes. After approximately 15 minutes we repeated the experiment.

In the second experiment we simulated the events involved in the appearance of a potential interloper during courtship by presenting 22 males one at a time with the following series of stimuli: First, we confined the male in a small (22 x 24 x 22 cm) screened test chamber. We then played one to five minutes of recorded calling song from ca. 25 cm away at an intensity of approximately 75 dB at 10 cm. Males often began to call during this treatment, but if the playbacks did not stimulate calling, we produced simulated wing-flick signals in response to the speaker using an electrical switch held within view of the male on the outside of the cage; signals from the switch always induced a call-walking approach (see below) from the male. Once the male began calling, we turned off the speaker and responded to the male's calls with the switch until the male had approached; once he reached the switch (on the opposite side of the screen), we ceased responding to his calls. Once the male stopped his calling or courtship songs (some males began CII or CIII calling during this duet), we resumed playbacks of calling song. We noted the context of any buzzes produced by the male during the trial.

In the third test, we confined four unmated female *M. septendecim* in the 22 x 24 x 22 cm test chamber and played a sequence of 30 call pairs each consisting of (a) one model pure-tone call phrase (main element pitch 1.4 kHz) played from one speaker followed by (b) one model call played from the same speaker along with a 0.25 s model 1.4 kHz pure-tone "buzz" played from a second speaker and superimposed over the model call downslur. We recorded the number of females responding with wing flick signals to each call and repeated the experiment 6 times, using different females each time. We compared the number of females responding to obscured and unobscured calls using a Friedman Two-Way analysis of variance.

Results

The nature, timing, and species-specificity of the female signal

We documented female wing-flick signals in all *Magicicada* species except *M. tredecula*, which we have not collected in sufficient numbers to study. A female signals in response to a calling conspecific male by moving her wings in a single, quick motion which produces a broad-frequency sound of approximately < 0.02 s duration (Fig. 3a-c). The motion and sound are distinct from wing flutters produced in response to disturbance, which consist of multiple wing movements. The signal's visual and acoustical components appear unspecialized but its timing in relation to the male's call is species-specific. In *M. septendecim*, females signal an average of 0.387 ± 0.106 s (mean ± SD, n = 235) after the end of the male calling phrase (Fig. 3a). The delay in *M. cassini*, 0.705 ± 0.112 s (mean ± SD, n = 16), is nearly twice as long (Fig. 3b). Qualitative observations of *M. neotredecim, M. tredecim* and *M. tredecassini* indicate that the signal timing in these species is similar to that of their 17-year counterparts. *M. septendecula* females produce individual wing-flick signals in one or more of the brief silences between subphrases (Fig. 3c); we expect that a similar signal and timing is present in *M. tredecula*.

In all species studied, once a male has perceived wing-flick signals, he begins to approach the signaling female, who produces wing-flicks in response to each of the male's song phrases (Fig. 1; CI of Dunning et al. 1979). In the -decim and -cassini species, this "duet" continues until the male switches to CII courtship. We have not determined what stimuli cause a male to begin CII courtship; he usually does so once within approximately 1-15 cm of the female, perhaps upon making close visual contact. Female -decim and -cassini only rarely produce wing-flicks during CII; however, if a -decim male ceases CII courtship, leaving a silent gap, the female may respond with a timed wing-flick. In -decula, no homologous CII courtship song is known. In all species the male switches to

CIII courtship soon after positioning himself next to the female, at which point he attempts to mount her. Females of all species studied do not wing-flick during CIII; wing-movements after the onset of mounting interrupt or terminate courtship and apparently indicate mating rejection by the female. The pair-forming sequence always occurs in the stereotyped sequence just described (see also Table 1 and Fig. 1) unless (1) the male fails to locate the female or (2) the female ceases wing-flicking at any point during CI or (3) the female rejects the male upon his first attempt to mount. In situations #2 and #3 the courtship may become prolonged, involving long waits, series of CI calls, and occasional attempts to mount with or without CII and CIII courtship calling (see Chapter 2).

M. septendecim females routinely responded to the *M. septendecim* call phrases in each trial. Only one of 25 *M. septendecim* females ever responded to a heterospecific playback; the responsiveness to conspecific and heterospecific calls was significantly different (Fisher's Exact two-tailed test: $P \leq 0.01$). The three *M. cassini* females in the experiment responded only to conspecific calls and never responded to heterospecific playbacks. Females of *M. tredecim* and *M. neotredecim* have also been shown to respond preferentially to models of conspecific calls (Cooley 1999, Marshall and Cooley 2000).

Female signals and sexual receptivity

Females first signaled to the *M. septendecim* calls 6.5 ± 1.1 (mean ± SD for 6 collections) days after emerging. This delay is roughly consistent with previously reported teneral periods (Karban 1981, Maier 1982, Young and Josephson 1983), but somewhat later than the onset of sexual maturity observed in some of our other experiments (e.g. Experiments C and E of Chapter 2); females might have responded earlier had a more appropriate call model been chosen. In 1996, none of the mated females responded to playbacks, while at least half of the unmated, mature females responded each day with wing-flick signals (Table 3). This difference was significant in each of the four days.

The effects of female signals on male chorusing behavior

Only wing-flicks produced after the downslur caused males to respond positively (Table 4). Males usually responded to such stimuli by walking toward the stimulus while calling. In this behavior, termed "call-walking", males stopped walking for approximately one second immediately following each downslur. This pattern is distinct from chorusing behavior, which involves bouts of stationary calling alternating with flights or silent walks. Males were equally unresponsive to the control treatment and to simulated wing-flicks produced during the main element or during the downslur (Table 4).

Males responded to single nearby and distant simulated wing-flick signals in a manner suggesting an attempt to localize the stimulus (Table 5). Both kinds of stimuli caused males to increase the number of calls in the current calling bout compared to control males, but, as in chorusing behavior, most males then flew to a new calling perch instead of call-walking toward the stimulus. Whether walking or flying after the calling bout, males presented with either stimulus were more likely to move in the direction of the stimulus than control males were. In control trials, males were more likely to move away from the stimulus than toward it, probably because the presence of the experimenter tended to disturb the cicadas.

Male responses to components of the female signal

Timed click sounds did not affect chorusing male *M. septendecim* behavior, but males of *M. cassini* for which click sounds were played flew significantly shorter distances between calls (Table 6); no males of either species attempted courtship in response to the clicks. Males of *M. septendecim* courted pen cap models that were moved silently to imitate wing-flick signals, but they were less likely to engage in late-stage courtship with

white colored caps than with black colored caps (Table 7), indicating that while movement alone is sufficient to provoke male responses, males also respond to color components of the stimulus. Anecdotal trials with *M. tredecassini* in a flight cage confirmed that males of this species will also court the silent moving pen model. In the trials directly comparing movement and sound stimuli, the model that moved and clicked simultaneously was most attractive to *M. septendecim* and *M. cassini* (Fig. 4).

The functions of -decim call components

Although call fragments elicited some female responses, females were more likely to respond to whole calls than to partial calls at all background chorus intensities (Fig. 5). As the background chorus intensity increased, female responsiveness to whole calls and main elements of whole calls declined, while females became more responsive to slurs (Fig. 5), such that at the highest intensity, females were more responsive to slurs alone than to main elements alone.

Male-male acoustical interference behavior

In trial 1 of the crowded-male test, no males produced buzzes prior to the production of simulated wing-flick signals, but two buzzes were heard once we began to respond to the males with simulated wing-flick signals. In trial 2, one buzz was heard during the first 20 minutes, but over 20 were heard after we engaged males in wing-flick duets. Males called often and frequently landed within centimeters of each other during both parts of each trial, and although some males courted or attempted to mount other males, these interactions did not lead to male buzzes.

In the second test, simulating the appearance of an interloper during courtship, males never produced buzzes in response to the initial series of speaker playbacks, never

produced buzzes during artificial duets between the speaker and the experimenter, and never produced buzzes while duetting with the experimenter. However, in 16 of the 22 trials, the male began producing buzzes once playbacks had resumed following the termination of his duet, usually in response to the first or second playback call, but sometimes not until 3 or 4 calls had played. The responses of males before and after simulation of an interloper are significantly different (Fisher's Exact two-tailed test, $P \leq 0.001$). Males producing buzzes did so only during the downslur of the recorded calling song phrases. In five of the trials, we again began producing simulated wing-flick signals to the speaker while the male buzzed; in four of these five cases this caused the male to cease buzzing and begin call-walking near the simulated wing-flick stimulus. In the fifth case the male walked while buzzing after each playback call.

In the third test, females were significantly less likely to respond to calls obscured by model buzzes than to unobscured calls (Table 8). Under the conditions of this experiment, buzzes halved the likelihood of a female response.

Discussion

Wing-flick signals in Cicadidae

Communicative wing-flicking (sometimes called wing-tapping, -banging, -clapping, -clacking, or -clicking) may be widespread in cicadas. We use the term "wing-flick" in this paper because it connotes movement and sound, both of which are perceived by male *Magicicada*; other terms emphasize only the acoustic component of the signal. Male wing-flicking during close-range courtship interactions with females has been reported in Australian and New Zealand *Kikihia* and *Amphipsalta*, (Dugdale & Fleming 1969, Lane 1995) North American *Okanagana* (Davis 1919, Alexander 1957, Cooley 1999), and European *Tibicina* (Fonseca 1991), while males combine wing-flicks with long-range

calling song in Asian *Cicadetta* (Popov 1981), Australian and New Zealand *Amphipsalta* (Dugdale & Fleming 1969, Lane 1995), and Western North American Platypediinae (Moore 1968). Female wing flick signaling is known in North American *Magicicada* (this study) and *Okanagana* (Davis 1919, Cooley 1999), Australian *Cystosoma* (Doolan 1981, Doolan & Young 1989), *Cicadetta* (Gwynne 1987), and *Amphipsalta* (Dugdale & Fleming 1969), and New Zealand *Amphipsalta*, *Kikihia*, *Maoricicada*, *Notopsalta*, and *Rhodopsalta* (Lane 1995, Dugdale & Fleming 1969); the most detailed published reports of female wing-flick signaling involve *Kikihia* spp. (Lane 1995), *Amphipsalta cingulata* (Lane 1995), *Cystosoma saundersii* (Doolan 1981, Doolan & Young 1989), and *Cicadetta quadricinctata* (Gwynne 1987). In each species studied in detail, female wing-flick signals elicit male courtship behavior and appear to have a specific temporal relationship to the male's song. In *Magicicada*, synchronized visual and acoustical stimuli are most effective in eliciting male responses, and the timing of the signal must be more important than other characteristics of the stimuli, since the click and movement of an ordinary electric light switch are sufficient to provoke male courtship. In a few species female wing-flick signals, while present, are apparently not always prerequisites for mating: Although sometimes both sexes of *Okanagana canadensis* and *O. rimosa* appear to use wing-flicks to signal their presence, females more often signal receptivity simply by approaching stationary calling males (Cooley 1999).

The evolution of *Magicicada* chorusing behavior

In *Magicicada*, it is apparently the sound of the entire chorus that attracts a female, rather than the song of any one male (Alexander 1975). Therefore, an individual male *Magicicada* with chorusing behavior that is less effective in long-range attraction but more likely to be detected by nearby stationary receptive females might realize a fitness advantage relative to those males with chorusing behaviors optimal for long-range attraction, and male

sexual behaviors are likely to be most strongly influenced by selection for effectiveness in local searching for receptive females. For *Magicicada*, the discovery that receptive females respond to the calls of individual males with timed wing-flicks resolves the question (Alexander 1975), of why males do not silently parasitize the mate-attracting abilities of others by conducting series of searching flights without intervening calling bouts: Males who search without signaling sacrifice the opportunity to cause females to respond and reveal their presence.

Magicicada chorusing lies at one extreme of the range of pair-forming behaviors found in cicadas (see Alexander 1960). Males alternate unusually brief bouts of calling (and unusually short call phrases, especially in -decim and -cassini) with short flights, a pair-forming strategy that is centered around male searching for females who have moved to the chorus and become stationary. Only one other well-studied cicada species, the Australian Tick-Tock cicada (*Cicadetta quadricinctata*) has a similar pair-forming system, although calling bouts in this species are still three to four times longer in duration than those of *Magicicada* (Alexander & Moore 1962, Gwynne 1987). Gwynne (1987) noted the similarities of male behavior in *Magicicada* and *Cicadetta quadricincta* and all but predicted that female wing-flick signals would be found in *Magicicada*. At another extreme are species in which males advertise with a continuous long-range acoustical signal (with or without separate phrases) from a single location for relatively long periods, such as in North American *Okanagana canadensis* and *O. rimosa* (Cooley 1999). A comparable range of pair-forming strategies is found in other singing insects such as Phaneropterine katydids (Spooner 1968, Heller & von Helversen 1993). The *Magicicada* pair-formation system is similar to those of fireflies (see Lloyd 1966, 1979), substrate-vibrating leafhoppers (see Claridge 1985, Hunt & Nault 1991, Hunt et al. 1992), and lacewings (see Wells & Henry 1992, Henry 1994).

Because the phylogenetic relationship of *Magicicada* to other cicada genera is poorly known, little information is available on the pair-forming behavior of the most recent non-

periodical ancestor of *Magicicada*. However, the uniqueness of the *Magicicada* system alone suggests that periodical cicadas probably evolved from ancestors with relatively stationary, advertising males and comparatively mobile females; females of these ancestors may have signaled to nearby males with wing-flicks, as suggested by the widespread occurrence of such signals in the Cicadidae. In the hypothesis below, we suggest that the evolution of the unique aspects of *Magicicada* pair-forming behavior from such ancestors can be understood as an outcome of adaptation to high population densities.

Upon the evolution of partial or complete periodicity (see Lloyd & Dybas 1966a,b; Cox & Carlton 1991, Long 1993, Yoshimura 1997) high population density and low predation risk (Williams et al. 1993) arising from lack of ecological control by predators and parasites became a consistent feature of *Magicicada* ecology. This presumably led to two important effects with dramatic consequences for the *Magicicada* mating system: First, males and females began to experience reduced risks associated with movement and/or signaling. Second, the greater density of receptive females increased the chances that a male could encounter a receptive female while moving through the environment. One or both of these factors could have improved the potential payoff to individual males of increasing the amount of time spent searching, at the expense of advertisement. Increased male searching would have caused females to gain from moving to areas of loud male chorus sound and remaining still once there, in part because increased male movement would reduce the ability of females to approach individual males. The new female strategy of moving to a chorus and becoming stationary would have favored a tendency in males to be attracted to the choruses of other males, completing the evolution of the basic elements of the *Magicicada* pair-forming system. An environment of abundant, locally aggregated, stationary females who respond to the call terminus with a wing-flick would select males further for a local search strategy emphasizing short calling bouts and short flights. The density of male aggregations would allow the chorus sound itself to assume the female-attraction function of the male's call, allowing the evolution of calling song structure more

effective for eliciting responses from nearby females in loud, dense choruses, possibly resulting in (1) shorter and quieter calls, and (2) calls with terminal downslurs enhancing recognizability in the –decim and -cassini species or in their common ancestor. Finally, the crowding and confusion of dense *Magicicada* choruses could have favored the elaboration or enhancement of the female wing-flick signal to reduce pair-forming delays, perhaps by refinement of its timing in relation to the male's call.

The best tests of the above scenario will likely come from comparative study of sexual pair formation systems in other Cicadidae, but at present few species have been studied in detail. One pattern within *Magicicada* offers initial support for the theory: Two *Magicicada* species, *M. septendecula* and *M. tredecula*, are consistently less abundant than the other *Magicicada* with whom they are synchronized. Compared to –decim and –cassini, –decula have longer calling bouts and a longer call unit that contains multiple temporal "windows" for female wing-flick responses, as expected from this hypothesis; this hypothesis does not, however, explain the progressive nature of the -decula calling song, which changes in the middle from repeated "tick-buzz" subphrases to repeated "tick" subphrases. Our playback experiments confirm a different aspect of the hypothesis, that male calling song has evolved under selection for distinctiveness in loud choruses. The two-part structure of –decim and possibly –cassini calling songs may provide a record of the evolution of song distinctiveness from an ancestral call without a downslur: The downslur functions at least in part to mark the end of a call against a loud background chorus.

Male-male competition in *Magicicada* mating aggregations

The *Magicicada* mating system appears to be an extreme form of scramble polygyny, perhaps unusually extreme even for insects (see Chapter 2). The extreme density of sexually active males and the potency of the wing-flick signal likely means that newly-

receptive females are detected and engaged in latter stages of courtship almost immediately by one or more males, making wing-flick duets brief and difficult to separate from the din and activity of a natural chorus. In addition, unless a teneral female's readiness to mate is activated all at once, fully formed, a newly adult female may be expected to pass through a period of partial receptivity in which (1) she produces the wing-flick signal very weakly and/or inconsistently and (2) she is more likely to reject males as a result of fluctuations in her mating readiness. If so, males can be expected to have evolved to respond to very weak or intermittent wing-flicking in females. Such a period of partial or weak sexual receptivity could be involved in observations of lengthy courtships involving repeated rejections before eventual copulation and the apparent coyness of *Magicicada* females (Alexander 1968, Dunning et al. 1979; see Chapter 2). Subtlety of wing-flick responses in newly-matured females, in addition to rapid detection of signaling females by chorusing males, could explain why the *Magicicada* wing-flick signal has for so long remained undiscovered. Selection on males to detect the earliest manifestations of wing-flicking could explain apparently wasted courtship effort directed toward mated or teneral females; if the hypothesis is correct, these courtships will always turn out to have been caused by incidental female movements coincidentally timed at the end of a nearby male's call.

The intensely competitive environment of a *Magicicada* chorus places any courting male in jeopardy from interlopers. In -decim and perhaps -cassini , when a close-range male-female duet or a prolonged courtship is interrupted by the arrival of a calling (and potentially interloping) male competitor, the courting male emits short buzzes coincident with the downslurs of his rival's calls. These buzzes obscure the downslur of the rival's call, reduce the likelihood that the female will perceive and respond to them, and thereby increase the likelihood that the interloper will continue chorusing and depart without detecting the female. One potential objection to this hypothesis is that the buzz itself could reveal the presence of the nearby receptive female to the interloper. However, male *M. septendecim* and *M. cassini* hearing sensitivity is reduced 5-15 dB during calling by the

action of muscles that reduce tension on the tympana (Simmons et al. 1971), a phenomenon also described for other cicada species (e.g., Pringle 1954, Hennig et al. 1994). Thus calling interlopers may have difficulty perceiving that their signals are being jammed by buzzing males. Buzzing males terminate the signal precisely at the end of the interloper's song (Fig. 2), suggesting that males have evolved to produce the buzz only when it is undetectable by the potential interloper. The reduced hearing ability of calling cicadas and the specific timing of the buzz do not support an alternative hypothesis -- that the buzz is used by a male to deflect mistaken courtship attention from another male by revealing the sex of the courted individual.

Competitive acoustic interactions are well-documented in insects, but these commonly involve the calling song or a part of the calling song believed to serve only a male-male competitive function (e.g. Shaw 1968, Feaver 1983, Greenfield and Roizen 1993). Specialized male-male competitive signals such as the *Magicicada* interference buzz are rarer. While calling, some male katydids produce accessory ticks that are timed in relation to their own calls in the same manner as female responses, possibly to confuse potential interlopers (Grove 1959, Alexander 1975). A similar function could be served by male wing-clicking during calling in cicadas such as *Amphipsalta cingulata*, in which males click to their own calling songs with a timing identical to that of female wing-flick responses (Lane 1995). Male-male interaction sounds have also been reported in two other cicadas, *Fidicina mannifera* (Cocroft & Pogue 1996) and *Cicada barbara lusitanica* (Fonseca 1991), but the signals are not yet well understood. The *Magicicada* interference buzz appears unique in that the sound apparently deceives a rival male by preventing signals from the unwitting courted female.

Literature Cited

Alexander, R. D. 1957. Sound production and associated behavior in insects. Ohio J. Science 57: 101-113.

Alexander, R. D. 1968. Arthropods. Pp. 167-216 in: T. A. Sebeok (ed). Animal Communication. Techniques of Study and Results of Research. Indiana University Press, Bloomington, IN.

Alexander, R. D. 1975. Natural selection and specialized chorusing behavior in chorusing insects. Pp. 35-77 in: D. Pimentel (ed). Insects, Science, and Society. Academic Press, New York.

Alexander, R. D., and G. Borgia. 1979. On the origin and basis of the male-female phenomenon. Pp. 417-440 in: M. S. Blum and N. A. Blum (eds). Sexual selection and reproductive competition in insects. Academic Press, New York.

Alexander, R. D., and T. E. Moore. 1958. Studies on the acoustical behavior of seventeen-year cicadas (Homoptera: Cicadidae: *Magicicada*). Ohio J. Science 58: 107-127.

Alexander, R. D., and T. E. Moore. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species. (Homoptera: Cicadidae, *Magicicada*). Univ. Mich. Mus. Zool. Misc. Publ. 121.

Bradbury, J. W. 1981. The evolution of leks. Pp. 138-169 in: R. D. Alexander & D. W. Tinkle (eds). Natural selection and social behavior. Chiron Press, New York.

Bradbury, J. W. 1985. Contrasts between insects and vertebrates in the evolution of male display, female choice, and lek mating. Fortschr. Zool. 31: 273-289.

Claridge, M. F. 1985. Acoustic signals in the Homoptera: Behavior, Taxonomy, and Evolution. Ann. Rev. Entomol. 30: 297-317.

Cocroft, R. B., and M. Pogue. 1996. Social behavior and communication in the neotropical cicada *Fidicina mannifera* (Fabricius) (Homoptera: Cicadidae). J. Kansas Entomol. Soc. 69(4) suppl.: 85-97.

Cooley, J. R. 1999. Sexual behavior in North American cicadas of the genera *Magicicada* and *Okanagana*. PhD. Dissertation, The University of Michigan.

Cooley, J. R., G. S. Hammond, and D. C. Marshall. 1998. The effects of enamel paint marks on the behavior and survival of the periodical cicada, *Magicicada septendecim* (L.) (Homoptera) and the lesser migratory grasshopper, *Melanoplus sanguinipes* (F.) (Orthoptera). Great Lakes Entomologist 31: 161-168.

Cox, R. T., and C. E. Carlton. 1991. Evidence of genetic dominance of the 13-year life cycle in periodical cicadas (Homoptera: Cicadidae: *Magicicada* spp.). Am. Midl. Nat. 125: 63-74.

Davis, W.T. 1919. Cicadas of the genera *Okanagana, Tibicinoides*, and *Okanagodes*, with descriptions of several new species. J. N. Y. Entomol. Soc. 27: 179-223.

Doolan, J. M. 1981. Male spacing and the influence of female courtship behavior in the bladder cicada, *Cystosoma saundersii* Westwood. Behav. Ecol. Sociobiol. 9:269-276.

Doolan, J. M., and D. Young 1989. Relative importance of song parameters during flight phonotaxis and courtship in the bladder cicada *Cystosoma saundersii*. J. Exp. Biol. 141: 113-131.

Dugdale, J. S., and C. A. Fleming. 1969. Two New Zealand cicadas collected on Cook's Endeavour voyage, with a description of a new genus. New Zealand J. Sci. 12: 929-957.

Dunning, D. C., J. A. Byers, and C. D. Zanger. 1979. Courtship in two species of periodical cicadas, *Magicicada septendecim* and *Magicicada cassini*. Anim. Behav. 27: 1073-1090.

Dybas, H. S., and M. Lloyd. 1962. Isolation by habitat in two synchronized species of periodical cicadas (Homoptera: Cicadidae: *Magicicada*). Ecology 43: 444-459.

Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. Am. Nat. 139: s125-s153.

Endler, J. A. 1993. Some general comments on the evolution and design of animal communication systems. Phil. Trans. Roy. Soc. Lond. B. 340: 215-225.

Feaver, M. 1983. Pair formation in the katydid *Orchelimum nigripes* (Orthoptera: Tettigoniidae). Pp. 205-239 in: D. T. Gwynne and G. K. Morris (eds). Orthopteran mating systems: sexual selection in a diverse group of insects. Westview Press, Colorado.

Fonseca, P. J. 1991. Characteristics of the acoustic signals in nine species of cicadas (Homoptera: Cicadidae). Bioacoustics 3: 173-182.

Greenfield, M. D., and I. Roizen. 1993. Katydid synchronous chorusing is an evolutionarily stable outcome of female choice. Nature (London). 364: 618-620.

Grove, D. G. 1959. The natural history of the angular-winged katydid *Microcentrum rhombifolium* Ph.D. Dissertation, Cornell University.

Gwynne, D. T. 1987. Sex-biased predation and the risky mate-locating behavior of male tick-tock cicadas (Homoptera: Cicadidae). Anim. Behav. 35: 571-576.

Heller, K., and D. von Helversen. 1993. Calling behaviour in bushcrickets of the genus *Poecilimon* with differing communication systems (Orthoptera: Tettigonioidea, Phaneropteridae). J. Insect Behav. 6: 361-377.

Hennig, R. M., T. Weber, F. Huber, H. U. Kleindienst, T. E. Moore, and A. V. Popov. 1994. Auditory threshold change in singing cicadas. J. Exp. Biol. 187: 45-55.

Henry, C. S. 1994. Singing and cryptic speciation in insects. Trends Ecol. and Evol. 9: 388-392.

Huber, F., D. W. Wohlers, and T. E. Moore. 1980. Auditory nerve and interneurone responses to natural sounds in several species of cicadas. Physiol. Entomol. 5: 25-45.

Hunt, R. E., and L. R. Nault. 1991. Roles of interplant movement, acoustic communication, and phonotaxis in mate-location behavior of the leafhopper *Graminella nigrifrons*. Behav. Ecol. Sociobiol. 28: 315-320.

Hunt, R. E., J. P. Fox, and K. P. Haynes. 1992. Behavioral response of *Graminella nigrifrons* (Homoptera: Cicadellidae) to experimentally manipulated vibrational signals. J. Ins. Behav. 5: 1-13.

Karban, R. 1981. Effects of local density on fecundity and mating speed for periodical cicadas. Oecologia 51: 260-264.

Lane, D. H. 1995. The recognition concept of species applied in an analysis of putative hybridization in New Zealand cicadas of the genus *Kikihia* (Insecta: Hemiptera: Tibicinidae). Pp. 367-421 in: D. M. Lambert & H. G. Spencer (eds). Speciation and the Recognition Concept: Theory and Application. John Hopkins University Press.

Lloyd, J. E. 1966. Studies on the flash communication system in *Photinus* fireflies. Univ. Mich. Mus. Zool. Misc. Publ. 130.

Lloyd, J. E. 1979. Sexual selection in luminescent beetles. Pp. 293-342 in: M. S. Blum & N. A. Blum (eds). Sexual selection and reproductive competition in insects. Academic Press, New York.

Lloyd, M., and H. S. Dybas. 1966a. The periodical cicada problem. I. Population ecology. Evolution 20: 133-149.

Lloyd, M., and H. S. Dybas. 1966b. The periodical cicada problem. II. Evolution. Evolution 20: 466-505.

Lloyd, M., J. White, and N. Stanton. 1982. Dispersal of fungus-infected periodical cicadas to new habitat. Environ. Entomol. 11:852-858.

Long, C. A. 1993. Evolution in periodical cicadas: A genetical explanation. Evol. Theory 10: 209-220.

Maier, C. T. 1982. Observations on the seventeen-year periodical cicada, *Magicicada septendecim* (Hemiptera: Homoptera: Cicadidae). Ann. Entomol. Soc. Amer. 75: 14-23.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with a description of a new species, 13-year *Magicicada neotredecim*. Evolution in press (slated for August 2000).

Moore, T. E. 1968. The evolution of cicadas and their acoustical behavior. Thirteenth Internat. Congr. Entomol. Abstracts of Papers: 173 (Moscow).

Otte, D. 1974. Effects and functions in the evolution of signaling systems. Ann. Rev. Ecol. Syst. 5: 385-417.

Parker, G. A., 1979. Sexual selection and sexual conflict. Pp. 123- 167 in: M. S. Blum & N. A. Blum (eds). Sexual selection and reproductive competition in insects. Academic Press, New York.

Parker, G. A., 1983a. Mate quality and mating decisions. Pp. 141-166 in: P Bateson (ed). Mate Choice. Cambridge University Press, Cambridge.

Popov, A. V. 1981. Sound production and hearing in the cicada *Cicadetta sinuatipennis* Osh. (Homoptera: Cicadidae). J. Comp. Physiol. 142: 271-280.

Pringle, J. W. S. 1954. A physiological analysis of cicada song. J. Exp. Biol. 34: 525-560.

Shaw, K. C. 1968. An analysis of the phonoresponse of males of the true katydid *Pterophylla camellifolia* (F.) (Orthoptera: Tettigoniidae). Behaviour 31: 203-260.

Simon, C., J. Tang, S. Dalwadi, G. Staley, J. Deniega, and T. Unnasch. 2000. Genetic evidence for assortative mating between 13-year cicadas and sympatric "17-year cicadas with 13-year life cycles" provides support for allochronic speciation. Evolution in press (slated for August 2000).

Simmons, J. A., E. G. Wever, W. F. Strother, J. M. Pylka, and G. R. Long. 1971. Acoustic behavior of three sympatric species of 17-yr cicadas. J. Acoust. Soc. Amer. 49: 93.

Soper, R. 1974. The genus *Massospora* entomopathogenic for cicadas. Part I. Taxonomy of the genus. Mycotaxon 1: 13-40.

Soper, R., A. J. Delyzer, and L. F. R. Smith. 1976. The genus *Massospora* entomopathogenic for cicadas. Part II. Biology of *Massospora levispora* and its host *Okanagana rimosa*, with notes on *Massospora cicadina* on the periodical cicadas. Ann. Entomol. Soc. Amer. 69: 89-95.

Speare, A. T. 1921. *Massospora cicadina* Peck, a fungous parasite of the periodical cicada. Mycologia 13: 72-82.

Spooner, J. D. 1968. Pair-forming acoustic systems of Phaneropterine katydids (Orthoptera: Tettigoniidae). Anim. Behav. 16: 197-212.

Wells, M. M., and C. S. Henry. 1992. The role of courtship songs in reproductive isolation among populations of green lacewings of the genus *Chrysoperla* (Neuroptera: Chrysopidae). Evolution 46: 31-42.

White J., 1973. Viable hybrid young from crossmated periodical cicadas. Ecology 54: 573-580.

White, J., and M. Lloyd. 1983. A pathogenic fungus, *Massospora cicadina* Peck (Entomophthorales), in emerging nymphs of periodical cicadas (Homoptera: Cicadidae). Environmental Entomology 12: 1245-1252.

Williams, K. S., and C. Simon. 1995. The ecology, behavior, and evolution of periodical cicadas. Annu. Rev. Entomol. 40: 269-295.

Williams, K. S., K. G. Smith, and F. M. Stephen. 1993. Emergence of 13-yr periodical cicadas (Cicadidae: *Magicicada*): phenology, mortality, and predator satiation. Ecology 74: 1143-1152.

Yoshimura, J. 1997. The evolutionary origins of periodical cicadas during ice ages. Am. Nat. 149: 112-124.

Young, D. and R. K. Josephson. 1983. Pure-tone songs in cicadas with special reference to the genus *Magicicada*. Journal of Comparative Physiology 152: 197-207.

Table 1.1. The pair-forming sequence in *Magicicada* species; note that temporal characteristics vary with temperature.

	Chorusing			Courtship								
Species	Call Structure	Male Behavior	Female Signal	CI Call Structure	Male Behavior	Female Signal	CII Call Structure	Male Behaviors	Female Signal	CIII Call Structure	Male Behaviors	Female Signal
-decim	Roughly pure-tone, musical buzz terminating in a noticeable drop in pitch; ca. 2-4 sec.	Bouts of 1-3 calls most common, with ca. 1-1.5 sec. silent gap between calls; fly or walk between calling bouts	Wing-flick timed ca. 0.4 sec. after each call	Same as chorus call; sometimes shortened	While locating responding female, or during prolonged courtship	Wing-flick timed ca. 0.4 sec. after each call	Shortened, concatenated call phrases (variable in number) without intervening silent gaps	As male completes approach, and/or prior to mounting; foreleg vibration at transition to mounting.	Wing-flick timed at end of CII	Short (ca. 0.05 sec.) buzzes with frequency content similar to main part of call phrase; repeated at ca. 4-7 per sec. until genitalia engaged	Foreleg vibration, mount, engage genitalia	None
-cassini	Series of ticks followed by high-pitched, broad-spectrum buzz that rises and then falls in intensity and pitch; ca. 2.4 sec.	Bouts of 1-3 calls most common (1 call per bout when synchronized), with ca. 1-1.5 sec. silent gap between calls; fly or walk between calling bouts	Wing-flick timed ca. 0.7 sec. after each call	Same as chorus call; sometimes shortened	While locating responding female, or during prolonged courtship	Wing-flick timed ca. 0.7 sec. after each call	Shortened, concatenated "inverted" call phrases (ticks following the buzz) with shorter intervening silences	As male completes approach, and/or prior to mounting; foreleg vibration at transition to mounting.	Wing-flick timed at end of CII, rarely during CII	Short (ca. 0.05 sec.) two-part buzzes with frequency content similar to main part of call phrase; repeated at ca. 6-9 per sec. until genitalia engaged	Foreleg vibration, mount, engage genitalia	None
-decula	Ca. 20 rhythmic, high-pitched, broad-spectrum tick-buzz subphrases, followed by ca. 20 subphrases containing only ticks; ca. 7-14 sec.	Bouts of 1-3 calls (1 call most common), with ca. 1-2 sec. silent gap between calls; fly or walk between calling bouts	Wing-flick during brief silent intervals within call	Same as chorus call; sometimes shortened	While locating responding female, or during prolonged courtship	Wing-flick during brief silent intervals within call	None known	N/A	N/A	Short (ca. 0.05 sec.) buzzes with frequency content similar to main part of call phrase; repeated at ca. 6-9 per sec. until genitalia engaged	Foreleg vibration, mount, engage genitalia	None

Table 1.2. Study Sites, 1995-1999.

Year	Brood	Life Cycle	Location	County	State	Characteristics
1995	I	17	Alum Springs	Rockbridge	VA	Logged site
1996	II	17	Horsepen Lake SWMA	Buckingham	VA	Logged site
1997	III	17	Siloam Springs SP	Brown, Adams	IL	Old field
1998	XIX	13	Harold Alexander WMA	Sharp	AR	Powerline cut, slope
1999	V	17	Tar Hollow State Forest	Ross	OH	Recently cleared slope

Table 1.3. Mated and unmated female *M. septendecim* responses to a 2-minute playback of male calls. Females responding positively (+) produced at least one wing flick signal in response to playbacks of male songs, while nonresponding females (-) did not.

Day	Mating Status	n	Response (+)	(-)	*P* (Fisher Exact Test)
1	Mated	22	0	22	
	Unmated	17	9	8	≤ 0.001
2	Mated	22	0	22	
	Unmated	16	8	8	≤ 0.001
3	Mated	20	0	20	
	Unmated	15	7	8	≤ 0.001
4	Mated	18	0	18	
	Unmated	14	7	7	≤ 0.001

Table 1.4. Responses of individual male *M. septendecim* to simulated wing-flick signals produced at different times in relation to their calls. We scored males as responding positively if they moved toward the clicking device and began late-stage courtship behaviors such as CII or CIII call, foreleg-vibration, or mounting behavior. We used Fisher's Exact two-tailed tests to compare treatments to 46 controls in which the clicking device was presented to the male, but no click was made.

		Response		
Timing	n	(+)	(-)	*P* (vs. Control)
Control	46	6	40	
End of call	83	66	17	≤ 0.001
During call	41	3	38	≤ 0.498
During slur	13	0	13	≤ 0.326

Table 1.5. Effect of single nearby (25 cm) or distant (1.3m) artificial wing-flick signal on male chorusing behavior. Direction of movement was recorded as a value from 1-12 as on a clock face with the observer at 12. In the analysis of movement direction, only males that moved in directions 11, 12, 1 (toward stimulus) or 4, 5, 6 (away from stimulus) were considered, to avoid biased interpretation of ambiguous lateral movements. Males that paused for longer than 20 seconds were not monitored further.

Single nearby simulated wing-flick signal

	Control	With signal	*P*
Call Number	2.28±1.16 (n=43)	3.70±2.46 (n=53)	Z= 3.247, ≤ 0.001 (Wilcoxon)
Likelihood of flight	(n=43)	(n=53)	
Fly after signal	36	38	
Do not fly after signal	7	15	*P* ≤ 0.223 (Fisher's Exact Test)
Distance of flight (cm)	25.75±19.4 (n=40)	22.60±29.8 (n=47)	Z= -0.926, *P*≤ 0.355 (Wilcoxon)
Movement direction	(n= 19)	(n=20)	
Toward Observer	3	13	
Away from Observer	16	7	*P* ≤ 0.003 (Fisher's Exact Test)

Single distant simulated wing-flick signal

	Control	With signal	*P*
Call Number	1.94±0.83 (n=17)	3.53±1.26 (n=19)	Z= 2.838, *P* ≤ 0.005 (Wilcoxon)
Likelihood of flight	(n=17)	(n=19)	
Fly after signal	13	14	
Do not fly after signal	4	5	*P* ≤ 1.000 (Fisher's Exact Test)
Distance of flight (cm)	35.76±26.55 (n=13)	20.98±15.14 (n=15)	Z= -1.225, *P* ≤ 0.221 (Wilcoxon)
Movement direction	(n=7)	(n=6)	
Toward Observer	0	4	
Away from Observer	7	2	*P* ≤ 0.02 (Fisher's Exact Test)

Table 1.6. Changes in male *M. septendecim* and *M. cassini* chorusing behavior in response to simulated wing-flick sounds produced after every call by a motionless mechanical relay held 10 cm away during each of two call bouts. In controls males were approached but no click sounds were made. Results were analyzed with Kruskal-Wallis one-way analysis of variance.

M. septendecim	n	mean	
Number of calls, first bout			
With click	64	3.3±2.1	
Control	46	3.0±2.5	H_{11} =1298.0, $P \leq 0.277$
Distance, first flight (cm)			
With click	50	25.2±25.3	
Control	37	24.8±17.0	H_{11} =990.5, $P \leq 0.573$
Number of calls, second bout			
With click	45	2.7±1.8	
Control	34	2.2±0.84	H_{11} =729.0, $P \leq 0.712$
Distance, second flight (cm)			
With click	39	30.0±27.4	
Control	32	37.0±28.5	H_{11} =744.0, $P \leq 0.165$

M. cassini	n	mean	
Number of calls, first bout			
With click	24	1.4±0.93	
Control	26	1.3±0.45	H_{11} =311.0, $P \leq 0.980$
Distance, first flight (cm)			
With click	23	7.8±5.6	
Control	25	31.0±33.9	H_{11} =458.5, $P \leq 0.001$*
Number of calls, second bout			
With click	18	1.3±0.69	
Control	22	1.5±0.80	H_{11} =223.0, $P \leq 0.399$
Distance, second flight (cm)			
With click	15	8.7±6.4	
Control	19	44.7±57.6	H_{11} =237.0, $P \leq 0.001$*

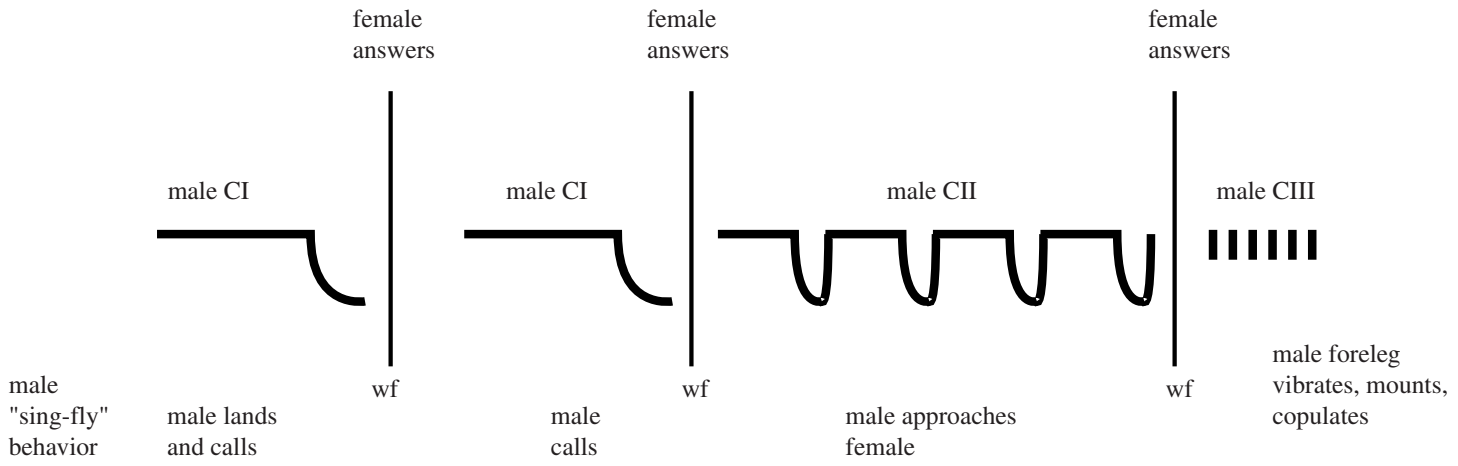
Table 1.7. Male *M. tredecim* courtship behaviors directed toward black- or white-colored models moved to simulate wing-flick responses without sound, or held still (controls). Model was held 25 cm away initially. Male responses, including call-walk towards, CII call, CIII call, and foreleg-vibration were compared using Fisher's Exact two-tailed tests.

Contrast	Call-Walk Towards	Court II Call	Court III Call	Foreleg Vibration
Black/move vs. black/still	15/22 vs. 3/22 ($P \leq 0.001$)*	10/22 vs. 1/22 ($P \leq 0.004$)*	4/22 vs. 0/22 ($P \leq 0.108$)	10/22 vs. 2/22 ($P \leq 0.001$)*
Black/move vs. white/move	15/22 vs. 9/22 ($P \leq 0.129$)	10/22 vs. 3/22 ($P \leq 0.045$)*	4/22 vs. 1/21 ($P \leq 0.345$)	10/22 vs. 0/22 ($P \leq 0.001$)*
White/move vs. white/still	9/22 vs. 0/21 ($P \leq 0.001$)*	3/22 vs. 0/21 ($P \leq 0.233$)	1/21 vs. 0/21 ($P \leq 1.00$)	0/22 vs. 0/21 ($P \leq 1.00$)
Black/still vs. white/still	3/22 vs. 0/21 ($P \leq 0.233$)	1/22 vs. 0/21 ($P \leq 1.00$)	0/22 vs. 0/21 ($P \leq 1.00$)	2/22 vs. 0/21 ($P \leq 1.00$)

Table 1.8. Effects of -decim "interference buzz." We confined four unmated female *M. septendecim* in a test chamber and played a sequence of 60 calls, alternating normal calls and calls with buzzes, recording the number of females responding to each call, and comparing the responses using Friedman two-way analyses of variance. We repeated the experiment six times.

Replicate	Buzz	Average number of females responding to each call (mean ± SD)	*P*
A	Yes	1.13 ± 0.86	
	No	2.23 ± 0.77	F=12.033, $P \leq 0.001$
B	Yes	0.57 ± 0.57	
	No	2.43 ± 0.68	F= 26.133, $P \leq 0.001$
C	Yes	0.80 ± 1.00	
	No	1.73 ± 1.08	F= 10.800, $P \leq 0.001$
D	Yes	1.27 ± 1.93	
	No	1.93 ± 0.58	F= 7.500, $P \leq 0.006$
E	Yes	0.93 ± 0.87	
	No	1.53 ± 0.78	F= 4.033, $P \leq 0.045$
F	Yes	0.47 ± 0.57	
	No	2.07 ± 0.98	F= 17.633, $P \leq 0.001$

Figure 1.1. Stylized sonogram of -decim male call/female wing flick courtship duet. Court I (CI) calls are produced as the male attempts to locate a responding female, or during prolonged courtship; if responsive, the female answers each call with a wing flick. Upon reaching the female, or shortly before attempting to mount, the male begins court II (CII) calling, which consists of repeated phrases of the same general type as CI calling, but shortened and without intervening silence. The female does not respond during CII. As he begins to mount, the male begins court III (C III) calling, which consists of repeated short buzzes. -Cassini and -decula courtship sequences are similar, except that -decula lack clearly defined CII calls.



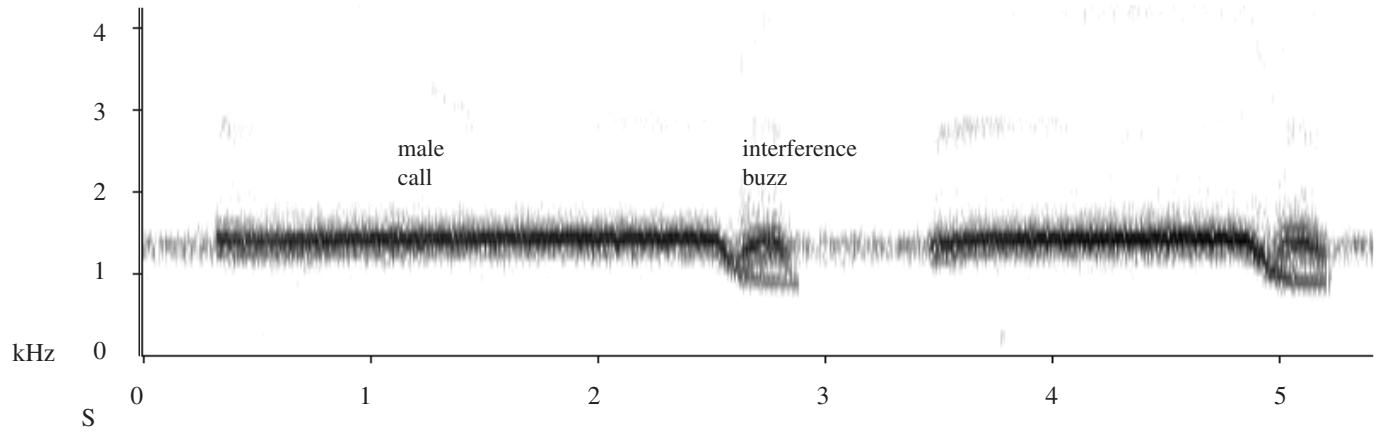


Figure 1.2. Sonogram of male -decim call with interference buzz of nearby male. Interference buzz overlaps calling male's terminal downslur.

Figure 1.3. Sonogram of male -decim call phrase (A), -cassini call phrase (B), and fragment from middle of -decula call (C), each with female wing flick response. Female response (marked with asterisk) produces a broad-frequency sound. Wing flick sounds are enhanced for clarity; note that -decim background chorus noise is visible on -decim and -decula sonograms.

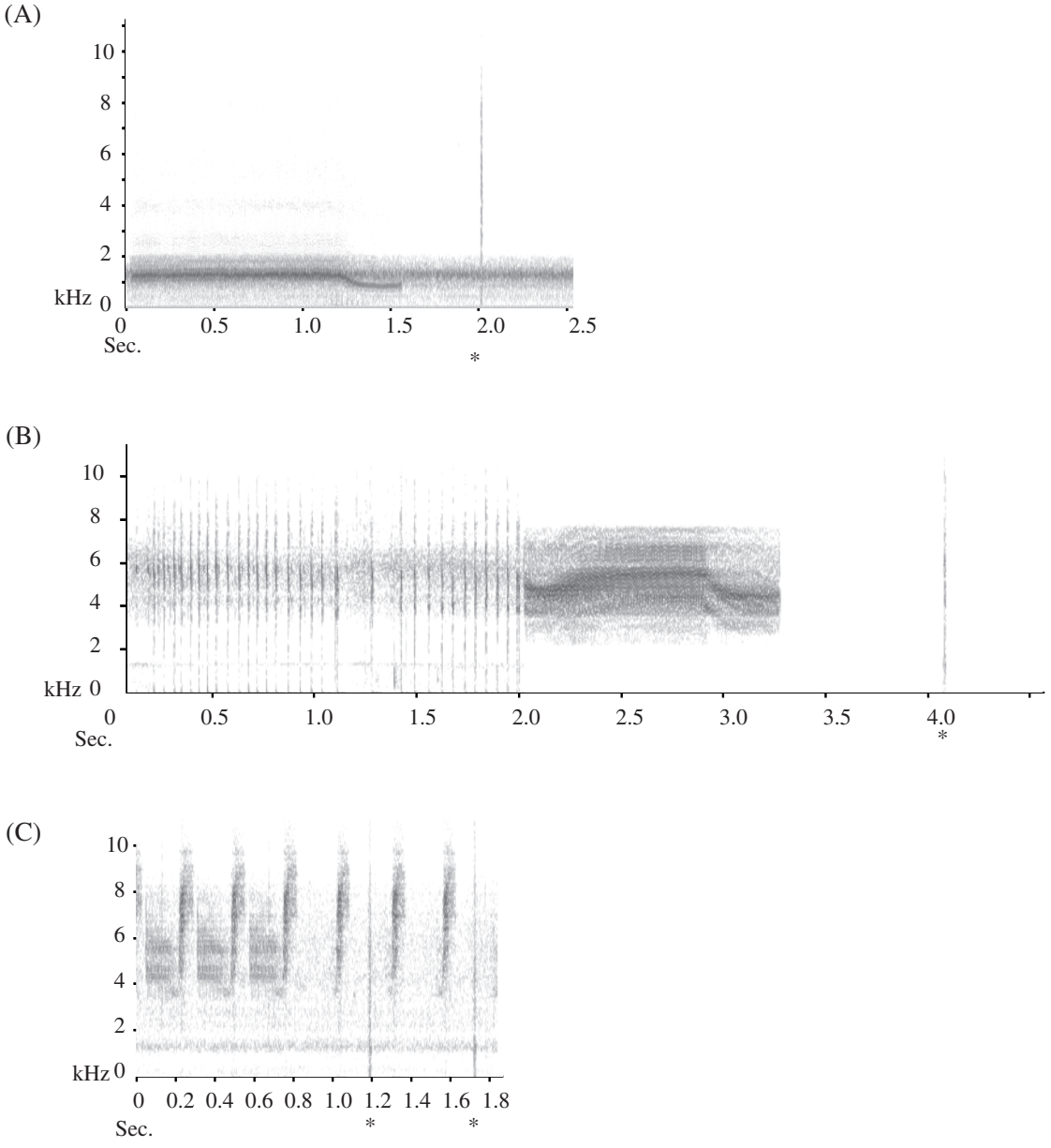


Figure 1.4. Male responses to actions of model. Males were scored as responding positively if they produced Court II or Court III courtship songs, or if they attempted to mount and copulate with the model. Positive responses marked with shading, negative responses unshaded. Within *M. septendecim*, the model that moved and clicked was more effective than the model that clicked only (P 0.001, Fisher's Exact two-tailed test) or the model that moved only (P 0.001, Fisher's Exact two-tailed test). For *M. cassini*, the results were similar; the model that moved and clicked was more effective than the model that clicked only (P 0.001, Fisher's Exact two-tailed test) or the model that moved only (P 0.001, Fisher's Exact two-tailed test). Responses to click only and move only treatements did not differ in either species.

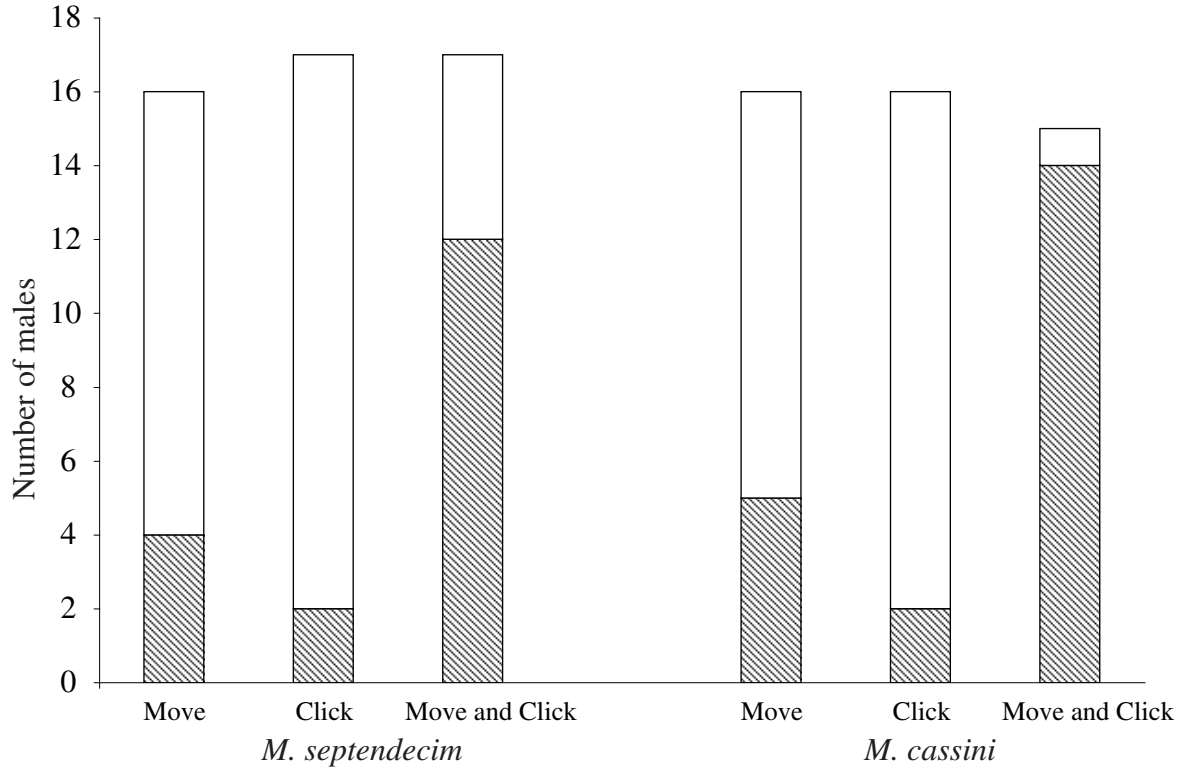
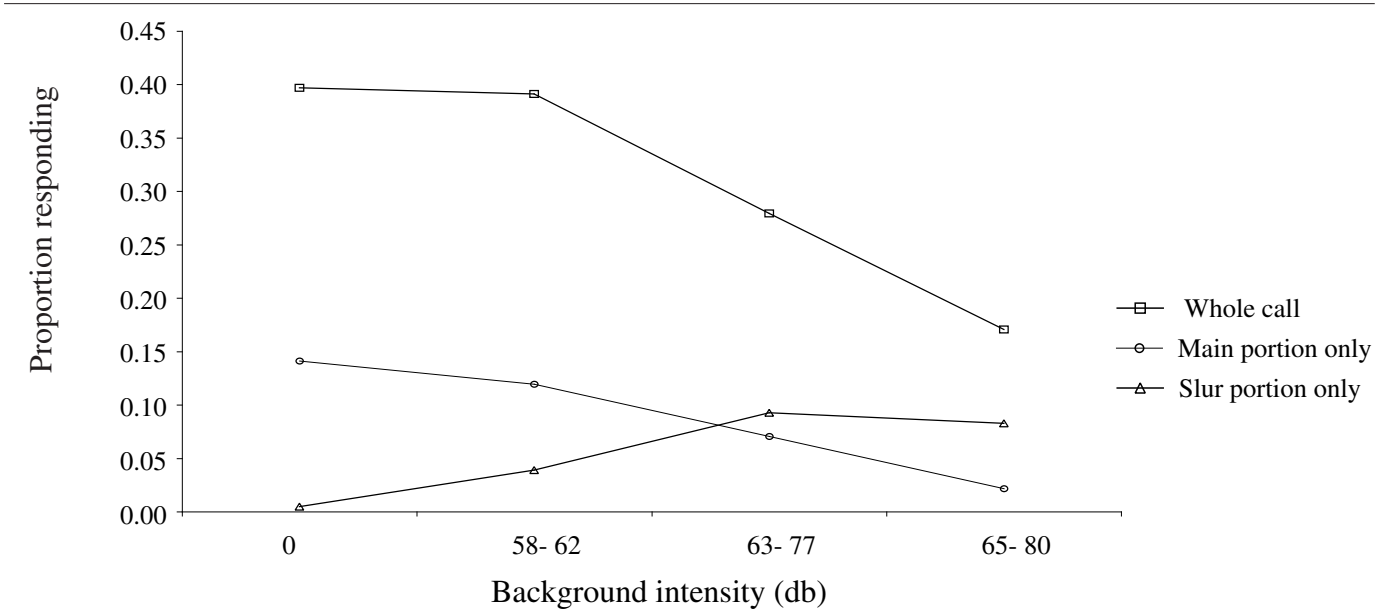


Figure 1.5. Responses of female *M. septendecim* to playbacks of artificial, pure tone calls and to portions of calls against artificial background choruses of different intensities. Females were scored as responding positively if they produced one or more wing flick signals in response to a playback. Data presented as proportion of positive responses for specific call and background condition given the total number of trials with those experimental conditions.

Contrast (Fisher's Exact two-tailed tests)	0 db	58-62 db	63-77 db	65-80 db
Whole vs. Slur	0.001	0.001	0.001	0.005
Whole vs. Main	0.001	0.001	0.001	0.001
Main vs. Slur	0.001	0.004	0.465	0.012

At all background intensities, whole calls (square markers) were more likely to elicit responses than either main portion only (circles) or slur portion only (triangles). At higher background intensities, the effectiveness of whole calls was reduced (Kruskal-Wallis One Way ANOVA $Z = 420$, P 0.001), as was the effectiveness of main portion only (Kruskal-Wallis One Way ANOVA $Z = 64$, P 0.001), while the effectiveness of slurs alone was increased (Kruskal-Wallis One Way ANOVA $Z = 44$, P 0.001).



CHAPTER 2

MATING BEHAVIOR AND MATE CHOICE IN THE PERIODICAL CICADA *MAGICICADA SEPTENDECIM*

Abstract

Periodical cicada mating aggregations meet the criteria for a lek mating system, but female mating behavior and the pattern of male mating success in *Magicicada septendecim* depart strongly from the characteristics of classical bird and mammal leks. Females mate promptly upon completing the post-ecdysis teneral phase, and most females mate only once. Variance of male mating success in a flight cage population was slightly but not significantly greater than that expected if all males have an equal probability of mating; variation of mating success among mated males was no more skewed than expected at random. Males showed no tendency to mate in the same order when the same group of males was mated twice to the same group of females. However, female mating order was significantly repeatable, perhaps indicating differences in vigor due to variation in storage cage effects. These results suggest that females employ threshold mate choice mechanisms, and that most males meet the minimal criteria for mating. Observed female "coyness" and a high failure rate of courtships result in large part from mistaken courtships

by males of mated, teneral, or heterospecific females. *Magicicada* mating aggregations are principally characterized by intense scramble competition among males for rare unmated, mature females.

Introduction

Adults of a wide variety of organisms form dense aggregations or "leks" on which most mating occurs (see Höglund and Alatalo 1995). Males of many such species exhibit extremes of sexual ornamentation or courtship behavior, while females appear to exercise potent mate choice leading to high variance in male mating success (mating skew). Female choosiness on leks, however, seems unlikely to be explained by direct or immediate benefits of mate choice, which increase a female's fecundity or the potential investment in her offspring, because males of such species appear to offer little more than gametes. Thus, lek mating systems have attracted much attention in the study of the evolution of more controversial evolutionary hypotheses of female choice based on indirect or "genetic" benefits, in which gains to female reproduction derive from the expression in the offspring of desirable genotypic elements from the father (Bradbury and Gibson 1983; Kirkpatrick and Ryan 1991).

Much of the theoretical development of the lek concept and the theory of mate choice on leks has involved studies of long-lived, iteroparous organisms such as mammals and birds (e.g. Snow 1963, Kruijt and Hogan 1967, Clutton-Brock et al. 1989, Wiley 1991; see also Andersson 1994, Höglund and Alatalo 1995). Leks or lek-like mating systems also exist in invertebrates, however, and some attempts have been made to compare the leks of these diverse organisms in light of the substantial life history differences between the groups (e.g. Bradbury 1985, Shelley and Whittier 1997). Alexander et al. (1997) argued that the life history differences of insects and vertebrates predict important differences in the development of mating criteria (see also Alexander 1975): With their long

lives, overlapping generations, iteroparity, and sometimes extended parental care, many birds and mammals experience extended social contact with parents or other adults during development, and thus may evolve to develop or modify mate criteria adaptively during juvenile life on the basis of these interactions; this may lead to evolution of additional learned adjustment of mate criteria based on comparison of prospective mates as an adult. Such "best of N" mate choice (Janetos 1980) can facilitate strong sexual selection if many mates are evaluated prior to copulation. In contrast, most insects live short lives with nonoverlapping generations and thus are more likely to develop the set of minimal mate criteria in the absence of contact with adult conspecifics. Insects are consequently less predisposed to evolve adaptive adjustment of mate criteria during adult life, and more likely to exercise Janetos' (1980) "threshold" mate choice. Threshold mate choice mechanisms can effect the substantial variance in male mating success that characterizes lek mating only under more restrictive conditions.

Short adult life spans in insects may be maintained in part by a high-risk environment that makes delays costly. Together, the shortness of life, semelparity, and increased significance of predation suggest a second general difference between insects and vertebrates of relevance to sexual selection: Direct costs associated with mating behavior will weigh more heavily on average in the economics of insect life histories than in comparable decisions of longer-lived bird and mammal species. In all organisms, additional time spent comparing mates, or in remating, brings additional risk of death or other impairment prior to oviposition or weaning. The greater significance of such costs for insects means that potential gains from increased female choosiness will have to be greater in order for such behavior to be favored by selection. In other words, indirect selection favoring increased mate choice (as a result of increased reproductive success of offspring) will more often be outweighed by direct selection against the behavior (resulting from lower parental fecundity or survival) (Kirkpatrick 1985, 1996; Kirkpatrick and Ryan 1991).

Candidate lek mating systems among insects therefore merit special scrutiny, because mating aggregations in different groups may have distinct underlying causes reflecting underlying life history differences. Female choice for indirect gains may be restricted to insect species for whom direct costs of mating are comparatively low, as when large numbers of potential mates are readily comparable and when predator risks are especially low.

The mating behavior of periodical cicadas (*Magicicada* spp.) meets the criteria for a lek mating system (Table 1) and is characterized by features superficially suggesting female choice for indirect, genetic gains. Adult males, who offer only sperm to their mates, alternate bouts of singing with short flights that attract both females and other males. Females fly into these aggregations and remain motionless; when receptive to mating they respond to individual calling males with timed wing-flicks (Chapter 1). Previous observations (Dunning et al. 1979) have indicated that courtship interactions may last for hours and usually do not lead to mating, suggesting coyness on the part of females. In addition, courtship in *Magicicada*, while highly stereotyped, is the most complex known for any acoustical insect, and includes two distinct courtship sounds (CII, CIII) produced in addition to the calling song (CI) and other nonacoustic courtship behaviors (including a male "foreleg vibration" that occurs prior to mounting of the female) (Alexander 1968, Alexander and Moore 1962, Dunning et al. 1979; see also Chapter 1). While the elements of courtship and the nature of the acoustical signals have been known for decades, no observations have been made of patterns of mating, including the degree of male mating skew, within *Magicicada* mating aggregations.

Female choice in *Magicicada* may be especially profitable for study because of the possibility that direct costs of mating are alleviated to an unusual extent for insects. Individuals in *Magicicada* populations are reproductively synchronized with extraordinarily dense adult populations ranging from 8,355 (Maier 1982a) to 3,700,000 per hectare (Dybas and Davis 1962). Consistently high adult population density appears to reduce the

risk of predation for individual cicadas (Lloyd and Dybas 1966a, Karban 1982, Williams et al. 1993), and results in continuous availability of large numbers of adult males. In these ways periodical cicada life history appears unusually well-suited among insect life histories for the evolution of female choice by indirect selection.

In the research described here, the nature of female choice in *Magicicada* was investigated by a combination of observational and experimental approaches. First, observations of captive cicadas were conducted to determine the phenology of female mating behavior and the likelihood of female remating. Second, captive mated and unmated captive females were observed to determine the effect of mating and oviposition on life length. Third, the extent of male mating skew and the likely mechanism of female mate choice (threshold, best-of-N) were investigated in two types of mating experiment. The latter two experiments also afforded separate estimates of the likelihood of female remating.

Because *Magicicada* females appear to control the onset of mating, female mating preferences should result in a distribution of matings among males that is more skewed than expected if each male has an equal probability of mating with any given female. Furthermore, if female preferences take the form of Janetos' (1980) "threshold mate choice", then the distribution of matings among *mated* males should be random. Finally, if females exercise choice using a "best-of N" mechanism, mating success among mated males should also be skewed more than expected at random.

Materials and Methods

General methods

Study locations and dates -- Most of the experiments and observations described here were conducted from 1995-1997 in the emergences of 17-year Broods I-III.

Brood I was studied in 1995 at the Alum Springs Youth Camp in Lexington Co., VA. Brood II cicadas were studied in 1996 at the Horsepen Lake Wildlife Management Area, Buckingham Co., VA. In 1997, Brood III cicadas were studied at Siloam Springs State Park, Brown Co., IL. One additional experiment was performed in 1998 using Brood XIX *Magicicada tredecim* at the Harold G. Alexander Wildlife Management Area, Sharp Co., AR.

Collection and storage of cicadas -- Although mated female *Magicicada* usually possess a copulatory "plug" of solidified seminal fluid (White 1973, Cooley 1999), this plug is sometimes absent, and individual cicadas cannot be aged. To be certain that the female cicadas in all experiments were of known age and mated status, female *M. septendecim* were collected from low grass and shrubs the morning after their emergence from the ground; such "teneral" cicadas are easily recognized by their soft, dull exoskeletons and yellow ovipositors. Teneral females were stored in ca. 50 liter cages formed by wrapping flexible screen material around a tree branch, which allowed sunlight to penetrate and provided the cicadas with appropriately sized twigs for feeding on xylem fluids. Ages of females are given below as days since emergence; the morning following the evening of emergence begins "Day 1".

For some of the procedures below, males were collected and stored in the same manner as the teneral females. However, adult males tend to be more active in storage cages, with the result that males that have been stored past the teneral period tend to become weakened. In general, when adult males were needed they were collected opportunistically while observed singing and flying in the ambient chorus; only adult males show this "chorusing" behavior (Maier 1982b).

Experimental cages -- Three types of cages were used in these studies. First. "storage cages" (described above) were used for long-term storage of unmated females

collected as tenerals. "Arena cages" were constructed of the same flexible Fiberglas material as storage cages but were larger (ca. 150 liter and 1.4 meters tall) and placed vertically over an entire deciduous stump sprout or small sapling. Finally, when a larger arena was desired a large "flight cage" was used, consisting of a ca. 3 meter (width) x 3 meter (width) x 2.5 meter (height) commercial outdoor screen tent with the originally opaque ceiling removed and replaced by screening material. Flight cages were placed over two or three tall saplings or stump sprouts at once. All cages were placed in the sunlight, which could penetrate the screening material.

Statistical analysis **--** All statistical analyses were conducted using Systat Version 5.0 (Macintosh).

Experimental and observational procedures

Female remating tendencies can be difficult to estimate, especially because of the potential that disturbance, handling effects, or other treatment effects may alter female behavior from its natural state. Therefore, mating behavior was investigated using a variety of approaches with complementary biases; the actual incidence of female remating may be expected to lie somewhere within the range observed under these methods.

Experiment A: Effect of mating and age on recruitment of chorusing males -- Several techniques were used to determine the relationship of age and mated status on female mating receptivity. In 1996, four arena cages (A, B, C, E) were set up in a cut-over field 8 m from a woods edge in which *M. septendecim* males were actively chorusing; males could be observed singing and flying in the regenerated stump sprouts surrounding the cages. One additional cage (D) was placed in another location approximately 50 m from the nearest chorus; although the ambient chorus was much

quieter at Cage D, males were observed flying on the surrounding vegetation. On the morning of May 20, thirteen Day-10 *M. septendecim* females that had completed a normal mating on the previous day as part of another experiment were placed in cage A. Thirteen Day 1 *M. septendecim* females, immature and unmated, were placed into cage B. Cages C and D were each filled with thirteen unmated *M. septendecim* females of the same age as those in Cage A. Cage E contained no cicadas. From 1:00-5:15 PM, and continuing from 9:00 AM - 12:00 P.M. the following day, the exteriors of the cages were scanned approximately every half-hour for male *M. septendecim*; this resulted in a total of 17 scans. Cage D was not scanned after 10:30 the following day, for a total of 12 scans. In each scan the males present were counted, and courtship behaviors were noted, including wing-flicking by the caged females, production of courtship songs by the males, or "male trains" that sometimes form when several males in close proximity mistakenly court one another. After each scan all males were collected and released ten meters to the north of each cage (away from the woods edge). No females were observed on the exteriors of the cages, although on two occasions a perched cicada flew before its sex could be determined.

Experiment B: Duration of remating resistance -- Experiment A judged female remating tendency by examining recruitment of sexually active males to female cages for two consecutive days after mating. A second experiment was completed in 1997 to judge female remating tendency using playbacks of recorded male calling song, over a longer time period. As part of a separate experiment (described below as Experiment F), four cohorts of individually marked *M. septendecim* females were allowed to mate once in arena cages. Cohorts A, B, and C each contained 16 females that emerged on 30/31 May, 1 June, and 3 June, respectively; cohort D contained 12 females that emerged on 7 June. The caged cohorts were mated to conspecific males on 12 June (A), 13 June (B), 15 June (C), and 18 June (D) as part of Experiment F; in each case the males were removed the following day. Beginning on 15 June, on each day with weather appropriate for cicada

activity (15, 16, 17, 19, 20, 21, and 22 June), a recorded male *M. septendecim* calling song phrase was played repeatedly for one minute at an intensity of ca. 75 dB (at 25 cm) just outside each of the arena cages from a 3" Radio Shack midrange speaker connected to a SONY Professional Walkman. The male song was then played in the same manner to unmated females of the same age, which were kept in nearby storage cages. Females were watched for timed wing-flick responses.

Experiment C: Observations of individual pair-formation and courtship sequences **--** From 14-24 May, 1996, individual *M. septendecim* males captured while chorusing in the surrounding vegetation were released one at a time into a flight cage population of individually marked, unmated females of varying ages (Table 2). All data were recorded during these individual "introductions". As each male began to call, an observer recorded (by speaking into a videotape microphone) the identities of any females observed wing-flicking to the male's calls and the approximate distances (judged by eye) of those females from the calling male. Observed female wing-flick responses to males landing on the outside of the cage were noted and included in the study as well. Although all parts of the cage could be observed from any given position, some females were always obscured by vegetation; however, this was not expected to bias the outcome toward any given age-class of females. In addition, in some situations too many females responded at once for all to be noted. As the male began to localize and approach a particular female, the courtship was videotaped until either (1) the pair began copulation or (2) the pair ceased activity for more than one minute.

Some information about the mode of female mate choice may be inferred from the phenology of wing-flicking and mating behaviors. If females always choose "best-of-N", then a given female should never mate with the first male that courts her, and many males should fail to copulate on their first approach to a female. Also, if females require

experience with many courting males prior to mating, as might be expected with "best-of-N" mate choice, then wing-flicking behavior should appear earlier in life than mating.

Experiment D: Effect of mating on female senescence rate -- Senescence is apparently triggered by reproduction in some semelparous organisms (e.g. salmon). Mature, unmated *M. septendecim*, *M. cassini*, *M. tredecim*, and *M. tredecassini* females begin ovipositing a few days after becoming sexually receptive whether mating has occurred or not (DCM and JRC unpublished obs.). This suggests that *Magicicada* females do not usually suffer an appreciable risk of failing to mate promptly, and raises the question of whether failure to mate delays the onset of senescence in *Magicicada*. If not, this would increase the costliness of delays associated with mate choice. In 1998, the following experiment was conducted to determine if mated status affects life length in -decim[1] females: 90 unmated females (presumed to be 13-year *M. tredecim*, see below) of the same post-emergence age were divided into three treatment groups and marked accordingly. Group A females were mated on 17-18 May, 5-6 days after emergence; Group B was mated on 22 May, 10 days after emergence, and Group C individuals were kept unmated for the duration of the experiment. The 30 cicadas of the three groups were distributed evenly among six arena cages for the duration of the experiment, except that individuals of Group A and Group B were kept in separate cages containing males during their respective mating treatments. Each day, dead individuals were located and removed and their identities recorded.

Prior to 1998, only two -decim siblings were recognized (*M. septendecim* and *M. tredecim*), and no differences were known between them other than life cycle length and geography; because of this it was assumed that the results of the experiment could be

[1] The seven *Magicicada* species fall into three sibling groups that share strong similarities in morphology, ecology, and behavior: -decim (17-year *M. septendecim*, 13-year *M. neotredecim*, 13-year *M. tredecim*); -cassini (17-year *M. cassini*, 13-year *M. tredecassini*); and -decula (17-year *M. septendecula*, 13-year *M. tredecula*).

applied to 17-year *M. septendecim*. After this experiment was begun, a new 13-year -decim species was discovered at the study site (*M. neotredecim*; Marshall and Cooley 2000). It is likely that individuals from both 13-year -decim species were used in the senescence experiment; however, given the observed proportions of the species at the site (Chapter 3), a large majority (ca. 85%) of the females used are likely to have been *M. neotredecim*. Because the new species appears to be derived recently from *M. septendecim* (Chapter 3, Simon et al. 2000), the results of the experiment may still generalize to the 17-year species.

Patterns of male mating success and mechanisms of female choice

In this study, female choice was investigated using a pattern-based approach, rather than by seeking phenotypic correlates of male mating success. Two methods were used here: (1) observation of mating success in a large undisturbed population of individually marked *M. septendecim* males and females in a flight cage (Experiment E), and (2) observation of repeatability of male mating order in small groups of male *M. septendecim* mated to females, immediately separated, and remated on the following day (Experiment F).

Experiment E: Patterns of mating and courtship in a large caged population -- In 1995, 87 males and 119 female *M. septendecim* were individually marked and placed in a flight cage according to the schedule shown in Table 3. A small number of male and female *M. cassini* were included in the cage for casual observation but are not discussed further in this paper. Beginning with the onset of adult activity in the cage (as indicated by male singing and flying) on 23 May, and continuing until 3 June, the population was scanned visually for mating pairs continuously during hours of peak activity (ca. 10 AM - 2 PM) and approximately every 15-30 minutes for the remainder of

the day (beginning about 8:00 AM and continuing until about 7 PM); the identities of these individuals were noted along with the current time. In addition, the identities of courting individuals and their positions with respect to one another were also noted; individuals were judged to be courting when a male and female were found less than 5 cm apart with the male oriented toward the female. Courtships accompanied by acoustic courtship signals (CII, CIII), male "foreleg vibration" behavior, male mounting attempts, or eventual mating were recorded as "unambiguous" courtships. Because copulation takes 270 minutes on average (Cooley 1999), no complete copulation bouts should have escaped notice. At the end of each day, the identities of dead individuals were noted and these were removed from the cage.

In order to determine the variance in male mating success expected under random mating (i.e. equal probability of mating across males), a computer program (Appendix A) was designed in Think Pascal 4.0 (Macintosh) to simulate random mating in a model population of the same demographics and mating frequency as the natural cage population. Two modifications of this program were used, one to simulate random mating across the entire male sample, and one to simulate random mating across only mated males. In all simulations, all males older than 5 days post-emergence were considered adult, males were assumed able to mate only once in a day (an assumption violated only once by one male during the study), and mated males become available for remating the following day. A single simulation consisted of a number of rounds corresponding to the number of days in the field experiment. In each round, a number of males were drawn at random without replacement from the list of available males, with the number of drawings corresponding to the number of matings that occurred in the corresponding day of the field trial. Each male drawn had a value of 1 added to its running total of "copulations" for the simulation. After the completion of a each simulation, the program calculated the variance in the total number of "copulations" across males, stored the value, reset the copulation totals for each male to zero, and began again. The program repeated the simulation 10,000 times, and generated a

cumulative frequency distribution of the resulting variance values. By comparing the observed variance in mating success among the 1995 flight cage males to this distribution, one can determine whether the hypothesis of random mating can be rejected.

Accumulating deaths of mated males will tend to affect the final variance in male mating success in a population. Therefore, the program was designed to draw, at random from the model population in each round, a number of males matching the number of additional deaths that had occurred up the corresponding day in the 1995 population, and mark these males as "dead"; males so marked were not assigned any additional matings.

Experiment F: Repeatability of male mating rank -- If a male and female are pulled apart within minutes after copulation begins, no apparent damage is caused and both individuals will remate if given the opportunity; female tendency to mate does not appear to be affected by such aborted copulations (Cooley 1999). If females prefer some males over others and exercise at least partial control over the initiation of mating, the order of male mating should not be random when the same male and female groups are used twice.

In 1996, the repeatability of male mating order was investigated using the following protocol: (1) Nine chorusing males were captured from the surrounding trees on the morning of a trial and marked individually; (2) Between 10:30 AM and 12:30 PM, under weather conditions appropriate for mating, these males were placed into an arena cage with nine individually marked, unmated Day 5 or Day 6 females; (3) As individual male-female copulating pairs formed, they were immediately removed from the cage, separated, and placed in single-sex holding cages; the identities of the cicadas and the time of mating were recorded; (4) On the following day, the process was repeated with the same males and females; (5) Each male was assigned a first-day rank and a second-day rank reflecting his position in the mating order on the two different days of a complete trial. If the order of two or more matings could not be determined (because both began while the observer was temporarily absent) they were assigned an appropriate tied rank; unmated males were also

assigned a tied rank reflecting their position at the bottom of the hierarchy. This process was repeated for a total of four replicates, referred to here as Trials 1A-1D. At 5 PM of each trial, the remaining unmated individuals were removed and stored in the single-sex holding cages.

In 1997, a different version of the mating order experiment was conducted, using one larger group of (at first) 20 individually marked males. This group of males was mated on three separate days (10, 13, and 15 June) to different groups of females each day. As in Trials 1A-1D, the order of male mating was recorded, but in this trial all pairs were allowed to complete copulation normally. Mated females were moved to other arena cages and monitored as part of Experiment B. Sixteen of the 20 males (fifteen that mated and one that did not) were used in the second mating bout (Trial 2B), which was completed from 13 June to 14 June; these same sixteen males were used in the third bout (Trial 2C) on 15 June.

Computer-generated model simulations (Appendix B) were used to determine if male mating order occurred with greater repeatability than expected at random. For each trial, two simulation statistics were measured to quantify the repeatability of male rank. The first statistic, FHLH (First-Half, Last-Half), quantified the degree to which individuals tended to mate in the same half of the rankings in both parts of a trial; to calculate FHLH, the summed first-day ranks of the last four males to mate on the second day were subtracted from the summed first-day ranks of the first four males to mate on the second day. On average, if first-day mating order does not predict second-day mating order, FHLH should be zero. If early-mating males tend to retain their high mating rank, FHLH will be negative. The second statistic, SR (Specific Rank), measured the tendency for males to mate in the same specific rank position on both days of a trial; to calculate SR, the difference between first-day rank and second-day rank was summed across all males. All values of SR are positive; SR becomes smaller as male mating order becomes more repeatable.

Two model simulations were completed for each 1996 trial, one simulation using FHLH and one simulation using SR. Then, two global simulations were completed which considered all four trial groups at once, one using FHLH and one using SR. All simulations used the same general random model: Nine males in a simulated sample were assigned first-day rank values matching those assigned to the males in a given 1996 trial and placed into an array in numerical order; these males were drawn at random from the array, without replacement, until all had been chosen. As each male was chosen, his first-day rank value was placed into a second array in a sequence reflecting the selection order. Each male remaining in the sample had an equal probability of being selected. The model simulation calculated FHLH or SR from these arrays, stored the simulation statistic, and repeated the simulation. After 10,000 simulations, the program sorted the resulting values and returned the cumulative frequency distribution of the statistic. The values of SR and FHLH observed in the 1996 trials were compared to these frequency distributions to determine if the null hypothesis of random mating could be rejected. The 1997 trials were similarly modeled, but without a global analysis.

Results

Experiment A: Effect of mating and age on recruitment of chorusing males

Male *M. septendecim* were rarely observed on the surfaces of the cages containing mated *M. septendecim* females (Cage A), teneral *M. septendecim* females, (Cage B), or no cicadas (Cage E). By contrast, in nearly every scan males were observed sitting or walking on the surfaces of Cages C and D, which contained mature, unmated females (Table 4). The combined scan counts differed strongly across treatments (Kruskal-Wallis test statistic = 48.729, $P<0.001$). Interestingly, the scan counts of the two cages containing unmated, mature females did not differ (Mann-Whitney U=118.0, $P=0.461$), suggesting that local

chorus intensity does not affect female receptivity to males in the pair-formation stage, at least not at an age of ten days after emergence. In addition, the few males found on Cages A, B, and E were never observed to perform courtship-related behaviors, and the females in these cages (A and B) were not observed to wing-flick, while such behaviors were repeatedly observed in association with the cages containing unmated females.

Experiment B: Duration of remating resistance

Only one of the 60 mated females (female "I" in Cohort B, on 16 and 19 June) ever responded with wing-flicks to the *M. septendecim* call playbacks during the eight days of the experiment. In contrast, on each day multiple unmated females in the control cages wing-flicked to the recorded calls.

Experiment C: Observations of individual pair-formation and courtship sequences

Phenology of wing-flicking and mating behavior -- Males were released into the flight cage population of unmated females on nine days (May 11, 13, 14, 17, 18, 19, 20, 21, 22, 23, and 24). In all, 33 males were introduced, 25 of which mated. Some of these males were left in the cage after the first mating and copulated more than once; at no time was the cage left unattended long enough to miss a copulation of normal duration.

149 different wing-flick responses were logged, involving 76 different females; 67 of the 149 were responses to males outside the cage. Initial distances between males and responding females varied from one to 120 cm; distances of 50 cm or so were common. Females less than five days old (post-emergence) did not wing-flick or mate. Both wing-flicking and mating appeared five days after emergence (Fig. 1), despite a relatively low total number of copulations, and despite the fact that early in the study (11-16 May), the

cicadas were not allowed to mate. Restricting the analysis to cohorts that matured after 16 May (Cohorts 3-8), yields essentially the same pattern -- for these cohorts only one wing-flick was observed before the day on which the first mating occurred (Fig. 2).

To determine the statistical significance of the absence of wing-flick observation in young adult cicadas, the population was divided into two groups - cicadas of ages 1-4 days and unmated cicadas older than four days, taking into account the changing proportions of these two groups during the study. These totals were used to calculate the expected number of wing-flick responses for each group under the null assumption that cicadas of all ages are equally likely to respond. A Fisher Exact test indicates that the absence of responses in young females is statistically significant ($P<0.001$; Table 5).

Once adult females began to show wing-flick behavior, they continued to do so during the remainder of the study as long as they remained unmated. Mated females were never observed to wing-flick, despite the fact that they comprised 29% of the cage population by the end of the study. A chi-square test (Table 6) shows that this is far less than the number expected if mated and unmated females are equally likely to wing flick.

Role of wing-flick in pair-formation and courtship -- 24 of the 25 observed matings were initiated when the female wing-flicked in response to the target male's calling song. In nine cases, the female began her wing-flick responses in response to the first call the male produced after being released into the cage. Each of these 24 matings followed completion of the normal, stereotypical acoustic courtship sequence (see Chapter 1). The remaining copulation occurred when a female responded to a courting male on the outside of the cage and remained still while the target male inside the cage located her and copulated without a sound.

All but one observed mating occurred without apparent delay. In the exceptional case, the male flew and landed on the female as he approached, apparently disturbing her temporarily because she flapped her wings until he moved. She wing-flicked to his next

call, however, and the courtship and mating proceeded as expected. In six complete videotaped sequences an average of 55 seconds passed from the time the male localized the female (arrived within 15-30 cm on the same branch) to the time the pair had completed external genitalic attachment (69, 45, 45, 38, 45, and 89 seconds). The latter male took only 29 seconds to begin genitalic attachment, but then needed another 60 seconds to finish.

Males often received wing-flick responses to their calling songs but failed to hear or locate the female. Contact is lost sometimes when the male moves in the wrong direction and ceases to receive responses from the female, perhaps because his calling song ceases to be loud enough due to distance or obstruction. However, this was not the only cause of failure to mate: In five other complete pair-forming sequences (17% of observed completed approaches), a target male received wing-flick responses from a female, located her, and approached her in apparently normal fashion, only to have to female either (1) cease wing-flicking at the last moment (four cases) or (2) flap her wings as he attempted to mount (one case), despite her having wing-flicked at all appropriate times previously. These five females were somewhat younger (average 9.2 days) on average than the females who mated immediately after an observed approach (average 11.3 days); the sample size is too small to test statistically, but immediate mating appears more likely with older females.

Experiment D: Effect of mating on female senescence rate

Two problems prevented the completion of Experiment D: The experiment was interrupted because of time constraints, and two replicate cages were damaged by birds, causing loss of some cicadas. Treatments A, B, and C lost 7,4, and 6 cicadas, respectively, from these two cages. However, the results from the remaining cicadas were clear; deaths occurred at nearly the same rate across treatments (Fig. 3).

Experiment E: Patterns of mating and courtship in a flight-caged population

After a period of teneral-phase inactivity in which both males and females remained largely quiescent on the vegetation and inner cage surface, the flight cage population became very active beginning on May 23. Males chorused (combined singing bouts with short flights) in the cage, and females mated, on all subsequent days except May 27-28 and June 1-2, during which rain, overcast conditions, and cool temperatures suppressed nearly all activity in the cage and surrounding woods. Only dates with weather conditions appropriate for cicada activity are included in the analysis below. Oviposition was monitored only casually, but was observed first on 25 May.

Because Experiment E was completed before the discovery of the female wing-flick signal (Chapter 1), no data were gathered on the incidence of this behavior.

Courtship behavior **--** Male *M. septendecim* courted both conspecifics and heterospecifics, male and female. 392 courtships were observed between male *M. septendecim* and female *M. septendecim* that mated; 118 of these were "unambiguous". In Table 7 these courtship observations, both ambiguous and unambiguous, are sorted according to their timing in relation to the onset of mating behavior in each female and standardized across days to account for the changing numbers of females of different "ages". Note that these female "age" values are different from those used elsewhere in this chapter. Females tended to become engaged in courtships as soon as they were placed in the cage (for most, Day -4, four days prior to their first mating). This tendency increased from Day -2 to Day 0, and then dropped, although courtships with mated females did not become rare until Day 3. Courtship durations ranged from 1 minute (the minimum value by default) to 345 minutes, and the average courtship duration remained between nine and 19 minutes from Day -4 to Day 4, with a decrease occurring after Day 0; recall that these values generally underestimate the true courtship duration.

The pattern of courtship frequency is approximately the same whether one considers all courtships, unambiguous courtships alone, or lengthy courtships alone. Most courtships were unsuccessful (did not lead to mating), regardless of duration. The longest unambiguous courtship lasted 345 minutes and was unsuccessful. The durations of all successful courtships were 1, 1, 1, 1, 3, 8, 19, 20, 23, 25, 27, 59, 69, 78, 82, 122, 142, and 174 minutes.

Female mating pattern **--** Females mated from 0 to 3 times (Table 8), most often on the fifth through seventh days after emergence (excluding cold rainy days) (Table 9). Some females (15) mated for the first time on the last day of observation and thus had no opportunity to remate; these were excluded from Table 8 and from the analysis below. Of the females who mated, but not on the last day, 82% mated just once. The one female who mated three times behaved unlike all other mating females: None of her copulatory bouts was observed in more than one cage scan, and the copulating male on one occasion appeared to have difficulty attaching despite an apparent lack of resistance by the female; this female may have been physically incapable of mating. Three other remating females were observed *in copula* only briefly with one male. The remaining eight females mated for an average minimum of 195 minutes with the first mate and 185 minutes with the second. Because all but four of the 13 rematings occurred on the day following the previous copulation (mean 1.4 days after prior copulation, range 1-3; Table 10), and because the average mating occurred with 3.1 days remaining in the observation period (range 0-7, mode 3), most females had ample opportunity to remate during the study.

The large number of unmated females probably reflects poor storage treatment of the earliest cohorts: 22 of the 29 females collected on May 16 or 17 (the earliest collection dates) did not mate; males that did not mate were also disproportionately represented among these early cohorts.

Male mating pattern **--** Males mated from 0 to 6 times (Table 8); one unusual male mated twice on the same day. Unlike females, males tended to remate throughout the study (Table 10); the average number of days by which a copulation followed a prior copulation was 2.8 (range 0-7).

Analysis of variance in male mating success **--** The variance in male mating success in the flight cage population was somewhat high compared to the variance estimated by the model simulation under random mating expectations, although the trend was not statistically significant (Table 11a). Because there was a tendency for the unmated males to belong to the earliest collected cohorts (67% of the unmated males came from the May 16 and 17 cohorts), the weakly nonrandom mating skew may have resulted from the inclusion in the study of a small number of moribund males weakened by comparatively poor storage conditions. In addition, some males not recorded as dead during the study were not found in the cage after the final day; these may have escaped, or they may have been eaten by ants shortly after falling to the ground; this reduces the accuracy of the model simulation. For these reasons, the model simulations were recalculated using only those males accounted for by the end of the study. In this reanalysis, neither the variance in mating success among all males, nor the variance in success of mated males alone, differed significantly from random expectations (Table 11b,c).

Experiment F: Repeatability of male mating rank

Trials 1A-1D, 1996 **--** Male singing and searching (walking and necessarily short flights), as well as mating, occurred readily in three of the four 1996 replicates, often with the first matings beginning minutes after the addition of the males (Table 12a). Females were always observed wing-flicking to calling males before mating, if the interaction was observed prior to the onset of CII courtship calling, and no apparently forced copulations

were seen. Matings always occurred more readily on the second day. The first and last matings in Groups A, B, and D occurred at about the same time, while mating occurred much less readily in Group C. In Group B all cicadas mated in both trials, but one male who mated died during the night. In Group A one male did not mate because one female died in the evening. In Group D one male and one female remained unmated in the first trial, while both mated with the rest of the cicadas in the second trial. In Group C only five pairs mated in the first trial; however, all but two mated in the second trial, the remaining male and female not mating because one male became moribund and died. The early Group A matings occurred so rapidly that in the confusion one cicada was accidentally placed in the wrong storage cage and included in the wrong group (Group B) the next day. This individual was removed from consideration because he did not "compete" with the same males in both trials. Correction for this error and for the deaths reduced the total sample size for males in groups A-C from nine to eight. For the one group with nine males, FHLH was calculated using the ranks of the first four and last four males, with male #5 not assigned to either half.

Male mating order as measured by FHLH and SR did not deviate significantly from random mating expectations, either when replicates were considered individually, or when all four replicates were simulated as a whole (Table 13a). FHLH values did tend to lean in the direction indicating positive mating order correlation (negative FHLH values). To investigate the possibility that sample size could have limited the ability of the experiment to reveal mating order correlations, the experimental dataset was duplicated, creating a new dataset that retained the original pattern with double the sample size. Repetition of the analysis using this dataset again failed to reject the null hypothesis of random mating. Observed values of SR did not tend to fall on one side of the frequency distribution of random mating values.

During the analysis it became apparent that mating order in females might be significantly repeatable, so the computer simulations were remodeled to allow analysis of

female mating pattern. Groups B and D showed a significant correlation in female mating order between trials, while Group C females showed a weak but nonsignificant correlation in the same direction (Table 13a). Both the FHLH and SR correlation values of Groups B and D were at least weakly significant (at least $P<.08$) in two-tailed tests, with the SR random mating model being somewhat more strongly rejected in both groups. Only the females of group A showed no indication of mating order repeatability between trials.

Trials 2A-2C, 1997 **--** Mating occurred in 1997 in the same manner as in the 1996 trials (Table 12b). Most males mated in each of the three trials, and copulations appeared to be of approximately normal duration, although this parameter was not measured carefully. Three of the sixteen males did not mate in Trial 2B, and one of the sixteen males did not mate in Trial 2C. As in the 1996 trials, neither simulation statistic revealed significant repeatability of male mating order between consecutive trials (2A-2B, or 2B-2C; Table 13b). Male order did repeat significantly across the set of three trials (2A-2C), meaning that the males who mated first in the first pairings tended to do so again on the third pairing, although there is no immediately obvious significance of this for female mate choice.

Discussion

Female behavior and male mating success on *Magicicada* leks bear only superficial resemblance to that observed on the classical leks of birds and mammals. Females do not appear to delay the mating process for extensive comparison of mates, and they do not appear to delay oviposition to revise mate choice through remating. Copulation is almost entirely restricted to a period of one to three days beginning about five days after emergence under good weather conditions; the five-day delay is consistent with prior measurements of the *Magicicada* post-ecdysis teneral period and matches the onset of first chorusing

behavior in males (Maier, 1982b; DCM/JRC unpublished observations). In addition, wing-flick signaling and female mating behavior appear at about the same time in the female's adult life. Once mated, females are unlikely to copulate again or to respond again with wing-flicks to calling song, despite continuing courtship attempts by males. The low frequency of remating in the 1995 flight cage population (Experiment E) is especially significant because mated females could not move as far away from chorusing males as they might normally do while seeking oviposition sites; this experiment might therefore be expected to have yielded an overestimate of the true remating frequency. Because not all females were watched until death, the results in this paper cannot exclude the possibility of remating late in adult life; however, such behavior would have comparatively little impact on the variance in male mating success.

Absence of obvious mating delays and of remating do not alone prove that potent female choice is absent -- females could use information gathered during the teneral period to select a superior first mate soon after the onset of adulthood, especially given the abundance of adult males. However, analysis of male mating success shows little evidence of the expected male mating skew. In the 1995 flight cage population, which included both teneral-collected and chorus-captured males, only a weak, nonsignificant departure from random mating expectations was evident at best, and this appears to be explainable by the presence of a small number of moribund males damaged during storage. Mating success among mated mates (a majority of the male population) was not skewed at all from random expectations. The 1996 and 1997 mating order trials (Experiment F), which used only males captured as adults from the surrounding chorus, found no tendency for males who mated first to do so again when remated to the same females or to different females. Both Experiments E and F involved females at or near the normal age of onset of mating receptivity. Equal probability of mating among mated males is consistent with the operation of a threshold mate choice mechanism; a "best-of-N" mechanism cannot be ruled

out, but if such a mechanism has evolved in *Magicicada septendecim* then there must be little variation in male quality.

Additional observations suggest that adult female behavior may not be designed to maximize mate choice options: First, as discussed below, some evidence of female coyness apparently derives from a high frequency of mistaken courtships by males. Second, females kept away from singing males during the post-emergence teneral period will wing-flick to the first song played back to them (unpublished observations of Day 6 *M. septendecim*, 1997), and females caged well away from the main chorus will mate without apparent additional delay (unpublished observations using Day 10 *M. septendecim*, 1996). Third, in 13-year populations containing both *M. tredecim* and *M. neotredecim* in the same woods, individuals of these similar species do not appear to segregate themselves locally into different choruses (Marshall and Cooley 2000), as expected if female gains within choruses derive from mate choice; in contrast, the more ecologically divergent -decim, -cassini, and -decula species are more strongly segregated during an emergence, both in time and space (Alexander and Moore 1962, Dybas and Lloyd 1974).

Perhaps the best evidence in this study for the potential for female choice was anecdotal -- in five of 30 complete pair-forming sequences initiated by the release of a chorusing male into a cage of unmated females (Experiment C), the female ceased to respond to the male as he arrived and mating did not immediately occur. Females who did not immediately mate tended to be younger on average, so it is possible that the proportion of initially "failed" courtships would have been higher in a population of younger females; on the other hand, disturbance by the videotaping experimenter or other conditions likely accounts for some of the observed "failed" courtships. Males in such situations never immediately abandon the female, and the ensuing protracted courtships sometimes lead to mating (Experiment E); the absence of mating skew in the Experiment E population suggests that such occasional hesitancy by females does not increase the variance in male mating success. Nonetheless, because females mate early in their adults lives, the behavior

of early-adult females may be especially relevant (see "Scramble Competition" section below).

Although willing to accept most males as mates, females apparently require certain "minimal criteria" (Alexander et al. 1997) from courting males; females will "wing-flick" only to sounds resembling conspecific male calling song (Chapter 1), and every copulation observed in the study was preceded by the stereotypical acoustic courtship sequence. The one near-exception occurred when a female mated with a silent male while another male, on the opposite side of the cage screen, completed the acoustic courtship sequence. Because courtship signals (CII, CIII) are potently attractive to potential interlopers (R. D. Alexander unpubl. data), it is likely that females require these signals for mating. Females appear to possess substantial control over the onset of mating and need not accept any given male; teneral and mated females routinely reject courting males by moving away, flapping wings, or pushing the courter away with a leg. Whatever the minimal criteria may be, it appears that most adult *Magicicada* males meet them.

The absence of female selectivity on *Magicicada* leks weighs against arguments for the general significance of female choice for indirect, genetic gains in insects. Among other Cicadidae at least, direct costs of mating behavior for females are not likely to be lower than they are in *Magicicada*, where females control the onset of mating, predator risks are comparatively low (due to "predator satiation"; see below), and mates are continuously available in huge numbers; despite this, the evidence of direct selectivity by females is weak. Direct costs associated with the more expensive mechanisms of female choice, such as remating, may remain generally prohibitive in many insect groups; this possibility raises questions for female choice theories that depend on costly remating, such as the cryptic female choice theory of the evolution of insect genitalia (Eberhard 1985, 1997). A rough calculation illustrates the potential problem for the evolution of female choice posed by direct costs associated with remating: For *Magicicada*, the limited data here suggest that unmated females do not live longer than mated females, and a complete mating bout lasts

approximately 4.5 hours (Cooley 1999). If individuals live on average for approximately three weeks (as suggested by Maier 1982 data and by Experiment B here), then the typical female may have approximately ten days to complete oviposition, assuming five days for the teneral period, one day for mating, and four or more days lost to poor weather. If females require much of this time to complete oviposition, then just one extra bout of pair-formation and mating could cost 5% of the average female's direct reproductive output in lost time alone; indirect gains would have to be substantial to override such a disadvantage, especially so if they accrued only to male offspring (as in "sexy son" evolution). The comparable costs of remating for a long-lived, iteroparous female mammal, on average, are likely to be smaller.

If copulation in *Magicicada septendecim* were not so lengthy, remating costs would be substantially lowered, and the plausibility of female mate choice revision by this mechanism would increase. In comparison, Cooley (1999) found that mating in two other Spring cicadas of North America, *Okanagana rimosa* and *O. canadensis*, lasts on average only 19 minutes. Perhaps female *Magicicada septendecim* have evolved to employ multiple mating as a mate choice strategy for a limited period of time, and males have evolved lengthy copulation in order to retain control of the female until the point at which remating ceases to be a cost-effective option for the female. Alexander and Moore (1958) and Maier (1982b) found that mating in *M. septendecim* occurs primarily between 9:00 AM and 3:00 PM, and that male chorusing activity drops sharply after 3 PM; the average mating female thus becomes free to remate only after most chorusing has ceased for the day. This hypothesis predicts that mating durations should be briefer in *M. cassini*, in which chorusing behavior peaks in the mid to late afternoon. Anecdotal observations of this species from Experiment E are consistent with this prediction, but a larger sample size will be necessary to accurately measure mating duration in these species. In addition, matings initiated later in the day should be shorter if only males gain from lengthy copulations.

Male scramble competition for mates, female "coyness", and the evolution of acoustic courtship

Because most females mate only once, the operational sex ratio of *Magicicada* choruses must be extremely male-biased during much of the emergence. Although this must be true for most species with single-mating females (e.g. Lloyd 1979 notes skewed operational sex ratios in fireflies), the skew is exaggerated in *Magicicada* by the synchrony of the emergence, especially among males. Except perhaps during a short period occurring approximately five days after the female emergence peak, nearly all of the females a male encounters during the day will be either teneral or mated and therefore unreceptive. Perhaps surprisingly, males do not appear to have evolved to distinguish these classes: Females of all types, as well as males and heterospecifics, were courted frequently in Experiment E, sometimes for very long periods. This observation matches descriptions by Dunning et al. (1979) and Alexander (1968, 1975).

The explanation of this puzzling male "strategy" may derive from the extreme nature of male scramble competition and nature of the *Magicicada* pair-forming signal system (see Chapter 1). If chorusing males are very abundant (several males per branch is common; Cooley 1999), then most wing-flicking females are likely to be located and courted almost immediately by one or more males. Because chorusing males appear nearly equivalent as potential mates (see Experiments E and F), on average the most successful males will be those who are most successful at detecting the first wing-flick signals of newly-receptive females. Unless mating readiness develops in females all at once, like a switch, the signals of such females may be produced weakly and/or inconsistently for some transitional period as fluctuations in sunlight, temperature, and disturbance move the female in and out of a receptive state. This situation would select males to respond to the slightest signs of timed movements in stationary individuals, likely resulting in (1) fruitless courtships, perhaps sometimes lengthy, when coincidental movements lead males to mistakenly approach

mated, same-sex, or heterospecific cicadas, and (2) courtships of varying length, ultimately leading to mating, when males detect early signals of responses in females but are prevented from mating at first by inconsistency in the female's response. If attention from males is costly for females who are not yet ready to mate, selection will act to shorten the period of transition to mating receptivity, but even an extremely short developmental transition (ca. 60 minutes) could create the effects described above for *Magicicada* if calling males are always present nearby and if females are present in the chorus prior to the onset of mating readiness. The hypothesis yields several predictions: (1) For females just entering the age of mating readiness, mating will occur more rapidly on average under good conditions (bright sun, high temperature, low wind) than bad, and this effect will decrease rapidly with age; (2) If male-female pairs are collected just after wing-flicking attracts the male, separated for a period of time, and then allowed to re-pair, longer delays will lead to more rapid second matings; also, after a delay corresponding to the length of the transition period, additional delay will not further reduce mating speed; (3) Mating speed should be independent of male identity: A chorus-caught male who mates quickly with a young female, if later paired with another young female, should be no less likely to become engaged in a lengthy courtship than a given chorus-caught male who mated more slowly the first time; (4) Acoustic courtship signals will be more prevalent in species in which male and female often come together when the female is only marginally receptive (e.g. species in which female must approach the male all the way for mating).

The hypothesis that males are selected primarily on the basis of their interactions with females just entering mating readiness may also help to explain the existence of acoustic courtship until copulation in *Magicicada*. Females presumably must remain stationary while wing-flicking for males to locate and approach them most efficiently; if so, nearby male calling song might be expected to increase a female's tendency to remain stationary. If a female's willingness to remain immobile for copulation is also influenced by the calling song, even if only incidentally, then a male engaging a responding female on the "edge" of

receptivity will surely gain by continuing to produce acoustic stimulation of a similar nature until the genitalia are engaged. If this effect benefits females, as by reducing mating delays, females will evolve to strengthen the response to song; if the effect is detrimental under certain circumstances, females will evolve to reduce it in that context, which could lead to antagonistic coevolution of the sort described by Holland and Rice (1998). If the effectiveness of such a courtship stimulus increases with its intensity, as seems likely if the females involved are often marginally receptive, then the hypothesis explains why males have not evolved to reduce interloping by producing quieter courtship signals. On the other hand, loudness of courtship signals may simply reflect the fact that loud background noise is characteristic of the mating situation in *Magicicada*.

Given an explanation for continuous courtship sound in *Magicicada*, all that remains is to explain the evolution of a stereotyped sequence of two distinct courtship signals. The simplest hypothesis would explain the courtship signals as modifications of the calling song[1] (CI). This is not difficult for the CII courtship song -- it seems likely that the effectiveness of calling song in eliciting immobility would increase if the phrases were strung together without silent gaps as in CII (R. D. Alexander pers. comm.). Thus males should switch from calling song to a CII-type song as soon as wing-flick responses are no longer needed to guide the male to the female[2] . Once the male begins to mount the female, a continuous signal (i.e., no silent gaps) becomes necessary for a second reason: to avoid

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[1] Alexander (1967) has suggested that long-range calling song may generally evolve from close-range courtship signals. The arguments here do not conflict with Alexander's hypothesis, which applies to the initial evolution of long-range calling songs in Cicadidae. By the hypothesis offered here, *Magicicada* courtship signals are comparatively derived traits that evolved after the process suggested by Alexander, and after the early courtship signals involved were lost.

[2] The strung-together phrases in CII courtship calling can be explained alternatively as an attempt by the male, upon locating the female but before attempting to mount, to maintain the courtship stimulus while preventing wing-flick signals that could reveal the location of the courting pair to potential interlopers (J. R. Cooley pers. comm.); females wing-flick only if silent gaps are left between song phrases. Potential interlopers are attracted both to CII calling and to wing-flick responses to other males' calls, but if interlopers identify the location of courting pairs most effectively by visual cues (Chapter 1) then CII may be less likely to lead to detection than wing-flicks. Lloyd (1979) describes a similar change in *Luciola lusitanica* male call structure at a late pair-forming stage, from discrete phrases that elicit female responses to continuous glows "up to 10 seconds in duration", although he does not note if females respond during continuous glows; individual females are sometimes approached by multiple males simultaneously in this species.

eliciting wing-flick movements from the female that would interfere with the male's attempt to climb onto the female. At this point, however, a CII-type call may not suffice for the male, because the frequency modulation in the downslur appears to require abdominal movements that could delay the male in engaging the genitalia. Therefore, as soon as the male begins to align the abdomen to connect the genitalia (this sometimes occurs coincident with mounting, and sometimes later), the male should begin to produce continuous courtship sound without the downslurs, in a manner similar to the CIII call today. The temporal patterning of the modern CIII call could be explained by selection to increase recognizability of the steady-pitch "proto-CIII" signal against the background chorus, a hypothesis proposed by Cooley and Marshall (Chapter 1) to explain the downslur of the calling song: If frequency modulation is not available to the male because of the need to hold the abdomen stationary, then only temporal patterning remains as an option. Alternatively, production of courtship sound in short bursts may facilitate its continued production at high intensity during the sometimes-lengthy process of engaging the genitalia.

One appealing aspect of this hypothesis is that it accounts for the fact of continuous acoustic courtship, as well as the number and form of courtship signals, in the absence of stringent or changing female mating criteria, and in the absence of indirect selection on female mating preferences. Complex courtship-related traits, especially those of lekking species in which males offer no direct benefits to females, are often viewed as "ornaments" evolved under directional selection resulting from (1) preference by females for ever-more-effective indicators of male genetic quality, (2) a Fisherian runaway process, or, in a somewhat different vein, (3) antagonistic coevolution driven by male sensory exploitation (Holland and Rice 1998) and/or male-female conflicts of interest (Alexander et al. 1997). These models usually predict divergent change in courtship signals among closely-related species. However, calling and courtship signals of *Magicicada* life cycle siblings are nearly indistinguishable, and the courtship differences existing between the -decim, -cassini, and -

decula groups are no greater than the differences in their calling songs, which have diverged mainly in frequency content. All -decim and -cassini species employ structurally similar CI, CII, and CIII signals in the same stereotyped sequence; the -decula species share the CIII-type signal, but differ in not possessing an obvious CII signal and in having evolved a multi-stage calling song that is approximately five times longer (Alexander and Moore 1962). The apparent evolutionary stability of *Magicicada* courtship signals, combined with the weak evidence of female choice in *M. septendecim*, suggests that novel explanations such as the one above may be more appropriate.

The evolution of *Magicicada* leks

The above discussion has shown that mating behavior and mate choice on *Magicicada septendecim* leks is distinct from that apparently characterizing many vertebrate leks, and that many signs of intense female choice discussed in previous research may be attributable to the special nature of male-male scramble competition. This leaves the problem of understanding the evolutionary forces that underlie lek behavior in *Magicicada*.

The evolution of leks depends fundamentally on the emancipation of males from parental duties (Snow 1963, Bradbury 1981, Höglund and Alatalo 1995). Additionally, given the prevalence of resource- and female-defense strategies across both vertebrate and invertebrate systems (see Emlen and Oring 1977, Thornhill and Alcock 1983), one might assume that lek mating systems are likely to evolve only when resources important to females cannot be effectively monopolized by males and when receptive females do not cluster in a manner that facilitates male defense of such female groups (Bradbury 1981). Although no firm conclusion can yet be drawn, it seems a reasonable guess that these assumptions hold in *Magicicada*, where the known resources of interest to females, such as pencil-sized deciduous twigs for oviposition and sunlit branches for basking and feeding, appear widely available. These resources certainly vary in space, but they do not appear

clumped to a degree sufficient to facilitate resource defense by male cicadas. However, by themselves these conditions explain only why males should abandon attempts at resource- and female-defense in favor of pure advertisement; they do not explain why males should profit by advertising in groups (Bradbury 1981), and they therefore do not explain why males should be attracted to the signals of other males, as in *Magicicada* (Alexander and Moore 1958).

Other than resource- or female-defense polygyny, male aggregations can be explained in two additional ways deriving from underlying variation in resources, and in both hypotheses males may evolve attraction to the signals of other males. First, when optimum signaling arenas are limited (e.g. locations of optimum transmission properties), males might cluster if the display arenas are sufficiently superior to offset competition costs and if an individual male cannot defend the site against other males. *Magicicada* advertisement does not appear to depend on choice of optimal display arenas, because males move continuously during advertisement and because the individual male songs do not appear designed to maximize long-range attraction, at least not compared to other cicadas. The hypothesis is also inconsistent with the observation that local *Magicicada* choruses shift in space over a period of weeks (Williams and Smith 1991). Second, when female densities vary according to an underlying resource distribution, but males cannot defend resource centers, some degree of spatial clustering of males is likely. This is the "resource-based lek" of Alexander (1975). This form of lek differs significantly from that defined by Bradbury (1981, see Table 1 here), who emphasized the absence of resources other than males at the lek site; Alexander (1975) argues that even resource-based aggregations merit inclusion if males are attracted to other males. The observation that *Magicicada* choruses shift in space during an emergence (Williams and Smith 1991) is again a potential difficulty, unless these changes can be shown to track changes in resource distribution; such changes could occur, for example, if preferred oviposition sites (see White 1980) are initially more common and later more rare in the vicinity of male choruses. The extent of

flagging (breakage and death of twigs overburdened with eggnest cuts), which causes death of eggs, in locations of dense chorusing suggests that this possibility at least merits investigation. The resource-based lek concept may be more useful in understanding aggregations of the -decula siblings, which are often found in association with one of a small number of tree species (see Dybas and Lloyd 1974).

If male aggregations in many *Magicicada* species are non-resource-based, then it becomes more difficult to explain why males should evolve to advertise with other males and suffer increased intrasexual competition. Bradbury (1981) has argued that, generally speaking, clustered males cannot attract more females per male simply by summing their signal efforts (to make a louder or more continuous sound, for example). Therefore, the problem of non-resource-based lek evolution reduces to that of understanding why females should evolve to prefer males in groups *per se*. Once females have evolved a special preference for clusters of males, males are more likely to profit by joining those aggregations. Tendencies for females to prefer grouped males can be classified into by-product preferences and direct preferences.

Males in groups could outperform lone males if female responses evolved in one context incidentally cause a disproportionate response to grouped males. For example, recent description of signal "precedence effects" (e.g. Wyttenbach and Hoy 1993) and "leading male effects" (e.g. Minckley and Greenfield 1995, Snedden and Greenfield 1998), show that in many species females move preferentially toward the first of two signals occurring in close temporal proximity, even if the leader is somewhat weaker. It is at possible that bias for leading males could provide sufficient advantage to grouped males, although such a hypothesis could explain male aggregations only in species with discontinuous songs (R. D. Alexander pers. comm.). There is little evidence for or against this hypothesis as an explanation for *Magicicada* aggregations.

Direct female preference for grouped males may evolve for a number of reasons. Alexander (1975) proposed perhaps the most general explanation for such female

tendencies: that females in species with non-resource-based leks have evolved to prefer clusters of males because in doing so they gain the opportunity to select a high quality mate from the largest sample at the least expense. In this hypothesis, the primary benefit to females of joining mating aggregations derives from mate choice; Alexander (1975) noted that *Magicicada* behavior on leks includes features suggestive of strong female choice, such as lengthy copulation and time-consuming courtship bouts. The results of this study, however, suggest that these features may be attributable to other processes. As an additional test, the observation of caged females mating readily outside the chorus (discussed above) should be repeated with younger females, and the speed of mating outside of the chorus should be compared with that of caged females within choruses.

A simpler version of Alexander's (1975) hypothesis could operate if females that move to aggregations gain primarily by reducing the time spent waiting for the first males to find them. This hypothesis may be especially relevant for periodical cicadas given their unusual mode of pair-formation. In many organisms with long-range acoustic signals, females move toward individual signaling males, either approaching all the way to the male or approaching only part-way and then signaling the male to complete the approach (e.g. Alexander 1967, 1975; Spooner 1968); in *Magicicada*, however, females wait for individual males to come within range of a wing-flick response (see Chapter 1 for discussion of the evolution of this strategy in *Magicicada*). In such a system, movement to areas of high male density is likely to reduce waiting time and thereby reduce the cost of pair-formation. A similar mode of pair-formation has been suggested for Tick-Tock cicadas (*Cicadetta quadricincta*; Gwynne 1987) and for many fireflies (Lloyd 1971), which might be expected to show aggregation by both sexes for the same reason.

Early discussions of lek mating systems (e.g. Lack 1968, Spieth 1974) suggested that lekking behavior may afford participants greater protection from predators. This argument was questioned by Alexander (1975), who noted that some evidence cited in favor of the hypothesis, such as specialized anti-predator behavior by lekking males,

instead suggests increased risks associated with leks, which may be expected to attract increased predator attention (see also Höglund and Alatalo 1995. However, the unusual ecology of *Magicicada* may create a rare exception to this general rule, in which predation risk decreases with increasing local density: Williams et al. (1993) found that predation rates by birds (described as percentage of the "standing crop" eaten) are highest early and late in the emergence when cicadas are least abundant, indicating that the populations "satiate" avian predators when most dense. Further evidence of the general significance of high population density for *Magicicada* is found in observations of birds annihilating large numbers of transplanted or off-schedule cicadas (e.g. Marlatt 1923; Beamer 1931; Alexander and Moore 1962; Dybas 1969), by the maintenance of strictly periodical emergences in the face of straggling, and by the consistent temporal synchrony of the different species within 13- and 17-year broods. These observations suggest that, at minimum, individual cicadas suffer increased predation risks when located in an area of unusually low adult density; this effect may be most significant for those individuals that emerge earliest. Risks may decrease continually as density increases, or risks may decrease until some threshold is reached and then level off. Either pattern should favor aggregation by cicadas of both sexes.

The predation hypothesis predicts that *Magicicada* individuals should move toward chorusing centers as soon as possible after eclosion; such movement should be most strongly favored for adults present in the early days of an emergence. In contrast, if periodical cicadas do not benefit from reduced predation in aggregations, then risky movement early in life should be strongly disfavored, especially in the first days of the emergence. The female choice hypothesis does not predict movement toward aggregations until near the time of sexual receptivity, unless females gain by comparing males during the post-emergence teneral phase.

Literature Cited

Alexander, R. D. 1967. Acoustical communication in arthropods. Annu. Rev. Entomol. 12: 495-526.

Alexander, R. D. 1968. Arthropods. In: T. A. Sebeok (ed). Animal Communication. Techniques of Study and Results of Research. Bloomington, Indiana: Indiana Univ. Press, pp. 167-216.

Alexander, R. D. 1975. Natural selection and specialized chorusing behavior in acoustical insects. In Pimental, D. ed. Insects, Science, and Society. New York: Academic Press. pp. 35-77.

Alexander, R. D., and T. E. Moore. 1958. Studies on the acoustical behavior of seventeen-year cicadas (Homoptera: Cicadidae: *Magicicada*). Ohio J. Sci. 58: 107-127.

Alexander, R. D., and T. E. Moore. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species (Homoptera, Cicadidae, *Magicicada*). Univ. of Mich. Mus. Zool. Misc. Pub. 121: 1-59.

Alexander, R. D., D. C. Marshall, and J. R. Cooley. 1997. Evolutionary perspectives on insect mating. pp. 4-31 In Choe, J. C., and B. J. Crespi, eds. The evolution of mating systems in insects and arachnids. Cambridge Univ. Press, Cambridge, U.K.

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton.

Beamer, R. H. 1931. Notes on the 17-year cicada in Kansas. J. Kans. Entomol. Soc. 4:53-58.

Borgia, G. 1979. Sexual selection and the evolution of mating systems. In M. S. Blum and N. A. Blum, eds. Sexual selection and reproductive competition in insects. pp. 19-80. Academic Press, New York.

Bradbury, J. W. 1981. The evolution of leks. In R. D. Alexander and D. W. Tinkle, eds. Natural selection and social behaviour: Recent research and new theory. pp. 138-173. Chiron Press, New York.

Bradbury, J. W. 1985. Contrasts between insects and vertebrates in the evolution of male display, female choice, and lek mating. Fortschr. Zool. 31: 273-289.

Bradbury, J. W., and R. M. Gibson. 1983. Leks and mate choice. In P. Bateson, ed. Mate Choice. pp. 109-138. Cambridge Univ. Press, Cambridge, U.K.

Clutton-Brock, T. H., M. Hiraiwa-Hasegawa, and S. D. Albion. 1989. Mate choice on fallow deer leks. Nature 340: 463-465.

Cooley, J. R. 1999. Sexual behavior in North American cicadas of the genera *Magicicada* and *Okanagana*. Ph.D. Dissertation, The University of Michigan.

de Vos, G. J. 1979. Adaptedness of arena behaviour in black grouse Tetrao tetrix and other grouse species (Tetraoninae). Behaviour 68: 277-314.

Dunning, D. C., J. A. Byers, and C. D. Zanger. 1979. Courtship in two species of periodical cicada, *Magicicada septendecim* and *Magicicada cassini*. Anim. Behav. 27: 1073-1090.

Dybas, H. S. 1969. The 17-year cicada: A four-year mistake? Field Mus. Nat. Hist. Bull. 40:10-12.

Dybas, H. S., and D. D. Davis. 1962. A population census of seventeen-year periodical cicadas (Homoptera: Cicadidae: *Magicicada*). Ecology 43:432-444.

Dybas, H. S., and M. Lloyd. 1974. The habitats of 17-year periodical cicadas (Homoptera: Cicadidae: *Magicicada* spp.). Ecol. Monogr. 44:279-324.

Eberhard, W. G. 1985. Sexual selection and animal genitalia. Harvard Univ. Press, Cambridge, MA.

Eberhard, W. G. 1997. Female control: sexual selection by cryptic female choice. Princeton Univ. Press, Princeton, NJ.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197: 215-223.

Gwynne, D. T. 1987. Sex-biased predation and the risky mate-locating behavior of male tick-tock cicadas (Homoptera: Cicadidae). *Anim. Behav.* 35: 571-576.

Höglund, J., and R. V. Alatalo. 1995. Leks. Princeton Univ. Press, Princeton.

Holland, B., and W. R. Rice. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. Evolution 52: 1-7.

Janetos, A. C. 1980. Strategies of female mate choice: a theoretical analysis. Behav. Ecol. Sociobiol. 7: 107-112.

Karban, R. 1982. Increased reproductive success at high densities and predator satiation for periodical cicadas. Ecology 63: 321-328.

Kirkpatrick, M. 1985. Evolution of female choice and male parental investment in polygamous species: The demise of the sexy son. Am. Nat. 125: 788-810.

Kirkpatrick, M. 1996. Good genes and direct selection in the evolution of mating preferences. Evolution 50: 2125-2140.

Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350: 33-38.

Kruijt, J. P., and J. A. Hogan. 1967. Social behavior on the lek in black grouse. Ardea 55: 203-240.

Lack, D. 1968. Ecological adaptations for breeding in birds. Methuen, London.

Lloyd, J. E. 1971. Bioluminescent communication in insects. Annu. Rev. Entomol. 16: 97-122.

Lloyd, J. E. 1979. Sexual selection in luminescent beetles. Pp. 293-342 *In:* M. S. Blum and N. A. Blum, eds. Sexual selection and reproductive competition in insects. Academic Press, New York.

Lloyd, M., and H. S. Dybas. 1966a. The periodical cicada problem. I. Population ecology. Evolution 20: 133-149.

Maier, C. T. 1982a. Abundance and distribution of the seventeen-year periodical cicada, *Magicicada septendecim* (Linnaeus) (Hemiptera: Cicadidae - Brood II), in Connecticut. Proc. Entomol. Soc. Wash. 84:430-439.

Maier, C. T. 1982b. Observations on the seventeen-year periodical cicada, *Magicicada septendecim* (Hemiptera: Homoptera: Cicadidae). Ann. Entomol. Soc. Am. 75:14-23.

Marlatt, C. L. 1923. The periodical cicada. U.S. Dept. Agric. Bur. Entomol. Bull. 71.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with a description of a new species, 13-year *Magicicada neotredecim*. Evolution in press (slated for August 2000).

Minckley, R. L., and M. D. Greenfield. 1995. Psychoacoustics of female phonotaxis and the evolution of male signal interactions in Orthoptera. Ethology, Ecology, and Evolution 7: 235-243.

Shelley, T. E., and T. S. Whittier. 1997. Lek behaviour of insects. In Choe, J. C., and B. J. Crespi, eds. The evolution of mating systems in insects and arachnids. Cambridge Univ. Press, Cambridge, U.K. pp. 273-293.

Snedden, W, A., and M. D. Greenfield. 1998. Females prefer leading males: relative call timing and sexual selection in katydid choruses. Animal Behaviour 56: 1091-1098.

Snow, D. W. 1963. The evolution of manakin displays. Proc. 13th Intl. Ornith. Congr., Vol. I, pp. 553-561.

Spieth, H. T. 1974. Courtship behavior in *Drosophila*. Annu. Rev. Entomol. 19: 385-405.

Spooner, J. D. 1968. Pair-forming acoustic systems of Phaneropterine katydids (Orthoptera: Tettigoniidae). Anim. Behav. 16: 197-212.

Thornhill, R.A., and J. Alcock, 1983. The evolution of insect mating systems. Harvard University Press, Cambridge.

White, J. 1973. Viable hybrid young from crossmated periodical cicadas. Ecology 54: 573-580.

White, J. 1980. Resource partitioning by ovipositing cicadas. Am. Nat. 115: 1-28.

Wiley, R. H. 1991. Lekking in birds and mammals: behavioral and evolutionary issues. Adv. Stud. Behav. 20: 201-291.

Williams, K. S., and K. G. Smith. 1991. Dynamics of periodical cicada chorus centers (Homoptera: Cicadidae: *Magicicada*). J. Insect Behav. 4: 275-291.

Williams, K. S., K. G. Smith, and F. M. Stephen. 1993. Emergence of 13-yr periodical cicadas (Cicadidae: *Magicicada*): phenology, mortality, and predator satiation. Ecology 74: 1143-1152.

Wyttenbach, R, A., and R. R. Hoy. 1993. Demonstration of the precedence effect in an insect. J. Acoust. Soc. Am. 94: 777-784.

Table 2.1. Criteria distinguishing the “classical” lek mating system, according to Bradbury (1981).

1) There is no male parental care.

2) There is an arena or lek to which females come and on which most of the mating occurs. An arena is a site on which several males aggregate and that does not fill the habitat normally used by the species for other activities such as feeding, roosting, etc.

3) The display sites of males contain no significant resources required by females except the males themselves. This stipulation includes food, water, roosts, nest sites, egg deposition sites, etc.

4) The female has an opportunity to select a mate once she visits the area.

Table 2.2. Composition of *M. septendecim* flight cage population for Experiment C. Numbers are the original total for each cohort minus the nunber of observed deaths in that cohort to date.

	Cohort and emergence date								
	A 7 May	B 8 May	C 9 May	D 10 May	E 11 May	F 12 May	G 17 May	H 18 May	I 20 May
Exp. Date									
11 May	15	15	14	15	15	0	0	0	0
12 May	15	15	14	15	15	15	0	0	0
13 May	15	15	14	15	15	15	0	0	0
14 May	14	15	14	15	15	15	0	0	0
15 May	14	15	13	15	15	15	0	0	0
16 May	14	15	13	15	15	15	0	0	0
17 May	13	15	13	14	15	15	0	0	0
18 May	13	15	13	14	14	15	0	0	0
19 May	13	12	11	13	14	14	14	0	0
20 May	13	8	10	12	13	14	13	14	0
21 May	13	8	10	11	12	14	12	14	0
22 May	13	8	10	11	11	12	12	14	14
23 May	13	7	10	10	11	12	10	13	14
24 May	13	7	10	10	10	11	8	11	14

Table 2.3. Initial composition of 1995 flight cage population of *M. septendecim.*

Emergence date	Number added	Date added
Females		
16 May	9	19 May
17 May	15	19 May
17 May	5	20 May
18 May	7	20 May
21 May	12	21 May
22 May	15	22 May
23 May	15	23 May
24 May	16	24 May
25 May	10	25 May
25 May	15	26 May
Total	119	
Males		
16 May	5	19 May
17 May	14	19 May
17 May	15	20 May
18 May	8	20 May
20 May	26	20 May
Adult-unknown	9	21 May
Adult-unknown	10	22 May
Total	87	

Table 2.4. Effect of female age or mated status on attractiveness to chorusing males. Counts are of male *M. septendecim* observed on cages in Experiment A. Cages containing unmated, mature females attracted more chorusing males, and courtship behaviors were observed only on these cages. Notations indicate courtship-related behaviors observed: WF = wing-flick responses; CII = stage II courtship calls; CIII = stage III courtship calls; TR = train of males in mutual courtship.

Time:	Cage A Mated	Cage B Teneral	Cage C Unmated	Cage D Unmated - Far	Cage E Empty
May 20					
1:08	0	0	1 WF	0	0
1:37	0	0	2 WF	1 WF	0
2:27	0	0	2 CIII	2 CIII	0
2:39	0	0	0	1 WF	0
3:20	0	0	4	3 CIII	0
4:09	0	0	3 WF	2 WF	0
4:31	0	1	2 WF	2 WF	0
5:15	0	0	0	2 WF	0
May 21					
9:00	0	0	5 WF	3 WF	1
9:31	0	0	5 WF	2	0
10:01	2	0	6 TR	5 WF	1
10:30	0	1	4	2	0
10:50	0	0	5 TR	-	0
11:07	0	0	2 CII	-	0
11:17	0	0	0	-	0
11:30	0	0	2 CIII	-	0
11:42	0	0	2 WF	-	1
Total observed	2	2	45	25	3
Average per scan	0.1	0.1	2.6	2.1	0.2

Table 2.5. Effect of age on *M. septendecim* female tendency to wing-flick to calling males. Wing-flick responses occurred significantly less often in young females than would be expected if age had no effect on tendency to respond (Fisher Exact test; $P<0.001$). Expected values are calculated using the proportions of females of the two age-classes observed in the cage throughout the study.

Age	Observed	Expected
1-4 Days	0	24
5+ Days	149	125

Table 2.6. Effect of mating on *M. septendecim* female tendency to wing-flick to calling males. Wing-flick responses occurred significantly less often in mated females than would be expected if mating history had no effect on tendency to respond (Fisher Exact test; $P<0.001$). Expected values are calculated using the proportions of females of the two groups observed in the cage throughout the study.

Mating Status	Observed	Expected
Mated	0	24
Unmated	149	125

Table 2.7. Courtships observed in 1995 *Magicicada septendecim* flight cage population (Experiment E). Courtship is defined as male and female less than 5 cm apart, with male oriented toward female. Unambiguous courtships are those for which acoustic courtship, male foreleg-vibration, mating attempts, or mating were observed. Courtship duration is an underestimate because most scans were 15-30 minutes apart. Courtships observed in only one scan were assigned a value of one minute. "Available females" is the number of females of a given "age" (in relation to onset of mating) that were present during the observations.

Number of days before (-) or after female's 1st mating	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7
Avg. courtship length (min.)	n/a	1.0	16.7	23.1	17.4	16.6	19.0	9.2	12.8	1.0	13.0	15.0	n/a	n/a
St. Dev.	n/a		30.4	55.3	33.6	35.5	37.2	27.1	34.2	0.0	26.8		n/a	n/a
Maximum	n/a	1	115	345	157	173	174	151	163	1	61	15	n/a	n/a
Minimum	n/a	1	1	1	1	1	1	1	1	1	1	15	n/a	n/a
Number of courtships	0	1	15	46	77	100	64	39	38	6	5	1	n/a	n/a
Number unambiguous	0	0	2	12	20	28	31	11	12	1	1	0	0	0
>10 min.	0.0	0.0	4.0	14.0	22.0	24.0	23.0	5.0	6.0	0.0	1.0	1.0	0.0	0.0
>30 min.	0.0	0.0	3.0	11.0	14.0	17.0	10.0	3.0	4.0	0.0	1.0	0.0	0.0	0.0
>30 min. and unamb.	0.0	0.0	1.0	5.0	10.0	7.0	8.0	3.0	1.0	0.0	0.0	0.0	0.0	0.0
>60 min.	0.0	0.0	1.0	6.0	11.0	12.0	6.0	3.0	3.0	0.0	1.0	0.0	0.0	0.0
Available females (AF)	1	2	24	57	65	71	73	60	53	43	20	9	5	1
Courtships per AF	0.0	0.5	0.6	0.8	1.2	1.4	0.9	0.7	0.7	0.1	0.3	0.1	0.0	0.0
Unambig. courtships per AF	0.0	0.0	0.1	0.2	0.3	0.4	0.4	0.2	0.2	0.0	0.1	0.0	0.0	0.0
>10 min. per AF	0.0	0.0	0.2	0.2	0.3	0.3	0.3	0.1	0.1	0.0	0.1	0.1	0.0	0.0
>30 min. per AF	0.0	0.0	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0
>30 min. and unamb. per AF	0.0	0.0	0.0	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
>60 min. per AF	0.0	0.0	0.0	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0

Table 2.8. Mating frequency in male and female *Magicicada septendecim* in a 1995 flight cage population (Experiment E). Note: Fifteen additional females mated on the last day of the study and thus did not have an opportunity to remate; these are excluded from the data below.

Number of Matings	Males	Females
0	30	37
1	22	55
2	10	11
3	14	1
4	1	0
5	0	0
6	1	0

Table 2.9. Phenology of *M. septendecim* female mating in the 1995 flight cage population (Experiment E). Numbers are of matings observed by date (23 May - 3 June), sorted by the emergence date of the mating female. Rain and cold temperatures suppressed cicada activity in the cage and surrounding woods on 27-28 May and on 1-2 June. Italicized values for the May 17 cohort on 26 and 29 May are the 2nd and 3rd matings of the one female who mated three times. The italicized value for the 25 May cohort on 29 May is likely to represent a female that was not teneral when collected.

		Observation Date											
		23	24	25	26	27	28	29	30	31	1	2	3
	16	1	1										
	17	1	2	2	*1*			*1*					
Female	18	1	2	3	1								
Emergence	21			1	6			5					
Date	22				3			11	1				
	23							12	3	1			
	24								5	6			4
	25							*1*	1	1			2
	26												12
						Rain					Rain		

Table 2.10. Number of days by which *M. septendecim* rematings followed the previous copulation, in the 1995 flight cage population, Experiment E. Remating females tended to mate over a total period of 2-3 days, while males continued to mate throughout the study.

Number of days after 1st mating	0	1	2	3	4	5	6	7
Male re-matings	1	8	9	16	6	4	1	1
Female re-matings	0	9	3	1	0	0	0	0

Table 2.11. Parameters and results of flight cage population model simulations, Experiment E. (a) Simulation of both mated and unmated males. (b) Simulation of both mated and unmated males, using only males accounted for by the end of the study. (c) Simulation of mated male subpopulation, using only males accounted for by the end of the study. Rounds 1-8 correspond to the eight study days with good weather, starting with 23 May, the day of first mating. Males that died in the flight cage prior to this date were not included in the simulations. All males were mature by May 23.

a) Simulation of all mated and unmated males (79 total). Actual 1995 variance statistic = 1.62 ($0.1>P>0.05$).

Parameters:

Round	1	2	3	4	5	6	7	8
New Deaths	0	4	0	2	0	1	2	10
New Matings	1	7	8	11	30	11	8	18

Frequency distribution of variance statistic:

P value	Variance	*P* value	Variance	*P* value	Variance
0.01	0.91	0.30	1.19	0.80	1.47
0.05	1.01	0.40	1.24	0.90	1.57
0.10	1.06	0.60	1.34	0.95	1.68
0.20	1.14	0.70	1.39	0.99	1.86

b) Simulation of both mated and unmated males accounted for by the end of the study (60 total). Actual 1995 variance statistic = 1.65 ($0.6>P>0.4$).

Parameters:

Round	1	2	3	4	5	6	7	8
New Deaths	0	4	0	2	0	1	2	10
New Matings	1	6	7	9	28	10	7	18

Frequency distribution of variance statistic:

P value	Variance	*P* value	Variance	*P* value	Variance
0.01	1.08	0.30	1.48	0.80	1.88
0.05	1.21	0.40	1.55	0.90	2.01
0.10	1.31	0.60	1.68	0.95	2.15
0.20	1.41	0.70	1.78	0.99	2.41

Table 2.11. (Continued).

c) Simulation of mated males alone (43 total), using only individuals accounted for by the end of the study. Actual 1995 variance statistic = 1.2 (0.6>*P*>0.4).

Parameters:

Round	1	2	3	4	5	6	7	8
New Deaths	0	0	0	1	0	1	2	6
Males Mating First Time	1	6	6	6	19	1	0	4
Males Remating	0	0	1	3	9	9	7	14

Frequency distribution of variance statistic:

P value	Variance	*P* value	Variance	*P* value	Variance
0.01	0.79	0.30	1.12	0.80	1.49
0.05	0.88	0.40	1.16	0.90	1.63
0.10	0.98	0.60	1.30	0.95	1.72
0.20	1.07	0.70	1.40	0.99	2.00

Table 2.12. Mating pairs and order of mating observed in *M. septendecim* in Experiment F, (a) 1996, (b) 1997. Rank values shown for second day reflect position in the order of mating from the first day; ties are used for simultaneous pairings and unmated cicadas.

(a) 1996: Trials 1A-1D

Trial 1A

First Day - Start 12:30

Time	Male I.D.	Female I.D.
1:40	RY	WG
1:41	RS[a]	WX
2:00	RRR	WY
2:02	YW	WB
2:06	YP	WP
2:59	RP	W
3:25	YY	WW
4:59	RB	WS

Second Day - Start 12:05

Time	Male 1st Rank	Male I.D.	Female 1st Rank	Female I.D.
12:11	3	YW	8	WS
12:18	7	RB	2	WX
12:27	6	YY	5	WP
12:32	1	RY	7	WW
12:37	8	RW	1	WG
12:43	5	RP	4	WB
12:48	4	YP	6	W
2:21	2	RRR	3	WY

[a] RS male not used after first day.

Trial 1B

First Day - Start 12:30

Time	Male I.D.	Female I.D.
1:25	RR	SP
1:36	YB	SR
1:37	RG[b]	SX
1:43	YYY	SB
2:21	YX	S
2:25	YS	SW
2:49	R	SS
3:27	RX	SY
4:57	Y	SG

Second Day - Start 12:13

Time	Male 1st Rank	Male I.D.	Female 1st Rank	Female I.D.
12:14	6	R	3	SX
12:17	3	YYY	2	SR
12:25	4	YX	1	SP
12:30	2	YB	6	SW
12:30	7	RX	4	SB
12:33	5	YS	8	SY
12:35	1	RR	5	S
12:53		RS[b]	7	SS
2:22	8	Y	9	SG

[b] RG male died after first day, male RS added for second day (not used in simulation).

Table 2.12. (Continued).

(a) 1996 (continued).

Trial 1C

First Day - Start 10:57			Second Day - Start 10:33				
Time	Male I.D.	Female I.D.	Time	Male 1st Rank	Male I.D.	Female 1st Rank	Female I.D.
12:05	1	8	10:47	4	3	7	9
12:07	2dot	1dot	10:51	7	2	1	8
2:10	8	7	11:18	5	7	2	1dot
2:35	3	3	12:15	3	8	4	3
2:56	7	2	1:25	2	2dot	7	1
none	9	9	1:54	7	9	7	6
none	4	6	2:18	7	4	3	7
none	2	2	2:44	1	1	7	2dot
none	6[c]	1	none	7	6	5	2[c]

[c] 6 male unmated both days (dropped from simulation); 2 female unmated second day

Trial 1D

First Day - Start 10:59			Second Day - Start 10:31				
Time	Male I.D.	Female I.D.	Time	Male 1st Rank	Male I.D.	Female 1st Rank	Female I.D.
11:25	6	7	10:34	8	9	1	7
11:41	4	2	10:46	5	5	2	2
12:23	3	9	10:49	1	6	3	1
12:23	2	1	10:50	2	4	5	8
1:19	5	4	10:53	7	8	7	5
1:19	1	8	11:03	3	3	3	9
1:43	8	5	11:29	3	2	5	4
4:09	9	3	11:46	9	7	9	6
none	7	6	2:40	5	1	8	3

Table 2.12. (Continued).

(b) 1997: Trials 2A-2C

2A Order	2B Order	1st Rank	2C Order	1st Rank	2nd Rank
K	G	16	K	1	3
V	V	2	M	5	8
Q	K	1	V	2	2
X	F	12	B	10	13
M	X	4	H	15	13
O	D	11	Q	3	13
Y	S	13	X	4	5
R	M	5	C	9	10
C	O	6	S	13	7
B	C[t]	9	R	8	13
D	Y[t]	7	F	12	4
F	A	14	D	11	6
S	B[n]	10	Y	7	10
A	H[n]	15	O[n]	6	9
H	Q[n]	3	A[n]	14	12
G	R[n]	8	G[n]	16	1

[t] Tied
[n] Not mated

Table 2.13. Statistical tests of repeatability of *M. septendecim* male and female mating order in Experiment F. FHLH and SR values are empirical results of mating order experiments in (a) Trials 1A-D,1996 and (b) Trial 2, 1997. *P* values or ranges are two-tailed significance levels of deviation of FHLH or SR from null hypothesis of random mating; the *P* values are derived from repeated computer simulation of random mating in model populations of the same composition (see Table 10 and Appendix B). The FHLH statistic is negative when mating order tends to be repeated, positive when male mating order tends to be reversed. SR is always positive and becomes smaller as mating order becomes more repeatable

(a) 1996 Trials 1A-1D

Trial	FHLH	*P*	SR	*P*
Male mating order:				
All groups	-9	0.52 - 0.58	92	0.82 - 0.94
Group A	-2	0.68 - 0.86	26	0.28 - 0.46
Group B	-6	0.34 - 0.46	18	0.46 - 0.68
Group C	+3	0.58 - 0.82	22	0.82 - 1.00
Group D	-4	0.58 - 0.68	26	0.78 - 1.00
Female mating order:				
All groups	-32	0.02 - 0.04	62	<.01
Group A	+8	0.20 - 0.34	24	0.64 - 0.77
Group B	-17	0.02	12	0.01
Group C	-9	0.20 - 0.26	16	0.10 - 0.15
Group D	-14	0.06 - 0.08	10	0.01
Groups B-D	-40	0.01	38	<0.01

(b) 1997 Trial 2

Trial	FHLH	*P*	SR	*P*
2A-2B	-8	0.70	74	0.48
2B-2C	+5	0.80	75	1.0
2A-2C	-38	0.06	51	0.01

Figure 2.1. Distritbuion of mating and wing-flicking observations in a flight cage population of *Magicicada septendecim* females, showing that both behaviors appeared at about the same time developmentally (Day 5). Numbers are not corrected for relative abundances of cicadas of different ages; young and old females were less common.

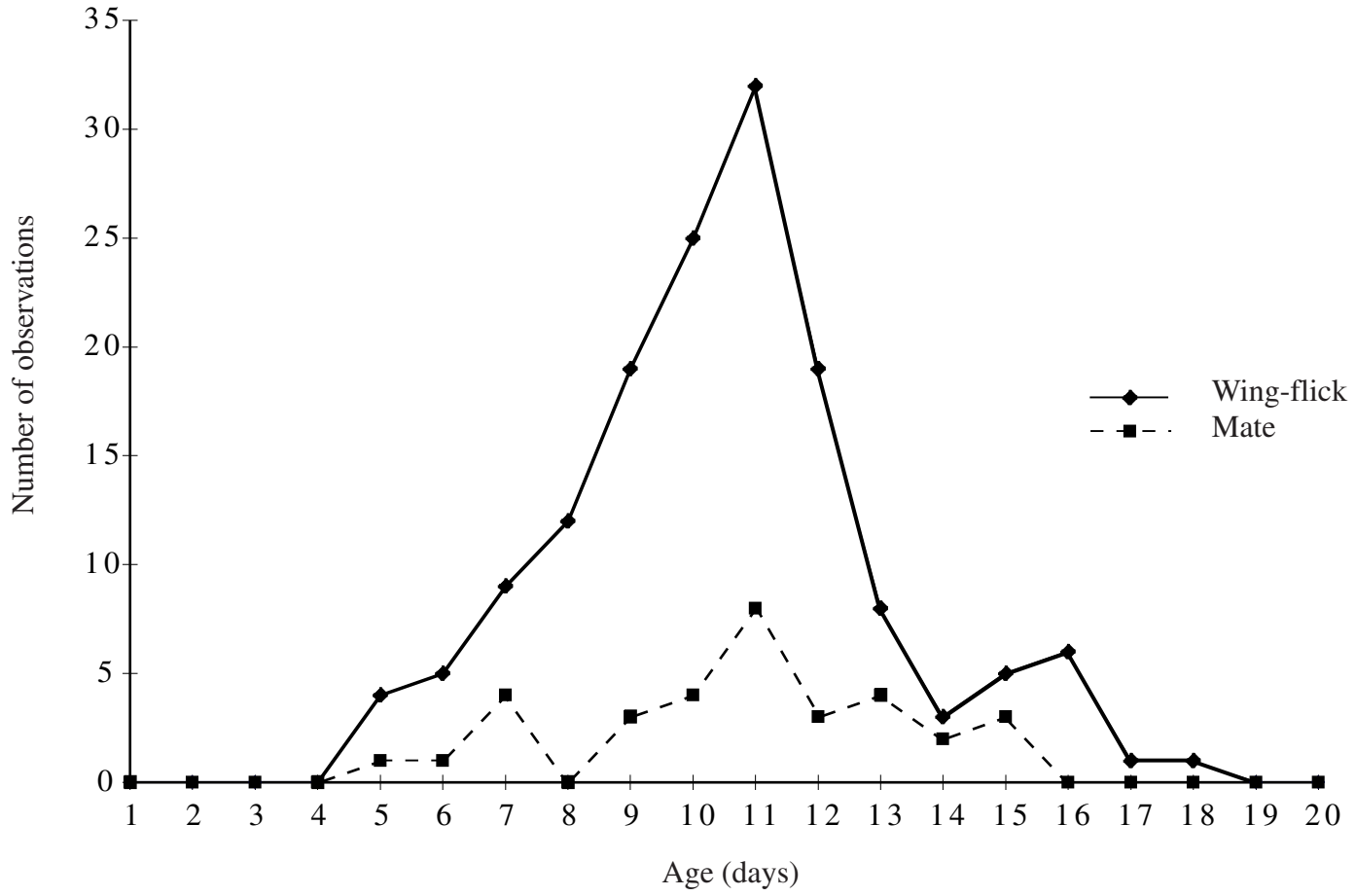


Figure 2.2. Distribution of observations of wing-flicking and mating, for cohorts 3-8 only, in a 1996 flight cage population of *Magicicada sepetndecim* females. Both behaviors appeared at about the same time developmentally (Day 5). Numbers are not corrected for differences in abundance of cicdas of different ages; young and old females were less common.

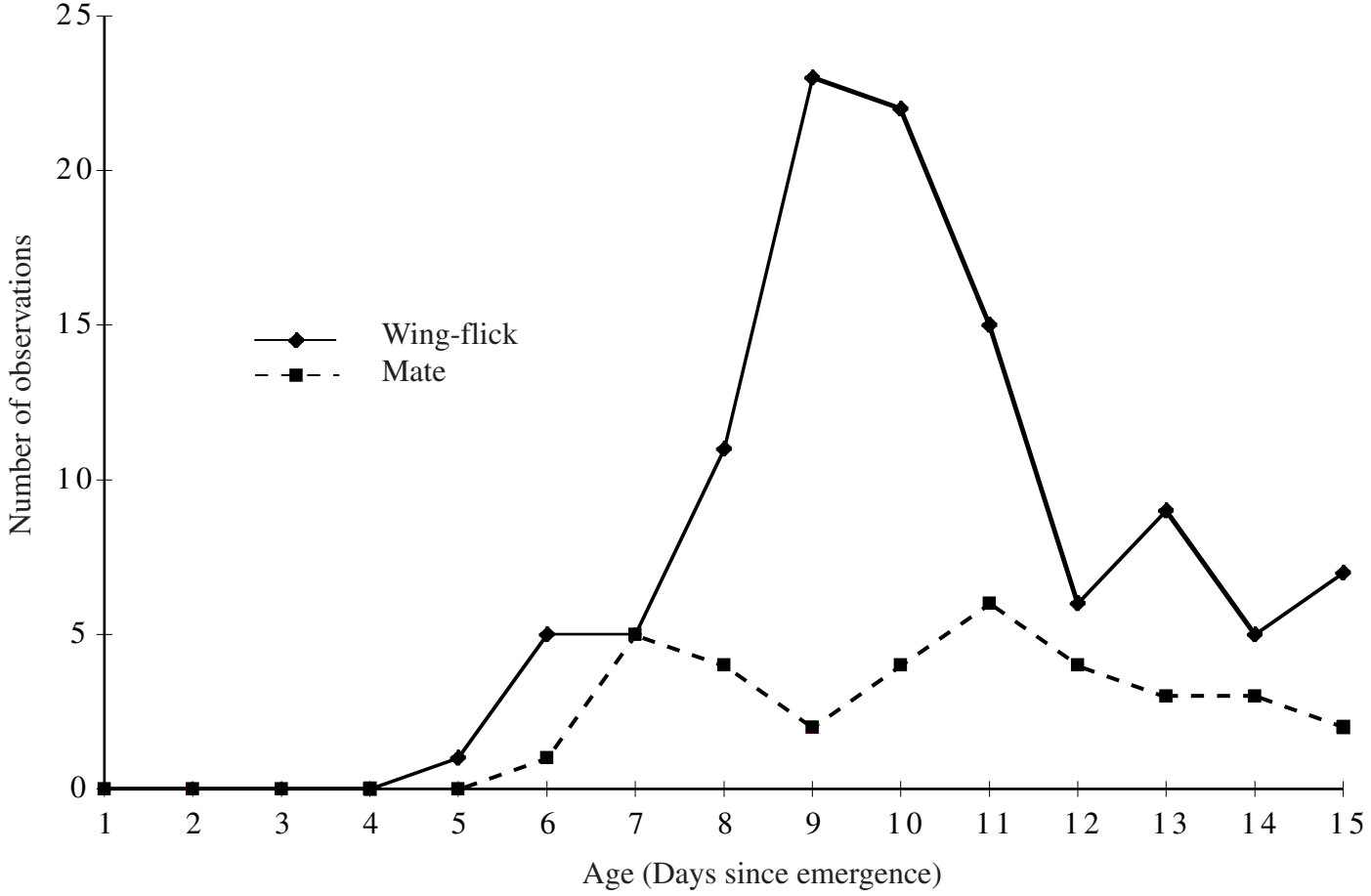
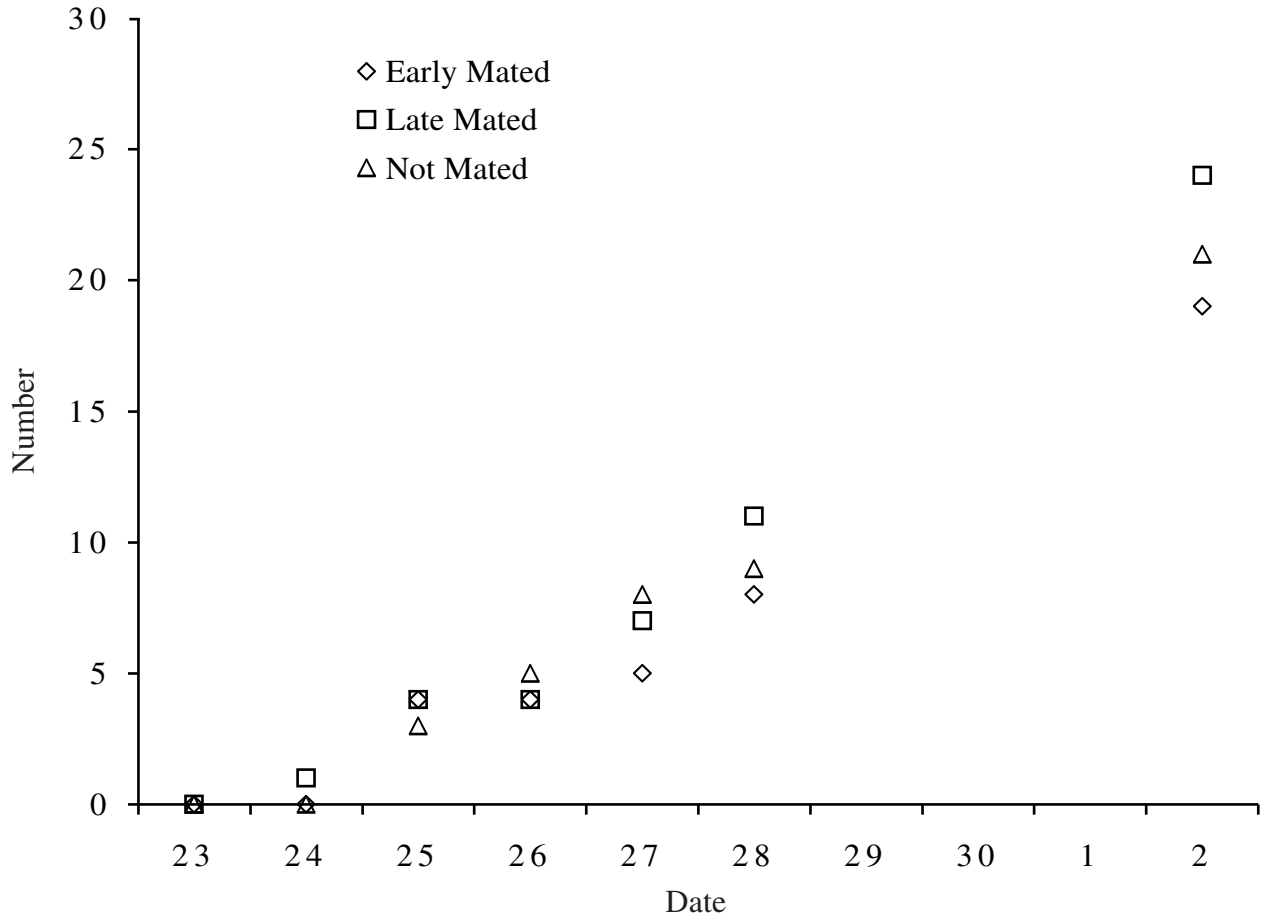


Figure 2.3. Pattern of mortality in *M. tredecim*, Experiment D, shows no effect of mated status on life length. No counts were made from May 29 - June 1, and the experiment could not be completed (cicadas remained alive in all treatments on June 2), so the possibility of later-acting effects has not been excluded.



CHAPTER 3

REPRODUCTIVE CHARACTER DISPLACEMENT AND SPECIATION IN PERIODICAL CICADAS

Abstract

Acoustic mate-attracting signals of related sympatric, synchronic species are always distinguishable, but those of related allopatric species sometimes are not, suggesting that such signals may evolve to "reinforce" premating species isolation when similar species become sympatric. This hypothesis predicts divergences restricted to regions of sympatry in partially overlapping species, but such "reproductive character displacement" has rarely been confirmed. We report such a case in the acoustic signals of a previously unrecognized 13-year periodical cicada species, *Magicicada neotredecim*. Where *M. neotredecim* overlaps *M. tredecim* in the central U.S., the dominant male call pitch (frequency) of *M. neotredecim* increases from ca. 1.4 to 1.7 kHz, while that of *M. tredecim* remains comparatively stable. The average preferences of female *M. neotredecim* for call pitch show a similar geographic pattern, changing with the call pitch of conspecific males. *M. neotredecim* differs from 13-year *M. tredecim* in abdomen coloration, mtDNA, and call pitch, but is not consistently distinguishable from 17-year *M. septendecim*; thus, like other

Magicicada species, *M. neotredecim* appears most closely related to a geographically-adjacent counterpart with the alternative life cycle. Speciation in *Magicicada* may be facilitated by life cycle changes that create temporal isolation, and reinforcement could play a role by fostering divergence in premating signals prior to speciation. Temporal founders, whether formed by mutation or the expression of developmental plasticity, may be more likely to succeed if they emerge synchronously with an overlapping brood of the same life cycle type; such a "nurse brood" could shield the temporal founders from predation.

Introduction

Periodical cicadas (*Magicicada* spp.) live underground as juveniles for either 13 or 17 years, after which they emerge for a brief adult life of approximately three weeks (Williams and Simon 1995). In northern and plains states, three morphologically and behaviorally distinct species coexist and emerge together once every 17 years (Fig. 1). These species are reproductively isolated in part by distinctive male acoustic signals and female responses (Alexander and Moore 1958, 1962). In the Midwest and South, three similar 13-year species have been described. Each species appears most closely related to another with the alternative life cycle; some of these species pairs can be distinguished only by life cycle length (Table 1). This pattern suggests that speciation in *Magicicada* may involve a combination of geographic isolation and life cycle changes that create temporal isolation (Alexander and Moore 1962; Lloyd and Dybas 1966; Lloyd and White 1976). Speciation involving allochronic isolation has been proposed for other organisms (e.g. field crickets: Alexander and Bigelow 1960, Alexander 1968; green lacewings: Tauber and Tauber 1977a,b), but remains controversial (e.g. Harrison 1979; Harrison and Bogdanowicz 1995).

The male sexual advertisement songs (or “calls”) of sympatric *Magicicada* species are readily distinguishable, while those of the parapatric life cycle siblings (e.g. 17-year *M.*

cassini and 13-year *M. tredecassini*) are similar or indistinguishable (Alexander and Moore 1962). This relationship between sympatry and song distinctiveness is common in groups with long-range sexual signals, and it suggests a process in which costly heterospecific sexual interactions lead to selection reinforcing differences that promote premating isolation (Dobzhansky 1940; Blair 1955). Selection of this form, long discussed as a potentially significant factor in speciation (Butlin 1989; Rice and Hostert 1993), also predicts greater reproductive trait differences in sympatry when species' ranges only partly overlap, a pattern termed "reproductive character displacement" (Brown and Wilson 1956; sensu Loftus-Hills and Littlejohn 1992). Waage (1979) argued that four criteria must be demonstrated to make a convincing case for reproductive character displacement: (1) The character(s) involved must play a significant role in aspects of premating isolation and they must be perceptible to the species across the range of phenotypic displacement observed in sympatry. (2) The allopatric character states must represent the precontact condition. (3) The apparent displacement in sympatry must not be explainable as part of a trend established for one or both species in allopatry. (4) The displacement must have occurred as a result of the interaction of the species in sympatry, and not as a result of interactions with other features of the environment in sympatry.

Few cases of reproductive character displacement have been demonstrated (Alexander 1967; Walker 1974; Howard 1993); for example, just one set of related examples (Hawaiian *Laupala*: Otte 1989) is known from the singing Orthoptera (grasshoppers, crickets, and katydids), a large, well-studied group with prominent acoustic signals. The small number of examples is surprising, especially given other evidence of reinforcing selection (Coyne and Orr 1989, 1997). Some authors point to a lack of adequately studied cases (e.g. Walker 1974; Howard 1993; Gerhardt 1994), or suggest that character divergence often becomes fixed too rapidly for transitional states to be observed (Alexander et al. 1997), while others suggest that sexual signal evolution may be driven mainly by within-species processes (e.g. West-Eberhard 1983; Paterson 1993).

Here we report the discovery of a new 13-year periodical cicada species, *Magicicada neotredecim*, that shows reproductive character displacement in male call pitch and female call pitch preferences in the central U.S. where it overlaps its closest 13-year relative, *M. tredecim* (Fig. 1). *M. neotredecim* appears most closely related to a 17-year counterpart, *M. septendecim*, from which it may have originated by a life cycle change (see also Martin and Simon 1988, 1990; Simon et al. 2000). These findings allow further refinement of hypotheses of life cycle evolution and allochronic isolation in *Magicicada* and suggest a way in which reinforcement of signal differences in sympatry may facilitate speciation in *Magicicada*.

Materials and Methods

Documenting sympatric 13-year –decim species with calls and morphology

Periodical cicada populations are extremely large; estimates of population density range from 8,355 (Maier 1982) to 3,700,000 per hectare (Dybas and Davis 1962). Most populations contain three species, a –decim[1] species that produces a narrow band of sound frequencies with a single dominant pitch between 1 and 2 kHz, and –cassini and –decula species that each produce broad-spectrum sounds above 3 kHz. While observing 13-year *Magicicada* in northern Arkansas in 1998, we found choruses (aggregations of singing males) with two peak frequencies in the –decim range (ca. 1.1 and 1.7 kHz), suggesting the presence of two –decim species, one previously undescribed (Fig. 2, background of Fig. 3). The location of this discovery suggested that the sympatric –decim would correspond to two forms of *M. tredecim* previously described using mitochondrial DNA

[1]For convenience we refer to *Magicicada* sibling species groups using the following shorthand: –decim: *M. septendecim* (17), *M. tredecim* (13), and *M. neotredecim* (13); –cassini: *M. cassini* (17) and *M. tredecassini* (13); –decula: *M. septendecula* (17) and *M. tredecula* (13).

(mtDNA) and abdomen coloration (amount of orange on the sternites) and found to meet along a zone reaching from Arkansas to Indiana (Martin and Simon 1988).

To determine if the sympatric –decim call types correspond to these morphs, we recorded the calls of 150 males collected from a mixed chorus and tested for association of call pitch and abdomen color. We collected the males from privately-owned woods on County Rd. 51 ca. 0.25 mi. S. of County Rd. 62, at a powerline right-of-way, just outside the northwest boundary of the Harold E. Alexander Wildlife Management Area, Sharp Co., AR; we will refer to this location as the "powerline" site.

All recordings were made using a Sony Professional Walkman cassette recorder with a Sony microphone and parabola, or a Sony 8mm videocassette recorder with built-in microphone. Because an individual male's calls do not vary significantly in dominant pitch, we isolated one call of each individual for spectral analysis. For each recording we generated a power spectrum (plot of sound intensity vs. frequency) using Canary 1.1.1 (Cornell Bioacoustics Laboratory) on a Macintosh computer and obtained the dominant pitch. Individual –decim calls consist of a 1-3 second steady-pitch and nearly pure-tone "main element" followed by a quieter 0.5 second frequency "downslur" ending about 500 Hz lower than the main element pitch (Fig. 2) (Alexander and Moore 1958; Weber et al. 1987). The "main element" contains most of the sound energy; therefore, chorus recordings are dominated by the main element pitch. We scored the abdomen color of each individually-recorded male using the method of Martin and Simon (1988), assigning each male a value from 1 (ca. 50% black) to 4 (all orange).

We tested for an overall relationship between call pitch and abdomen color class using a Kruskal-Wallis test. Because the preliminary chorus recordings suggested two call types with few intermediates (a bimodal distribution of chorus sound energy), we also divided our male sample by an intermediate pitch of 1.4 kHz and tested for a difference in abdomen coloration using a Mann-Whitney test. All statistical analyses were conducted using Systat Version 5.2.1, Macintosh version (SPSS, Inc.).

Measuring female call pitch preferences in sympatry

Sexually receptive female *Magicicada* produce timed "wing flick" signals in response to conspecific male calls; conspecific males respond to this signal by dropping out of the chorus, approaching the responding female, and beginning late-stage courtship behavior (Chapter 1). Most such courtships lead to mating in studies using captive cicadas (Cooley and Marshall unpublished data; Chapter 2). We used this signal as an assay of female mating receptivity to determine whether female preference for call pitch was correlated with abdomen color, using 74 –decim females collected from the Sharp Co., AR, powerline site. Using Sound Edit Pro (MacroMedia), we produced 14 pure-tone model calling phrases differing only in dominant pitch (1.0 kHz to 2.3 kHz main element pitch, in 0.1 kHz increments); models were designed to mimic the form of normal calls (described above) but contained no pulse structure. In previous experiments, we have found that females respond similarly to playbacks of recorded and artificial calls (Cooley 1999). We played the models to individually-marked caged females in both haphazard and ordered sequences using a Macintosh Powerbook computer connected to an amplified portable speaker positioned 25 cm away from the cage (68-75 dB, as determined by a Radio Shack sound level meter with A weighting). The playback experiments were carried out between 11:00 and 16:00 in bright overcast or sunny conditions against an acoustic background of a *Magicicada* chorus located in woods ca. 8 meters away and containing all four 13-year species. Females were tested in groups of four, in random order with respect to abdomen coloration; each was exposed to the entire model set from 2-10 times as time and mortality allowed. About a third (26/74) of the females did not respond to any call; these were dropped from the analysis. This response rate is similar to that observed in studies of 17-year *M. septendecim* females in Virginia and Illinois (Cooley 1999; also Chapters 1, 2, 5 here), where only one –decim species is known. For each female, we averaged all model

call pitches that elicited one or more wing-flick responses to determine the average pitch preference.

We scored female abdomen color using the method described above for males and tested for association between average pitch preference and abdomen color class by a Kruskal-Wallis test. In addition, using the intermediate pitch value (1.4 kHz) observed in the male sample, we divided the female sample in two by average pitch preference and tested for a difference in abdomen coloration using a Mann-Whitney test.

Estimating species distributions and geographic variation in calls

Once we had demonstrated the existence of two sympatric 13-year –decim species differing in call pitch, we estimated the species' distributions and measured geographic variation in dominant chorus pitch using recordings (15-30 seconds in duration) taken from 80 locations distributed throughout the 1998 *Magicicada* emergence. The 17- and 13-year life cycle groups each have formed several largely allopatric broods that emerge in different years; the broods are numbered according to year-class, from I-XVII for 17-year cicadas and from XVIII-XXX for 13-year cicadas. There are 12 extant 17-year broods and just three 13-year broods, so many year-classes are empty (see individual brood maps in Simon 1988 or Chapter 6). The 1998 13-year emergence involved the large Brood XIX, which reaches from Maryland to Oklahoma. Recording dates are given in Table 2.

For mixed choruses, we used the relative intensities of the two species-specific –decim dominant chorus pitches to estimate relative proportions of the species; these intensities were measured from the power spectrum of each chorus recording. This approach assumes that both species show the same relationship between male abundance and chorus intensity. Because field conditions did not allow direct comparisons of male sound output of the two –decim species, we tested the assumption indirectly: In the mixed-species "powerline" chorus from Sharp Co., AR, we compared the distribution of

individual call pitches of a random collection of 123 males to the distribution of acoustical energy in the chorus power spectrum, using a Kolmogorov-Smirnov test. The effectiveness of using chorus recordings to estimate –decim chorus composition is further improved if the two species do not form mutually exclusive spatial aggregations. To test this assumption, we recorded a continuous chorus sample along a 200m woodside trail in the Harold E. Alexander Wildlife Management Area, Sharp Co., AR, while pointing the parabola/ microphone assembly into the treetops at a 45 degree angle. We recorded one side while walking in one direction and then recorded the other side while returning. From samples of this recording taken at seven meter intervals, we measured the intensities of the *M. neotredecim* and *M. tredecim* frequency bands from power spectra; this yielded 47 samples because of gaps in the forest on one side (350m total). If the *M. neotredecim* and *M. tredecim* at the site were not uniformly distributed with respect to one another on a local scale, we would expect to observe significant variation among locations in the relative intensities of the two species' chorus bands.

Additional tests for demonstrating reproductive character displacement

As described below in Results, geographic sampling of choruses revealed an apparent pattern of reproductive character displacement in *M. neotredecim* call pitch, with more southern populations (those overlapping *M. tredecim*) exhibiting higher call pitch. Further confirmation of the pattern necessitated additional tests deriving from Waage's (1979) criteria #1 and #3 (see Introduction).

To determine if female call pitch preferences change geographically with male call pitch in *M. neotredecim*, a predicted pattern if male call pitch functions in mate recognition, we measured average pitch preferences of 33 *Magicicada neotredecim* females collected from a woodlot 0.8 miles south of White Heath, IL, on Rt. 1300E (Piatt Co.), beyond the range of *M. tredecim*. Twelve of these females were responsive during the test; the

remainder were discarded. We completed the playback experiments at nearby Lodge Park County Forest Preserve against a background chorus containing *M. neotredecim*, *M. tredecassini*, and *M. tredecula*. We used a Mann-Whitney test to determine if the average pitch preference of the Piatt Co. females differed from that of the Sharp Co., AR, powerline site females. Because of time constraints, we were unable to study allopatric *M. tredecim* females.

To test the alternative possibility that call pitch variations could be explained as a secondary effect of a north-south cline in male size, we compared the call pitches and body sizes of 61 *M. neotredecim* and 26 *M. tredecim* males from sympatry at the Sharp Co., AR, powerline site with those of 17 *M. neotredecim* males collected in Allerton Park, Piatt Co., IL, where no *M. tredecim* are present. We used three characters to estimate size: right wing length, thorax width between the wing articulations, and 1st abdominal sternite width between the sutures that join the sternite to the terga. We conducted pairwise comparisons among populations using Mann-Whitney-U tests. For each population, we tested for associations between size-related traits and call pitch using linear regressions.

Results

Behavioral and morphological evidence for sympatric 13-year –decim species

The Kruskal-Wallis test indicated a strong relationship between male call pitch and abdomen color class at the Sharp Co. AR powerline site (Table 3). Furthermore, the 150 individual male call pitches fell into two distinct groups with no intermediate pitch values from 1.20-1.42 kHz (Fig. 3), confirming the bimodal chorus energy distribution observed in chorus recordings. A Mann-Whitney comparison showed that these two groups differed significantly in abdomen coloration (Table 3): Males producing calls with low dominant

pitch had the orange abdomen color characteristic of Martin and Simon's (1988) "mtDNA lineage B," now recognized to be the previously described *Magicicada tredecim* (Alexander and Moore 1962). –Decim males producing higher-pitch calls had the darker abdomen color of Martin and Simon's "mtDNA lineage A," and constitute a new species, *Magicicada neotredecim* (description in Marshall and Cooley 2000). Among approximately 250 male cicadas observed during our study, we found just four putative intermediates: two high-pitch males with orange abdomens (category 4), one low-pitch male with a darker abdomen (category 2), and one male with an intermediate call (1.43 kHz).

Female call pitch preferences and morphology in sympatry

Most responding females wing-flicked (WF) to model calls of several different pitches (mean = 6.8 different call pitches, SD = 3.2). The average range of response (highest pitch eliciting WF - lowest pitch eliciting WF) was similar (mean = 7.4, SD = 3.5), because most females responded to a continuous rather than fragmented range of frequencies. There was a strong relationship between average pitch preference and abdomen color (Table 4). The bimodal phenotypic distribution apparent in male –decim call pitch appeared in the distribution of average female pitch preferences as well, indicating two classes of females (Fig. 3). When the female sample was divided at the intermediate pitch of 1.4 kHz, the resulting female groups differed in abdomen coloration just as in the male sample: Females responding on average to low-pitch calls (*M. tredecim*) were significantly more orange-colored than females responding on average to high-pitch calls (*M. neotredecim*; Table 4).

Species distributions and geographic variation in male calls

Using chorus recordings to estimate species abundance **--** The random sample of individual male calls from the Sharp Co., AR powerline population indicated a

strong relationship between relative abundance of the –decim species and the distribution of sound energy in the chorus: The standardized histogram of call pitches of individually-recorded males was indistinguishable from the standardized quadratic chorus power spectrum (Kolmogorov-Smirnov test, $P > 0.05$.; Fig. 3).

Although the proportions of the two –decim species vary on a scale of miles (e.g. Fig. 4 insets), the 13-year –decim species do not appear to cluster significantly within a location. In the 350m continuous recording the proportion of –decim chorus sound produced by the rarer species (*M. neotredecim*) remained between 10% and 36% of the total chorus sound output (mean = 19.0%, SD = 6.0, n = 47), and the chorus intensities of the two species were not significantly negatively correlated (Pearson coefficient = -0.229, $P \leq 0.121$).

Geographic overlap and reproductive character displacement in male call pitch between M. neotredecim and M. tredecim -- We found *M. neotredecim* in Missouri, Illinois, western Kentucky, and northern Arkansas (Fig. 4; see also Simon et al. 2000). The southernmost *M. neotredecim* populations overlap *M. tredecim* in a zone 50-150 km wide reaching from northern Arkansas into southern Missouri, southern Illinois, and western Kentucky. The remainder of Brood XIX contains *M. tredecim* and not *M. neotredecim*.

Geographic variation in dominant chorus pitch of *M. neotredecim* occurs in a pattern of reproductive character displacement (Fig. 5). *M. neotredecim* choruses have the highest dominant pitch (ca. 1.7 kHz) in sympatry with *M. tredecim*; in this region individual *M. neotredecim* males have call pitches as high as 1.9 kHz. North of the overlap zone, *M. neotredecim* dominant chorus pitch decreases to approximately 1.4 kHz in Illinois and 1.5 kHz in Missouri, a statistically significant shift (Table 5). Most of the change occurs immediately north of the zone of *M. tredecim/M. neotredecim* sympatry.

Call pitch variation in *M. tredecim* is more subtle (Fig. 6), less than 25% of that observed in *M. neotredecim*. *M. tredecim* choruses in deep sympatry with *M. neotredecim* have a low dominant pitch, and *M. tredecim* dominant chorus pitch slightly increases south and east in the overlap zone in Missouri and Illinois. However, some allopatric *M. tredecim* choruses in the southeast also contain very low-pitch calls, and there is no overall difference between choruses in sympatry and allopatry with *M. neotredecim* (Table 5).

Most of the chorus samples likely included the calls of hundreds or thousands of males. However, many of the populations from Missouri and Alabama were recorded late in the emergence when comparatively few males remained (Table 2). For these locations the chances of overlooking a rare species were greater.

Additional tests of reproductive character displacement

Female *M. neotredecim* call pitch preferences change geographically with male call pitch: In sympatry with *M. tredecim*, (powerline site; Sharp Co., AR) female *M. neotredecim* were most responsive to an average pitch of 1.72 ± 0.15 kHz (n = 38), while in allopatry (Piatt Co., IL) female preference averaged 1.31 ± 0.10 kHz (n = 12; Mann-Whitney U = 451, $P \leq 0.001$). Allopatric *M. neotredecim* also differed significantly (U = 104, $P \leq 0.003$) in pitch preference from the Arkansas (powerline site) *M. tredecim* (mean 1.19 ± 0.06 kHz, n = 10).

M. tredecim and *M. neotredecim* in sympatry were significantly different in all size measurements, although the magnitudes of these differences were not as great as those observed in call pitch (Fig. 7). *M. neotredecim* populations from Illinois and Arkansas differed in call pitch but not in size (Fig. 7). We found no significant relationship between call pitch and any measure of body size within species in any population using linear regressions.

Discussion

Call pitch and 13-year –decim species

The conclusion that *M. tredecim* and *M. neotredecim* are the 13-year –decim forms identified by Martin and Simon (1988, 1990) is supported by the correlation of call pitch differences with abdomen coloration differences and by the fact that the species' distributions within Brood XIX as determined using call phenotypes closely match those estimated by Martin and Simon using morphology and mtDNA (Martin and Simon 1988, 1990). The scarcity of call and preference intermediates (Figs. 2, 3) suggests that viable adult hybrids are rare (see also Simon et al. 2000); this could be due to hybrid failure or lack of interbreeding.

Because females of the two 13-year –decim species were able to distinguish call models varying only in dominant pitch, and because female call pitch preferences correlate with abdomen coloration types, call pitch differences are likely an important cause of species-specificity in –decim mate recognition. In addition to the dominant pitch, natural calls contain temporal patterns that result from individual tymbal contractions and the buckling of tymbal ribs (Young and Josephson 1983; Weber et al. 1987); our model calls did not contain such patterns. However, differential responses to our model calls demonstrate that such temporal characteristics are not required for mate recognition, and the call pitch differences are unlikely to be explained as secondary effects of differences in tymbal pulse rate. Variations in *M. septendecim* tymbal contraction rate do not alter dominant call pitch, which may be determined by physical properties of the resonating abdomen and its large air sac (Young and Josephson 1983). Furthermore, we found no relationship between air temperature (which affects tymbal contraction rate) and chorus pitch in 11 recordings taken from the same location at different times (Fig. 8). Little is known of the relative roles of temporal patterning and frequency content in cicada calls in

general, although both function in Australian bladder cicadas (*Cystosoma*; Doolan and Young 1989), each in a different context.

Reproductive character displacement in *M. neotredecim*

The increase of *M. neotredecim* call pitch in sympatry with *M. tredecim* (a change of nearly 25%) meets the criteria established by Waage (1979) for reproductive character displacement (see INTRODUCTION). The model call playback experiments demonstrate that the difference in 13-year –decim call pitch in sympatry likely plays a role in mate recognition, and that the range of variation is perceptible to the species. The fact that allopatric populations of *M. neotredecim* in Illinois are indistinguishable in call pitch from 17-year *M. septendecim* (dominant chorus pitch 1.30 - 1.45 kHz; unpublished data), the new species' closest relative (Martin and Simon 1988, 1990), supports the conclusion that the high call pitch of *M. neotredecim* in the overlap zone is derived. No trends exist in allopatry that can explain the pattern of displacement; rather, the displacement is associated with the zone of sympatry. In Illinois and eastern Missouri, nearly all of the geographic change in dominant chorus pitch occurs in a ca. 50 km zone immediately north of the *M. tredecim* range limit, and variation among allopatric populations or among sympatric populations is comparatively minor (Fig. 5); the pattern in central Missouri is less striking, however (see below). In addition, the change in call pitch does not appear to be an incidental effect of a latitudinal cline in body size (Fig. 7). Finally, the requirement that the divergence be attributable to reproductive interactions of the species is indirectly supported by the fact that average *M. neotredecim* and *M. tredecim* calls in sympatry differ just enough to avoid frequency overlap, with *M. neotredecim* downslurs ending at approximately the dominant call pitch of *M. tredecim* (Fig. 2).

A potential challenge to the conclusion of reproductive character displacement arises because some central Missouri populations apparently well outside the range of *M.*

tredecim have a partially elevated –decim dominant chorus pitch (ca. 1.5 kHz). This pattern could be explained if (1) *M. neotredecim* colonized Missouri from Illinois populations that were themselves adjacent to the range of *M. tredecim*, or (2) undiscovered *M. tredecim* populations exist in Missouri near the locations we sampled. Future surveys should investigate the latter possibility. Because *M. tredecim* appears to reach its northern limits on Mississippi and Wabash lowlands, it may be found only in restricted locations near rivers elsewhere in the northern part of its range.

Also of interest is that the dominant chorus pitch of *M. neotredecim* does not correlate with the relative abundance of the two 13-year –decim species in mixed populations (linear regression, $r^2 = 0.053$, $P > 0.3$, n = 21). This appears to undermine the conclusion that the displacement is attributable to reproductive interactions of the two species (Waage 1979, criterion #4), if the strength of reinforcing selection on one species is expected to depend on the abundance of the other (Howard 1993; Noor 1995). However, the prediction of frequency-dependence is not appropriate under some circumstances. Average *M. neotredecim* calls in sympatry are displaced just enough to avoid frequency overlap with *M. tredecim* (Fig. 2), suggesting that reinforcing selection may cease at that point; if only small numbers of *M. tredecim* are necessary to drive this change in *M. neotredecim*, then a correlation between relative abundance and degree of displacement would be detectable only among populations with extremely rare *M. tredecim*. In addition, if conditions influencing the relationship between relative abundance and displacement vary across regions, then the correlation may have been obscured by our combined analysis of all mixed populations; a more local scale of analysis could reveal the expected relationship. The data from southern Illinois (Fig. 5 inset), for example, suggest greater displacement in southern populations where *M. neotredecim* is more rare; however, this possibility will not be resolved without additional data.

M. neotredecim call pitch has changed much more than that of *M. tredecim*; such asymmetries are not unusual in cases of reproductive character displacement (e.g. Littlejohn

1965; Littlejohn and Loftus-Hills 1968; Fouquette 1975; Waage 1979; Noor 1995). In general, because the strength of selection on each species depends on factors that can differ between them, symmetrical displacement is probably unlikely (Grant 1972; Howard 1993). Possible explanations in the *Magicicada* case include the following: (1) greater numerical abundance of *M. tredecim* relative to *M. neotredecim* during critical stages of the interaction, perhaps because *M. neotredecim* originally invaded established *M. tredecim* populations and not vice versa; (2) greater *M. tredecim* female selectiveness upon initial contact; (3) greater constraints on the evolution of lower call pitch.

Reinforcement and speciation

The criteria for reproductive character displacement (sensu Howard 1993) as established by Waage (1979) reflect an expected outcome of natural selection reducing inefficiencies arising from heterospecific sexual interactions; such selection is sometimes referred to generally by the terms "reinforcement" or "reinforcing selection." In general, the reproductive inefficiencies driving such selection could range from interbreeding with partial hybrid success and limited introgression, to interbreeding with complete hybrid failure, to simple reproductive interference (e.g. crossmating with morphological incompatibility, or signal interference without crossmating). Because reinforcing selection can reduce gene flow between populations under certain conditions (Rice and Hostert 1993; Liou and Price 1994), such selection has been considered a process of speciation (Dobzhansky 1940; Blair 1955; Butlin 1995; Kelly and Noor 1996). In accordance with this view, Butlin (1987, 1989) argues for a redefinition of terms: "Reinforcement" should apply only when premating isolation is enhanced despite interbreeding and gene flow, and the term "reproductive character displacement" should refer to the divergence of mate recognition systems without gene flow (e.g., when hybrids are sterile). Under this

terminology, only reinforcement is a candidate speciation mechanism because speciation is already completed if gene flow is not possible (Butlin 1989).

This approach has two weaknesses. First, reinforcement may be best viewed not as a mechanism of speciation, but instead as a process that only species undergo. Most species definitions reflect a general concept of species as "population-level evolutionary lineages" (de Queiroz 1998); the best evidence (when available) of the distinctiveness of such lineages is their ability to remain distinct and/or diverge even in sympatry. Reinforcement occurs only if, prior to contact, changes in allopatry or allochrony have caused two populations to accumulate reproductive incompatibilities sufficient to make divergence irreversible; thus, the occurrence of reinforcement is itself evidence that the populations were species (able to remain distinct in sympatry/synchrony) before contact. When species are defined in terms of the irreversibility of their divergence, reinforcement becomes more an effect of speciation than a cause, whether or not the populations exchange genes at any point. This view of reinforcement and speciation does not require distinctions based on degree of hybrid failure. Furthermore, this approach is compatible with evidence of widespread natural hybridization (e.g. Grant and Grant 1992, Arnold 1997), which suggests that species status should not be rejected simply on the basis of incomplete hybrid sterility or naturally-occurring gene flow.

Second, the new definitions may be difficult or impossible to apply in many cases, because neither term can be applied if the extent of past gene flow between the species is unknown. We may not ever know if *M. neotredecim* and *M. tredecim* exchanged genes upon first contact. Evidence that hybridization does not occur now does not prove that it did not occur in the past, and evidence of past introgression may have been lost by selection and/or drift. Therefore, there may be value in retaining a general concept of reinforcement as a process of reproductive character divergence driven by selection against wasteful heterospecific sexual interactions, without assumptions of crossmating, gene

flow, or even relatedness of interactants; the term is used in this manner for the remainder of this paper.

Allochronic isolation and speciation in *Magicicada*

Because 13-year *M. neotredecim* and 17-year *M. septendecim* have parapatric distributions and are consistently distinguishable only in life cycle length, one of these two species likely originated from ancestral populations of the other (Martin and Simon 1988, 1990). Derivation of *M. neotredecim* from *M. septendecim* is supported by the comparatively restricted range of *M. neotredecim* (Fig. 1) and the likely recent nature of its contact and reinforcement with *M. tredecim* (see also Simon et al. 2000).

Speciation almost certainly begins with some form of partial isolation that facilitates genetic divergence by reducing interbreeding between populations. The alternative, evolution of assortatively mating forms initiated by strong disruptive selection alone, has been extensively modeled (e.g. Dieckmann and Doebelli 1999, Kondrashov and Kondrashov 1999), but appears to depend on restrictive or unrealistic conditions. Speciation models that begin with isolation fall into two categories: In the first, initial isolation is effected by phenotypic change in a subpopulation; such intrinsic changes can arise by mutation or by the cueing of phenotypic plasticity by novel environmental stimuli. In the second category, isolation is effected by changes extrinsic to the organisms. The appearance of a new geographic barrier (Mayr 1963) and the introduction of reproductive-incompatibility-inducing intracellular symbionts like *Wolbachia* (see Werren 1998) are examples of such extrinsic changes, so long as the division induced is not ultimately attributable to new changes in organismal phenotypes. Allochronic isolation always arises by intrinsic change, specifically by changes in developmental or other phenological timing. Ironically, although Mayr's (1963) original conception of allopatric or geographic speciation implies an extrinsic cause, even allopatric speciation may be initiated by

phenotypic (intrinsic) change that facilitates spatial isolation. Thus, it is unfortunate that models of speciation in which isolation arises initially via phenotypic change are often discussed under the concept of "sympatric" speciation, because spatial segregation can remain a key component of such models (e.g. speciation by "host shifts"; Bush 1992).

Once populations become partially isolated, genetic differences may accumulate that lead to irreversibility of evolutionary divergence, or speciation. Because selection drives genetic change more rapidly than other evolutionary mechanisms (mutation, drift), selection is likely the primary cause of divergence leading to speciation. The probability of speciation in a given case is therefore related principally to (1) the magnitude of the initial isolation, (2) the persistence of the initial isolating change and (3) the degree to which the partially isolated populations are subject to divergent selective pressures. Furthermore, the importance of selection in accelerating evolutionary divergence suggests that speciation may be more likely when isolation is mediated by intrinsic differences, if these are more likely to be accompanied by differences in selection.

Because all *Magicicada* sibling species pairs differ in life cycle length, and because life cycle changes can isolate populations in time, allochronic isolation may play a central role in periodical cicada speciation (see also Alexander and Moore 1962, Simon et al. 2000). This plausibility of this mechanism as an explanation of shifts between 13- and 17-year cycles in particular is increased by (1) the discovery that that the life cycle difference between 13- and 17-year species can be explained by an early 4-year developmental dormancy period found only in 17-year cicadas (White and Lloyd 1975) and (2) observations of apparently facultative 4-year accelerations in 17-year populations, sometimes involving large numbers of cicadas (e.g. Dybas 1969, Kritsky and Simon 1996). However, plausible models of allochronic speciation in *Magicicada* must accomodate a special challenge: Periodical cicadas apparently cannot survive and reproduce unless population densities remain on the order of thousands per acre, at densities that can "satiate" avian predators and reduce the risk for individuals (Marlatt 1923; Beamer 1931;

Alexander and Moore 1962; Dybas 1969; Williams et al. 1993); this suggests strong selection for developmental synchrony.

Life cycle change, like any intrinsic change mediating isolation, can arise either (1) by mutation or (2) by the repeated expression of developmental plasticity that is subsequently made permanent by genetic change (West Eberhard 1989). The mutation model is unlikely to be an important general cause of speciation because mutants are usually rare and therefore unlikely to find contemporaneous counterparts. However, in *Magicicada*, the possibility of simultaneous parallel mutations is increased by high population density (as high as 3.7 million per hectare: Dybas and Davis 1962) so the hypothesis cannot be entirely disregarded. The developmental plasticity model more easily accounts for multiple simultaneous founders, and is therefore more likely to have general significance. In *Magicicada*, occasional observations of large numbers of off-schedule "stragglers" (e.g. Dybas 1969) indicate that as-yet-unknown environmental conditions can trigger the expression of new life cycle length. If such extreme climatic conditions were to persist for generations, selection would favor genes tending to canalize the new phenotype, as long as cicadas expressing the old phenotype failed to satiate predators or find mates. If the climate returned to the initial conditions gradually, canalizing selection could lead to the evolution of cicadas that express the new life cycle even under the original conditions (Fig. 9; see Waddington 1953). Furthermore, if different *Magicicada* species possess similar life cycle plasticity, a climate shift causing a change in the life cycle of one species could change sympatric species in a similar fashion, resulting in simultaneous, parallel speciation events. Thus, we might expect to find that similar undiscovered 13-year –cassini and/or –decula species coexist with *M. neotredecim*.

The unique requirement of predator satiation for *Magicicada* is not a difficulty under the developmental plasticity model if an environmental stimulus of large magnitude can cause large numbers of cicadas to switch life cycle. In contrast, a small number of temporal founders, whether produced by mutation or developmental plasticity, will not

survive predation unless special circumstances can reduce the risk. The observation that broods of different life cycles sometimes overlap one another in the same woods (Lloyd et al. 1983; Simon 1988) suggests such a mechanism: Temporal founders from one brood could survive if their initial appearance were fortuitously timed with the emergence of a geographically overlapping brood of the same life cycle type as the founders (Fig. 10), who would then remain synchronized with the "nurse" brood (see also Lloyd and Dybas 1966, Simon et al. 2000). This mechanism has the potential to explain the synchronization of *M. neotredecim* with both adjacent 13-year broods (see Simon et al. 2000 for Brood XXIII evidence).

When allochronic speciation initiated by mutation or developmental plasticity is fostered by the nurse brood effect, temporal migrants are more likely to become established if the nurse brood does not already contain a confusingly similar species; otherwise the presence of such a species could result in the new founders being lost to interspecific hybridization or failed mate-location. However, reinforcement of premating isolation within a brood, prior to the initial isolation of temporal founders, could alleviate this difficulty (Fig. 11) by increasing the differences between allopatric/allochronic forms prior to contact. For example, reinforcement in 13-year *M. neotredecim* has incidentally caused some populations of this species to differ from nearby 17-year *M. septendecim* populations in dominant chorus pitch by approximately 300 Hz. If future 17-year life cycle mutants from a population of call-displaced *M. neotredecim* co-emerge with 17-year cicadas where 13- and 17-year broods overlap, the preexisting pitch differences might facilitate assortative mating of the new incipient 17-year species (Fig. 11). In this model reinforcement operates prior to speciation by causing divergence in allopatry that incidentally facilitates successful coexistence upon establishment of sympatry.

Nurse brood facilitation of temporal founders with new life cycles, whether formed by mutation or by the expression of developmental plasticity, thus suggests an explanation for the remarkable tendency of *Magicicada* to repeatedly evolve species-pairs with the same

alternative life cycles (Alexander and Moore 1962), as well as the synchrony of species of the same life cycle.

Literature Cited

Alexander, R. D. 1967. Acoustical communication in arthropods. Annu. Rev. Entomol. 12:495-526.

Alexander, R. D. 1968. Life cycle origins, speciation, and related phenomena in crickets. Q. Rev. Biol. 43:1-41.

Alexander, R. D, and R. S. Bigelow. 1960. Allochronic speciation in field crickets, and a new species, *Acheta veletis*. Evolution 14:334-346.

Alexander, R. D., D. C. Marshall, and J. R. Cooley. 1997. Evolutionary prespecitives on insect mating. Pp. 4-31 *In:* Choe, J. C., and B. J. Crespi, eds. The evolution of mating systems in insects and arachnids. Cambridge Univ. Press, Cambridge.

Alexander, R. D., and T. E. Moore. 1958. Studies on the acoustical behavior of seventeen-year cicadas (Homoptera: Cicadidae: *Magicicada*). Ohio J. Sci. 58:107-127.

Alexander, R. D., and T. E. Moore. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species. (Homoptera: Cicadidae, *Magicicada*). Univ. Mich. Mus. Zool. Misc. Publ. 121.

Arnold, M. 1997. Natural hybridization and evolution. Oxford, New York.

Beamer, R. H. 1931. Notes on the 17-year cicada in Kansas. J. Kans. Entomol. Soc. 4:53-58.

Blair, W. F. 1955. Mating call and stage of speciation in the *Microhyla olivacea- M. carolinensis* complex. Evolution 9:469-480.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. Syst. Zool. 5:49-64.

Bush, G. L. 1992. Host race formation and sympatric speciation in *Rhagoletis* fruit flies (Diptera: Tephritidae). Psyche 99:335-357.

Butlin, R. K. 1987. Speciation by reinforcement. Trends Ecol. Evol. 2:8-13.

Butlin, R. K. 1989. Reinforcement of premating isolation. Pp. 158-179 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Butlin, R. K. 1995. Reinforcement: an idea evolving. Trends Ecol. Evol. 10:432-434.

Cooley, J. R. 1999. Sexual behavior in North American cicadas of the genera *Magicicada* and *Okanagana*. Ph.D. Dissertation, The University of Michigan.

Coyne, J. A., and H. A. Orr. 1989. Patterns of speciation in *Drosophila*. Evolution 43:362-381.

Coyne, J. A., and H. A. Orr. 1997. “Patterns of speciation in *Drosophila*” revisited. Evolution 51:295-303.

de Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation. In: D. J. Howard and S. H. Berlocher, eds. Endless Forms: Species and Speciation. Oxford, New York.

Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. Nature (London) 400:354-357.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74:312-321.

Doolan, J. M., and D. Young. 1989. Relative importance of song parameters during flight phonotaxis and courtship in the bladder cicada *Cystosoma saundersii*. J. Exp. Biol. 141:113-131.

Dybas, H. S. 1969. The 17-year cicada: A four-year mistake? Field Mus. Nat. Hist. Bull. 40:10-12.

Dybas, H. S., and D. D. Davis. 1962. A population census of seventeen-year periodical cicadas (Homoptera: Cicadidae: *Magicicada*). Ecology 43:432-444.

Dybas, H. S., and M. Lloyd. 1974. The habitats of 17-year periodical cicadas (Homoptera: Cicadidae: *Magicicada* spp.). Ecol. Monogr. 44:279-324.

Fouquette, M. J. 1975. Speciation in chorus frogs. I. Reproductive character displacement in the *Pseudacris nigrita* complex. Syst. Zool. 24:16-22.

Gerhardt, H. C. 1994. Reproductive character displacement of female mate choice in the grey treefrog, *Hyla chrysoscelis*. Anim. Behav. 47:959-969.

Grant, P. 1972. Convergent and divergent character displacement. Biol. J. Linn. Soc. 4:39-68.

Grant, P. R., and Grant, B. R. 1992. Hybridization of bird species. Science 256: 193-197.

Harrison, R. G. 1979. Speciation in North American field crickets: Evidence from electrophoretic comparisons. Evolution 33:1009-1023.

Harrison, R. G., and S. M. Bogdanowicz. 1995. Mitochondrial DNA phylogeny of North American field crickets: perspectives on the evolution of life cycles, songs, and habitat associations. J. Evol. Biol. 8:209-232.

Howard, D. J. 1993. Reinforcement: Origin, dynamics, and fate of an evolutionary hypothesis. Pp. 46-69 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford, New York.

Kelly, J. K., and M. A. Noor. 1996. Speciation by reinforcement: A model derived from studies of *Drosophila*. Genetics 143:1485-1497.

Kondrashov A. S., and F. A. Kondrashov. 1999. Interactions among quantitative traits in the course of sympatric speciation. Nature (London) 400:351-354.

Kritsky, G., and S. Simon. 1996. The unexpected 1995 emergence of periodical cicadas (Homoptera: Cicadidae: *Magicicada*) in Ohio. Ohio J. Sci. 96:27-28.

Liou, L. W., and T. D. Price. 1994. Speciation by reinforcement of premating isolation. Evolution 48:1451-1459.

Littlejohn, M. J. 1965. Premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). Evolution 19:234-243.

Littlejohn, M. J., and J. J. Loftus-Hills. 1968. An experimental evaluation of premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). Evolution 22:659-663.

Lloyd, M., and H. S. Dybas. 1966. The periodical cicada problem. II. Evolution. Evolution 20:466-505.

Lloyd, M., and J. White. 1976. Sympatry of periodical cicada broods and the hypothetical four-year acceleration. Evolution 30:786-801.

Lloyd, M., G. Kritsky, and C. Simon. 1983. A simple Mendelian model for the 13- and 17-year life cycle of periodical cicadas, with historical evidence for hybridization between them. Evolution 37:1162-1180.

Loftus-Hills, J. J., and M. J. Littlejohn. 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *G. olivacea* (Anura: Microhylidae): A reexamination. Evolution 46:896-906.

Maier, C. T. 1982. Abundance and distribution of the seventeen-year periodical cicada, *Magicicada septendecim* (Linnaeus) (Hemiptera: Cicadidae - Brood II), in Connecticut. Proc. Entomol. Soc. Wash. 84:430-439.

Marlatt, C. L. 1923. The periodical cicada. U.S. Dept. Agric. Bur. Entomol. Bull. 71.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with a description of a new species, 13-year *Magicicada neotredecim*. Evolution in press (slated for August 2000).

Martin, A. P., and C. Simon. 1988. Anomalous distribution of nuclear and mitochondrial DNA markers in periodical cicadas. Nature 336:237-239.

Martin, A. P., and C. Simon. 1990. Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography. Evolution 44:1066-1080.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, MA.

Noor, M. A. 1995. Speciation driven by sexual selection in *Drosophila*. Nature 375:674-675.

Otte, D. 1989. Speciation in Hawaiian crickets. Pp. 482-526 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Paterson, H. E. H. 1993. Evolution and the recognition concept of species. Johns Hopkins Univ. Press, Baltimore, MD.

Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: What have we learned in 40 years? Evolution 47:1637-1653.

Simon, C. 1988. Evolution of 13- and 17-year periodical cicadas (Homoptera: Cicadidae: *Magicicada*). Bull. Entomol. Soc. Am. 34:163-176.

Simon, C., J. Tang, S. Dalwadi, G. Staley, J. Deniega, and T. Unnasch. 2000. Genetic evidence for assortative mating between 13-year cicadas and sympatric "17-year cicadas with 13-year life cycles" provides support for allochronic speciation. Evolution in press (slated for August 2000).

Tauber, C. A., and M. J. Tauber. 1977a. A genetic model for sympatric speciation through habitat diversification and seasonal isolation. Nature 268:702-705.

Tauber, C. A., and M. J. Tauber. 1977b. Sympatric speciation based on allele changes at three loci: evidence from natural populations in two habitats. Science 197:1298-1299.

Waage, J. K. 1979. Reproductive character displacement in *Calopteryx* (Odonata: Calopterygidae). Evolution 33:104-116.

Waddington, C. H. 1953. Genetic assimilation of an acquired character. Evolution 7:118-126.

Walker, T. J. 1974. Character displacement in acoustic insects. Am. Zool. 14:1137-1150.

Weber, T., T. E. Moore, F. Huber, and U. Klein. 1987. Sound production in periodical cicadas (Homoptera: Cicadidae: *Magicicada septendecim, M. cassini*). Pp. 329-336 *in* Proc. 6th Auchen. Mtg., Turin, Italy, 7-11 Sept. 1987.

Werren, J. H. 1998. *Wolbachia* and speciation. Pp. 245-260 in: D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, New York.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Q. Rev. Biol. 58:155-183.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. Syst. 20:249-278.

White, J., and M. Lloyd. 1975. Growth rates of 17- and 13-year periodical cicadas. Am. Midl. Nat. 94:127-143.

Williams, K. S., and C. Simon. 1995. The ecology, behavior, and evolution of periodical cicadas. Annu. Rev. Entomol. 40:269-295.

Williams, K. S., K. G. Smith, and F. M. Stephen. 1993. Emergence of 13-yr periodical cicadas (Cicadidae: *Magicicada*): phenology, mortality, and predator satiation. Ecology 74:1143-1152.

Young, D., and R. K. Josephson. 1983. Pure-tone songs in cicadas with special reference to the genus *Magicicada*. J. Comp. Physiol. 152:197-207.

Table 3.1. Traits distinguishing *Magicicada neotredecim* and other *Magicicada* species. "Pronotal extension" is the lateral extension of the pronotum behind the eye. For additional description and color photographs see Alexander and Moore 1962.

Species	Life Cycle (years)	Abdominal Sternite Color (each)	Dominant Call Pitch (kHz)	Pronotal Extension Color	Length of Call (seconds)
M. neotredecim Marshall and Cooley	13	orange with black lateral band or center	1.25 - 1.90	orange	1.5 - 4 [§]
M. tredecim (Walsh and Riley)	13	mostly orange	1.00 - 1.25	orange	1.5 - 4 [§]
M. septendecim (L.)	17	orange with black lateral band or center	1.25 - 1.50	orange	1.5 - 4 [§]
M. cassini (Fisher)	17	black, rarely with weak [††] orange lateral band	> 3.00	black	2 - 4 [§§]
M. tredecassini Alexander and Moore	13	black, rarely with weak [††] orange lateral band	> 3.00	black	2 - 4 [§§]
M. septendecula Alexander and Moore	17	black with orange lateral band	> 3.00	black	7 - 14 [†]
M. tredecula Alexander and Moore	13	black with orange lateral band	> 3.00	black	7 - 14 [†]

[§] Roughly pure-tone, musical buzz terminating in a noticeable drop in pitch; no ticks. Usually 2-3 calls between flights.

[§§] Rapid series of ticks followed by high-pitched, broad-spectrum buzz that rises and then falls in intensity and pitch. Usually 1-2 calls between flights.

[†] Repeated, rhythmic, high-pitched, broad-spectrum tick-buzz phrases, followed by repeated phrases containing only ticks. Usually 1 call between flights.

[††] Orange band, if present, often interrupted medially.

Table 3.2. Dates of 1998 chorus recordings by region.

Locations	Dates
Alabama, Kentucky, Mississippi, North Carolina, Tennessee:	
All sites	31 May - 3 June
Arkansas:	
Clark and Pike Counties	31 May
Sharp, Fulton and Lawrence Counties	12 - 25 May
Other sites	28 May
Illinois:	
Randolph, Monroe, Jersey, Sangamon and Piatt Counties	29 - 30 May
Other sites	9 - 14 June
Maryland:	
All sites	29 May - 1 June
Missouri:	
All sites	1 - 7 June

Table 3.3. Distinguishing 13-year -decim species by male abdomen color and call pitch; formal description of *M. neotredecim* is in Marshall and Cooley (2000). a) Kruskal-Wallis test, with call pitch as dependent variable, indicates overall relationship between pitch and abdomen coloration (test statistic = 45.969, $P < 0.001$); the break between the two species occurs in abdomen color class 3. b) Dividing the bimodal male -decim pitch sample by an intermediate pitch (1.4 kHz) yields two groups differing significantly in abdomen color (Mann-Whitney U = 3104.0, $P < 0.001$).

a)

Abdomen Color Class	Count	Call Pitch (mean ± SD)	Rank-Sum
1	11	1.73 ± 0.09	1138.5
2	103	1.70 ± 0.08	8863.5
3	15	1.46 ± 0.33	880
4	21	1.16 ± 0.20	443

b)

Dominant Call Pitch in kHz (mean ± SD)	Abdomen Color	Species Designation	n
1.10 (± 0.04)	3.69 (± 0.55)	*M. tredecim*	26
1.70 (± 0.07)	2.02 (± 0.48)	*M. neotredecim*	124

Table 3.4. Distinguishing 13-year -decim species by female abdomen color and call pitch preference; formal description of *M. neotredecim* is in Marshall and Cooley (2000). a) Kruskal-Wallis test, with pitch preference as dependent variable, indicates overall relationship between pitch preference and abdomen coloration (test statistic = 10.58, $P \leq 0.014$). b) Dividing the bimodal female -decim pitch preference sample (Fig. 3) by an intermediate pitch preference (1.4 kHz) yields two groups differing significantly in abdomen color (Mann-Whitney U = 317.000, $P \leq 0.001$).

a)

Abdomen Color Class	Count	Average Pitch Pref. (mean ± SD)	Rank-Sum
1	5	1.67 ± 0.15	136.5
2	22	1.69 ± 0.22	633.5
3	14	1.62 ± 0.26	341
4	7	1.28 ± 0.19	65

b)

Call Pitch Preference in kHz (mean, SD)	Abdomen Color	Species Designation	n
1.19 (± 0.06)	3.40 (± 0.84)	*M. tredecim*	10
1.72 (± 0.15)	2.24 (± 0.71)	*M. neotredecim*	38

Table 3.5. *M. neotredecim* populations sympatric with *M. tredecim* differ significantly in dominant chorus pitch from populations that are allopatric with *M. tredecim* (Mann-Whitney U = 21, $P \leq 0.001$) while no similar pattern appears within *M. tredecim* (U = 120.5, $P \leq 0.824$). The comparison is conservative because some apparently "allopatric" populations of *M. neotredecim* were recorded late in the emergence when cicadas were sparse and rare *M. tredecim* may have been missed.

	M. neotredecim		*M. tredecim*	
	Sympatry	Allopatry	Sympatry	Allopatry
Dominant Chorus Pitch in kHz (mean ± SD)	1.71 ± 0.05	1.52 ± 0.09	1.12 ± 0.03	1.12 ± 0.04
Range (kHz)	1.65 - 1.78	1.36 - 1.74 *	1.06 - 1.17	1.06 - 1.16
n	23	34	23	11

* Only two of the 34 allopatric *M. neotredecim* populations have dominant pitch values higher than 1.62 kHz, both from southern Missouri.

Figure 3.1. Distribution of the seven periodical cicada (*Magicicada)* species, including one new species described in Marshall and Cooley (2000). Ranges are summarized from county-level maps in Simon (1988) and from 1993-1998 field surveys in Illinois. The 17-year species are sympatric except in peripheral populations: *M. cassini* alone inhabits Oklahoma and Texas, while only *M. septendecim* is found in some northern populations (Dybas and Lloyd 1974). Two 13-year species (*M. tredecassini* and *M. tredecula*) are sympatric across the entire 13-year range, while the remaining 13-year species, *M. tredecim* and the new species *M. neotredecim*, overlap only in the central U.S. County-level maps overestimate distribution limits, hence the overlap between the 13- and 17-year populations is probably exaggerated. The overlap of *M. tredecim* and *M. neotredecim* is plotted from recent field surveys (this study; Simon et al. 2000). Characters distinguishing -decim species are noted; the -cassini and -decula siblings are distinguishable only by life cycle. "Call pitch" is dominant pitch of male call phrase; "mtDNA lineage" refers to types described in Martin and Simon (1990).

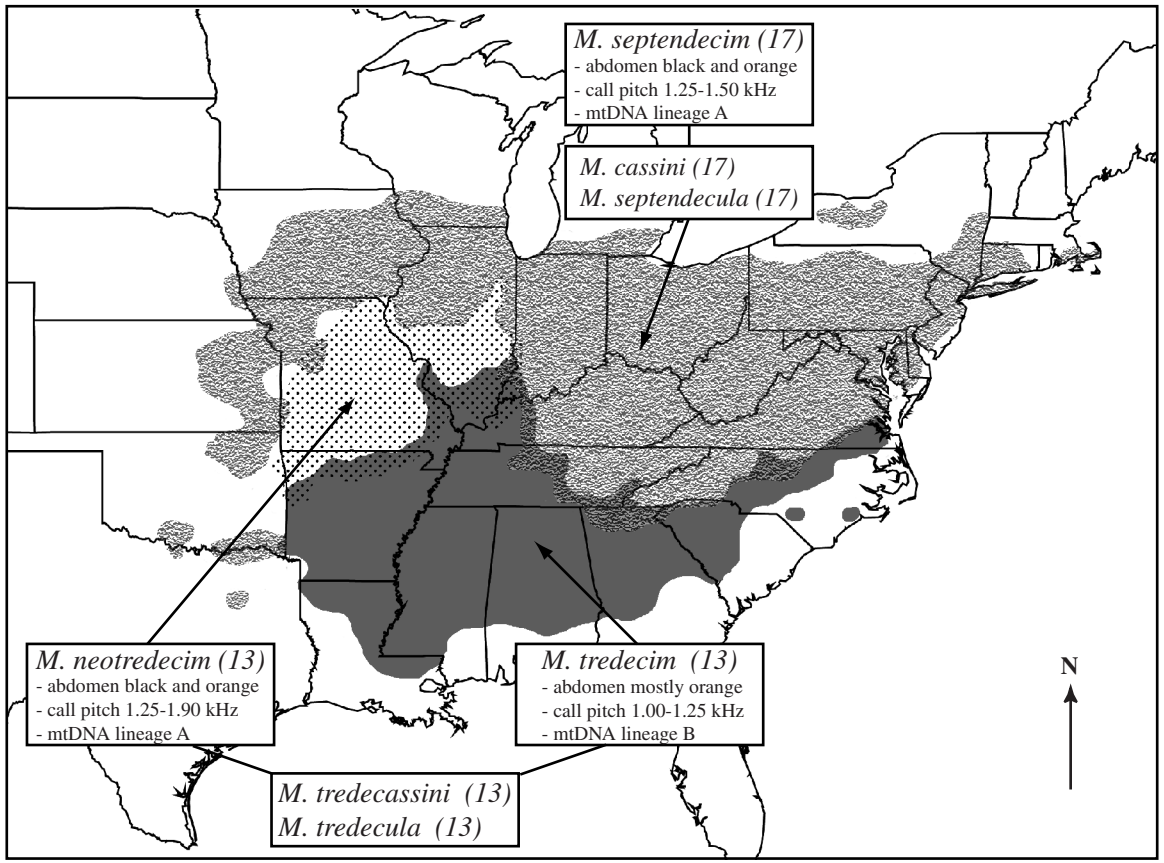


Figure 3.2. Spectrogram (power spectrum vs. time) showing a two-banded, mixed-species chorus of male -decim calls from Sharp Co., AR, with one call of each species standing out against the background chorus. Individual calls end with a downslur. Comparatively faint downslurs of background chorus males overlap and are not visible.

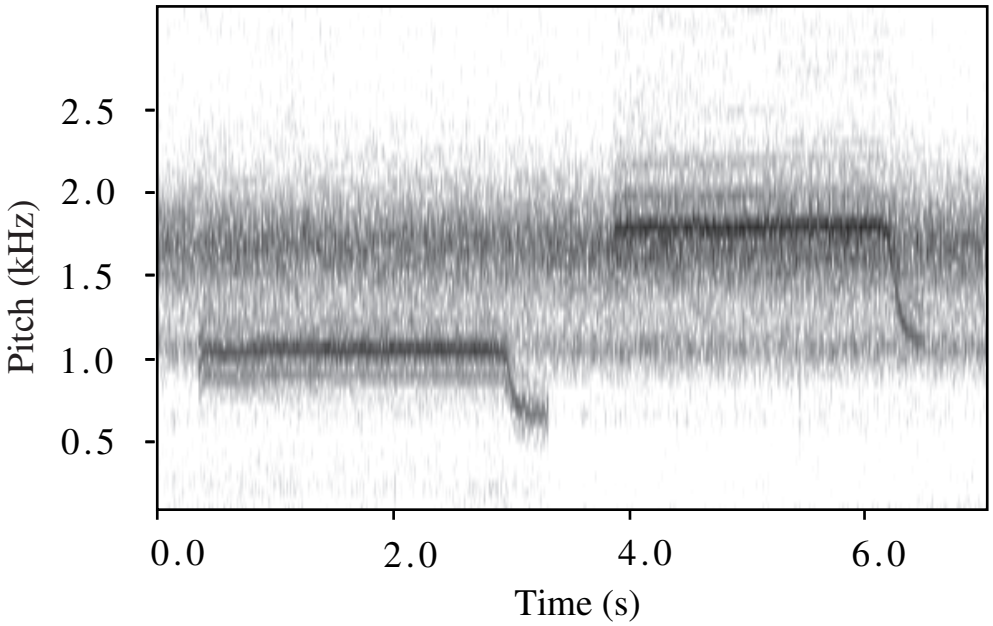


Figure 3.3. Power spectrum (shaded area) of mixed *M. neotredecim* and *M. tredecim* chorus at "powerline" study site, Sharp Co., AR, showing bimodal sound energy distribution with peaks at approximately 1.1 and 1.7 kHz. Accompanying frequency histograms are for male call pitches (black bars) and female average pitch preferences (white bars) of individuals collected at the site. Males were selected at random; females were selected for the playback experiment separately and with some bias toward the rarer species, which constitutes approximately 8% of the population.

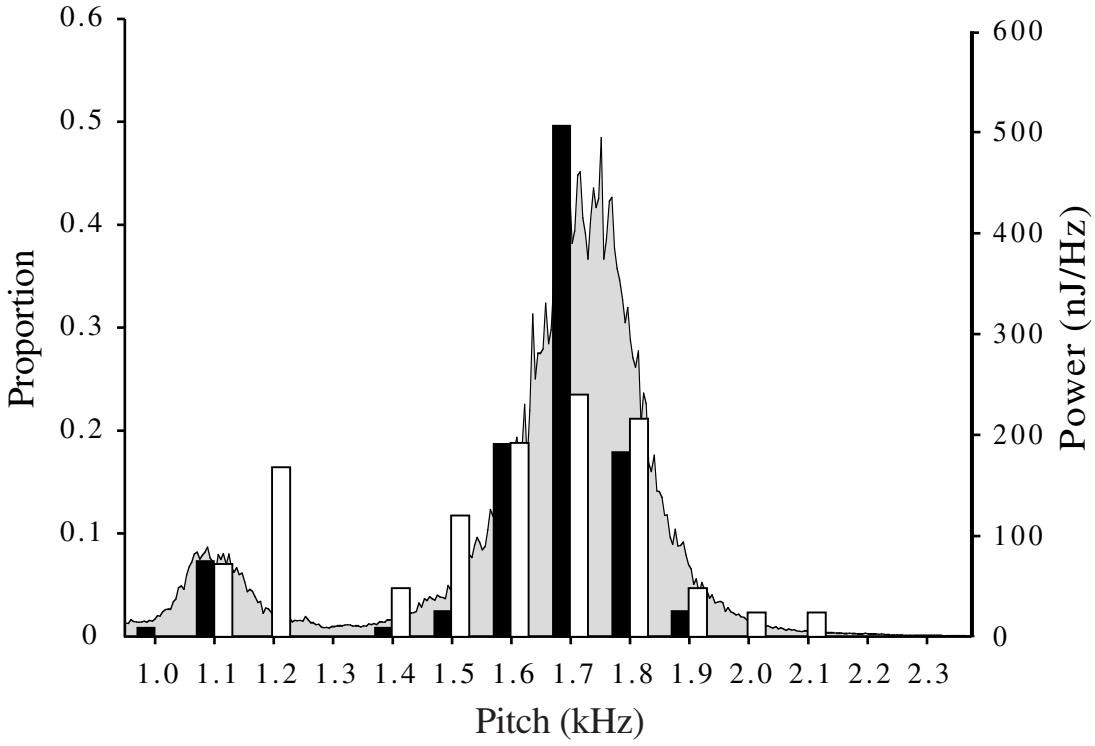


Figure 3.4. Relative proportions of *M. neotredecim* (black) and *M. tredecim* (white) estimated from chorus recordings of the 1998 emergence of 13-year Brood XIX.

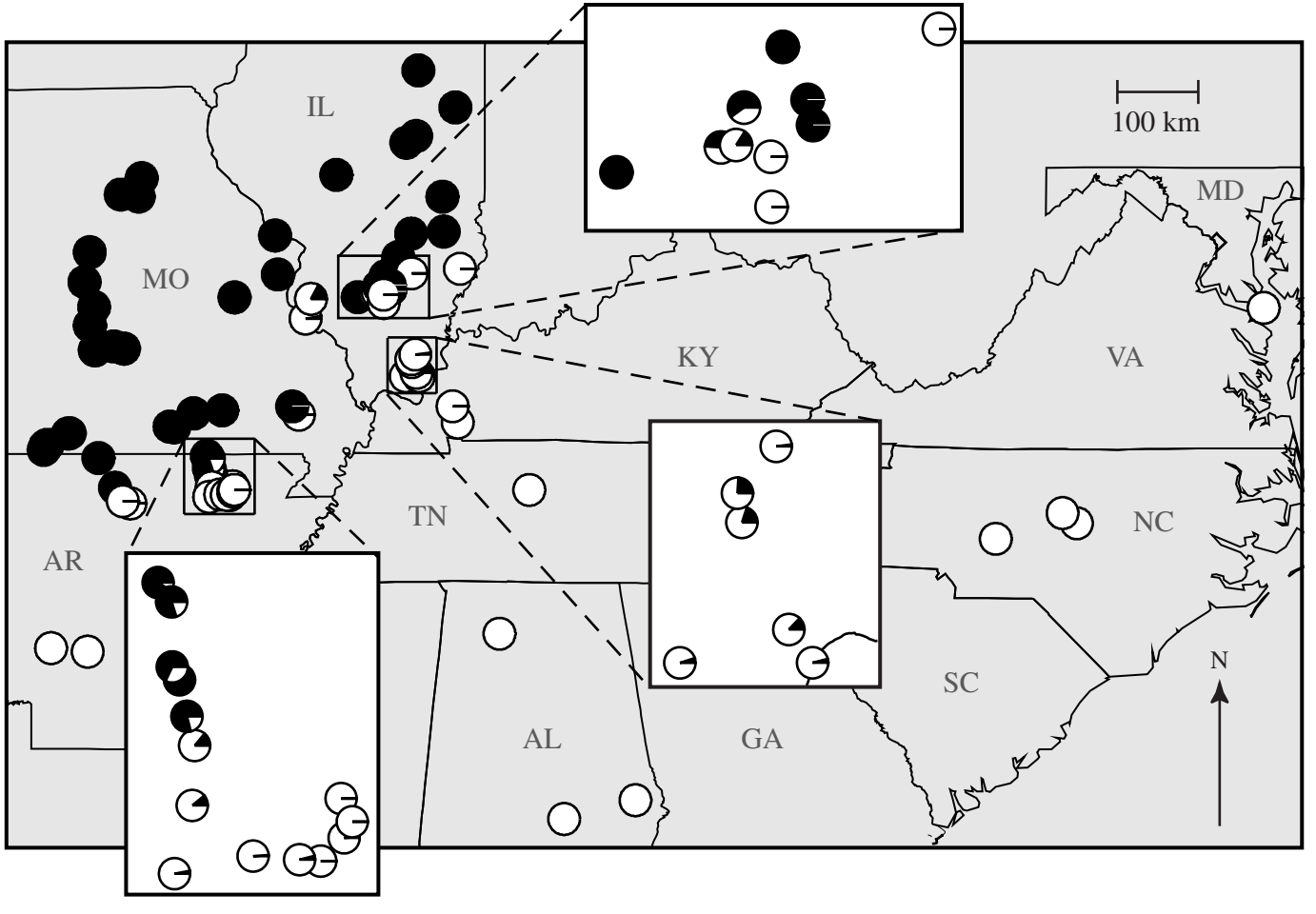


Figure 3.5. Geographic variation in dominant chorus pitch of *M. neotredecim*, showing higher-pitch calls in and near the region of overlap with *M. tredecim*. Lighter shaded circles indicate higher-pitch calls. Shaded region is approximate *M. tredecim* range. Weak choruses not plotted.

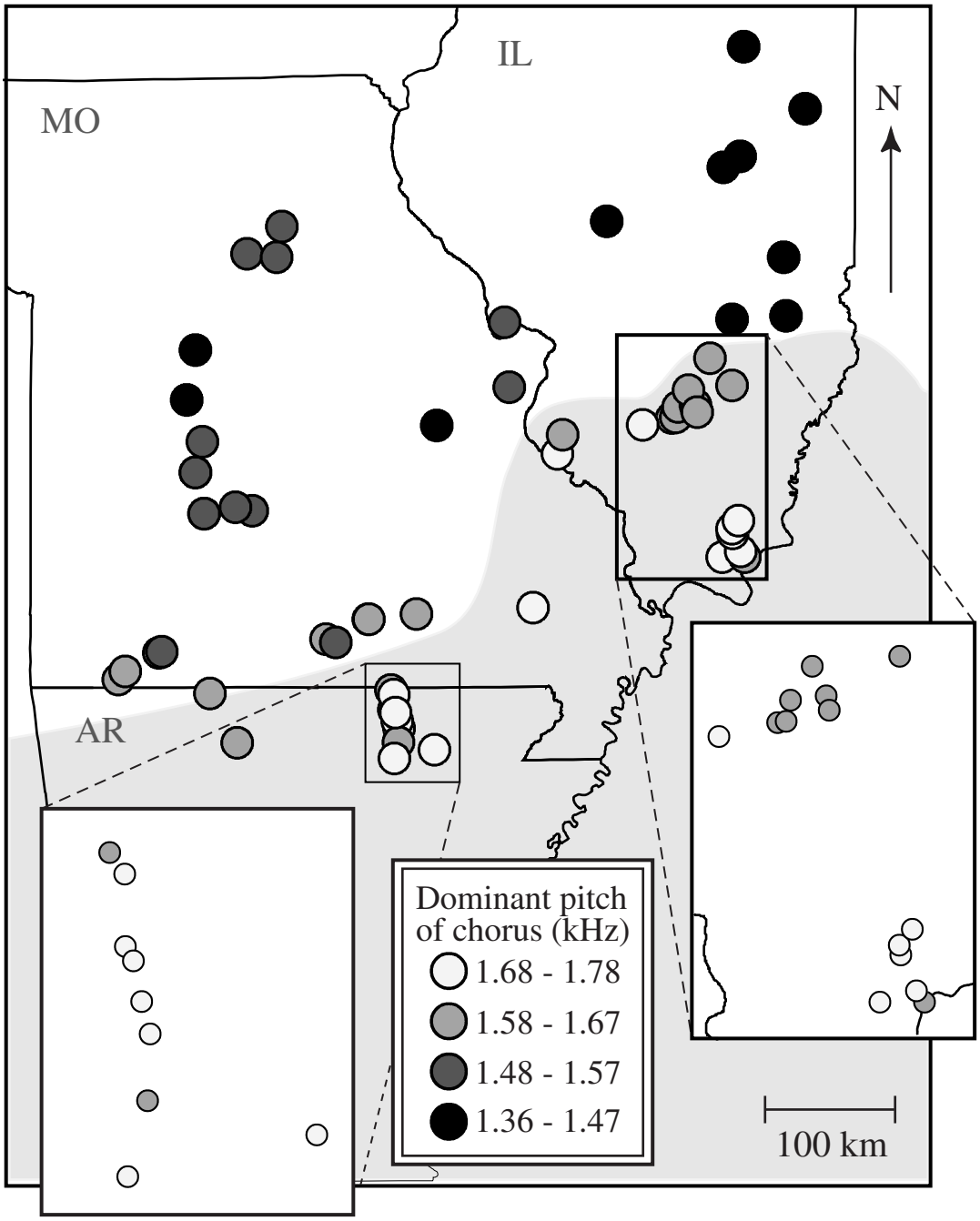


Figure 3.6. Geographic variation in dominant chorus pitch of *M. tredecim*, showing lower-pitch calls in sympatry with *M. neotredecim*. Lighter shaded circles indicate lower-pitch calls. Shaded area is approximate *M. neotredecim* range. Note that range of variation is only 1/4th of that shown in Fig. 3.5. Weak choruses not plotted.

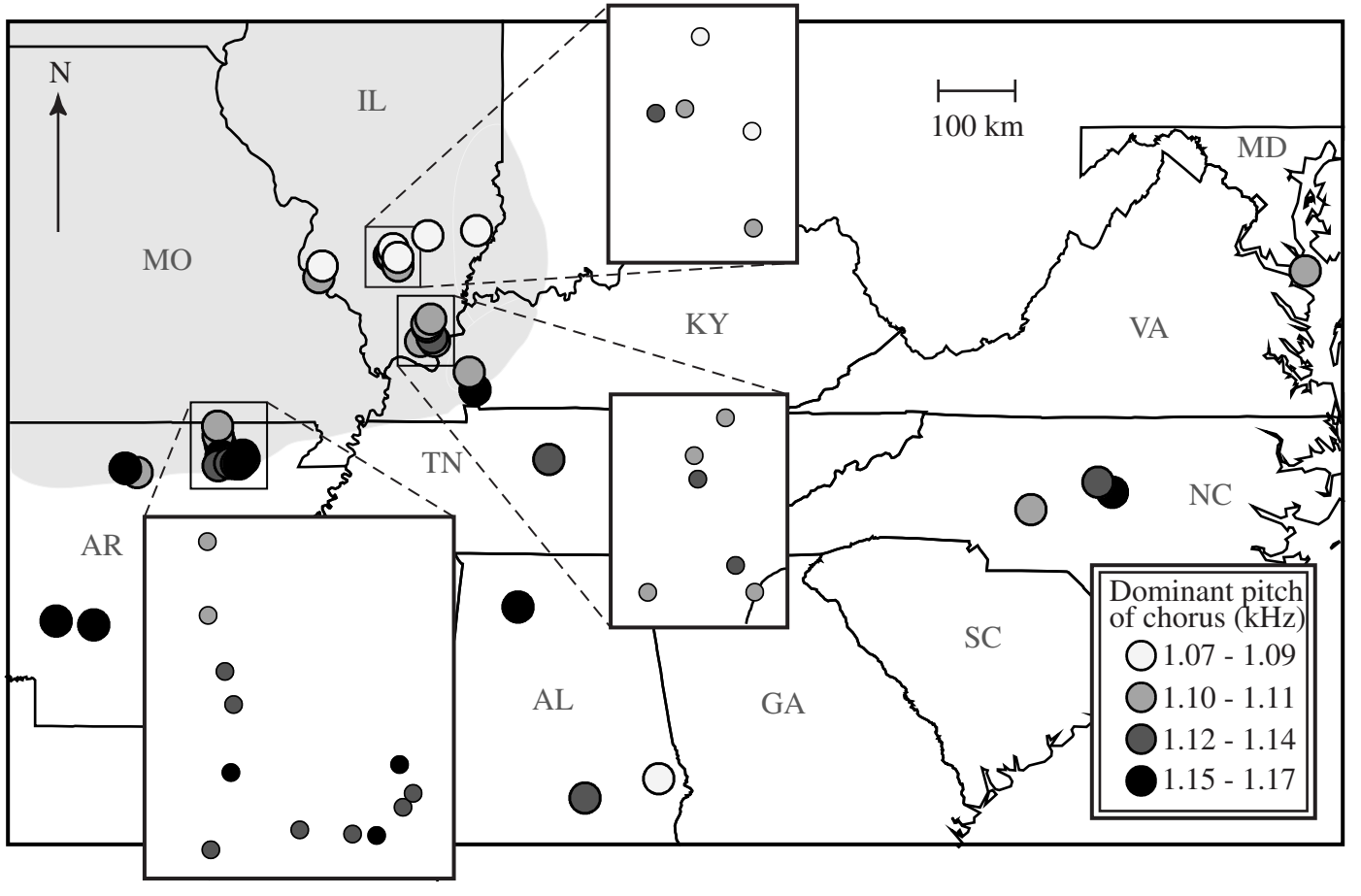


Figure 3.7. Box plots of male call pitch (A), right wing length (B), thorax width (C), and first sternite width (D) of *M. tredecim* ("T" -- Sharp Co., AR; n = 26), *M. neotredecim* in sympatry with *M. tredecim* ("NS" -- Sharp Co., AR; n = 61), and *M. neotredecim* in allopatry ("NA" -- Piatt Co., IL; n = 17). Shown for each sample are the median, the lower and upper hinges (first and third quartiles), the inner fences (± 1 step from hinge, a "step" = [1.5 * difference between hinges]), and outliers within (*) or beyond (o) the outer fence (± 2 steps from hinge). Male call pitch samples are significantly different ($P < 0.001$, Mann-Whitney) in all pairwise combinations, while size measurements show no significant differences within *M. neotredecim*. *M. tredecim* is significantly larger than *M. neotredecim* in all size traits (for each, $P < 0.001$; Mann-Whitney).

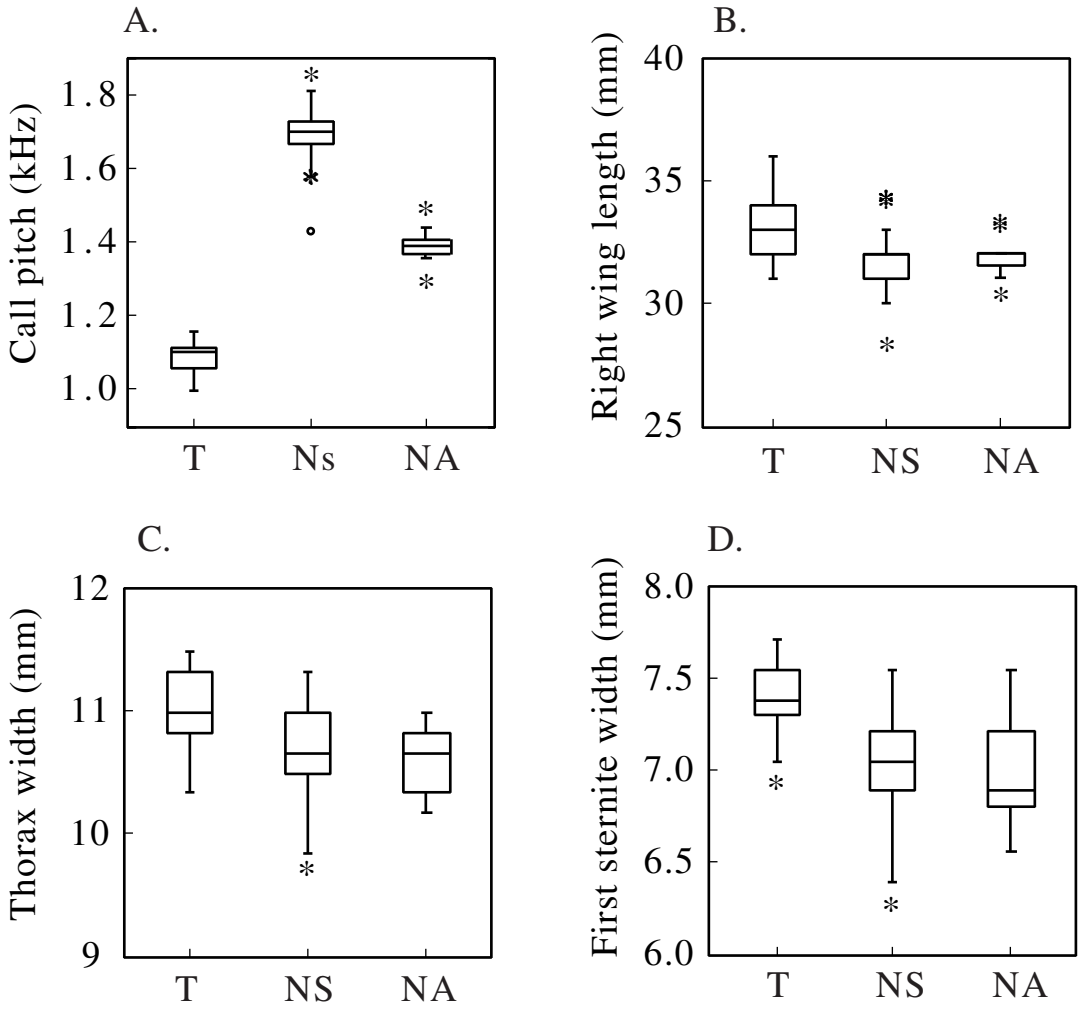


Figure 3.8. *M. neotredecim* and *M. tredecim* dominant chorus pitches plotted from ten recordings taken at different ambient temperatures in the same mixed-species chorus (H. G. Alexander WMA, Sharp Co., AR; May, 1998). Linear regression indicates no significant relationship between temperature and chorus pitch within either species (*M. neotredecim* r2 = 0.008, *P* 0.804; *M. tredecim* r2 = 0.15, *P* 0.274).

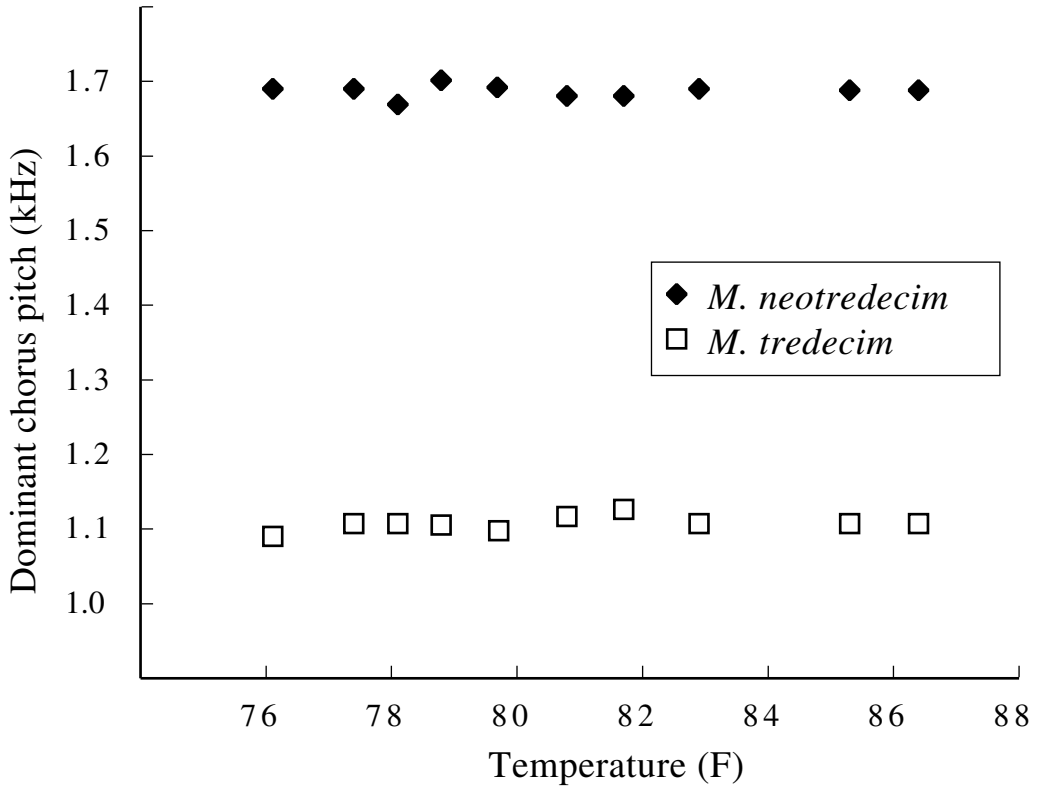


Figure 3.9. A model of *Magicicada* life cycle evolution via canalization of a climate-induced life cycle shift. Graph shows temporal change in a climate parameter such as temperature. Pie charts indicate proportion of cicadas emerging in 17 years (light) and 13 years (dark). During stage A, all cicadas emerge on a 17-year cycle but are capable of expressing life cycle length plasticity and emerging in 13 years under unusual climatic conditions. During stage B, the climate changes suddenly and dramatically such that the majority of cicadas are induced to express the 13-year cycle. During stage C, climatic conditions slowly ameliorate, imposing canalizing selection for the majority life cycle phenotype of 13 years because 17-year stragglers are never abundant enough to survive predation. By stage D, the population has evolved to express the new 13-year cycle even under the original conditions.

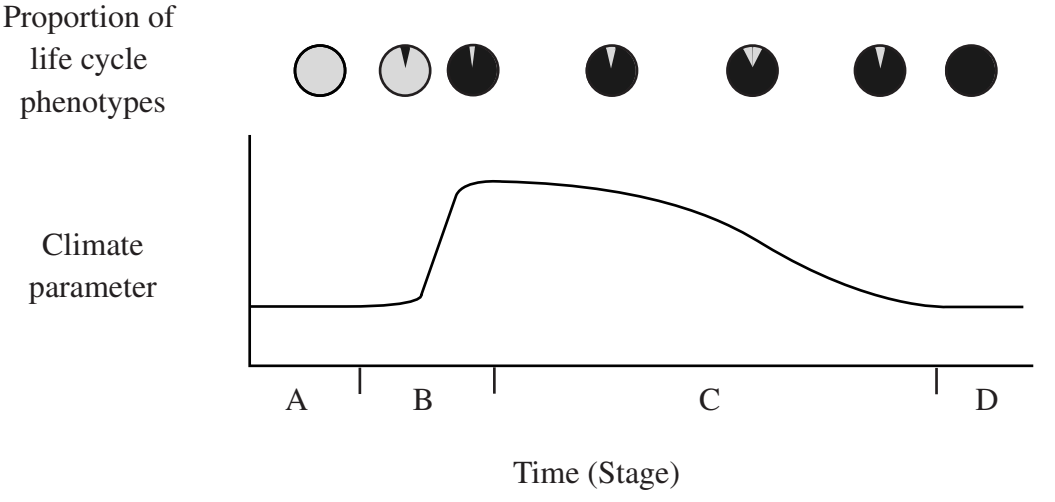


Figure 3.10. Formation of an incipient *Magicicada* species by "nurse brood facilitation" of life cycle variants (mutants or developmental variants). Three 17-year species each have a similar 13-year counterpart, but the 13-year counterpart of species 17 A is not present where the life cycle types overlap geographically. In this situation 13-year life cycle founders from 17 A (curved arrow) can establish a new incipient species (13 A') in the overlap zone if they emerge synchronously with the 13-year "nurse" brood (13 B + 13 C). Success of life cycle variants from 17 A is facilitated in the life cycle overlap zone because 13 B and 13 C provide the rare founders with numerical protection from predators. Rare life cycle variants from 17 B and 17 C are likely to be lost to interspecific hybridization with the similar and abundant 13 B and 13 C.

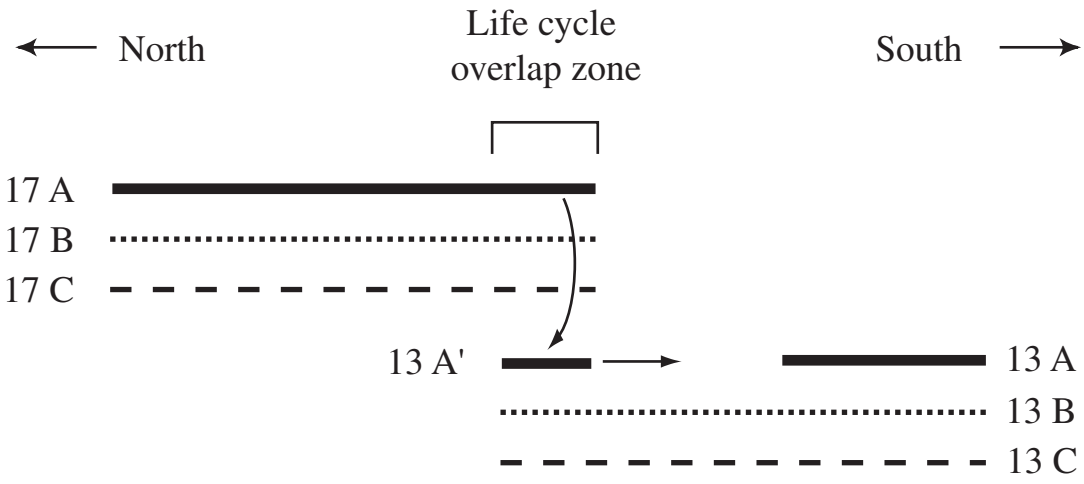
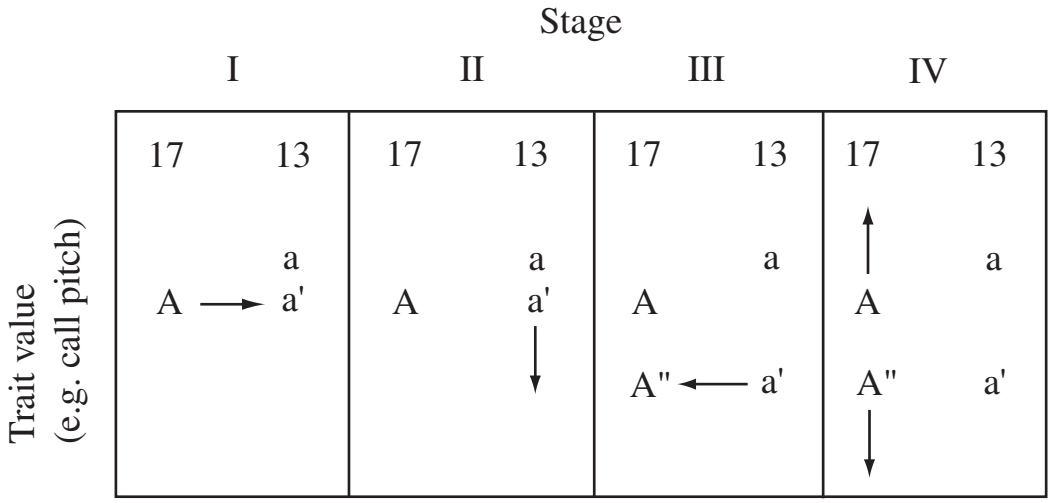


Figure 3.11. A model of *Magicicada* speciation involving nurse brood facilitation and reinforcement to explain pairs of similar life cycle cognates. Vertical dimension reflects degree of character divergence. I. Life cycle variants from 17-year species A join an overlapping, co-emerging 13-year brood, forming a'. II. Reinforcement occurs between a' and a in the 13-year brood. III. Life cycle variants from 13-year a' join the 17-year brood, forming A". IV. Reinforcement occurs between A and A" in the 17-year brood. Our data suggest that steps I and II may have occurred. Steps III and IV are plausible by extension of the model.



CHAPTER 4

GEOGRAPHIC VARIATION AND BIOGEOGRAPHY OF THE *MAGICICADA* –DECIM COMPLEX (*M. SEPTENDECIM, M. NEOTREDECIM, M. TREDECIM*)

Abstract

Recent discoveries in *Magicicada* systematics and biogeography have been based primarily on male calling songs, female song pitch preferences, and mtDNA haplotype. These characters have revealed a new 13-year species (*Magicicada neotredecim*), apparently derived from a 17-year relative (*Magicicada septendecim*) by a life cycle change, that has evolved reproductive character displacement in a calling song trait upon establishment of synchrony and sympatry with its closest 13-year relative, *M. tredecim*. New morphological data, primarily from abdomen color, indicate that (1) on average, *M. neotredecim* samples are indistinguishable from western populations of *M. septendecim* but distinct from eastern populations, supporting the hypothesis of a recent Midwestern origin for the new species; (2) *M. neotredecim* and *M. tredecim* are not more similar in sympatry than in allopatry, suggesting that little gene flow has occurred between these species during their evolutionary interaction. The new data are consistent with prior estimates of the

ranges of each –decim species. No additional morphological traits useful for distinguishing the –decim species were identified.

Introduction

Recent studies of periodical cicada (*Magicicada* spp.; Table 1) male calling songs, female song pitch preferences, morphology, and mtDNA haplotypes has shown that Midwestern periodical cicadas once recognized as part of 13-year *Magicicada tredecim* instead belong to a fourth 13-year species, *M. neotredecim* (described in Marshall and Cooley 2000; see also Martin and Simon 1988, 1990; Simon et al. 2000). Like other *Magicicada*, the new species appears to be most closely related to a counterpart with the alternative life cycle, in this case 17-year *M. septendecim*. This discovery has led to modification of one of the intriguing general rules of *Magicicada* biogeography, that species with the same life cycle type share nearly coincident ranges: Geographic sampling of male song pitch throughout the 13-year range indicates that the two 13-year –decim[1] species overlap across only a small fraction of their ranges; *M. tredecim* inhabits the southeastern and south-central states and *M. neotredecim* inhabits the Midwestern states, with only a 50-150 km region of overlap extending from northern Arkansas into southern Illinois (Fig. 1 of Chapter 3). In sympatry, the –decim species are reproductively isolated in part by distinctive male calling song pitches and female song pitch preferences. Although these traits differ for all populations of *M. tredecim* and *M. neotredecim*, the difference is most pronounced within the overlap zone. Many populations of *M. neotredecim* outside the overlap zone have a calling song that is indistinguishable from that observed in *M. septendecim*, the species' closest relative. Marshall and Cooley (2000) attributed the higher

[1] For convenience the periodical cicada sibling species groups are named using the following shorthand: –decim: *M. septendecim* (17), *M. tredecim* (13), and *M. neotredecim* (13); –cassini: *M. cassini* (17) and *M. tredecassini* (13); –decula: *M. septendecula* (17) and *M. tredecula* (13).

song pitch of *M. neotredecim* populations in the overlap zone to selection for reinforced premating barriers to reduce wasteful heterospecific sexual interactions.

Most information on the geographic distributions of *M. neotredecim* and *M. tredecim* within Brood XIX[2], which contains most of the 13-year range, is based upon calling song data (Marshall and Cooley 2000), which can be gathered rapidly across a wide region. The only other traits known to distinguish these species are mtDNA haplotype and abdomen color, although the latter distinguishes only population averages, not individuals; the available data for these traits are limited to nine –decim populations sampled by Martin and Simon (1988). Published information on other morphological traits of the –decim species is limited to size measurements of individuals collected from Sharp Co., AR (both species) and Piatt Co., IL (*M. neotredecim* alone); these data show that *M. tredecim* are larger than *M. neotredecim* from both locations (Marshall and Cooley 2000).

Additional knowledge of geographic variation in abdomen color and other morphological traits would be valuable for several reasons. First, such information would further resolve the ranges of the 13-year –decim species, thereby increasing the accuracy and detail of hypotheses for the origin and histories of these species. Second, additional geographic sampling could determine whether the size difference between *M. tredecim* and *M. neotredecim* is consistent across populations, or it could reveal other as-yet-undetected differences.

Third, analysis of abdomen color across *M. septendecim* populations could test the hypothesis (Marshall and Cooley 2000, Simon et al. 2000) that *M. neotredecim* was formed recently from Midwestern populations of 17-year *M. septendecim*, after the post-Wisconsin distribution of *M. septendecim* had been established. Archie et al. (1985) found that allele frequencies at three allozyme loci consistently distinguish western *M.*

[2]Although species of the same life cycle emerge synchronously in any given location, the 13- and 17-year life cycle groups have become broken regionally into allochronic "broods". Each brood is given a Roman numeral indicating the timing of its emergence pattern relative to other same-cycle broods, from I-XVIII for 17-year broods and XVIII-XXX for 13-year broods. There are 12 extant broods of 17-year cicadas and three broods of 13-year cicadas (Simon 1988), so many year-classes are empty.

septendecim populations (Broods III and IV) from eastern *M. septendecim* populations (I, II, V, XIV), while Midwestern Brood XIII *M. septendecim* showed "unclear affinities". Morphometric analysis of wing vein traits (Simon 1983, 1990) also shows average differences between Brood XIII and the eastern Brood XIV. The available data on abdomen color in *M. septendecim* (Martin and Simon 1988) are few, but they weakly suggest that a similar pattern of geographic variation may exist in that trait. If *M. neotredecim* was recently formed *in situ* from Midwestern *M. septendecim* populations, the new 13-year species should be more similar in abdomen color to nearby *M. septendecim* populations than to distant, eastern populations.

Finally, additional data on geographic variation in abdomen color could help resolve the evolutionary processes underlying the remarkable geographic variation in *M. neotredecim* song pitch. Recent discussions of reinforcing selection (e.g. Butlin 1989, Howard 1993) recognize a distinction between (1) selection that derives from competitive inferiority of hybrids that achieve partial reproductive success, and (2) selection that derives from complete sterility of hybrids or inefficiency of heterospecific sexual interactions that do not or cannot lead to fertilization. This distinction is based on two conceptual foundations: First, some regard selection against inferior but fertile hybrids as a "mechanism of speciation" because the resulting character evolution may effectively complete the genetic isolation of two lineages (Butlin 1987, 1989, 1995; Liou and Price 1994); by contrast, when hybrids are sterile, reinforcing selection is viewed as occurring after speciation (genetic isolation of populations) is complete (but see Discussion). Second, theoretical models and laboratory selection experiments (Rice and Hostert 1993, Hostert 1997, Liou and Price 1994) suggest that reproductive character divergence is unlikely to evolve when gene flow is not greatly restricted, as in #1 above. Hybridization appears to be rare in the overlap zone today (Marshall and Cooley 2000, Simon et. al 2000); however, the absence of present-day hybridization does not prove that gene flow did not occur in earlier stages of the interaction between the species. Knowledge of

geographic variation in abdomen color in relation to the overlap zone could be used to determine the extent of current and/or past hybridization between *M. neotredecim* and *M. tredecim*: Although abdomen color is probably affected by selection of some sort, the trait seems unlikely to be involved in sexual interactions (described in Chapters 1, 2) and therefore unlikely to be affected by reinforcing selection. Thus, if substantial hybridization occurred between these species early in their interaction, populations of the two species in sympatry should be more similar in abdomen color than allopatric populations.

Materials and Methods

Specimens used

Pinned specimens of all three *Magicicada* –decim species were obtained from museum collections across the eastern United States (Table 2). Specimens in alcohol were obtained from the University of Michigan Museum of Zoology and the Chicago Field Museum only, the latter contributed no pinned specimens. Collections were chosen with the intent of emphasizing the Mississippi Valley from Missouri to Louisiana, which contains the most northern and southern 13-year –decim populations as well as the *M. neotredecim*/M. *tredecim* overlap zone. Northern 13-year populations in the east (North Carolina) were also emphasized, in an attempt to maximize the chances of detecting *M. neotredecim* populations.

Martin and Simon (1988) originally described population abdomen color averages using males alone. Early in this study it became apparent that abdomen color could not be scored in both sexes with the same scale. Because of this, female specimens were excluded from all analyses.

Measurement techniques

Alcohol-preserved specimens **--** Male *Magicicada* specimens preserved in alcohol were subject to analysis of the morphological measurements listed in Table 3. All measurements were made with an ocular micrometer in a dissecting microscope at sharp focus and 25X magnification from a view directly above the specimen. In addition, each specimen was scored for abdomen color. Martin and Simon (1988) scored abdomen color in Brood X (17-year, *M. septendecim*) and XIX (13-year) using a discrete scale with 1 representing "mostly black" abdominal sternites and 4 representing "all orange" sternites. However, even though specimens in this study were obtained from the same broods and general localities studied by Martin and Simon (1988), no specimens were ever observed to have a "mostly black" abdomen color phenotype; this raises the problem of repeatability and comparability of judgments. –Decim males with the darkest abdomens have sternites with approximately 50% black color, while the lightest-colored specimens have sternites that are entirely orange. Additionally, the lightest-colored specimens have lateral tergites that are entirely orange ventrally, while the darkest-colored specimens have lateral tergites that are nearly all black. To maximize repeatability of estimates of abdomen color, males in this study were scored using discrete, qualitative rules (Table 4) that partition the continuous range of abdomen color and remove the need for more subjective quantitative estimates. Condition of the abdominal sternites was used as the primary criterion; if a specimen was not easily scored using this criterion (as sometimes occurred with individuals having a marginal score of 3 based on sternite criteria), the condition of the lateral tergites was used to decide between the remaining two options.

Pinned specimens **--** Because pinned specimens are not flexible and are easily damaged by handling, pinned specimens were scored for abdomen color only, using the protocol described above for alcohol-preserved specimens.

Specimen identification

Because abdomen color score does not consistently distinguish individuals of the –decim species (all species contain individuals with scores of 3), populations were grouped according to species using prior range estimates based on mtDNA haplotype and calling song pitch (Martin and Simon 1988, Marshall and Cooley 2000, Simon et al. 2000). Specimens collected from populations located within the estimated 13-year –decim overlap zone could not be identified to species; data from these samples were mapped but not used further in analyses.

Analysis: Morphological differences between –decim species

Means from each morphological trait were compared across species using Mann-Whitney tests. Individual scores were inspected to determine if any variable consistently distinguished all members of the two species.

Analysis: Intraspecific variation in abdomen color

Testing the hypothesis of Midwestern derivation of M. neotredecim -- Intraspecific variation in abdomen color was analyzed in the pinned specimen dataset only. Because sample size varied greatly across populations, samples were combined by region and the resulting groups tested for differences in abdomen color. To determine if significant regional heterogeneity in abdomen color exists within *M. septendecim*, samples were lumped by state or by brood and tested for overall differences using a Kruskal-Wallis one-way ANOVA. Next, the *M. septendecim* specimens were lumped into "eastern" and "western" samples (see Results for details) approximating the geographic partition

described by Archie et al. (1985) using allozyme loci. *M. neotredecim* specimens from localities outside the estimated range of *M. tredecim* were combined to form the *M. neotredecim* sample; 13-year specimens from locations within the *M. neotredecim*/ *M. tredecim* overlap zone could not be used in this analysis because abdomen color alone is insufficient to distinguish all individuals of the two 13-year species. To determine if additional regional heterogeneity exists within either the eastern *M. septendecim* sample, the western *M. septendecim* sample, or the *M. neotredecim* sample, specimens of each group were again divided by brood or by state and tested for differences using a Kruskal-Wallis one-way ANOVA (for three or more groups) or a Mann-Whitney test (for two-group comparisons). Finally, the eastern *M. septendecim*, western *M. septendecim*, and *M. neotredecim* groups were tested for differences in abdomen color using Mann-Whitney tests.

Intraspecific variation of call pitch and abdomen color in M. neotredecim -- Marshall and Cooley (2000) noted that populations of *M. neotredecim* in allopatry do not exhibit uniform dominant chorus pitch; "intermediate" dominant chorus pitch was observed in populations throughout Missouri and in extreme western Illinois, while eastern Illinois populations exhibit a low dominant chorus pitch indistinguishable from that of many *M. septendecim* populations. No data were available at the time to determine if additional traits covary with the change in chorus pitch. To address this question, the *M. neotredecim* pinned specimen samples here were divided into two subgroups according to dominant chorus pitch, which were then tested for a difference in abdomen color using a Mann Whitney test.

Analysis: Abdomen color and interspecific gene flow between *M. neotredecim* and *M. tredecim*

Testing for gene flow effects on a population from sympatry in Sharp Co., AR **--** Interspecific hybridization of *M. neotredecim* and *M. tredecim* may be expected to reduce abdomen color differences between the species in sympatry. To test for the predicted geographic pattern within species, the abdomen color data from pinned specimens collected outside the overlap zone were used as estimates of pre-contact morphological population phenotypes for *M. neotredecim* and *M. tredecim*. These allopatric abdomen color samples were compared to a sample of each species (identified to species using calling songs) taken from the overlap zone in Sharp Co., AR in 1998 (Marshall and Cooley 2000), using a Mann-Whitney test.

Testing for gene flow effects on a population from sympatry in Union Co., IL **--** Evidence of interspecific hybridization may vary among locations within the *M. neotredecim*/ *M. tredecim* overlap zone. To reduce the likelihood that evidence of hybridization could be missed by focusing on the Arkansas locality, abdomen color variation was analyzed in a sample of 300 alcohol-preserved male 13-year –decim obtained from within the overlap zone at the Pine Hills region of Union Co., IL (ca. 250 km NW of the Sharp Co., AR, site). Because these preserved specimens could not be identified to species with certainty, a simple comparison of within-species variation in sympatry with that in allopatry could not be conducted. Instead, a resampling algorithm (Appendix C) designed in Think Pascal 4.0 (Macintosh) was employed to estimate the variance expected in a hypothetical mixed-species sample of the same mean and sample size drawn at random from the species in allopatry; interspecific hybridization is expected to reduce differences between species and therefore to reduce the variance observed in a mixed-species sample. If the *M. neotredecim* and *M. tredecim* populations present at the Union Co., IL, location

have the same distribution of abdomen color phenotypes as populations in the allopatric regions represented in the museum samples, the variance of the Union Co. sample should fall within the range of variances obtained by repeatedly resampling (from the allopatric datasets) mixed-species samples that have the same mean as the Union Co. sample.

Results

Morphological differences of the –decim species

Relatively few male *M. neotredecim* (n=21) and *M. tredecim* (n=43) were available in the UMMZ alcohol-preserved collection, compared to *M. septendecim* (n=136) (Table 5). The combined *M. tredecim* sample was significantly different from both *M. septendecim* and *M. neotredecim* in all traits measured, but only abdomen color showed consistently strong population differences between species. *M. neotredecim* and *M. septendecim* were distinguishable on average in abdomen color and thorax width only. Furthermore, none of the traits consistently distinguishes individuals of any species pair, and some of the significant differences between the lumped species samples disappear when only one or a few populations of *M. septendecim* are considered. For example, although *M. neotredecim* and *M. septendecim* differ on average in thorax width, the *M. neotredecim* sample is not different from the sample of 17 *M. septendecim* from Prince William County, VA.

Intraspecific variation in *M. septendecim* abdomen color

The pinned specimen collections contained 607 male –decim from 14 of the 15 *Magicicada* broods; specimens were lacking only from the westernmost *M. septendecim* populations (Brood IV) and from the southeastern range of *M. tredecim* (southeastern Brood XIX) (Table 6). Just two males with an abdomen color score of 4 (the most

common score for *M. tredecim*) were found from localities outside of the range of *M. tredecim* estimated by Marshall and Cooley (2000), one from Grafton, IL, and one from Pomme de Terre Lake S.P., MO; no males with scores of 2 or 1 (which are nearly always *M. neotredecim*) were found from localities outside the previously estimated ranges of *M. neotredecim* and *M. septendecim*. Individuals with scores of 1 and 3 were found across the ranges of both *M. neotredecim* and *M. septendecim*. None of the larger samples from outside the 13-year –decim overlap zone showed a bimodal distribution of abdomen color scores (e.g., scores 1 and 3 more common than 2, or 2 and 4 more common than 3), although it is admittedly difficult to detect bimodality with this variable.

On visual inspection, the geographic variation in abdomen color within *M. septendecim* (Fig. 1) appears to follow a pattern similar to that demonstrated by Archie et al. (1985) for allozyme frequencies, although the changes are of small magnitude. Abdomen color averages of samples from central Indiana eastward appear uniformly low (dark color), while west of this boundary the averages appear uniformly higher and generally similar to averages for *M. neotredecim* samples from central Illinois and Missouri, with little appearance of further geographic subdivision within each group. The western Indiana samples are all from Brood X, which was not studied by Archie et al. (1985). Because these Brood X samples appeared most similar to western *M. septendecim*, they were lumped with the samples from Broods III and XIII to form the "western *M. septendecim*" combined sample discussed below. Brood X specimens from east of Indiana were combined with specimens from the remaining eastern *M. septendecim* broods to form the "eastern *M. septendecim*" combined sample. Average differences are also apparent between Mississippi Valley *M. tredecim* and North Carolina *M. tredecim*, which were grouped separately for further analysis; the significance of this difference is unclear because of the lack of specimens from intervening areas.

The combined *M. septendecim* sample shows statistically significant geographic variation in abdomen color both among broods and among states (Table 7). This variation

appears to consist mainly of a general difference between eastern (more black) and "western" (more orange) *M. septendecim*: Neither of these subgroups alone shows significant variation among states or among broods (Table 7), and the two groups are significantly different from one another (Table 8). The *M. neotredecim* sample is also geographically uniform (Table 7), whether divided into two groups by state or into two groups by dominant chorus pitch ("intermediate" = MO specimens plus Grafton, IL, "low" = remaining IL specimens: Mann-Whitney U=1068, *P*=0.364); all *M. neotredecim* samples were collected from Brood XIX.

Pairwise comparisons of the regional groups suggest an affinity between *M. neotredecim* and western *M. septendecim* (Table 8). The *M. neotredecim* sample is statistically distinct in abdomen color from the combined *M. septendecim* sample and from the eastern *M. septendecim* subgroup, but not from the western *M. septendecim* subgroup. The *M. neotredecim* sample is also clearly distinct from the darkest-colored *M. tredecim* subsample, from North Carolina.

Abdomen color and interspecific gene flow

Testing the hypothesis of interspecific gene flow: Direct comparison of phenotypes from sympatry and allopatry

Comparison of *M. neotredecim* and *M. tredecim* from sympatry and allopatry yielded partially conflicting results (Table 9). *M. neotredecim* from Sharp Co., AR (in sympatry with *M. tredecim*) are significantly darker in abdomen color than the combined allopatric *M. neotredecim*, the opposite of the effect expected from hybridization with *M. tredecim*. The Sharp Co., AR, *M. tredecim* are significantly darker colored than the allopatric *M. tredecim* sample from the Mississippi Valley, a pattern predicted by the hybridization hypothesis, but they are not significantly different from the North Carolina allopatric *M. tredecim* sample. In addition, comparison of the abdomen color averages from the various

–decim groups (Fig. 2) shows that the difference between the Sharp Co., AR, *M. neotredecim* and *M. tredecim* averages (3.7 - 2.0 = 1.7) is approximately the same as the overall difference observed between the *M. neotredecim* and *M. tredecim* allopatric samples (3.9 - 2.3 = 1.6), suggesting no effect of sympatry.

Testing the hypothesis of interspecific gene flow: Resampling techniques

The Union Co., IL, alcohol-preserved collection contained 300 males with abdomen color phenotypes ranging from 1 to 4, a mean of 3.12, and a variance of 0.74 (Fig. 3). Because the MS and NC *M. tredecim* from outside the overlap zone exhibited significant differences in abdomen color, two separate simulations were completed to determine if the Pine Hills sample exhibits a variance different from that expected from a random mixture of the same mean drawn from *M. neotredecim* and *M. tredecim* samples from outside the zone of overlap.

Simulating mixed populations by resampling from allopatric M. neotredecim and NC allopatric M. tredecim datasets -- Because the average abdomen color of the *M. tredecim* museum sample from North Carolina was 3.6, and the average abdomen color of the allopatric *M. neotredecim* museum sample was 2.4, an average mixed sample of 300 (the Pine Hills sample size) with a mean of 3.12 (the Pine Hills average) drawn from these sources is expected to contain 104 (35%) *M. neotredecim* and 196 (65%) *M. tredecim*. For each simulation, the resampling algorithm drew 300 values from the observed allopatric *M. neotredecim* and NC *M. tredecim* samples (with replacement) in the above proportions and calculated the variance of the mixed sample; this simulation was repeated 10,000 times, and the resulting variance values were sorted to determine the frequency distribution of the variance statistic, shown in part A of Table 10. The observed variance of the Pine Hills collection, 0.74, is much greater than that expected

from a random mixture of the allopatric samples with the same mean (P<0.01), the opposite of that expected under the hypothesis of hybridization.

Simulation of mixed populations by resampling from allopatric M. neotredecim and MS allopatric M. tredecim datasets -- Because the average abdomen color of the Mississippi Valley *M. tredecim* was 3.9, and the average abdomen color of the allopatric *M. neotredecim* museum sample was 2.4, an average mixed sample of 300 with a mean of 3.12 drawn from these sources is expected to contain 149 (49.7%) *M. neotredecim* and 151 (50.3%) *M. tredecim*. Resampling of the source populations, completed 10,000 times, in these proportions yielded a variance statistic distributed as shown in part B of Table 10. The observed variance of the Union Co., IL, collection, 0.74, is not significantly different from that expected from a random mixture of the allopatric samples with the same mean (0.10 <P<0.20).

Discussion

Morphological differences between –decim species

The average differences observed between samples of *M. septendecim* and *M. tredecim* can be summarized by saying that *M. tredecim* individuals tend to be larger. Only one quantitative trait, the number of tymbal ribs, trends in the opposite direction. Individual *M. tredecim* calls are consistently lower in dominant pitch than those of *M. septendecim* (Marshall and Cooley 2000); in principle, one might expect that a decrease in the number of tymbal ribs could influence pulse rates in the calling song, and indirectly influence the dominant pitch. However, dominant pitch of the *Magicicada* calling song is primarily influenced by properties of the resonating abdominal air sac (Young and Josephson 1983; Weber et al. 1987), and the morphological difference measured (only 0.5

ribs on average, with considerable overlap) cannot alone explain a difference in call pitch that is consistent across individuals of the two species.

The *M. tredecim* sample from Hope, AR stands out from the remaining *M. tredecim*; individuals from this population tend to be more similar to *M. septendecim* than to other *M. tredecim* in all characters studied other than abdomen color, yet this locality is at least 80 km from the nearest known *M. septendecim* or *M. neotredecim* population (Simon 1988). In a few traits (wing length, abdomen width) the Hope, AR, cicadas are even smaller on average than the combined *M. septendecim* sample. This may be related to the fact that the Hope cicadas were recorded as "Brood XXIV", a year-class not currently recognized as containing any *Magicicada* populations (Simon 1988) -- because only Brood XXIII is otherwise known from the area, these cicadas were probably Brood XXIII individuals that emerged one year late as 14-year-old "stragglers" (see Marshall ms and Chapter 6). Although many causes for straggling have been proposed (see Alexander and Moore 1962, Lloyd and Dybas 1966), little is known about the phenomenon, in part because stragglers appear erratically and usually in numbers too small for study. Perhaps the size difference observed here indicates that slow developmental rate was involved in triggering the inhibition of normal emergence in the 13th year.

The origin of *M. neotredecim*

Like all *Magicicada* species, *M. neotredecim* appears most closely related to a species with the alternative life cycle, 17-year *M. septendecim* (Fig. 4). Because these siblings are indistinguishable in traits other than life cycle length and geography, with no divergence in mtDNA haplotype (Martin and Simon 1988, 1990), the speciation event by which they are related probably occurred recently. In contrast, there is considerable sequence divergence (2.6%) between the *M. tredecim* and *M. septendecim* + *M. neotredecim* lineages, suggesting that either *M. neotredecim* or *M. septendecim* predates the most recent

(Wisconsin) glacial maximum. The available data are not yet sufficient to determine cladistically which life cycle, 13-year or 17-year, characterized the common ancestor of *M. neotredecim* + *M. septendecim* (or the ancestors of the other life-cycle-sibling pairs). In other words, it is difficult to determine which species gave rise to the other. Marshall and Cooley (2000) and Simon et al. (2000) argue that 13-year *M. neotredecim* may have been formed *in situ* from Midwestern populations of *M. septendecim* after the last (Wisconsin) glacial maximum. They support this hypothesis with biogeographic evidence including (1) the much larger distribution of *M. septendecim*, which surrounds *M. neotredecim* on three sides (see Fig. 1 of Chapter 3), and (2) the restriction of high-pitch (character-displaced) populations of *M. neotredecim* to the region of sympatry with its 13-year relative *M. tredecim*, which would be unlikely if both species have undergone the range shifts associated with glacial cycles.

The hypothesis of post-Wisconsin Midwestern derivation of *M. neotredecim* is consistent with data on geographic variation in allozymes (Archie et al. 1985) and abdomen color (this study). Both allozymes and abdomen color show average differences between eastern and western populations of *M. septendecim*, and in both cases *M. neotredecim* is more similar to the western populations. This inference is strengthened in both datasets by the observation that no additional significant variation appears within the eastern and western *M. septendecim* subgroups; this tends to diminish the alternative hypothesis that the geographic variation in color is due to selection gradients. The eastern *M. septendecim* subgroup in particular has a wide distribution within which one might expect considerable habitat variation. Nonetheless, because significant average habitat differences exist between the eastern and Midwestern United States, a more careful study of possible habitat effects on *Magicicada* abdomen coloration would be useful. It is possible that sample sizes in this study are too small to detect variations between broods or states, or that analysis on a more local scale would reveal habitat effects.

The evolutionary interaction of *M. neotredecim* and *M. tredecim*

The *Magicicada* literature is dotted with disputes over the species status of the life cycle siblings (e.g. Alexander and Moore 1962, Lloyd and Dybas 1966, Lloyd 1984, Lloyd and White 1976). The members of most sibling species pairs cannot be consistently distinguished absent knowledge of life cycle length and/or geography; the problem is exacerbated by occasional observations of apparent life cycle plasticity (e.g. Dybas 1969), and the fact that sibling populations do overlap to a minor degree (Simon 1988, Alexander et al. in prep.) and coemerge once every 221 years. The significance of previous demonstrations of crossmating by –decim life cycle siblings (Alexander 1968, Lloyd and Dybas 1966) in coemergence years is unclear because (1) both studies were conducted in the Midwest before *M. neotredecim* was identified, and (2) no study has followed hybrid offspring to adulthood.

Fortunately, the discovery of *M. neotredecim* and its relationship to *M. septendecim* has revealed a natural test of the potential evolutionary independence of *M. septendecim* and *M. tredecim*, in which the two lineages have been brought into lasting temporal and spatial contact. The outcome, reproductive character displacement (Marshall and Cooley 2000), suggests that *M. septendecim* and *M. tredecim* have diverged sufficiently to make future amalgamation unlikely, in effect confirming a prediction by Alexander and Moore (1962). The likely recent (post-Wisconsin) derivation of the new 13-year species is significant here because comparatively little additional time has passed since speciation from *M. septendecim* for *M. neotredecim* to diverge further from *M. tredecim* on its own, therefore differences between *M. septendecim* and *M. tredecim* are likely to be similar in magnitude to those between *M. neotredecim* and *M. tredecim*. The –decim example therefore suggests that isolation by combined allochrony/parapatry is sufficient to facilitate divergence. An intriguing follow-up question, raised by many prior authors, is what should be expected of different broods of the same life cycle, which also experience

temporal isolation and at least partial geographic isolation but for whom selection may be less divergent.

The pattern of reproductive character displacement (Brown and Wilson 1956, but sensu Loftus-Hills and Littlejohn 1992) does not alone indicate whether *M. neotredecim* and *M. tredecim* exchanged genes in the past or continue to do so today. Available data from this and prior work, although far from conclusive, suggest that populations of these species could have been genetically isolated from the time of first contact. Such isolation would have facilitated the divergence in sexual signals (Rice and Hostert 1993). There is little evidence of intermediate phenotypes in the overlap zone (Marshall and Cooley 2000, Simon et al. 2000), and there is no statistical relationship between call pitch and abdomen color within either species in sympatry in Sharp Co., AR (linear regression of DCM/JRC "powerline site" data from 1998), as might be expected if hybrids are being produced and successfully backcrossing in sympatry. Furthermore, although *M. tredecim* in sympatry with *M. neotredecim* are darker colored (more *M. neotredecim*-like) than are *M. tredecim* from allopatry in the Mississippi Valley, the absolute difference in average color between populations of the two species in sympatry is the same as the difference in average color between the allopatric samples. Computer resampling of the allopatric *M. neotredecim* and *M. tredecim* datasets suggests that the large –decim sample from sympatry in Union Co., IL does not show the reduced variance in abdomen color expected if hybridization and introgression had reduced differences between the species. The latter outcome is important because of the possibility that Union Co., IL, may be located closer to the site of first contact between *M. neotredecim* and *M. tredecim*, where one might be most likely to find evidence of past hybridization. Marshall and Cooley (2000) noted that the allopatric *M. neotredecim* populations exhibiting call pitches most similar to those of *M. septendecim* (the closest relative of *M. neotredecim*) are found in eastern Illinois. This suggests that the eastern Illinois *M. neotredecim* populations could be those with the history of least contact

with *M. tredecim*. If so, then *M. neotredecim* originated in eastern Illinois and spread south and west, likely contacting *M. tredecim* first in southern Illinois.

Post-contact range expansion of *M. neotredecim* and *M. tredecim*

Without additional information it is impossible to determine if the class 4 males from Grafton, IL (1), and Pomme de Terre Lake S.P., MO (1) (outside the estimates range of *M. tredecim*) are *M. tredecim*. Marshall and Cooley (pers. obs.) recorded extensively near Grafton in 1998 in strong choruses but did not observe a single *M. tredecim* call; in addition, rare *M. neotredecim* (identified using song or mtDNA) can be found with an abdomen score of 4 (Marshall and Cooley 2000, Simon et al. 2000). However, *M. neotredecim* choruses from both of these locations exhibit a dominant pitch that is somewhat higher than the pitch of *M. neotredecim* choruses in eastern Illinois or that of *M. septendecim* choruses, suggesting the possibility that rare or isolated *M. tredecim* may be present and driving minimal character displacement (Marshall and Cooley 2000). Other than these two possible discrepancies, the morphological data obtained in this study suggest no changes to the prior range estimates for *M. neotredecim* and *M. tredecim*.

The apparent absence of *M. neotredecim* from more northern 13-year regions of the southeast (see Fig. 1 of Chapter 3) is interesting given (1) the similarity of this species to 17-year *M. septendecim*, which is northern in distribution, (2) the extension of *M. neotredecim* far to the north in the Midwest, to less than 100 km south of Chicago, and (3) the widespread sympatry of other *Magicicada* species of the same life cycle. The two 13-year species show a maximum overlap today of about 150 km. Perhaps the best hypothesis to explain this pattern is that *M. neotredecim* has had insufficient time since its formation to disperse farther into the 13-year range to the southeast, and that dispersal continues today. The current degree of overlap between *M. neotredecim* and *M. tredecim* is reasonably consistent with this simple hypothesis, even when correcting for uncertainty

in estimates of the amount of overlap or the number of years elapsed since the two species became compatible in sympatry. Assuming that from 8,000-12,000 years has elapsed since *M. neotredecim* and *M. tredecim* could coexist in sympatry, and that from 50-150 km of *M. neotredecim* dispersal into sympatry has occurred (part of the overlap may be explained by reciprocal dispersal by *M. tredecim*), then *M. neotredecim* has been extending its range into that of *M. tredecim* by 0.05 - 0.24 km per generation. Although it is difficult to know the real-world significance of such numbers, they do not appear unreasonable for such mobile insects; one or both of these species may be continuing to diffuse into the other's range today. Furthermore, if this simple calculation is reasonable, then the hypothesis of post-Wisconsin Midwestern derivation of *M. neotredecim* would require modification if *M. neotredecim* were ever to be discovered in eastern (North Carolina) Brood XIX populations -- dispersal to such locations, even over a generous estimate of 12,000 years, would require approximately 1 km range expansion per generation.

In contrast, *M. tredecim* may be more likely to be influenced in its northward dispersal by habitat or climate-related factors, because of its probable long history as a southern species. The *M. tredecim* lineage almost certainly predates recent glacial cycles (Martin and Simon 1990), so there is little reason to invoke limitation of dispersal distance by time. The fact that *M. tredecim* reaches its northern limits along Mississippi Valley lowlands also suggests that its northern boundary may be ecologically determined; perhaps this northern boundary approximates the 13-/17-year life cycle boundary that existed prior to the formation of *M. neotredecim* from Midwestern *M. septendecim*?

If *M. neotredecim* is not currently expanding its range southeastward into that of *M. tredecim*, two interacting explanations may be involved. First, Mississippi Valley lowlands separate most, but not all, *M. neotredecim* populations from regions to the southeast. These habitats are unlike those of the main *M. neotredecim* range in Missouri and central Illinois and might present a barrier to *M. neotredecim* dispersal. The fact that these regions contain not Brood XIX (which includes most of the range of *M. neotredecim*) but Brood

XXIII suggests an additional temporal mechanism: Although *M. neotredecim* is found in the northern portion of Brood XXIII (southern Illinois and Indiana), dispersal from this region to the southeast would first require Brood XXIII *M. neotredecim* to join overlapping or parapatric Brood XIX populations to the east by way of a fortuitous and perhaps implausible four-year acceleration of development (13-year cicadas emerging in nine years).

Magicicada and the study of populations

The remarkable qualities of periodical cicadas present excellent opportunities for the study of populations. No other organisms are known to exhibit such varied forms of geographic and temporal mechanisms of population isolation -- strict parapatry and imperfect allochrony of sibling species (in most cases), near-perfect sympatry and synchrony of same-cycle species, near-perfect allochrony of broods of the same life cycle, and now partial overlap of synchronous, same-cycle 13-year species recently derived from parapatric siblings. The prominent male calling songs, species-specific within a brood, facilitate precise mapping and estimates of species range limits possible with few other organisms. Their long life cycles, while contrasting usefully with other insect populations and their short generation times, do present serious practical difficulties; at least these are alleviated by the allochrony of the many broods.

Literature Cited

Alexander, R. D. 1968. Arthropods. *In:* Sebeok, T. A., ed. Animal communication: Techniques of study and results of research. Bloomington: Indiana Univ. Press.

Alexander, R. D., and T. E. Moore. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species. (Homoptera: Cicadidae, *Magicicada*). Misc. Publ. no. 121. Univ. of Michigan Museum of Zoology, Ann Arbor, MI.

Alexander, R. D., Richards, A. F., Marshall, D. C., and J. R. Cooley. In prep. The microdistributional relationships of Brood III 17-year *Magicicada*.

Archie, J., C. Simon, and D. Wartenburg. 1985. Geographical patterns and population structure in periodical cicadas based on spatial analysis of allozyme frequencies. Evolution 39: 1261-1274.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. Syst. Zool. 5:49-64.

Butlin, R. K. 1987. Speciation by reinforcement. Trends Ecol. Evol. 2:8-13.

Butlin, R. K. 1989. Reinforcement of premating isolation. Pp. 158-179 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Butlin, R. K. 1995. Reinforcement: an idea evolving. Trends Ecol. Evol. 10:432-434.

Dybas, H. S. 1969. The 17-year cicada: A four-year mistake? Bull. Field Mus. Nat. Hist. 40:10-12.

Hostert, E. E. 1997. Reinforcement: A new perspective on an old controversy. Evolution. 51: 697-702.

Howard, D. J. 1993. Reinforcement: Origin, dynamics, and fate of an evolutionary hypothesis. Pp. 46-69 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford, New York.

Liou, L. W., and T. D. Price. 1994. Speciation by reinforcement of premating isolation. Evolution 48:1451-1459.

Lloyd, M. 1984. Periodical cicadas. Antenna 8: 79-91.

Lloyd, M., and H. S. Dybas. 1966. The periodical cicada problem. II. Evolution. Evolution 20:466-505.

Lloyd, M., and J. White. 1976. Sympatry of periodical cicada broods and the hypothetical four-year acceleration. Evolution 30:786-801.

Loftus-Hills, J. J., and *M.* J. Littlejohn. 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *G. olivacea* (Anura: Microhylidae): A reexamination. Evolution 46:896-906.

Marshall, D. C. ms. Periodical cicada (*Magicicada*) life cycle plasticity, the historical record, and the geographic stability of brood distributions. Accepted by Annals of the Entomological Society of America.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with a description of a new species, 13-year *Magicicada neotredecim*. Evolution in press (slated for August 2000).

Martin, A. P., and C. Simon. 1988. Anomalous distribution of nuclear and mitochondrial DNA markers in periodical cicadas. Nature 336:237-239.

Martin, A. P., and C. Simon. 1990. Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography. Evolution 44:1066-1080.

Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: What have we learned in 40 years? Evolution 47:1637-1653.

Simon, C., J. Tang, S. Dalwadi, G. Staley, J. Deniega, and T. Unnasch. 2000. Restricted gene flow between 13-year cicadas and coexisting "17-year cicadas with 13-year life cycles." Evolution in press (slated for August 2000).

Simon, C. 1983. Morphometric differentiation in wing venation among broods of 13- and 17-year periodical cicadas. Evolution 37: 104-115.

Simon, C. 1988. Evolution of 13- and 17-year periodical cicadas (Homoptera: Cicadidae: *Magicicada*). Bull. Entomol. Soc. Am. 34:163-176.

Simon, C. 1990. Discriminant analysis of year classes of periodical cicada based on wing morphometric data enhanced by molecular information. In: J. T. Sorensen and R. G. Footit (eds). Ordinations in the study of morphology, evolution, and systematics: applications and quantitative genetic rationales. Elsevier: Elsevier.

Weber, T., T. E. Moore, F. Huber, and U. Klein. 1987. Sound production in periodical cicadas (Homoptera: Cicadidae: *Magicicada septendecim, M. cassini*). Pp. 329-336 *in* Proc. 6th Auchen. Mtg., Turin, Italy, 7-11 Sept. 1987.

Williams, K. S., and C. Simon. 1995. The ecology, behavior, and evolution of periodical cicadas. Annu. Rev. Entomol. 40:269-295.

Young, D., and R. K. Josephson. 1983. Pure-tone songs in cicadas with special reference to the genus *Magicicada*. J. Comp. Physiol. 152:197-207.

Table 4.1. The periodical cicada complex.

Species	Life Cycle
Magicicada septendecim (L.)	17
Magicicada neotredecim Marshall and Cooley	13
Magicicada tredecim (Walsh and Riley)	13
Magicicada cassini (Fisher)	17
Magicicada tredecassini Alexander and Moore	13
Magicicada septendecula Alexander and Moore	17
Magicicada tredecula Alexander and Moore	13

Table 4.2. Sources of preserved *Magicicada* -decim specimens used in morphological analyses, and numbers of specimens obtained.

Collection	Pinned	Alcohol
Chicago Museum of Natural History		300
Illinois Natural History Survey	14	
Illinois State Museum	33	
Mississippi Entomological Museum	144	
North Carolina State University, Raleigh	31	
Southern Illinois University	49	
University of Kentucky, Lexington	33	
University of Michigan	254	200
University of Missouri, Columbia	49	

Table 4.3. Morphological traits measured in analysis of alcohol-preserved *Magicicada*.

Trait	Definition or Restrictions
# Ribs on Tymbal (L,R)	Counted only those ribs attached to margin at one or both ends. Any protrusion from margin counts as a rib.
Right wing length	Distance from base of wing articulation to tip of most distal vein
Eye span	Shortest distance between interior eye margins
Pronotal width	Maximum width of pronotal collar
Abdomen width	Lateral distance across abdomen at 1st abdominal segment

Table 4.4. Criteria used to determine abdomen color score.

Score	Abdominal sternites	Ventral third of tergites
1	Complete dark bands on all segments	Nearly entirely dark
2	Incomplete dark bands or centers on all segments	More dark than orange
3	Incomplete dark bands or centers on some segments	More orange than dark
4	No dark bands or centers on segments	Orange

Table 4.5. Morphological comparison of *M. septendecim* , *M. neotredecim*, and *M. tredecim* alcohol-preserved specimens by locality and by species. *P* values are for pairwise comparisons of combined species samples using a Mann-Whitney test. "ns" indicates *P* > 0.05

M. septendecim						Eyes	Abd. Width	Abd. Color	Ribs L	Ribs R	Thor. Width	Wing Length
	County	Locality	Date	Brood		mm	mm	Index	#	#	mm	mm
NJ	Union	Scotch Plains	1-Jun-96	II	Mean	4.4	10.6	1.7	12.0	12.0	7.9	35.3
					St. Dev.	0.1	0.2	0.6	0	0	0.1	0.6
					N	3	3	3	3	3	3	3
IL	Brown		27-May-63	III	Mean	4.3	10.2	2.3	12.0	11.9	7.6	34.0
					St. Dev.	0.1	0.3	0.5	0.5	0.5	0.3	1.2
					N	29	29	29	29	29	29	22
IL	McDonough	Fandon	7-Jun-80	III	Mean	4.3	10.1	2.1	11.9	11.8	7.6	32.6
					St. Dev.	0.2	0.5	0.4	0.6	0.4	0.3	2.0
					N	10	10	7	10	10	10	10
IL	Randolph	NE of Chester	May-63	III	Mean	4.2	9.8	2.0	12.5	12.0	7.3	34.0
					St. Dev.	0.2	0.8	0.0	0.7	0.0	0.5	1.4
					N	2	2	2	2	2	2	2
IL	Brown	Ripley	25-Jun-80	III	Mean	4.3	10.3	2.5	12.5	13.0	7.6	
					St. Dev.	0.1	0.5	0.7	0.7	0.0	0.6	
					N	2	2	2	2	2	2	
OH	Ross		15-May-91	XIV	Mean	4.3	10.5	2.1	11.7	11.7	7.6	33.9
					St. Dev.	0.2	0.4	0.3	0.5	0.4	0.3	1.1
					N	39	37	39	39	39	39	39
PA	Centre	Centre Hall	Jun-91	XIV	Mean	4.2	9.8	2.0	12.0	12.0	7.5	33.2
					St. Dev.	0.3	0.6	0.0	0.0	0.0	0.3	1.2
					N	6	6	6	6	6	6	6
VA	Alleghany	U.S. 60	23-Jun-61	I	Mean	4.2	9.8	1.9	12.2	12.2	7.4	33.2
					St. Dev.	0.2	0.5	0.4	0.4	0.5	0.4	1.8
					N	28	28	28	28	28	28	28
VA	Pr. Willm.	Bull Run Mtn.	11-Jun-62	II	Mean	4.4	10.5	2.3	12.4	12.3	7.8	34.1
					St. Dev.	0.2	0.5	0.6	0.6	0.7	0.3	1.4
					N	17	17	17	17	17	17	14

Table 4.5. (Continued).

M. tredecim						Eyes	Abd. Width	Abd. Color	Ribs L	Ribs R	Thor. Width	Wing Length
	County	Locality	Date	Brood		mm	mm	Index	#	#	mm	mm
AL	Pickens	Aliceville	18-May-72	XIX	Mean	4.7	11.3	4.0	12.0	12.0	8.3	
					St. Dev.							
					N	1	1	1	1	1	1	
AL	Chambers	SE Part	23-May-72	XIX	Mean	4.3	10.5	4.0	12.0	12.0	7.8	35.0
					St. Dev.							
					N	1	1	1	1	1	1	1
MO	Butler		15-May-59	XIX	Mean	4.5	10.9	3.9	11.6	11.5	8.1	36.1
					St. Dev.	0.2	0.3	0.3	0.5	0.5	0.3	1.3
					N	13	13	13	13	13	13	13
NC	Chatham		13-Jun-72	XIX	Mean	4.4	10.6	3.9	11.6	11.4	7.9	35.0
					St. Dev.	0.1	0.3	0.2	0.7	0.5	0.3	0.0
					N	9	9	9	9	9	9	1
TN	Davidson	Rt. 12	1972	XIX	Mean	4.7	10.6	4.0	11.3	11.3	8.1	36.5
					St. Dev.	0.0	0.3	0.0	0.6	0.6	0.1	0.7
					N	3	3	3	3	3	3	2
TN	Davidson	Nashville	1972	XIX	Mean	4.5	10.5	4.0	11.0	11.0	7.8	36.0
					St. Dev.							
					N	1	1	1	1	1	1	1
TN	Henry	Paris	28-May-76	XXIII	Mean	4.3	10.5	4.0	11.0	11.0	8.0	33.0
					St. Dev.							
					N	1	1	1	1	1	1	1
TN	Shelby		29-May-76	XXIII	Mean	4.6	11.2	3.5	11.5	11.0	8.0	36.5
					St. Dev.	0.1	0.5	0.7	0.7	0.0	0.2	0.7
					N	2	2	2	2	2	2	2
AL	*Hempstead*	*Hope*		*XXIV*	*Mean*	*4.4*	*9.7*	*3.3*	*11.8*	*11.8*	*7.7*	*33.2*
					St. Dev.	*0.1*	*1.6*	*0.5*	*0.4*	*0.4*	*0.3*	*1.1*
					N	*12*	*12*	*12*	*10*	*11*	*12*	*9*

Table 4.5. (Continued).

M. neotredecim					Eyes	Abd. Width	Abd. Color	Ribs L	Ribs R	Thor. Width	Wing Length
County	Locality	Date	Brood		mm	mm	Index	#	#	mm	mm
IL Jersey	Grafton	5-Jun-59	XIX	Mean	4.3	10.2	2.6	12.1	12.2	7.8	33.9
				St. Dev.	0.2	0.4	0.4	0.5	0.4	0.3	1.4
				N	21	21	21	21	21	21	18

Summary by species		Eyes	Abd. Width	Abd. Color	Ribs L	Ribs R	Thor. Width	Wing Length
		mm	mm	Index	#	#	mm	mm
M. septendecim combined	Mean	4.3	10.1	2.1	12.0	12.0	7.6	33.7
	St. Dev.	0.2	1.3	0.4	0.5	0.5	0.3	1.5
	N	136	136	133	136	136	136	124
M. tredecim combined	Mean	4.5	10.8	3.9	11.5	11.5	8.0	35.9
(except Hope, AR sample)	St. Dev.	0.2	0.4	0.3	0.6	0.5	0.3	1.3
	N	31	31	31	31	31	31	21
M. neotredecim combined	Mean	4.3	10.2	2.6	12.1	12.2	7.8	33.9
	St. Dev.	0.2	0.4	0.4	0.5	0.4	0.3	1.4
	N	21	21	21	21	21	21	18

Statistical comparison of species (Max. *P* value, Mann-Whitney test)	Eyes	Abd. Width	Abd. Color	Ribs L	Ribs R	Thor. Width	Wing Length
	mm	mm	Index	#	#	mm	mm
M. septendecim vs. *M. neotredecim*	ns	ns	0.001	ns	ns	0.030	ns
M. septendecim vs. *M. tredecim*	0.001	0.001	0.001	0.001	0.001	0.001	0.001
M. tredecim vs. *M. neotredecim*	0.021	0.001	0.001	0.001	0.001	0.003	0.001

Table 4.6. *Magicicada* -decim abdomen color data by locality from pinned museum specimens.

Brood	Location	State	Year	Mean	S. D.	N	Latitude	Longitude
I	Arcadia	VA	1978	1.8	0.6	12	37.545	79.624
I	Augusta Co.	VA	1978	2.0	0.0	2	38.167	79.117
I	Bath Co.	VA	1978	2.0	0.0	2	38.067	79.733
I	Bedford Co.	VA	1978	2.0	0.0	2	37.376	79.546
I	Rockbridge Co.	VA	1978	1.5	0.7	2	37.800	79.433
II	Milldale	CT	1979	1.6	0.6	5	41.566	72.892
II	Cleveland	GA	1962	2.0	0.0	1	34.597	83.763
II	Grasmere	NY	1979	1.4	0.6	5	40.604	74.088
II	Greene Co.	NY	1979	1.5	0.6	4	42.267	74.217
II	Saylorsburg	NY	1979	1.5	0.7	2		
II	Belfast	PA	1979	2.0	0.0	3	40.781	75.278
II	Lehigh Co.	PA	1979	1.7	0.6	3	40.750	75.625
II	Swatara Gap	PA	1979	1.3	0.5	4	40.479	76.529
II	Williamstown	PA	1979	1.5	0.6	4	40.580	76.618
II	Catharpin	VA	1979	1.7	0.6	20	38.854	77.572
III	Mt. Sterling	IL	1980	2.0	0.0	7	39.987	90.763
V	Chardon	OH	1965	1.8	0.8	5	41.614	81.149
V	Hocking Co.	OH	1982	2.0	0.5	20	39.517	82.489
V	Knox Co.	OH	1982	2.0	0.0	2	40.400	82.372
V	Mohican S.F.	OH	1982	1.8	0.5	5	40.611	82.313
V	Lehigh Gorge	PA	1982	1.7	0.6	3	40.868	75.736
V	Mauch Chunk Dam	PA	1982	1.5	0.6	6	40.847	75.792
VI	Walhalla	SC	1932	2.0	0.0	2	34.765	83.064
VII	Levanna	NY	1984	2.0	0.8	20	42.784	76.714
VII	Union Springs	NY	1967	1.7	0.6	3	42.840	76.694
VIII	Newell	WV	1968	1.6	0.5	16	40.618	80.604
IX	Jefferson	NC	1935	2.0	0.0	1	36.420	81.474
IX	Mercer Co.	WV	1986	1.3	0.6	3	37.400	81.117
X	Bloomington	IN	1953	2.3	0.5	12	39.165	86.526
X	Brown Co. S.P.	IN	1953	2.0	0.0	2	39.114	86.265
X	Marshall	IN	1936	3.0	0.0	3	39.848	87.188
X	Orange Co.	IN	1919	2.7	0.7	9	38.556	86.468
X	South part of state	IN	1936	2.0	0.0	1		
X	Louisville	KY	1902	2.0	0.0	1	38.254	85.759
X	Ann Arbor	MI	1936	1.8	0.7	20	42.271	83.726
X	Davie Co.	NC	1987	1.4	0.6	5	35.933	80.533
X	Princeton	NJ	1936	1.0	0.0	1	40.349	74.659
X	Knox Co.	TN	1919	2.0	0.0	1	36.017	83.933
XIII	Chicago	IL	1956	2.0	1.4	2	41.850	87.650
XIII	Des Plaines	IL	1973	2.3	0.7	20	42.033	87.883
XIII	Forest Park	IL	1973	2.5	0.7	2	41.879	87.814
XIII	Ravinia	IL	1905	2.5	0.7	2	42.164	87.787
XIII	Villa Park	IL	1973	2.0	0.8	4	41.890	87.989
XIII	Brodhead	WI	1922	3.0	0.0	1	42.618	89.376

Table 4.6. (Continued.)

Brood	Location	State	Year	Mean	S. D.	N	Latitude	Longitude
XIV	Corydon	IN	1991	1.9	0.4	8	38.212	86.122
XIV	Berea	KY	1923	2.0	0.0	1	37.569	84.296
XIV	McCreary Co.	KY	1991	1.6	0.6	5	36.733	84.467
XIV	Buncombe Co.	NC	1957	3.0	0.0	1	35.600	82.517
XIV	Edgemont	NC	1974	2.0	0.0	2	36.002	81.775
XIV	Mitchell Co.	NC	1957	2.0	0.0	1	36.017	82.133
XIV	Old Fort	NC	1974	2.0	0.0	1	35.629	82.181
XIV	Ft. Ancient	OH	1991	1.7	0.6	3	39.408	84.090
XIV	Scioto Co.	OH	1940	2.0	0.0	1	38.800	82.983
XIV	Cades Cove	TN	1940	2.0	0.0	1	35.608	83.826
XIX	Jackson Co.	AL	1959	3.5	0.7	2	34.767	86.000
XIX	Catoosa Co.	GA	1972	3.0	0.0	1	34.900	85.150
XIX	Champaign Co.	IL	1972	2.5	0.6	4	40.133	88.200
XIX	Effingham	IL	1985	2.0	0.0	1	39.120	88.543
XIX	Grafton	IL	1959	2.5	0.6	20	38.970	90.431
XIX	Mahomet	IL	1959	2.7	0.5	12	40.195	88.404
XIX	Monticello	IL	1959	2.0	0.0	1	40.028	88.573
XIX	Pope Co.	IL	1972	3.4	0.6	5	37.417	88.567
XIX	Sangamon Co.	IL	1972	2.4	0.6	17	39.767	89.650
XIX	Springfield	IL	1946	2.2	0.5	5	39.802	89.644
XIX	White Heath	IL	1933	3.0	0.0	1	40.086	88.513
XIX	Caldwell Co.	KY	1985	3.4	0.6	19	37.150	87.850
XIX	Cliff Cave	MO	1907	4.0	0.0	3	38.462	90.288
XIX	Columbia	MO	1972	2.0	0.8	4	38.952	92.334
XIX	Hannibal	MO	1972	3.0	0.0	1	39.708	91.358
XIX	New Franklin	MO	1972	3.0	0.0	1	39.017	92.737
XIX	Pomme de Terre S.P.	MO	1972	2.5	0.6	16	37.901	93.318
XIX	Ranken	MO	1933	2.5	0.7	2	38.535	90.512
XIX	Versailles	MO	1972	2.1	0.5	15	38.431	92.841
XIX	Zalma	MO	1972	3.5	0.7	2	37.145	90.076
XIX	Chapel Hill	NC	1972	3.0	0.0	1	35.943	82.709
XIX	Chatham Co.	NC	1985	3.3	0.6	3	35.700	79.267
XIX	Durham Co.	NC	1985	3.3	0.6	3	36.033	78.867
XIX	Franklin Co.	NC	1985	4.0	0.0	4	36.067	78.333
XIX	Halifax Co.	NC	1985	3.8	0.5	4	36.250	77.650
XIX	Hertford Co.	NC	1985	4.0	0.0	1	36.350	76.983
XIX	Moore Co.	NC	1985	4.0	0.0	1	35.300	79.483
XIX	Neuse R.	NC	1933	4.0	0.0	1		
XIX	Northampton Co.	NC	1985	3.0	0.0	1	36.417	77.367
XIX	Vance Co.	NC	1985	3.0	0.0	1	36.367	78.400
XIX	Williamsboro	NC	1972	3.5	0.7	2	36.430	78.432
XIX	Winton	NC	1972	3.5	0.7	2	36.396	76.932

Table 4.6. (Continued.)

Brood	Location	State	Year	Mean	S. D.	N	Latitude	Longitude
XXII	Baton Rouge	LA	1975	3.8	0.5	4	30.451	91.154
XXII	Cantwell	LA	1949	4.0	0.0	1	29.454	89.812
XXII	E. Feliciana Parish	LA	1975	4.0	0.0	2	30.850	91.050
XXII	Livingston Parish	LA	1975	4.0	0.0	1	30.450	90.767
XXII	W. Feliciana Parish	LA	1975	4.0	0.0	1	30.850	91.367
XXII	Bovina	MS	1923	4.0	0.0	20	32.352	90.735
XXII	Fayette	MS	1923	4.0	0.0	2	31.711	91.061
XXII	McNair	MS	1923	4.0	0.2	20	31.638	91.042
XXII	Meadville	MS	1923	4.0	0.0	3	31.472	90.897
XXII	Natchez	MS	1962	4.0	0.0	1	31.560	91.403
XXII	Pickneyville	MS	1923	3.5	0.6	4	31.050	91.487
XXII	Sibley	MS	1923	3.8	0.4	15	31.379	91.399
XXII	Stephenson	MS	1923	4.0	0.0	3	31.278	91.056
XXII	Tillman	MS	1923	4.0	0.0	3	31.854	90.916
XXII	Utica	MS	1923	4.0	0.0	6	32.109	90.623
XXIII	Jonesboro	AR	1963	4.0	0.0	1	35.842	90.704
XXIII	Carbondale	IL	1976	4.0	0.0	1	37.727	89.217
XXIII	Giant City	IL	1963	4.0	0.0	1	37.673	89.172
XXIII	Murphysboro	IL	1963	4.0	0.0	1	37.764	89.335
XXIII	Pine Hills	IL	1963	4.0	0.0	1	37.561	89.439
XXIII	Columbus	KY	1911	3.5	0.7	2	36.760	89.103
XXIII	Farmington	KY	1924	4.0	0.0	3	36.669	88.526
XXIII	Madisonville	KY	1911	3.5	0.7	2	37.328	87.499
XXIII	Bentonia	MS	1924	4.0	0.2	20	32.641	90.365
XXIII	Crystal Springs	MS	1976	3.0	0.0	1	31.987	90.357
XXIII	Dumas	MS	1924	3.3	0.5	6	34.640	88.844
XXIII	Mechanicsburg	MS	1924	4.0	0.0	16	32.633	90.503
XXIII	Phoenix	MS	1924	3.9	0.3	14	32.581	90.563
XXIII	Pine Valley	MS	1924	4.0	0.0	12	34.068	89.520
XXIII	Memphis	TN	1976	3.6	0.5	10	35.149	90.049

Table 4.7. Tests for geographic variation in abdomen color by state or by brood within (a) all *M. septendecim*, (b) eastern *M. septendecim*, (c) western *M. septendecim*, (d) *M. neotredecim*. Significant regional variation within *M. septendecim* disappears when the sample is divided into eastern and western subgroups, which are spatially homogeneous. *M. neotredecim* also shows no regional variation by state. All *M. neotredecim* specimens are from Brood XIX. The one Wisconsin *M. septendecim* was included with Illinois Brood XIII. Only states or broods with five specimens or more were included.

a) All *M. septendecim*

State	Mean	S.D.	N	Rank-Sum
CT	1.6	0.55	5	528
IL	2.2	0.66	37	6189
IN	2.2	0.50	33	5753
KY	1.9	0.49	7	831
MI	1.8	0.70	20	2515
NC	1.8	0.60	11	1419
NY	1.8	0.70	34	4139
OH	1.9	0.52	36	4940
PA	1.6	0.51	23	2335
VA	1.7	0.55	40	4758
WV	1.5	0.51	19	1844

Kruskal-Wallis statistic = 37.024, $P<0.001$

Brood	Mean	S.D.	N	Rank-Sum
I	1.8	0.52	20	2543
II	1.6	0.54	51	5269
III	2.0	0.00	7	1057
V	1.8	0.54	41	5333
VII	1.9	0.73	23	3156
VIII	1.6	0.51	16	1611
X	2.0	0.67	53	7908
XIII	2.3	0.73	31	5374
XIV	1.9	0.45	24	3262

Kruskal-Wallis statistic = 28.691, $P<0.001$

b) Eastern *M. septendecim* (excludes specimens from localities west of central Indiana)

State	Mean	S.D.	N	Rank-Sum
CT	1.6	0.55	5	454
IN	1.9	0.35	8	934
KY	1.7	0.49	7	711
MI	1.8	0.70	20	2112
NC	1.8	0.60	11	1198
NY	1.8	0.70	34	3481
OH	1.9	0.52	36	4169
PA	1.6	0.51	23	2011
VA	1.7	0.55	40	4048
WV	1.5	0.51	19	1591

Kruskal-Wallis statistic = 8.252, $P=0.509$

Brood	Mean	S.D.	N	Rank-Sum
I	1.8	0.52	20	2115
II	1.6	0.54	50	4307
V	1.8	0.54	41	4422
VII	1.9	0.73	23	2574
VIII	1.6	0.51	16	1361
X	1.7	0.67	26	2539
XIV	1.9	0.46	23	2584

Kruskal-Wallis statistic = 9.410, $P=0.152$

Table 4.7. (Continued.)

c) Western *M. septendecim*

State	Mean	S.D.	N	Rank-Sum
IL	2.2	0.66	38	1168
IN	2.4	0.49	25	849

Brood	Mean	S.D.	N	Rank-Sum
III	2.0	0.00	7	165
X	2.4	0.49	25	849
XIII	2.3	0.73	31	1003

Mann-Whitney U = 426.5, *P*=0.438

Kruskal-Wallis statistic = 2.332, *P*=0.312

d) *M. neotredecim*

State	Mean	S.D.	N	Rank-Sum
IL	2.5	0.57	61	3187
MO	2.3	0.63	38	1764

Kruskal-Wallis statistic = 1296, *P*=0.265

Table 4.8. Mann-Whitney comparisons of abdomen color scores of *Magicicada* -decim subgroups, (see text for definitions) using data from pinned museum specimens. The comparisons suggest an affinity between *M. neotredecim* and western *M. septendecim* populations, consistent with the hypothesis that *M. neotredecim* was derived from these populations and not from eastern *M. septendecim*. Differences between *M. tredecim* and *M. septendecim* groups are all highly significant ($P<0.001$) and are therefore not included in this table.

Group 1	Mean	S.D.	n	Group 2	Mean	S.D.	n	U	*P*
M. septendecim, east	1.7	0.57	209	*M. septendecim*, west	2.3	0.60	63	3819	<0.001
M. neotredecim	2.4	0.59	99	*M. septendecim*	1.9	0.62	272	7672	<0.001
M. neotredecim	2.4	0.59	99	*M. septendecim*, east	1.7	0.57	209	4920	<0.001
M. neotredecim	2.4	0.59	99	*M. septendecim*, west	2.3	0.60	63	3485	=0.153
M. neotredecim	2.4	0.59	99	*M. tredecim*, NC	3.6	0.51	25	262	<0.001
M. tredecim, NC	3.6	0.51	25	*M. tredecim*, MS	3.9	0.29	145	2448	<0.001

Table 4.9. Within-species comparisons (Mann-Whitney) of samples of *M. neotredecim* or *M. tredecim* from within and outside the zone of overlap of the two species. *M. neotredecim* from the overlap zone are not more similar to *M. tredecim* than are allopatric *M. neotredecim*, as might be expected if the two species hybridize in sympatry. One *M. tredecim* comparison suggests greater similarity to *M. neotredecim* in sympatry, the other comparison does not.

M. neotredecim

Sample	Mean	S.D.	N	U	*P*
Sympatry -- 1998, Sharp Co., AR	2.0	0.48	125	8436	< 0.001
Allopatry -- Museum samples	2.4	0.59	99		

M. tredecim

Sample	Mean	S.D.	N	U	*P*
Sympatry -- 1998, Sharp Co., AR	3.7	0.55	26	2230	= 0.008
Allopatry -- MS Museum samples	3.9	0.29	145		
Sympatry -- 1998, Sharp Co., AR	3.7	0.55	26	275	= 0.258
Allopatry -- NC Museum samples	3.6	0.51	25		

Table 4.10. Cumulative frequency distributions of population variance observed in simulated mixed-species samples formed by resampling allopatric *M. tredecim* and *M. neotredecim* datasets at random. (a) Distribution obtained using allopatric *M. neotredecim* and North Carolina allopatric *M. tredecim*. (b) Distribution obtained using allopatric *M. neotredecim* and Mississippi Valley allopatric *M. tredecim*.

(a)

Percentile	Variance statistic
0.01	0.50
0.05	0.52
0.10	0.53
0.20	0.55
0.30	0.56
0.40	0.57
0.50	0.58
0.60	0.59
0.80	0.60
0.80	0.61
0.90	0.63
0.95	0.64
0.99	0.67

(b)

Percentile	Variance statistic
0.01	0.69
0.05	0.71
0.10	0.73
0.20	0.75
0.30	0.76
0.40	0.77
0.50	0.78
0.60	0.79
0.70	0.80
0.80	0.81
0.90	0.83
0.95	0.85
0.99	0.88

Figure 4.1. Geographic variation in *Magicicada* -decim abdomen color. Background shading indicates ranges of *M. septendecim* (textured), *M. neotredecim* (dots), and *M. tredecim* (solid gray). Pie chart indicates average abdomen color score of sample; all white = 4.0 (all orange); all black =1.0 (50% orange). Sample sizes vary from 1 to 20; see Table 6 for details.

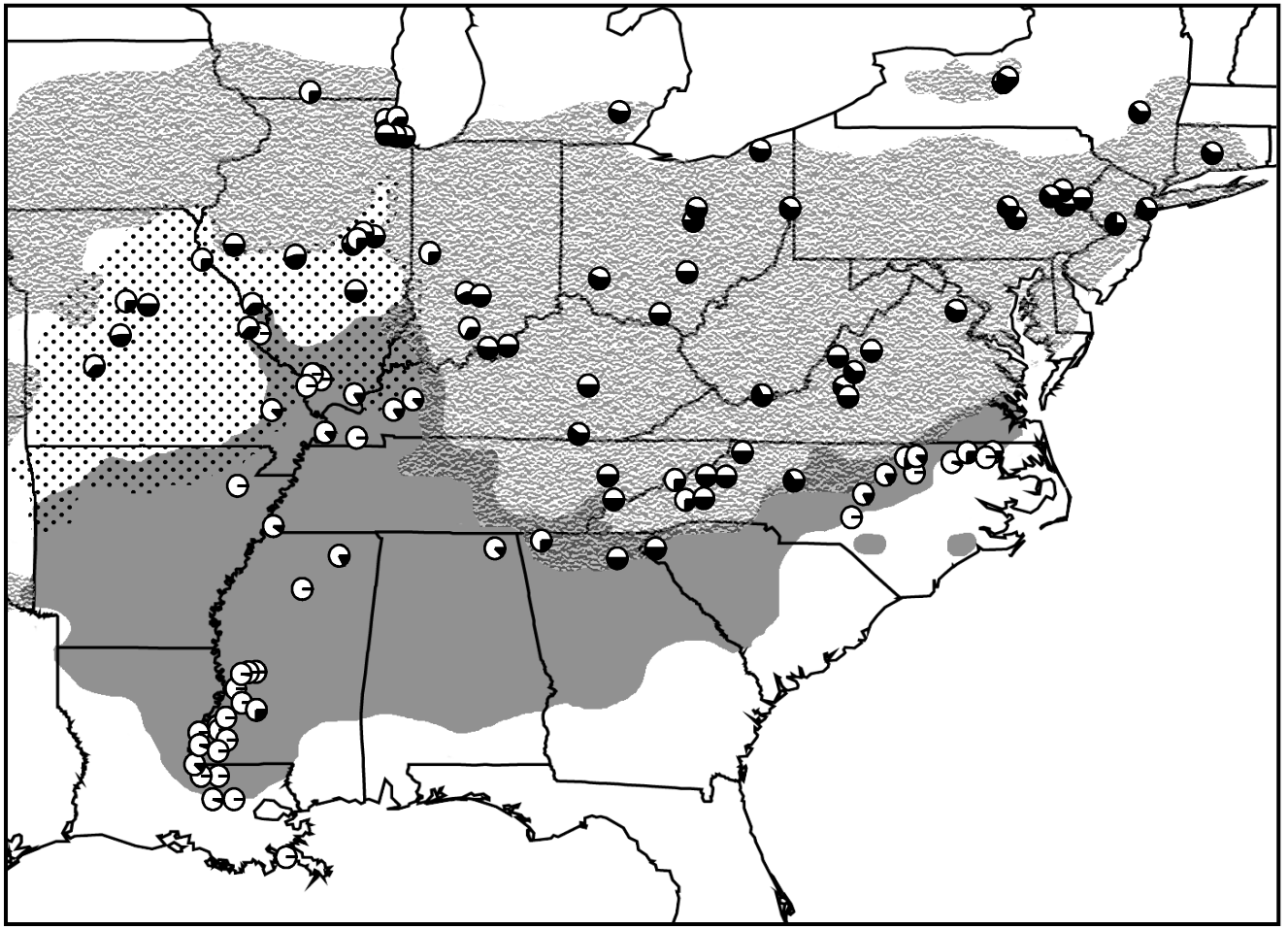


Figure 4.2. Comparison of average abdomen color scores of -decim whole-species samples (1 - *M. septendecim*, 4 - *M. neotredecim*, 9 - *M. tredecim*), regional combined samples (2 - eastern *M. septendecim*, 3 - western *M. septendecim*, 7 - Mississippi Valley *M. tredecim*, 8 - North Carolina *M. tredecim*), and sympatric *M. neotredecim* (5) and *M. tredecim* (6) from Sharp Co., Arkansas. The Sharp Co. samples are marked "S".

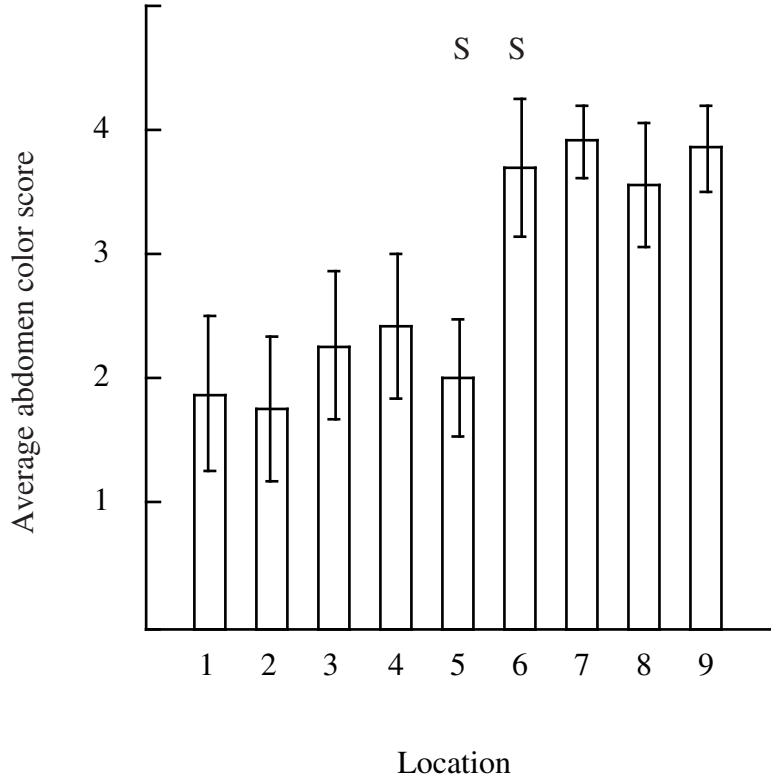


Figure 4.3. Histogram of abdomen color scores of 300 *Magicicada* -decim from Pine Hills, IL (Union Co.), within the overlap zone of 13-year *M. neotredecim* and *M. tredecim*.

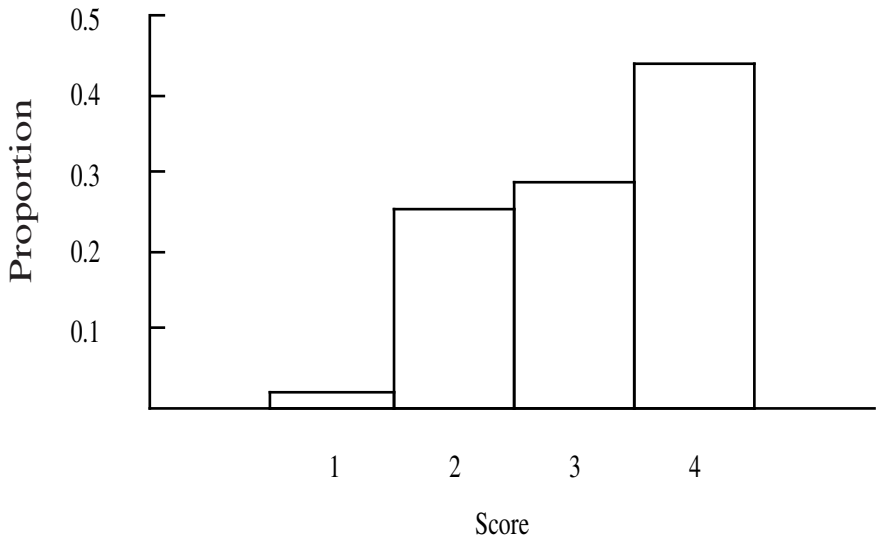
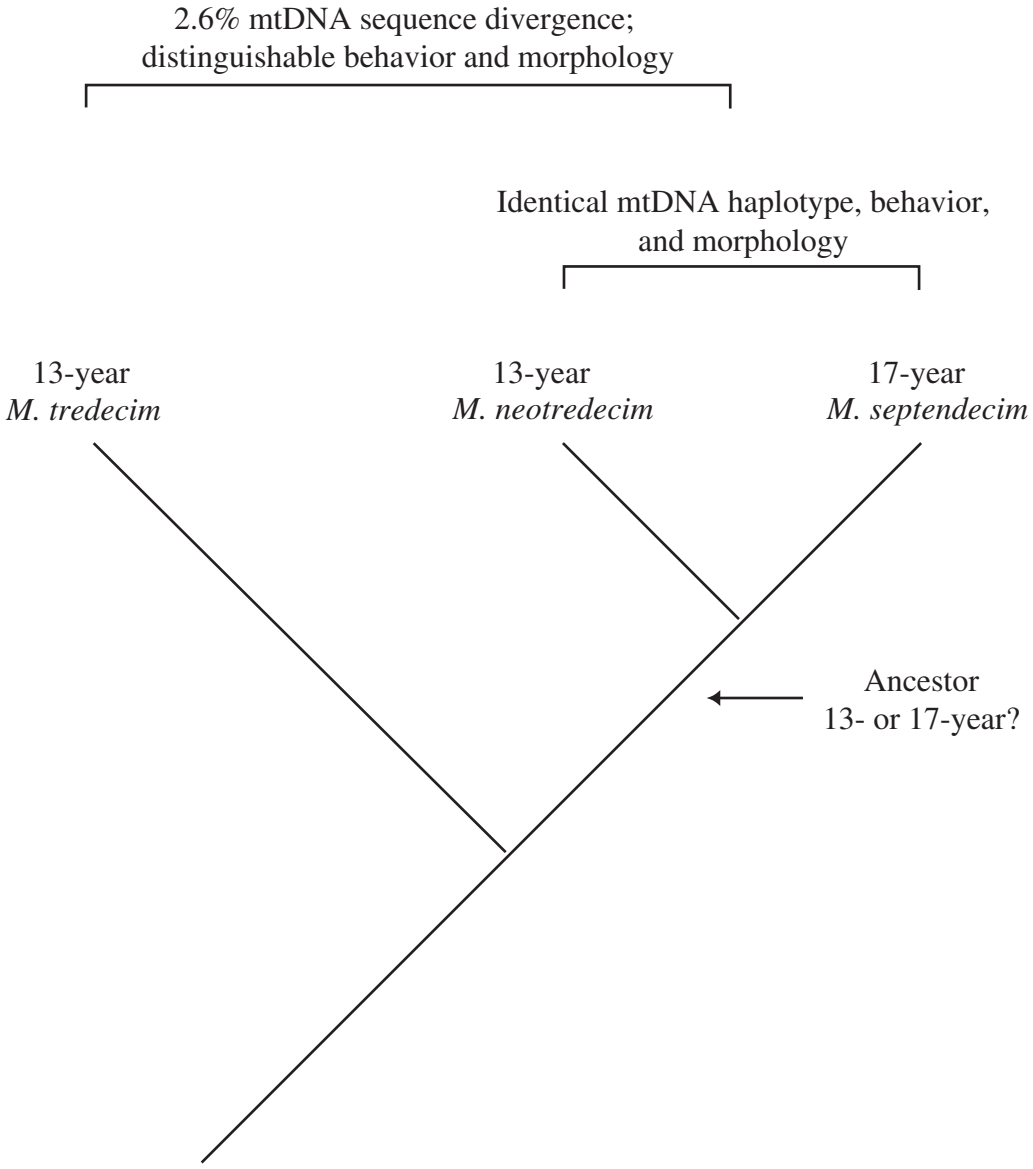


Figure 4.4. Phylogeny of the *Magicicada* -decim species. The life cycle of the ancestor of (*M. neotredecim* + *M. septendecim*) remains unknown. Because 13 and 17-year life cycles have evolved repeatedly in *Magicicada,* inference of a 13-year ancestor via parsimony is weakened. For summaries of supporting data see Simon et al. (2000), Marshall and Cooley (2000), and Williams and Simon (1995)



CHAPTER 5

FEMALE PREFERENCES, THE ACOUSTIC ENVIRONMENT, AND REINFORCEMENT OF PREMATING ISOLATION.

Abstract

Although signal-receiver coevolution is a fundamental aspect of reinforcement of premating isolation, studies of this process to date have not incorporated quantitative data on female preference functions, which describe the relationship between a male trait and the probability of acceptance of that trait by a female. In this study, the periodical cicada (*Magicicada septendecim*) female preference function for male dominant call pitch was measured using playbacks of model calls and used as an estimate of the pre-contact preference function of a close relative, *M. neotredecim*, which has undergone reinforcement upon contact with another periodical cicada species, *M. tredecim*. In addition, playbacks of model calls accompanied by an artificial *M. tredecim* background chorus were used to simulate the conditions of early sympatry in order to determine if acoustic background interference alone could have driven reinforcing selection in *M. neotredecim*. The peak of the *M. septendecim* female preference function closely matched

the most common male call phenotype, corroborating models of signal-receiver evolution that predict stabilizing selection on such traits. However, females exhibited a latent ability to respond to model calls containing sound energy well outside the frequency range, although these extreme model calls were not preferred. The data suggest that *M. neotredecim* females may have possessed the potential to respond to calls of *M. tredecim* when the species first became sympatric, and that evolution by reinforcement of *M. neotredecim* female preferences has consisted mainly of elaboration of preexisting response potential. The simulated *M. tredecim* background chorus attenuated female responses to low-pitch model calls and not responses to high-pitch models; however, this attenuation did not alter the preferred model call pitch.

Introduction

Ritchie (1996) suggests that the study of sexual selection, especially models pertaining to signal coevolution and animal speciation, can benefit from incorporating detailed information on what he termed the "shape" of female mating preferences. Ritchie defined the term as "the relationship between a male trait ... and the probability of acceptance of that trait value in a mating partner". Data on the shape of female mating preferences have been almost entirely lacking from studies of one of the more controversial speciation-related models of signal evolution, reinforcement of premating isolation (Blair 1955; sensu Loftus-Hills and Littlejohn 1992), in which selection against wasteful heterospecific sexual interaction drives premating signal divergence. Analyses of female preferences in cases of reinforcement usually involve only two-way choice tests using males from inside and outside of a zone of sympatry; such data do not reveal the underlying quantitative preference function. The absence of female preference-shape data from studies of reinforcement probably derives both from the rarity of convincing cases of reinforcement (Alexander 1967, Walker 1974; but see Howard 1993) and the methodological difficulties

of studying female preferences in the cases that are known. For example, among the singing Orthoptera, a large group in which signaling systems are especially easy to study, clear evidence of reinforcement is known in only one genus (Otte 1989).

Female response curves have gained attention recently in discussions of "sensory exploitation" (e.g., Ryan and Rand 1990, Basolo 1998) which emphasize the role of latent female "biases" in driving the evolution of male signals; such biases are believed to arise prior to the male trait mainly as a side-effect of selection on other aspects of the female's phenotype. Whether or not male signals ever evolve solely because of fixed nonadaptive, preexisting biases, sensory exploitation of the female preference curve should be regarded as a universal process in the evolution of male signals, if only because no trait can evolve as a signal prior to the existence of a potential receiver. Data on "preexisting" female preference functions can be applied to the study of reinforcement when the process interacts with geography to created a pattern of reproductive character displacement (Brown and Wilson 1956, but sensu Howard 1993), or signal divergence restricted to regions of sympatry. In such cases, measurements of the female preference function in allopatry can be used to estimate the phenotypic variation that existed prior to selection for divergence, which in turn helps determine the likely direction, rate, and extent of signal evolution.

Investigating the mechanics of reinforcement in *Magicicada* with female preference functions

In this chapter, data on female preference functions are used in the analysis of reinforcement of premating isolation in the –decim group of periodical cicadas (17-year *Magicicada septendecim*, 13-year *M. neotredecim*, 13-year *M. tredecim*; for taxonomy see Alexander and Moore 1962 and Marshall and Cooley 2000). A recently discovered 13-year species, *M. neotredecim* (described in Marshall and Cooley 2000), exhibits reproductive character displacement in male song pitch and female song pitch preference where it

overlaps a 13-year relative, *M. tredecim* (Chapter 3). In the overlap zone, *M. neotredecim* calls have a dominant pitch of ca. 1.7 kHz, while *M. tredecim* calls average about 1.1 kHz. Outside the overlap zone, *M. neotredecim* calls drop to ca. 1.3 - 1.5 kHz. *M. tredecim* exhibits little geographic variation in dominant pitch.

The *Magicicada* case offers an excellent opportunity for the detailed study of both male and female phenotypic variation in sexual traits and the application of these data to understanding reinforcement as a coevolutionary process. The opportunities derive from two general advantages, the first of which is methodological. Pair-formation in periodical cicadas involves a visually detectable female signal called a "wing-flick" that is produced by receptive females in response to the individual calls of a conspecific male (Chapter 1, Cooley 1999). In cage studies, the majority of females that respond with wing-flick signals to males mate after a brief courtship by the male. Knowledge of this system has facilitated the successful development and application of techniques using playbacks of computer-synthesized model calls in order to measure female response curves (e.g. Cooley 1999, Marshall and Cooley 2000).

A second advantage derives from *Magicicada* species relationships and biogeography. Biogeographic evidence suggests that *M. neotredecim* split from populations of an existing 17-year species, *M. septendecim*; northern populations of *M. neotredecim* are indistinguishable from populations of *M. septendecim* in behavior, morphology, and mtDNA haplotype, suggesting that the split was very recent (perhaps in the last 10,000 years; Marshall and Cooley 2000, Simon et al. 2000). If *M. neotredecim* is very recently derived from *M. septendecim*, data from *M. septendecim* may provide an accurate measure of pre-contact *M. neotredecim* female preferences, perhaps an even better measure than that obtainable from allopatric populations of *M. neotredecim* because of the greater certainty that populations of *M. septendecim* have not been influenced by evolutionary interaction with *M. tredecim*. In this study, a quantitative estimate of the *M. septendecim* female

preference function for male call pitch was obtained using playbacks of model calls; the resulting data were used to address the following questions:

(1) Does the range of *M. septendecim* female response overlap the range of phenotypes observed in male *M. tredecim* calls, as measured in 1998 in Brood XIX (Chapter 3)? The answer would help determine the risk of interspecific mating attempts and/or hybridization that existed between *M. neotredecim* and *M. tredecim* in the early stages of contact.

(2) Does the *M. septendecim* female preference function include the entire range of phenotypes observed in Brood XIX male *M. neotredecim* calls in sympatry, where call pitch has evolved to its most extreme? Information on this question is relevant to understanding the nature of the phenotypic evolution that has occurred -- To what extent has reinforcing selection modified the preexisting sensory phenotype?

Signal evolution and the effect of the acoustic environment

As discussed above, data on pre-contact female responses can help determine the extent to which males and/or females of the two 13-year –decim species were faced with the threat of interspecific mating upon the establishment of sympatry. Evidence suggests that viable hybrids of *M. neotredecim* and *M. tredecim* are currently rare in the overlap zone, if they exist at all (Marshall and Cooley 2000, Simon et al. 2000; Chapter 3); there is also little evidence of past interspecific gene flow (Chapter 4). The species may have interbred in early stages of contact without producing viable offspring, or past evidence of gene flow may have been lost to selection or drift; both of these hypotheses are extremely difficult to test in *Magicicada*. Alternatively, selection for reproductive signal and preference divergence may have been driven entirely by acoustic interference (Otte 1974), with no interspecific mating, if similarity in call frequencies of the species reduced efficiency in male advertisement or reduced female ability to respond to calling conspecific

males. *Magicicada* adult populations are extremely dense (Dybas and Davis 1962), and loud background noise is a constant feature of their mating aggregations. In this study we employed playback techniques to test this hypothesis in *Magicicada* by measuring the effect of a simulated *M. tredecim* chorus on female *M. septendecim* responses to model calls of varying pitch. Specifically, we asked the following questions:

(1) Does a background chorus with frequency characteristics of *M. tredecim* reduce response rates of *M. septendecim* to model male calls?

(2) Does the effect of the background chorus, if any, on female responsiveness vary across the frequency spectrum? If signal interference of the background chorus is sufficiently greater for model calls of lower frequencies, the resulting selection alone could drive directional change in the male call phenotype.

Some evidence suggests that range expansion and overlap of the two –decim species has occurred asymmetrically, with *M. neotredecim* dispersing into the range of *M. tredecim* more than vice versa (Chapters 3, 4). Thus, the background experiment was designed to mimic a scenario in which rare *M. neotredecim* adults are found within a dense chorus of *M. tredecim*.

Materials and Methods

Experimental procedures

Study location -- Playback experiments were conducted from 4-8 June 1999 in a small clearing at Tar Hollow State Forest, Ross Co., OH. All three 17-year *Magicicada* species (*septendecim*, *cassini*, *septendecula*) emerged and chorused in the surrounding woods.

Collection and storage of females -- *Magicicada septendecim* females were collected from low grass and shrubs the morning after their emergence from the ground; such "teneral" cicadas are easily recognized by their soft, dull exoskeletons, and yellow ovipositors. Teneral females were stored in ca. 50 liter cages formed by wrapping flexible fiberglass screen material around a tree branch, which allowed sunlight to penetrate and provided the cicadas with appropriately sized twigs for feeding on xylem fluids. Teneral females require at least 5 days of maturation before becoming receptive to mating (Cooley 1999, Maier 1982b; Chapter 2). Females were collected from 22-31 May and ranged in age from 6-16 days post-emergence during the playback experiments (mean 10 days ±3.4 for the entire test sample).

Collection and recording of males -- The natural chorus, which is composed almost entirely of male calling songs, was recorded for thirty seconds at Tar Hollow State Park with a Sony Professional Walkman cassette recorder connected to a Sony stereo microphone. The microphone was held at arm's length and head height at a distance of approximately 10 meters from the nearest calling males. The same equipment was used to record the individual calling songs of 27 *M. septendecim* adult males captured from a natural chorus; the males were each recorded immediately prior to capture, preserved in 75% EtOH, and individually marked. Using Canary 1.24 (Macintosh), power spectra were generated for the chorus recording and the individual male call recordings and used to obtain the dominant pitch.

Model calls and simulated background chorus -- Individual –decim calls consist of a 1-3 second steady-pitch and nearly pure-tone "main element" followed by a quieter 0.5 second frequency "downslur" that terminates at a pitch about 500 Hz lower than the main element pitch (Chapter 1; Alexander and Moore 1958, Young and Josephson 1983, Weber et al. 1987). Using SoundEdit Pro (Macromedia), sixteen model calls were

constructed using pure-toned (sine wave) sound structured to match the shape of normal calls but without internal temporal structure such as pulses. Each of the sixteen different model calls was scaled to contain a different dominant (main element) pitch. In prior work (Chapter 1), female –decim have been shown to respond similarly to recorded natural and pure-tone model calls. Because a chorus contains the sounds of many males whose calls vary in dominant pitch (Marshall and Cooley 2000), and because this experiment was intended to measure acoustic interference by the background, a pure-tone model would not be an appropriate simulation of a background chorus. Instead, a simulated chorus of *M. tredecim* was created by subjecting a loop recording of a normal *M. septendecim* chorus to the "bender" feature of SoundEdit Pro until the dominant pitch of the recording matched that of an *M. tredecim* chorus (ca. 1.1 kHz).

Cage and playback apparatus **--** Females were tested in two side-by-side 22x24x22 cm screen test chambers within which a few vertical sprigs of vegetation were placed for perching. Playback equipment for the model calls consisted of a Macintosh Powerbook computer (for model calls) or a Sony portable CD player (for the simulated background chorus) connected to Radio Shack amplified 3.5" speakers (cat. #32-2040), which were positioned 20 cm away on opposite sides of the test cage. Output volumes of the model calls and simulated background were adjusted to remain within 68-72 dB in the test cage. The intensity of the natural background chorus never exceeded 60 dB within the cage.

Experimental design **--** A single experimental protocol was used to measure the shape of *M. septendecim* female responses to call pitch and to estimate the effect of a simulated *M. tredecim* background chorus on the female response curve. The protocol contained three steps in the following order: (1) the "no-background pretest", during which the model calls were played in the absence of a simulated background; (2) the "background

test", during which the model calls were played along with the simulated background; (3) the "no-background posttest", identical to step #1.

In all, seven trials were conducted, with six females tested during each trial. From 4-5 June, individual females were judged receptive and used in the experiment when they responded with one or more wing-flicks to a model call of a randomly selected 1.2, 1.3, or 1.4 kHz dominant pitch. From 6-8 June, individual females were judged receptive and used in the experiment when they responded with one or more wing-flicks to a model call of pitch chosen at random from the entire test range. When not enough stored females responded for a given trial, additional females were taken at random from cohorts older than five days. All playback trials were completed between 10:00 AM and 4:00 PM in bright overcast or sunny weather in temperatures ranging from 68 F to 84.5 F (mean 76.2F).

Before each of the seven trials, the six individually-marked females were divided between the two test chambers and left undisturbed for ten to twenty minutes. During the no-background pretest the sixteen model calls were played from a speaker one at a time in a random order, each repeated six times in a row before proceeding to the next. All females were observed simultaneously during the playbacks; a given female was scored as responding positively to a model call if she produced one or more wing-flicks with the appropriate species-specific timing (see Chapter 1). During the background test, the model calls were played in the same manner and in the same random order but with the model *M. tredecim* background chorus playing simultaneously from the other speaker. Finally, during the no-background posttest the model call series was repeated once more without the background, again using the same random order. A different random order was established for each of the seven trials.

Analysis

Combining data across trials -- Sample sizes within each trial were low because of the need to observe the females in small groups, therefore the results of the seven trials were combined for analysis. All statistical analyses were conducted using Systat version 5.0 for the Macintosh; results are reported below in the format "mean ±1 S.D."

Order effects and sample size correction -- Repetition of the no-background treatment in each trial was intended to (1) allow detection of wholesale loss of female receptivity in response to disturbance or other environmental changes and (2) allow detection of and correction for effects of treatment order (e.g. females could become progressively less receptive as a trial proceeds, independent of treatment). In all trials, one or more females responded during each of the playback series, indicating that conditions remained at least minimally appropriate for testing throughout. To determine if an order effect was apparent in the data, the numbers of females responding and the numbers of positive responses across all calls were summed across the no-background pretests, the background tests, and the no-background posttests (Table 1). Because this tabulation showed no simple order effect, the data from the no-background tests (pre- and post-) were combined and compared as a whole to the results from the background tests. These procedures yielded separate female response curves for the no-background and background treatments.

Comparison of M. septendecim and Piatt Co., IL, M. neotredecim (1998 data) female response curves --The 1998 data on "allopatric" *M. neotredecim* female responses were obtained using a similar protocol (Marshall and Cooley 2000; Chapter 3), but with fewer model calls (no 0.8 and 0.9 kHz models). These data were not

divided into pitch groups; instead the individual responses of all Piatt Co. *M. neotredecim* females were combined into a single sample by assigning each response a "response frequency" corresponding to the pitch of the call eliciting the response. The response frequencies from the no-background *M. septendecim* tests were similarly lumped. The two samples were then compared for a difference in mean using a Mann-Whitney test and a difference in distribution shape using a Kolmogorov-Smirnov test.

Testing for background effects -- Because some model calls elicited very few responses, it was necessary to divide the frequency range into "low-pitch" (0.8-1.2 kHz), "intermediate-pitch" (1.3-1.7 kHz), and "high-pitch" (1.8-2.3 kHz) model groups and combine the female response data accordingly for the following statistical analysis.

Two statistical methods were used to test for effects of the background chorus on the female response curve. First, the data for each pitch group were considered separately. To determine if the background chorus altered female responsiveness to a given set of model calls, the numbers of responding and nonresponding females in the background test were compared to those observed in the no-background tests, using Fisher's Exact test. Then, in an alternative method emphasizing simultaneous analysis of the three model groups, the numbers of positive responses across the three model groups in the background test were compared to those observed in the no-background tests, this time with a Pearson chi-square test.

Results

The distribution of M. septendecim male call phenotypes -- The dominant pitch values for the 27 individually-recorded males at the Tar Hollow site averaged 1.33 kHz and were tightly clustered, with a range of 1.21-1.41 kHz. This distribution closely

matched the distribution of sound energy found in the natural *M. septendecim* chorus (Fig. 1), which exhibited a peak sound pitch of 1.34 kHz.

***M. septendecim* female response curve** -- 34 of the 42 females wing-flicked at least once during the no-background pretest or posttest series; these females responded on average to 4.4 ±2.8 different model calls. The average range of response (highest pitch eliciting a response minus lowest pitch eliciting a response) was 5.9 ±3.5 kHz, indicating that female responses tended to be clustered on the frequency band. Fig. 2 shows all of the 248 female responses combined into a single histogram that shows the "shape" of the female response curve. Females were most responsive to the 1.3 kHz model call, which contained a dominant pitch very similar to that of the local *M. septendecim* chorus (Fig. 2 background). 11 of the 13 model calls elicited at least one response, including models with dominant pitches much higher than those found in the natural chorus.

The response curve from the 1998 study (Marshall and Cooley 2000) of 12 Piatt Co., IL, *M. neotredecim* females is shown in Fig. 3. Across the frequency range studied in both 1998 and 1999, the average response frequency does not differ significantly between the 13-year *M. neotredecim* (1.36 ±0.25 kHz, n=49) and 17-year *M. septendecim* (1.38 ±0.26 kHz, n=236) samples (P=0.894, Mann-Whitney test). Similarly, the Kolmogorov-Smirnov test found no significant difference between the distributions of the two samples (P=0.363).

Effect of the simulated M. tredecim chorus on the female response curve -- 29 of the 42 females responded during the no-background pretests and 27 responded during the no-background posttests, compared to only 16 during the background test, a significant decrease for both pairwise comparisons (Fisher's exact test: pretest vs. background, P<0.008; posttest v. background, P<0.03). The females who did respond during the background treatment wing-flicked on average to only 3.0 ±1.9 model

calls, over an average range of 4.2 ±3.1 kHz. Although these ranges are smaller than the values observed in the no-background treatment (see above), the differences are not statistically significant (P=0.09 for number of calls and P=0.08 for range of response, Mann-Whitney test).

In Fig. 4, the 48 female responses from the background test (black bars) are combined into a single histogram and superimposed on the female response curve from the no-background treatment (gray bars); the latter is scaled to correct for the fact that twice as many no-background tests were conducted. The simulated background chorus, with a low dominant pitch of 1.1 kHz, appears to have an attenuating effect on female responsiveness that increases toward the lower portion of the frequency spectrum. Analysis of the attenuating effect by pitch-group (Table 2) shows that the background chorus significantly reduced female responsiveness to low- and intermediate-pitched model groups, but did not significantly reduce responsiveness to the high-pitched model group; the combined effect across all groups is statistically significant as well.

Discussion

The shape of female *M. septendecim* female preference for call pitch

On the scale of analysis of this study, the peak of the *M. septendecim* female response curve for call pitch closely matches the dominant pitch found in male *M. septendecim* calling song (Figs. 1, 2). The pattern fits the expectation of a coevolutionary match between male signal and female receiver, and indicates that in this population male call pitch is subject to stabilizing sexual selection imposed by female preferences.

A notable feature of the preference curve (Fig. 2) is its width compared to the corresponding degree of variation observed among males in the population (Fig. 1). At a basic level the data indicate a latent female "ability" to respond to calls well outside the

phenotypic range observed normally in *M. septendecim* males, although such extreme phenotypes are not preferred as observed in some recent studies of latent female preferences (Ryan and Rand 1990, Basolo 1998). However, because of the design of the experiment, the significance of the data for understanding the underlying female sensory system is unknown: Previous playback studies (Chapter 1) have shown that females will sometimes respond to pure-tone models containing only the "main element" or the "downslur" component of the call (although the experiments demonstrating this used older females on average); there is no absolute requirement that the model call contain both parts, although whole calls always elicit more responses. Therefore, because the model calls in this study were structured to match the form of natural calls, including both main element and downslur, we cannot be certain from these data that females are able to perceive sound energy at pitches greater than about 1.6 kHz, which is the lowest frequency contained in the 2.3 kHz model (at the end of the "terminal downslur" of that model). Similarly, we do not know if females can perceive sound at frequencies lower than about 0.8 kHz (the highest pitch contained in the 0.8 kHz model); incidentally, sound energy of ca. 0.8 kHz is contained at the end of the downslur in some naturally-occurring *M. septendecim* calls. Further analysis of this sort, using models varying in dominant pitch but without downslurs, will be necessary to make inferences concerning *Magicicada* female sound perception.

Female preferences and reinforcement between *M. neotredecim* and *M. tredecim*

The advantage of using whole-call models is that the data allow realistic inferences about the potential for sexual interactions between female *M. septendecim* and male –decim of a given call pitch; this information can be put to use in understanding the dynamics of reinforcement between *M. neotredecim* (closely related to *M. septendecim* -- see

Introduction) and *M. tredecim*. The *M. septendecim* female preference curve, incidentally, does not differ from that measured in 1998 using 12 *M. neotredecim* females from Piatt Co., IL, outside the zone of overlap with *M. tredecim* (Fig. 3); it remains true that the only known differences between *M. septendecim* and such allopatric *M. neotredecim* are in life cycle and geography.

Comparison of the *M. septendecim* female preference curve with the distribution of male call pitch types observed in Brood XIX *M. tredecim* (Fig. 5, data from Marshall and Cooley 2000 and Chapter 3) suggests that, prior to contact with *M. tredecim*, *M. neotredecim* females possessed the latent ability to respond to all male call phenotypes present in a population of *M. tredecim*. Model calls of 1.1 and 1.2 kHz, a range including the upper half of the *M. tredecim* call range, elicited from *M. septendecim* females only about 25% fewer responses than the preferred 1.3 kHz model call. Thus it is not possible from these data to reject the hypothesis that reinforcement between *M. neotredecim* and *M. tredecim* involved selection against heterospecific matings per se. However, because the observed phenotypic distributions of *M. tredecim* and allopatric *M. neotredecim* male call pitch do not overlap (Chapter 3), no *M. tredecim* males would have been more likely to elicit responses from *M. neotredecim* females than the least effective (ca. 1.2 kHz) *M. neotredecim* males.

These data, in conjunction with similar female preference data from the 13-year –decim overlap zone (Marshall and Cooley 2000; Chapter 3), suggest that reinforcing selection has not shifted *M. neotredecim* female preferences much beyond the latent range of response that existed prior to contact; the maximum male call pitch (ca. 1.9 kHz) and peak female response pitch (ca. 1.7 kHz) of *M. neotredecim* in sympatry (Marshall and Cooley 2000; Chapter 3) both fall well within the range of response observed here for *M. septendecim*. However, the relationship between female preference curve and male phenotypic distribution in *M. neotredecim* has remained similar to that in *M. septendecim*: Marshall and Cooley (2000) found that, at the upper limit of male call pitch variation in

sympatry (ca. 1.9 kHz), *M. neotredecim* female responses had dropped to about 80% of the peak level. Similarly, at the upper limit of male call pitch variation in *M. septendecim* (ca. 1.45 kHz), the female response curve drops to approximately 70% of the peak value. These results suggest an evolutionary process in which latent sensory biases are exploited and elaborated by selection in response to a changing environment, but the preexisting female "bias" alone cannot be said to have caused the male trait evolution.

Signal evolution in response to the acoustic environment

The background experiment was designed to explore the possibility that a change in the acoustic environment associated with the onset of sympatry may have created the directional selection that shifted *M. neotredecim* male signals and female preferences. Two primary results emerged from the experiment. First, the presence of a simulated *M. tredecim* background did not alter the preferred model pitch of 1.3 kHz (Fig. 4). In other words, the background did not alter the call pitch most likely to elicit a female wing-flick; on the basis of this result alone no change in male call pitch phenotype would be expected. However, the background did attenuate female responses increasingly toward the lower end of the model call spectrum. Selection deriving from pitch-dependent interference of this sort could drive change in male call pitch indirectly, via selection on females. Depending on (1) the nature and significance of pair-formation costs (such as lost time and increased risk of predation) for *Magicicada* females, (2) the frequency distribution of male call pitch phenotypes, and (3) the relationship (if any) between a male's call pitch and his quality as a mate, it is possible that background interference could result in selection favoring a different female preference phenotype. This could happen if the background interference increases the relative amount of time spent in costly pair-formation activity by females with lower preference curves, compared to females with higher preference curves who may be able to respond to more distant males or to respond more reliably (multiple

wing-flick responses are usually required for a male to locate a signaling female). Directional selection on the female preference curve would indirectly create similar directional selection on the male call pitch. There is some circumstantial evidence to suggest that direct costs of mate choice may be significant for *Magicicada* (Chapter 2); however, more detailed life history information will be necessary to sufficiently evaluate the plausibility of the above hypothesis.

It is important to note that the female preference data discussed in this paper are measures of phenotypic variance only. Presumably only a fraction of this phenotypic variance is attributable to genetic variations, and *Magicicada* are not well-suited to heritability studies. However, the existence of reproductive character displacement in *M. neotredecim* alone is a potent justification for assuming that evolutionarily significant levels of genetic variation exist in the female preference function.

Conclusion

Reinforcement of premating isolation is usually studied as a problem of population genetics, in part because interest is most often focused on reinforcement involving populations that are not genetically isolated (e.g. Butlin 1987, 1989, 1995). Many studies focus on understanding the extent to which selection against hybrids can favor divergence of coevolving signal-receiver systems in the face of genetic recombination (e.g. Liou and Price 1994, Kelly and Noor 1996); such studies often employ models characterized by abstract alleles and fixed selection coefficients. In contrast, few studies have approached the evolutionary problem of reinforcement at the level of the individual organism, and few examine mate search and advertisement costs or incorporate consideration of the ways such factors may differentially affect success of males and females. For example, two recent models (Dieckmann and Doebelli 1999, Kondrashov and Kondrashov 1999) of sympatric speciation by disruptive selection (which is similar to the problem of reinforcement in

hybrid zones) both assume random mating and explicitly hold costs of mating equal across genotypes as a simplifying assumption. Such costs surely play a central role in both male and female sexual evolution, and their incorporation would almost certainly alter the evolutionary outcome of sympatric speciation models. Furthermore, the discussion of acoustic background interference and its potential effects on *M. neotredecim* song evolution suggests that such traits cannot be considered in isolation; sexual signal evolution is a coevolutionary process, so changes in traits of one sex can alter selection coefficients on traits of the other in a complex feedback.

Literature Cited

Alexander, R. D. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species. (Homoptera: Cicadidae, *Magicicada*). Misc. Publ. no. 121. Univ. of Michigan Museum of Zoology, Ann Arbor, MI.

Alexander, R. D. 1967. Acoustical communication in arthropods. Annu. Rev. Entomol. 12:495-526.

Alexander, R. D., and T. E. Moore. 1958. Studies on the acoustical behavior of seventeen-year cicadas (Homoptera: Cicadidae: *Magicicada*). Ohio J. Sci. 58:107-127.

Basolo A. L. 1998. Evolutionary change in a receiver bias: A comparison of female preference functions. Proc. Roy. Soc. Lond. B: Biol. Sci. 265: 2223-2228.

Blair, W. F. 1955. Mating call and stage of speciation in the *Microhyla olivacea- M. carolinensis* complex. Evolution 9:469-480.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. Syst. Zool. 5:49-64.

Butlin, R. K. 1987. Speciation by reinforcement. Trends Ecol. Evol. 2:8-13.

Butlin, R. K. 1989. Reinforcement of premating isolation. Pp. 158-179 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Butlin, R. K. 1995. Reinforcement: an idea evolving. Trends Ecol. Evol. 10:432-434.

Cooley, J. R. 1999. Sexual behavior in North American cicadas of the genera *Magicicada* and Okanagana. PhD. Dissertation, The University of Michigan.

Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. Nature (London) 400:354-357.

Dybas, H. S., and D. D. Davis. 1962. A population census of seventeen-year periodical cicadas (Homoptera: Cicadidae: *Magicicada*). Ecology 43:432-444.

Howard, D. J. 1993. Reinforcement: Origin, dynamics, and fate of an evolutionary hypothesis. Pp. 46-69 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford, New York.

Kelly, J. K., and M. A. Noor. 1996. Speciation by reinforcement: A model derived from studies of *Drosophila*. Genetics 143:1485-1497.

Kondrashov A. S., and F. A. Kondrashov. 1999. Interactions among quantitative traits in the course of sympatric speciation. Nature (London) 400:351-354.

Liou, L. W., and T. D. Price. 1994. Speciation by reinforcement of premating isolation. Evolution 48:1451-1459.

Loftus-Hills, J. J., and M. J. Littlejohn. 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *G. olivacea* (Anura: Microhylidae): A reexamination. Evolution 46:896-906.

Maier, C. T. 1982b. Observations on the seventeen-year periodical cicada, *Magicicada septendecim* (Hemiptera: Homoptera: Cicadidae). Ann. Entomol. Soc. Am. 75:14-23.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with a description of a new species, 13-year *Magicicada neotredecim*. Evolution in press (slated for August 2000).

Otte, D. 1974. Effects and functions in the evolution of signaling systems. Ann. Rev. Ecol. Syst. 5: 385- 417.

Otte, D. 1989. Speciation in Hawaiian crickets. Pp. 482-526 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Ritchie, M. G. 1996. The shape of female mating preferences. Proc. Natl. Acad. Sci. USA 93: 14628-14631.

Ryan M J., and A. S. Rand. 1990. The sensory basis of sexual selection for complex calls in the tungara frog *Physalaemus pustulosus*: sexual selection for sensory exploitation. Evolution 44: 305-314.

Simon, C., J. Tang, S. Dalwadi, G. Staley, J. Deniega, and T. Unnasch. 2000. Restricted gene flow between 13-year cicadas and coexisting "17-year cicadas with 13-year life cycles." Evolution in press (slated for August 2000).

Walker, T. J. 1974. Character displacement in acoustic insects. Am. Zool. 14:1137-1150.

Weber, T., T. E. Moore, F. Huber, and U. Klein. 1987. Sound production in periodical cicadas (Homoptera: Cicadidae: *Magicicada septendecim*, *M. cassini*). Pp. 329-336 *in* Proc. 6th Auchen. Mtg., Turin, Italy, 7-11 Sept. 1987.

Young, D., and R. K. Josephson. 1983. Pure-tone songs in cicadas with special reference to the genus *Magicicada*. J. Comp. Physiol. 152:197-207.

Table 5.1. Responsiveness of *M. septendecim* females during the three parts of the background experiment, showing no continuous increase or decrease in overall female receptivity during the trials.

	No-background pretest	Background treatment	No-background posttest
# of Females Responding	29	16	27
# of Responses	118	48	128

Table 5.2. Effect of background chorus on *M. septendecim* female responsiveness to model calls of three different pitch-groups -- low (0.8-1.2 kHz), intermediate (1.3-1.7 kHz), and high (1.8-2.3 kHz). R = Number of responses, across all females tested, to models of a pitch-group; NR = Number of opportunities for responses to models of a given pitch group, across all females tested, minus R. Fisher's Exact Test shows that the background chorus significantly reduced female responsiveness in the low ($P<0.001$) and intermediate ($P<0.001$) groups, but not the high group ($P=0.64$). A Pearson chi-square comparison of the R values across all three categories indicates an overall interaction between background treatment and number of responses by pitch-group ($P=0.03$).

	Low Pitch		Intermediate Pitch		High Pitch	
	R	NR	R	NR	R	NR
No Background	9	201	31	179	8	244
Background	47	164	66	145	11	241

Figure 5.1. Distribution of *Magicicada septendecim* male dominant call pitch phenotypes (dark gray bars) superimposed on the distribution of sound energy in the natural *M. septendecim* male chorus (light gray background). The 27 individual male call pitch values are grouped into the following five categories: 1.21 -1.25, 1.26-1.30, 1.31-1.35, 1.36-1.40, and 1.41-1.45 kHz.

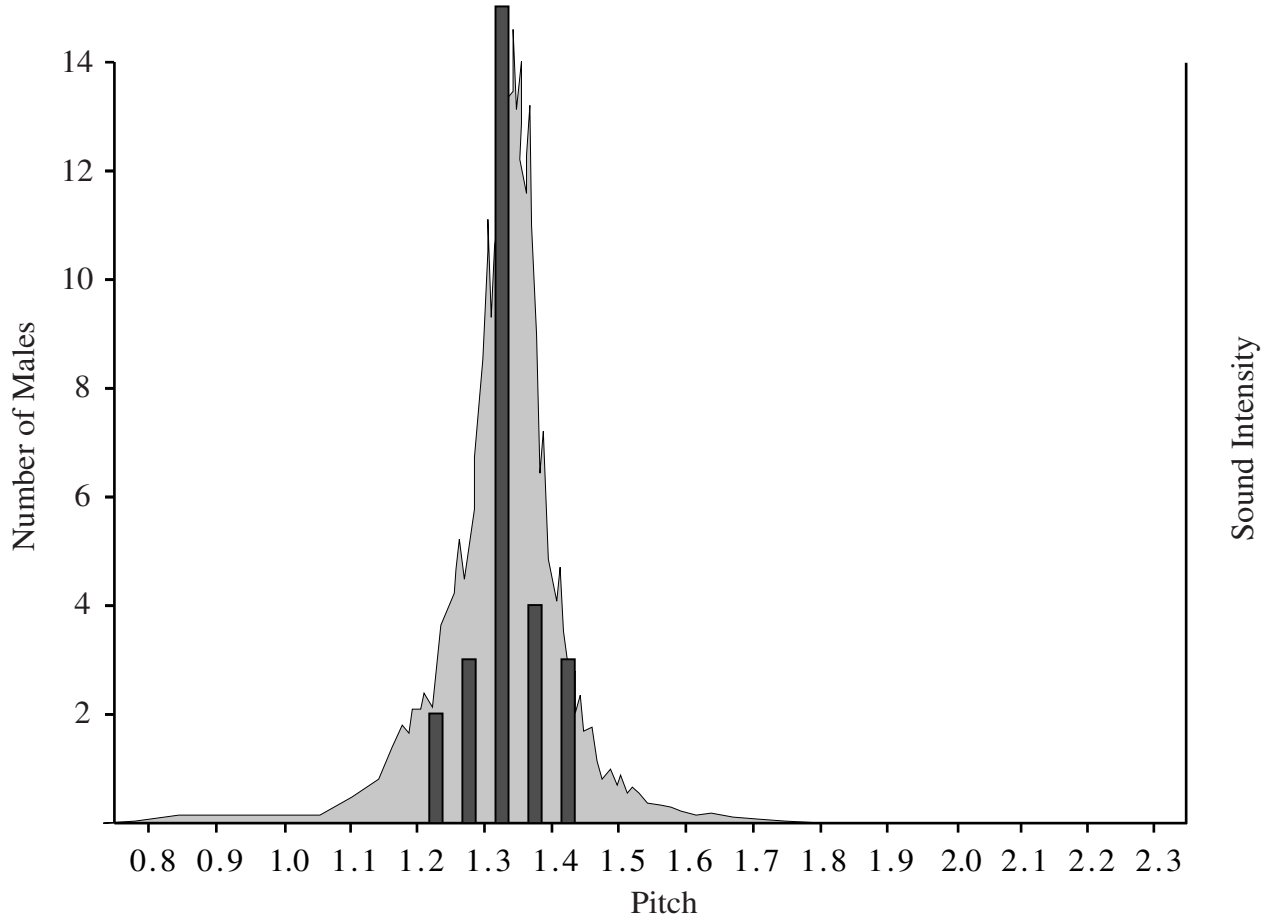


Figure 5.2. *Magicicada septendecim* female preference curve for male dominant call pitch (white bars) superimposed on the distribution of sound energy in the natural *M. septendecim* male chorus (gray background).

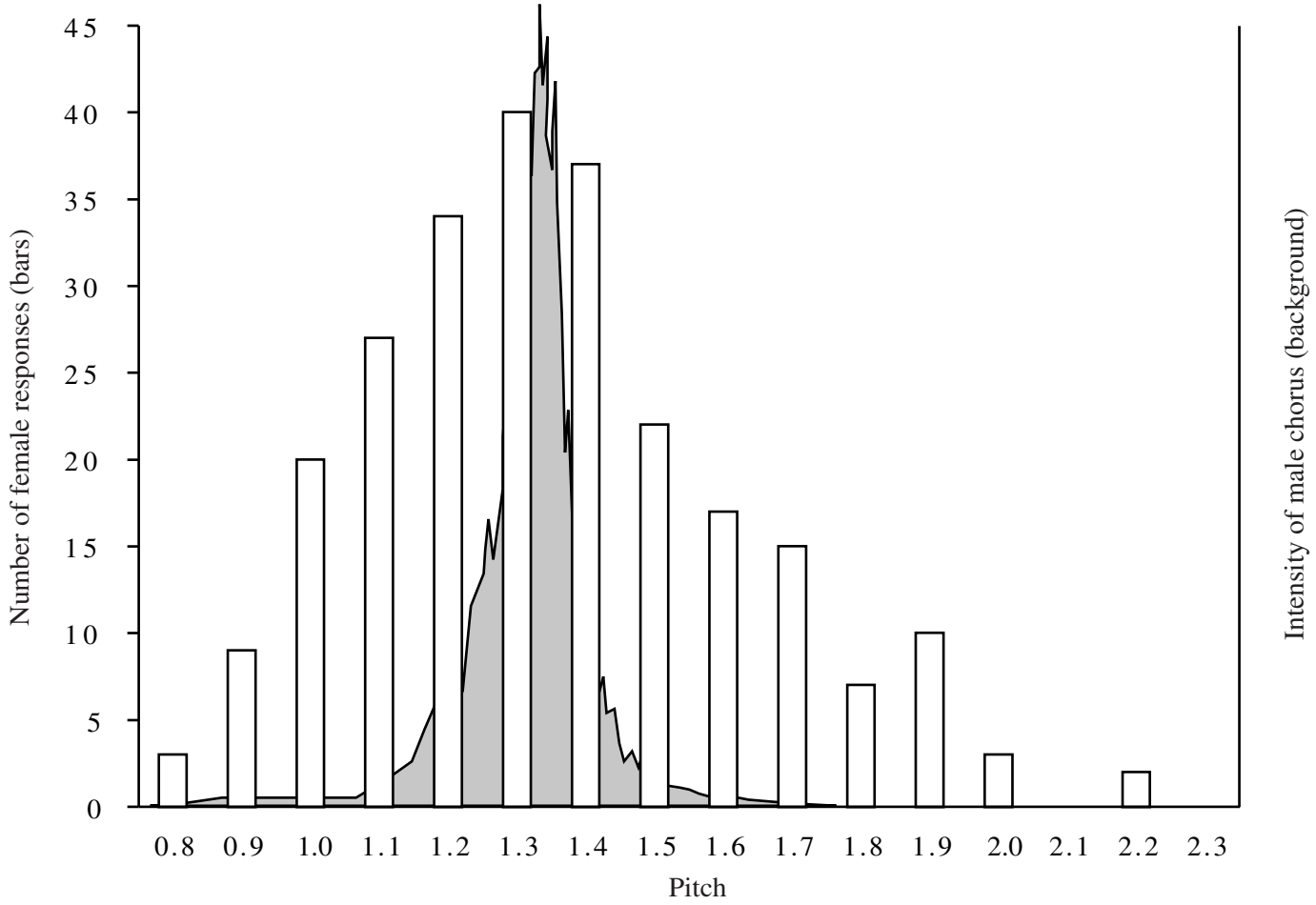


Figure 5.3. 13-year *M. neotredecim* female preference curve for dominant pitch of male calls, from a Brood XIX population in Piatt Co., IL, outside the zone of overlap with *M. tredecim* (data from Marshall and Cooley 2000). The female preference curve is not significantly different from that of 17-year *M. septendecim*. Males of *M. septendecim* and Piatt Co., IL, *M. neotredecim* exhibit approximately the same dominant call pitch, from 1.3-1.4 kHz.

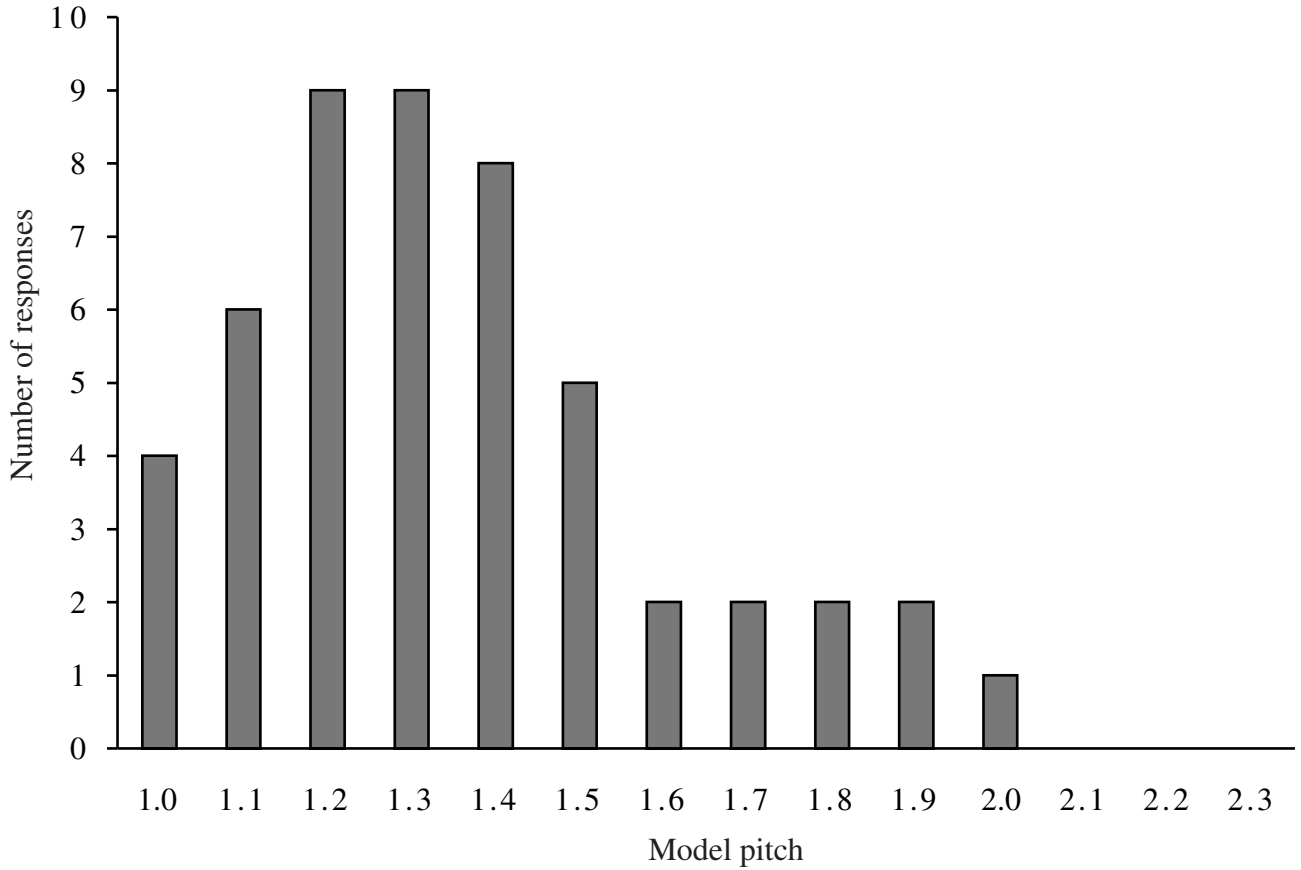


Figure 5.4. A simulated *M. tredecim* background chorus (dominant pitch 1.1 kHz) reduces *M. septendecim* female responsiveness to low and intermediate pitch calls, but not to high pitchcalls. Gray bars = without background; black bars = with background. Dominant pitch of male *M. septendecim* call is ca. 1.3 kHz at the study site.

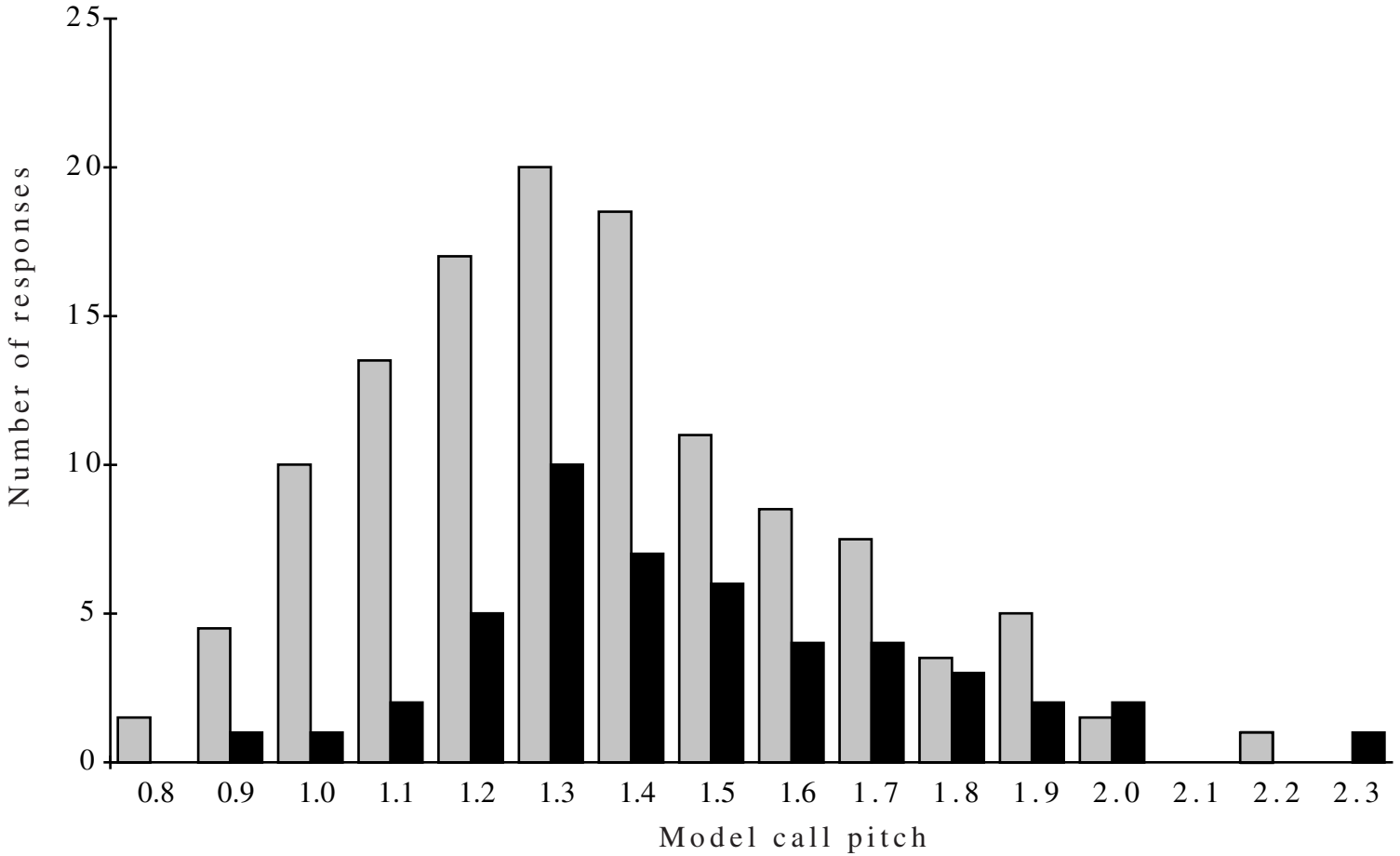
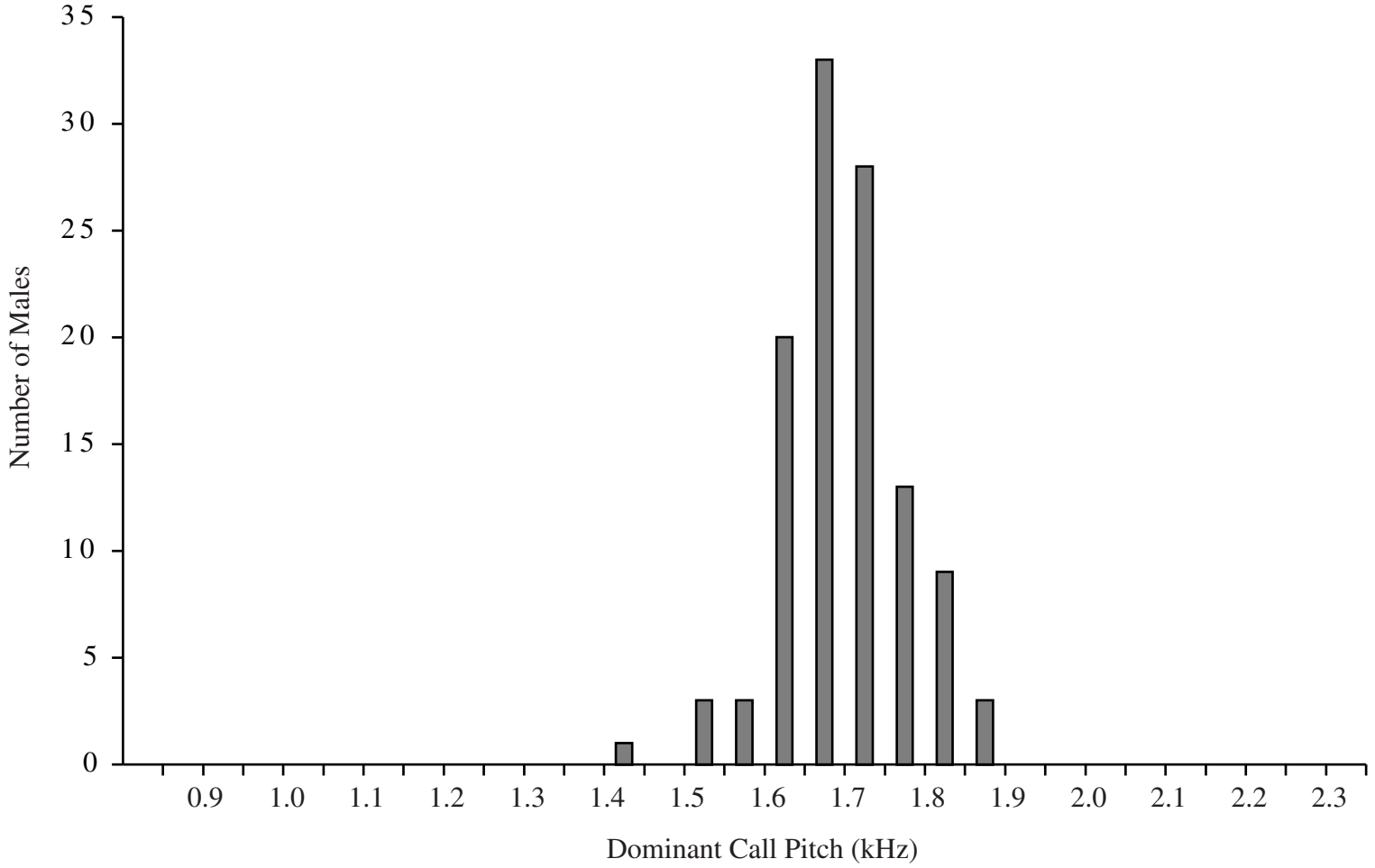


Figure 5.5. Distribution of 113 male *M. neotredecim* dominant call pitch phenotypes (calls above 1.4 kHz in dominant pitch) sampled at random from a mixed *M. neotredecim* / *M. tredecim* chorus at Sharp Co., AR. Data from Marshall and Cooley (2000).



CHAPTER 6

PERIODICAL CICADA LIFE CYCLE VARIATIONS, THE HISTORICAL EMERGENCE RECORD, AND THE GEOGRAPHIC STABILITY OF BROOD DISTRIBUTIONS

Abstract

The complex biogeography of the 13- and 17-year periodical cicadas offers important opportunities for testing hypotheses of *Magicicada* evolution and ecology. However, the historical record of *Magicicada* brood distributions has been complicated by misinterpretation of stragglers (cicadas that emerge off-schedule), a problem exacerbated by the use of cross-generation brood maps that lump records sharing a common 13- or 17-year pattern. Misinterpretation of stragglers as on-schedule emergences, combined with systematic biases in search effort, likely results in erroneous edge extension of adjacent broods and the appearance of sympatric shadow broods.

Substantial changes in brood distributions have been inferred from the *Magicicada* historical record, including the decline and extinction in the Midwest of 17-year Brood X, the widespread expansion and contraction of 17-year Brood VI, and the displacement of Brood XIII 17-year cicadas in Illinois by 13-year Brood XIX. Reanalysis of the historical

data with an awareness of straggler-induced error suggests instead that brood distributions in these cases have remained stable.

Introduction

The periodical cicadas (*Magicicada* spp.) of the eastern U.S. are characterized by long life cycles, dense populations, and synchronous development; all individuals emerge together as adults in the spring of one year and produce offspring that spend 13 or 17 years underground as juveniles (Marlatt 1907; Alexander and Moore 1962; Lloyd and Dybas 1966a, b; Williams and Simon 1995). Three morphologically and behaviorally distinct species of 17-year cicadas (Table 1) inhabit the northern and plains states sympatrically and synchronically, and four 13-year *Magicicada* species inhabit the southeastern and midwestern states. The 17- and 13-year life cycle groups each have formed several largely allopatric broods that emerge in different years (Fig. 1). Each brood is given a Roman numeral indicating its temporal relationship to other same-cycle broods, from I-XVII for 17-year cicadas and XVIII-XXX for 13-year cicadas. There are 12 extant 17-year broods and three 13-year broods (Table 2), so many year-classes are empty.

These complex biogeographic patterns offer important opportunities for testing hypotheses of life cycle evolution, brood formation, and the ecological and evolutionary interactions of 13- and 17-year cicadas (e.g. Alexander and Moore 1962; Lloyd and Dybas 1966b; Lloyd and White 1976; Lloyd et al. 1983; Martin and Simon 1988, 1990a). However, while brood distributions are well-known on a regional scale, there has been striking disagreement over the degree to which broods overlap, especially broods of different life cycle types (e.g. Marlatt 1907; Bryce and Aspinwall 1975; Simon 1988; Cox and Carlton 1988, 1991). In addition, surveys in different emergence years have sometimes yielded radically different distributions for the same brood, suggesting substantial range changes. As discussed below, analysis of these patterns suggests that

much of the confusion has been caused by misinterpretation of off-schedule cicadas that emerge out of synch with the rest of the brood. This paper first reviews and extends prior discussion (e.g. Lloyd and White 1976, Maier 1985, Moore 1993) of the ways in which such life cycle variants contribute to erroneous patterns in *Magicicada* distribution maps. Then, recent historical hypotheses of *Magicicada* brood range changes are reanalyzed and found to be based in part on misinterpretation of straggler emergences and other flaws in the historical record.

I. The problem of straggling and the *Magicicada* historical record

Although most cicadas in a *Magicicada* population emerge in synchrony, sometimes off-schedule *stragglers* appear; the term applies to both premature and delayed cicadas. Such individuals are usually rare, but they can be common on occasion, as might be expected given the extraordinary density of *Magicicada* emergences: Population density estimates range from 8,355 (Maier 1982) to 3,700,000 per hectare (Dybas and Davis 1962), so even a minuscule rate of straggling could result in noticeable numbers of cicadas in off-years, given the conspicuousness of male cicada calling song. One-year premature or delayed cicadas are most common (Kritsky 1987, Moore 1993), but various multiple-year errors can occur and should be expected in such long-lived organisms (see also Moore 1993). Apparent four-year premature 17-year cicadas have been observed on many occasions, sometimes in large numbers (Simon 1988, Williams and Simon 1995, Kritsky and Simon 1996). Maier (1985) suggests that 17-year Brood II has repeatedly produced four-year delayed stragglers; similar straggling occurred in Indiana in 1974 (Young 1974) and in Chicago in 1994 (DCM pers. obs.). A 1993 M. *cassini* (Fisher) male observed in Springfield, IL may have been 20 years old, and one in Chicago in 1995 may have been 22 (DCM pers. obs.). *Magicicada* appeared nine years late in Flossmoor, IL in 1999 (nymphal skin collected and adults observed by Susan White). As argued below, 13-year

cicadas may have emerged from 4-8 years late in Missouri at the turn of the century. Off-schedule 17-year cicadas were recorded on 10 separate years from 1944-1961 in southeast Ohio (Alexander and Moore 1962), where only three broods are known. Furthermore, on 7 June and 14-15 June 1994, small populations of 1-10 male *Magicicada cassini* were heard in six out of seven locations checked within the range of 17-year Brood XIII (DCM pers. obs.), suggesting that stragglers were widely distributed that year. Such life cycle variations could be caused by mutations influencing life cycle length or by environmental conditions that induce developmental plasticity (Martin and Simon 1990b); observations of cicadas emerging prematurely in recently-cleared plots and beneath a greenhouse (Marlatt 1907) implicate climate effects as one likely trigger.

Past straggling probably formed the temporally isolated broods of each life cycle (Alexander and Moore 1962, Lloyd and Dybas 1966b, Lloyd and White 1976, Simon and Lloyd 1982, Martin and Simon 1990b). However, because most woods contain only one brood, successful reproduction by off-schedule cicadas must be rare. *Magicicada* are unusually weak fliers and easily captured by predators. Flocks of birds have been observed annihilating comparatively small introduced or straggler populations of up to a few thousand (Marlatt 1907, Beamer 1931, Alexander and Moore 1962, Dybas 1969, Chilcote and Stehr 1984). Apparently, normal *Magicicada* emergences are dense enough to satiate local predator populations, which cannot respond numerically due to the long life cycle (Lloyd and Dybas 1966a, Karban 1982, Williams et al. 1993), and great numbers are required for successful establishment of a new population by straggling.

Magicicada broods of the same life cycle are defined entirely by their temporal relationships. Conspecific cicadas of different broods have diverged slightly in wing morphology (Simon 1983, 1990) and allozymes (Simon 1979, Archie et al. 1985) but no fixed differences are known, and cicadas cannot be aged. Therefore the number and timing of broods in a location can be determined only by analysis of historical emergence patterns, an effort greatly complicated by records of off-schedule emergences. Such straggler

records are made especially problematic because most published records are not accompanied by information on emergence density. Because sparse *Magicicada* populations apparently fail to reproduce, evidence of very low emergence density implies that the population is rapidly declining or that the cicadas are stragglers from another established brood. Unfortunately, many records in current maps are probably based on one-time appearances of small numbers of cicadas (e.g. 1-3 individuals); examples are discussed below.

Use of cross-generation brood maps

Straggler-related error in the *Magicicada* historical record is increased by the use of distribution maps (Fig. 1) that lump records sharing a common 13- or 17-year historical pattern (e.g. Brood I maps lump records from 1995, 1978, 1961, etc., while Brood II maps include 1996, 1979, 1962, etc.). Such *cross-generation* maps do not include emergence dates; a dot indicates only that periodical cicadas appeared in one or more normal emergence years of a given brood. This practice makes it difficult to determine if a given record can be explained as stragglers from a different brood. Examination of one unusual case in which the original data are available today reveals the potential flaws in these maps: Marlatt (1898b) listed all localities reporting *Magicicada* to the USDA in 1898. The emergences were each assigned to either 13-year Brood XXIII or 17-year Brood VI, both of which appeared that year (Table 2). Listed for Illinois were eight county records of 17-year Brood VI and 25 county records of 13-year Brood XXIII (Fig. 2). Because only life cycle distinguishes 13- and 17-year cicadas in central Illinois, these records must have been assigned using *a priori* range estimates. However, the range of Brood XXIII in Illinois was not well-established in 1898 (Marlatt 1898a, b), and Brood VI had been recorded only sporadically. Brood assignments were therefore made more or less arbitrarily, with more northern emergences assumed to be from the 17-year brood; at least

one of these estimates is known today to have been incorrect (DeWitt Co., Lloyd et al. 1983). Some populations from adjacent counties were attributed to different broods, and two Scott county records were assigned differently (Fig. 2). Furthermore, descriptive data accompanying the original records (Marlatt 1898b) show that many involved only a handful of cicadas (Fig. 2). Since the 1898 cicadas appeared four years after 13-year Brood XIX and four years before 17-year Brood X (Table 2), which today inhabit many of the same localities, four-year straggling may explain many of the observations, especially those of Brood VI which is not known from the region today (see below). The 1898 records survive in many cross-generation brood maps, separated from the information required for recognition of their potential missassignment and straggler origin.

Edge extension and shadow broods

Two important questions in *Magicicada* biology are the frequency of brood overlap and the relationship of overlap to temporal isolation. Some broods (e.g. 17-year III and XIII) appear parapatric, while others (13-year XIX and XXIII) appear broadly sympatric in some maps. Cox and Carlton (1991) discussed a "broad zone of overlap" between 13- and 17-year cicadas, using Marlatt's (1907) cross-generation maps, while recent fine-scale mapping in the Midwest indicates comparatively minor sympatry (Simon 1988). The apparent overlap of 13- and 17-year broods in cross-generation maps could be explained by *edge extension* caused by chronic misinterpretation of stragglers: When stragglers from one brood (Brood A) appear in the normal emergence year of an adjacent but nonoverlapping brood (B), Brood A stragglers observed close to the anticipated range of Brood B may be incorrectly identified as Brood B cicadas, extending the recorded distribution of Brood B. Brood A stragglers appearing far from the anticipated limits of Brood B may be disregarded, if they are noticed at all. The reciprocal process may extend the recorded distribution of Brood A, until the two broods appear to overlap

geographically. Since stragglers can appear many years early or late, adjacent 17- and 13-year broods could leak stragglers into each other's emergence year in many different generations during a 221-year cycle.

Broods of the same life cycle may also undergo edge extension; such brood pairs share only one temporal path for straggler exchange, but the opportunity occurs in every generation. Edge extension between same-cycle broods may follow predictable patterns. First, some broods may be too far isolated in time to exchange many stragglers (e.g. 17-year Broods III and XIII). Second, if some forms of straggling are more common (e.g. one- and four-year aberrations: Kritsky 1987, Moore 1993), then certain brood pairs will accumulate more distribution errors. Third, four-year straggling, being perhaps less often anticipated, may generate more erroneous records than one-year straggling. If true, the latter prediction could explain the observation that geographic overlap is generally limited to broods separated by four years (e.g. 13-year Broods XIX and XXIII and 17-year Broods II, VI, X, and XIV) (Lloyd and White 1976, Simon and Lloyd 1982). While a few cases of four-year brood sympatry appear proven (e.g. Lloyd and White 1976), other cases may involve a nonexistent *shadow brood* produced by recurring four-year stragglers (see also Brood XXIII discussion below). Maier (1985) suggested that repeated four-year delayed stragglers from Brood II have created the illusion of sympatric populations of Brood VI on Long Island; Lloyd and White (1976) proposed a similar explanation for Brood XV.

Biased search effort and the distribution of Brood VI

Uneven search effort may strongly influence patterns of straggler-induced error on *Magicicada* distribution maps. For example, stragglers may be more likely to be observed in heavily-populated regions and near university towns. Stragglers are also more likely to be noticed when periodical cicadas are expected to emerge nearby or when large-scale mapping efforts are conducted. The groups of 1-10 Brood XIII stragglers observed in

northern Illinois in 1994 could have been recorded as populations of Brood XVII by an inexperienced observer, but they were unlikely to be noticed at all because no broods were anticipated in 1994.

The apparently shifting distribution of 17-year Brood VI through history illustrates the interaction of nonrandom searching and straggler misidentification. Brood VI was once described as "more widespread than any other 17-year brood" (USDA 1932), with isolated populations described from many states, but today it is limited to Appalachian NC, SC, and GA (Fig. 1). The apparently changing distribution of Brood VI and the scattered nature of many of its populations suggest that many records derive from misidentified stragglers (see also Lloyd and White 1976, Maier 1985, Kritsky 1987). Nearly all of the questionable Brood VI records fall within the ranges of 17-year Broods II, V, and X -- all separated from Brood VI by one or four years. But if straggling has caused the many Brood VI records outside of NC, SC, and GA, then why have other year-classes with similar potential remained empty (e.g. Brood XV, which could accumulate four-year straggler records from Broods II and XIV)? The answer appears to be that greater search efforts were conducted in the Brood VI year-class, mainly as part of an unusually thorough 1898 USDA study (Marlatt 1898b) that yielded widespread records of stragglers and led to searches in future Brood VI emergence years.

Brood VI maps before 1898 showed only six (Riley 1885, Walsh and Riley 1868) to twelve (Marlatt 1898a) records from no more than six states outside WI and Appalachian NC, SC, and GA. The 1898 USDA search increased this number to 90, from 21 states and the District of Columbia. This newly widespread distribution may have sparked interest in Brood VI, because just two generations later the cumulative USDA list had grown to 310 localities (USDA 1932). At this point the inconsistency of the records was apparent, because only 84 of these localities actually reported cicadas in 1932, and these were concentrated in Appalachian NC, SC, and GA, and within the eastern part of Brood X. Except for areas in Wisconsin and Michigan, nearly all of the locations that reported

strong Brood VI emergences 100 years ago support the brood today (Simon 1988), while Brood VI has not been recorded again from many locations where it was first noted in 1898. The strongest evidence that the apparent Brood VI distribution was expanded by misinterpreted stragglers in 1898 is the data on emergence density for that year (Marlatt 1898b): Outside the modern Brood VI range, low numbers were mentioned for 56% (61/106) of the counties, while abundant cicadas were reported in only 17% (18/106). No data on abundance were available for the remaining counties. Of the 18 counties that reported dense populations outside the modern range, ten are northern localities in Michigan and Wisconsin where confusion with the related genus *Okanagana* can occur (Maier 1985, Moore 1993); cicadas of this genus are morphologically similar, emerge in the spring, and sometimes form dense aggregations. Thus, the unusual historical record of Brood VI is likely attributable to stragglers being recorded in a year of unusually thorough search efforts.

II. Hypotheses of recent changes in *Magicicada* brood distributions

Historical evidence of brood distribution shifts has been cited in discussions of many aspects of *Magicicada* ecology and evolution, including competition between broods (Lloyd et al. 1983), life cycle genetics and hybridization (Lloyd et al. 1983, Cox and Carlton 1991), and life cycle shifts (Martin and Simon 1988). The above discussion and earlier arguments suggest that one apparently declining brood (Brood VI) has instead remained historically stable in its geographic distribution, and that the appearance of historical change is in part due to straggler-related error. A re-examination of the supporting data in two additional cases leads to a similar conclusion.

Hypothesis 1 -- Decline and extinction of midwestern 17-year Brood X

Several papers (Lloyd et al. 1983; Martin and Simon 1988, 1990a; Cox and Carlton 1991) have cited historical evidence suggesting that 17-year Brood X declined to extinction across Missouri, Illinois, and northern Arkansas between 1868 and 1919, and that during this period it was replaced by 13-year cicada populations. One element of the hypothesis has been challenged by Kritsky (1989) and Cox and Carlton (1991), who noted records of 13-year Brood XIX from Illinois and Missouri before 1868. No one has yet challenged the central element of the hypothesis -- that 17-year Brood X existed in the Midwest before 1868 and disappeared after 1902. This paper adds to earlier arguments and shows that Brood X was probably never present in the Midwest. The discussion can be organized under three points:

1) 13-year Broods XIX and XXIII existed in Missouri and Illinois before 1868.

Kritsky (1989) noted that "twelve counties in Illinois and much of southwestern Missouri experienced periodical cicada emergences in 1829, 1842, and 1855 indicating that the 13-year life cycle was established before 1868". Other sources lend considerable additional data to this conclusion: Cox and Carlton (1991) cited newspaper accounts suggesting a long history of 13-year broods in eastern Missouri. Walsh and Riley (1868) described accounts of probable 13-year cicadas near St. Louis (Brood XXIII) in 1859. They also mention records for Brood XXIII in Jackson Co. and Union Co., IL. While discussing stragglers, Walsh and Riley referred to one-year premature 13-year cicadas in two Missouri counties (Luray Co. and Daviess Co. in 1854) and one county in western Illinois (Madison Co. in 1867). Marlatt (1907) included a chronology of records including some from "all southeast part" of Missouri in 1829, 1842, 1855, and 1868. In 1868 Walsh and Riley wrote that Brood XIX was found in "nearly the whole state" of Missouri

without qualification even though Brood X emerged in the same year. They apparently did not believe confusion with Brood X to be a problem in Missouri and western Illinois, presumably because of the consistency of the records listed above.

2) No historical evidence indicates that 17-year Brood X cicadas existed in Missouri or western Illinois before 1902.

None of the primary sources of 19th century distribution information (Marlatt 1898a, Walsh and Riley 1868, Riley 1885) included a single record of 17-year Brood X in Missouri or western Illinois for years prior to 1868. Poor sampling was not the reason: Each publication listed ample Missouri records of 13-year Broods XIX and XXIII (see above) and 17-year Broods III and IV. Cicadas appeared in the region in the Brood X years 1868 and 1885, but these emergences could not be assigned to a brood with certainty because Brood X emerged together with 13-year Brood XIX in 1868 and with 13-year Brood XXIII in 1885 (Table 2); 13- and 17-year cicadas are morphologically and behaviorally indistinguishable in the Midwest. Nonetheless, later solo emergences of the 13-year broods together demonstrated that the 1868 and 1885 populations were probably 13-year cicadas, as concluded by Marlatt (1907, 1919) and suggested by the pre-1868 13-year cicada records discussed above. The existence of 17-year Brood X in the region in the 19th century has been inferred entirely from historical records of 1902 and 1919, evidence disputed in #3 below.

3) Cicadas emerging in the Brood X years 1902 and 1919 were likely stragglers from 13-year Broods XIX and/or XXIII.

The uncertain records from 1902 and 1919. 1902 was first year after 1851 in which 17-year Brood X emerged alone. Recognizing this, the USDA solicited records widely (Marlatt 1898b). Marlatt considered emergences of Brood X in Illinois and Missouri a possibility, but only because he viewed the northern latitudes as more appropriate for 17-

year cicadas (Marlatt 1898b). The results apparently confirmed the pre-1868 historical record: Marlatt (1919) later wrote "In 1902, for the first time since very careful study of the cicada began, it was not accompanied by a 13-year brood, and its actual range was more nearly determined than before, although the old limits of distribution were pretty generally confirmed". Marlatt's subsequent distribution maps (1907, 1919) contain no Missouri records of Brood X, and just a few Illinois records where Brood X exists today along the Indiana border. If these were the only sources available there would likely be no controversy surrounding the historical distribution of this brood.

However, uncertainty regarding Brood X in Missouri exists because a different source described 1902 emergences from 32 counties, citing J. M. Stedman, who "after careful culling of the reports received . . . found the brood to be quite generally tho' lightly distributed through the eastern half of the state" (Haseman 1915; Fig. 3f here). (Incidentally, Haseman also stated that Brood X "has not heretofore been reported from this state", supporting the arguments made in #2 above.) Marlatt published Brood XXIII records from Stedman in 1898 (Marlatt 1898b), so it is unclear why his later Brood X maps did not include Stedman's 1902 data.

Most of the critical 1902 Missouri records were not replicated 17 years later, when just four counties reported *Magicicada* (Haseman 1919). Only four subsequent records of putative Brood X exist for Missouri (Simon 1988); because these records are of "small populations" (Simon 1988) and do not correspond to the counties reporting cicadas in 1919, they remain of uncertain origin. The apparent decline from 1902-1919 inspired the theory that Brood X became extinct after that period (Lloyd et al. 1983). However, the records from one of these two emergence years can be easily dismissed: Haseman (1919) noted that the 1919 cicadas emerged in small numbers and may have been one-year precursors of 13-year Brood XIX populations known from those counties. The evidence of Brood X in Missouri and western Illinois therefore reduces to records from a single year -- 1902. As argued below, the historical record suggests that the 1902 cicadas were

delayed stragglers from one or both of the 13-year Broods XIX and XXIII. This discussion will focus on the more complete Missouri records, but the same hypothesis should apply to western Illinois (see Moore 1993).

Brood XIX or Brood XXIII stragglers, or both? -- The 1902 Missouri Brood X cicadas appeared in the central, southern, and eastern parts of the state (Fig. 3f), areas inhabited today by 13-year Broods XIX and XXIII (Fig. 1). Therefore, if due to straggling, these records could have involved four-year delayed Brood XXIII cicadas, eight-year delayed Brood XIX cicadas, or a combination of both (Table 2) (assuming that Brood XIX individuals due in 1907 could not have emerged in just their eighth year). There have been no published examples of multiple-year delayed 13-year cicadas. However, because (a) one-year life cycle plasticity is documented for both life cycle types, and (b) multiple-year delayed emergences have been observed in 17-year cicadas (see Section I above), such straggling seems plausible in 13-year cicadas as well. Furthermore, as discussed below, the arguments here offer an explanation for the apparent lack of multiple-year delayed straggling in 13-year cicadas.

The simplest straggling hypothesis would explain the 1902 cicadas as 4-year delayed Brood XXIII. At first, this hypothesis appears plausible because the historical Brood XXIII data (Marlatt 1898b, Haseman 1915, USDA 1937, Fig. 3a-d here) do include most of the counties that reported cicadas in 1902. However, as argued below, the pattern of records in Missouri from 1872 to the present day suggests that the apparent distribution of Brood XXIII was expanded in Missouri around the turn of the century by widespread delayed Brood XIX straggling. If correct, this possibility would rule out Brood XXIII as a source for many of the 1902 records, but it would also indirectly support the central hypothesis that the unexpected Brood X records were caused by multiple-year delayed straggling in 13-year cicadas: If four-year delayed Brood XIX straggling occurred to an extent sufficient to cause widespread records of Brood XXIII in 1898 and 1911, then it is

possible that some of the same Brood XIX stragglers emerged eight years late in 1902. Therefore, the discussion below will first analyze the Missouri distribution of 13-year Brood XXIII and then return to the argument that 1902 Brood X cicadas were delayed 13-year stragglers.

Straggling and 13-year Brood XXIII in Missouri -- Simon's (1988) recent maps show 13-year Brood XXIII in 10 Missouri counties near the Mississippi and Missouri Rivers. However, earlier maps show Brood XXIII in two to six times as many counties, including all but three of the counties reporting Brood X in 1902 (Fig. 3): the list includes 26 counties in 1885 (USDA 1937), 50 in 1898 (Marlatt 1898b, USDA 1937), and 59 in 1911 (Haseman 1915, USDA 1937). In notable contrast to the apparently changing distribution of Brood XXIII, 13-year Brood XIX has remained stable; the 1907 (Haseman 1915) and 1933 (USDA 1933) records show 13-year Brood XIX in all of Missouri except the northwestern counties, which are inhabited by 17-year Broods III and IV (Fig. 1). This description matches the those of Walsh and Riley (1868), Riley (1885), and Simon (1988).

These data suggest at first that Brood XXIII became more widespread after 1885, peaked in 1898 and 1911, and then declined sharply while Brood XIX remained stable, but there are reasons for skepticism. Given that Midwestern populations of these two 13-year broods contain the same species, there is little reason to believe that one brood could uniformly expand in distribution and then decline while another remains stable throughout the same general region. Furthermore, Kritsky (1987) noted that Brood XXIII populations in Indiana have been historically stable; these populations also lie at the northern 13-year cicada range limit and share a similarly close geographic relationship to Brood XIX.

A simpler hypothesis would explain the apparently shifting distribution of Missouri Brood XXIII as an artifact of repeated four-year delayed straggling from Brood XIX from 1885-1911, a hypothesis supported by many details of the historical record: (1) The "core"

Brood XXIII distribution may have changed little since 1868. Except for a cluster of counties around Springfield in 1885, the pre-1898 records of Brood XXIII are all from locations near the modern Brood XXIII distribution (Fig. 3) (Walsh and Riley 1868, Riley 1885, USDA 1937). (2) All counties listed by Marlatt (1898b 1907) as containing dense Brood XXIII populations are found within or near the modern-day range (Fig. 3), as are the localities for all four Brood XXIII specimen records from Froeschner (1952); specimens may be more often obtained from dense populations. (3) Many of the 1898 and 1911 populations were sparse: In 1898, 86% (31/36) of the within-county Missouri localities reporting data on abundance (36/71) noted small numbers (most reported "one, "one or two", "two or three", "few", or "very few"); nearly all of these counties fall outside of the modern Brood XXIII distribution (Fig. 3c; Marlatt 1898b). In addition, Haseman (1915) described the 1911 Missouri emergence as "much lighter than in former visitations" and "much less abundant than in 1907", attributing the pattern to an unusually dry spring. (4) Most of the counties which have reported Brood XXIII at least once contain Brood XIX today (67 out of 75) or are located where Brood XXIII certainly exists today (five out of 75), as expected if the Brood XXIII distribution has not changed and Brood XIX straggling caused most of the 1898 and 1911 records. The three exceptions are all counties that today harbor either Brood III or IV 17-year cicadas; because the records from these counties all date to a Brood III or IV year, or to one or two years preceding a Brood III or IV year, emergences of 17-year cicadas are a likely explanation. (5) There is inconsistency in the Brood XXIII record across generations: Of the 77 counties listed by Marlatt (1898b) or Haseman (1915), 23 were reported only by Marlatt and 24 were reported only by Haseman.

Four-year delayed stragglers from Brood XIX may often be mistaken for Brood XXIII cicadas, especially when emergence density is not considered, because such stragglers always appear synchronously with the later brood, and because only emergence timing distinguishes cicadas of the two broods. Because more than half of all 13-year

populations are found in Brood XIX, errors of this sort could explain in part the lack of published cases of multiple-year delayed straggling by 13-year cicadas.

Straggling and 1902 Brood X in Missouri -- The discussion can now return to the question of 17-year Brood X in Missouri. If the above arguments are correct and Brood XXIII has always been restricted to central and southeastern Missouri, then many of the 1902 cicadas cannot be explained simply as four-year delayed stragglers from Brood XXIII because they appeared outside the range of that brood, in locations containing only Brood XIX. However, if many of the widespread records of Brood XXIII in 1898 were caused by four-year straggling from Brood XIX populations, then it is possible that other stragglers from the same populations delayed longer than four years and emerged eight years late in 1902 as the unexpected Brood X populations. The hypothesis that the 1902 straggler records were caused by a combination of four- and eight-year delayed straggling predicts that the 1902 counties should tend to be the same ones that observed apparent Brood XXIII emergences in 1898. This prediction is confirmed: 25 of the 32 Missouri counties reporting Brood X in 1902 also reported an apparent Brood XXIII emergence in 1898 (Marlatt 1898b), a statistically significant association within the 13-year Missouri range (Fisher Exact $P = 0.001$; Table 2); all of the 1902 counties today harbor either Brood XIX or XXIII. The 13-year straggler hypothesis also predicts the absence of 1902 Brood X records from northwestern Missouri counties (Fig. 3) (Marlatt 1898b), which are inhabited today only by 17-year Broods III or IV (Fig. 1). Cicadas from these broods are separated in time almost maximally from Brood X (Table 2) and would have to emerge in their 6th, 7th, 23rd, or 24th year of development (respectively) to produce false Brood X records. These patterns strongly indicate that straggling from the 13-year broods created false Brood X records in 1902.

The possibility of greater straggling by midwestern 13-year cicadas -- Each *Magicicada* species is most closely related to a counterpart with the alternative life cycle. The hypothesis that midwestern 13-year populations sometimes produce straggler cicadas as old as 21 years is made more plausible by the discovery that one 13-year cicada species from the region, *Magicicada neotredecim* (description in Marshall and Cooley 2000), shares an unusually recent common ancestry with its 17-year sibling *M. septendecim* (L.), from which it can be distinguished only by life cycle; in contrast, its most similar 13-year counterpart *M. tredecim* (Walsh and Riley) differs from *M. septendecim* in song, morphology and mitochondrial DNA (Martin and Simon 1988, 1990a; Marshall and Cooley 2000, Chapter 3) (Table 1). 21-year old stragglers from 17-year broods have been observed repeatedly (see above); perhaps 13-year *M. neotredecim* retains similar developmental plasticity. Most *M. neotredecim* are found in Brood XIX in Missouri and Illinois, where, as argued above, multiple-year delayed straggling has created false records of Brood XXIII and Brood X. Furthermore, unexpected Brood XXIII populations of likely straggler origin have appeared more often in the midwest (where *M. neotredecim* is present) than in the south or southeast (where only *M. tredecim* is present). Because Brood XXIII contains comparatively few *M. neotredecim* populations (Simon et al. 2000), the hypothesis could explain in part why similar delayed straggling from Brood XXIII has not created shadow populations of later broods in the historical record (e.g. Brood XXVII). This hypothesis could be tested more severely by comparing straggling rates of *M. neotredecim* and *M. tredecim* where they occur sympatrically (Simon et al. 2000) in northern Brood XXIII populations of Indiana and Illinois.

A note on other interpretations of unexpected Brood XXIII records -- Lloyd et al. (1983) proposed a single-locus model of *Magicicada* life cycle genetics and suggested that hybridization between 17-year Brood X (AA or A-) and a 13-year Brood XIX (aa) gave rise to widespread new populations of 13-year Brood XXIII in Illinois, Missouri, northern Arkansas, and Kentucky following F1 interbreeding and Mendelian segregation of F2 homozygotes. Historical records were cited showing the unexpected

appearance of Brood XXIII populations throughout the region in 1898, exactly 30 years after the co-emergence of 13- and 17-year Broods XIX and X. The above discussions suggest that (1) unexpected populations of Brood XXIII can be accounted for by four-year straggling from 13-year Brood XIX (see also Cox and Carlton 1991), and (2) there is little evidence that Brood X existed in much of the midwest during the 19th century. Other evidence cited in favor of the model was apparently not considered in light of the problem of straggling (see Moore 1993).

Hypothesis 2: 13-year competitive displacement of 17-year cicadas

Lloyd et al. (1983, p. 1170) proposed that the boundary between 13- and 17-year broods in Illinois has shifted northward during the past century. The historical hypothesis was developed by comparing the southern distribution limit of 17-year Brood XIII in Illinois as mapped in 1871 by LeBaron (1872) to the same boundary as mapped in 1973 by Stannard (1975). The boundaries (Fig, 4a) suggest recent northward retreat of about 50 km by Brood XIII in eastern Illinois and in the Knox Co./Henry Co. area in western Illinois. Lloyd et al. noted distribution records from Stannard (1975) indicating the presence of the 13-year brood in the apparently vacated region and suggested that Brood XIX cicadas had displaced the 17-year brood. However, neither the retreat of Brood 17-year XIII nor the advance of 13-year Brood XIX is supported by the modern data.

Stannard (1975) included both positive and negative search records in his maps. Fig. 4b shows the negative Brood XIII records (1973) consistent with Brood XIII retreat and the positive Brood XIX records (1972) consistent with Brood XIX invasion (those found on or north of the 1871 Brood XIII boundary). The negative records show that almost no data support the position of the southern boundary drawn by Stannard; just two such records for Brood XIII (in Knox and DeWitt counties) exist more than 10 km north of the 1872 limit estimated by LeBaron. Moreover, Stannard obtained two additional negative

Brood XIII records deep within the range of this brood in Lee and Lake counties (not plotted, but counties indicated in Fig. 4a), so it is not clear that the critical negative records indicate anything other than a patchy distribution.

The proposed advance of 13-year Brood XIX is also poorly supported, for two reasons. First, very few positive records were listed for the disputed region: Stannard noted only four such records for 1972 within the entire 12,000 km^2 region apparently vacated by Brood XIII (Fig. 4b). Second, three of these localities have probably contained 13-year cicadas for at least a century: Walsh and Riley (1868) recorded Brood XIX in eastern McLean Co. in 1868. Hyslop (1935) listed *Magicicada* in Wapella in Dewitt county for 1868 and 1885, consistent with either 17-year Brood X or 13-year Broods XIX and XXIII; both 13-year broods probably inhabit the county today (Lloyd et al. 1983, Simon 1988). The Livingston County record matches records from the USDA (USDA 1933) and Marlatt (1907), although Walsh and Riley did not list the county for 1868. Stannard's Livingston County record is located farther north than any other from LeBaron's Brood XIII line and is therefore most responsible for the appearance of northward Brood XIX dispersal.

The remaining Brood XIX 1972 record (Knox Co.) could be questioned because Brood XIII is found in approximately this location and emerged one year later in 1973. Stannard (1975) noted that the 1972 Knox county emergence could be interpreted as a one-year premature emergence of Brood XIII, although there remains some question because apparently large numbers were involved. Unfortunately, the locality was not searched for Brood XIII in 1973. Extensive searches in Knox County in 1998 did not locate Brood XIX *Magicicada* (Alexander et al. in prep.).

Even if more supporting records were available, the conclusion that brood distribution changes had occurred might not be warranted. Broods of periodical cicadas may not have simple margins on a local scale. LeBaron (1872, p. 132) described the 1871 Brood XIII (Northern Illinois Brood) range limit with this comment:

> "...Neither must it be understood that no locusts were seen outside of this range. The locust line is not a simple and straight one, but more or less zig-zag, being necessarily much governed by the presence or absence of the timber which constitutes the natural depository of the insects' eggs . . . I may here remark that the Northern Illinois brood of locusts of 1871 meets and interlocks more or less with the Southern Illinois brood of 1868...".

Finally, additional historical data cast some doubt on the thoroughness of Lebaron's Brood XIII survey. Riley (1885) published an apparently independent account of the 1871 Brood XIII emergence, stating that "there seem to be detachments extending farther south, especially in the eastern portions of the State, and they occurred as far south as Shelby County." These "detachments" were reported from well south and east of the southern limit of LeBaron's (1872) distribution. As late as 1923 Marlatt's maps showed Brood XIII as far south as Edgar and Shelby counties; neither of these counties was checked by Stannard (1975), who mapped just nine negative emergence records for Brood XIII in the entire eastern half of Illinois. The Edgar and Shelby county records could have been erroneous, or perhaps disjunct Brood XIII populations exist there today. The contrast between the LeBaron and Riley accounts is noted here only to illustrate the uncertainty inherent in the *Magicicada* record.

The stability of brood distributions

The most significant *Magicicada* brood distribution changes implied by the historical record (the fluctuating ranges of Broods VI, X, and XXIII and the northward displacement of the boundary between 13- and 17-year broods) appear to be false patterns resulting in part from erroneous interpretation of straggling cicadas. Instead, brood distributions seem

to have remained stable throughout recorded history. However, this conclusion is not intended to ignore important changes occurring on a more local scale: First, straggling events of large magnitude may sometimes lead to the establishment of new brood populations (Lloyd and White 1976, Simon and Lloyd 1982), although the rarity of brood sympatry suggests that these are not formed often or that they do not usually persist for many generations. Second, many authors (e.g. Marlatt 1907, Young 1958) have noted the widespread loss of periodical cicada populations to forest fragmentation; fortunately, *Magicicada* appear to thrive on woods edges and persist even in narrow strips of woods along prairie rivers. Finally, evidence suggests recent loss of a few very localized broods (Williams and Simon 1995), such as Brood XI in CT (Manter 1974) and Brood XXI in FL (Young 1958).

Literature Cited

Anon. 1932. Periodical cicada. USDA Bur. Ent., Ins. Pest Surv. Bull. 12: 424-425.

Anon. 1933. Periodical cicada. USDA Bur. Ent., Ins. Pest Surv. Bull. 13: 336-338.

Anon. 1937. Periodical cicada. USDA Bur. Ent., Ins. Pest Surv. Bull. 17: 111-116, 617-618.

Alexander, R. D. and T. E. Moore. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species (Homoptera, Cicadidae, *Magicicada*). Univ. Mich. Mus. Zool. Misc. Publ. 121: 1-59.

Alexander, R. D., A. F. Richards, D. C. Marshall, and J. R. Cooley. In prep. Observations of straggler *Magicicada* in Illinois.

Archie, J. W., C. M. Simon, and D. Wartenburg. 1985. Geographical patterns and population structure in periodical cicadas based on spatial analysis of allozyme frequencies. Evolution 39: 1261-1274.

Beamer, R. H. 1931. Notes on the 17-year cicada in Kansas. J. Kans. Entomol. Soc. 4: 53-58.

Bryce, D., and N. Aspinwall. 1975. Sympatry of two broods of the periodical cicada (*Magicicada*) in Missouri. Am. Midl. Nat. 93: 450-454.

Chilcote, C. A., and F. W. Stehr. 1984. A new record for *Magicicada septendecim* (L.) in Michigan (Homoptera: Cicadidae). Great Lakes Entomol. 17: 53-54.

Cox, R. T. and C. E. Carlton. 1988. Paleoclimatic influences in the evolution of periodical cicadas (Insects: Homoptera: Cicadidae: *Magicicada* spp.). Am. Midl. Nat. 120: 183-193.

Cox, R. T. and C. E. Carlton. 1991. Evidence of genetic dominance of the 13-year life cycle in periodical cicadas (Homoptera: Cicadidae: *Magicicada* spp.). Am. Midl. Nat. 125: 63-74.

Dybas, H. S. 1969. The 17-year cicada: A four-year “mistake”? Bull. Field Mus. Nat. Hist. 40: 10-12.

Dybas, H. S., and D. D. Davis. 1962. A population census of seventeen-year periodical cicadas (Homoptera: Cicadidae: *Magicicada*). Ecology 43: 432-444.

Froeschner, R. C. 1952. A synopsis of the Cicadidae of Missouri. J. N. Y. Entomol. Soc. 60: 1-14.

Haseman, L. 1915. The periodical cicada in Missouri. Univ. Mo. Agric. Exp. Sta. Bull. 137: 1-33.

Haseman, L. 1919. Brood X of the periodical cicada in Missouri. J. Econ. Entomol. 12: 467.

Hyslop, J. A. 1935. The periodical cicada. USDA Entomol. Plant Quar. Bur. Ser. E-364.

Karban, R. 1982. Increased reproductive success at high densities and predator satiation for periodical cicadas. Ecology 63: 321-328.

Kritsky, G. 1987. An historical analysis of periodical cicadas in Indiana (Homoptera: Cicadidae). Proc. Ind. Acad. Sci. 97: 295-322.

Kritsky, G. 1989. Periodical cicadas. Nature 341: 288-289.

Kritsky, G., and S. Simon. 1996. The unexpected 1995 emergence of periodical cicadas (Homoptera: Cicadidae: *Magicicada* spp.) in Ohio. Ohio J. Sci. 96: 27-28.

LeBaron, W. 1872. Periodical cicada, pp. 124-133. *In* Second annual report on the noxious and beneficial insects of the state of Illinois. Trans., Ill. St. Hort. Soc. for 1871.

Lloyd, M., and H. S. Dybas. 1966a. The periodical cicada problem. I. Population ecology. Evolution 20: 133-149.

Lloyd, M., and H. S. Dybas. 1966b. The periodical cicada problem. II. Evolution. Evolution 20: 466-505.

Lloyd, M., and J. White. 1976. Sympatry of periodical cicada broods and the hypothetical four-year acceleration. Evolution 30: 786-801.

Lloyd, M., G. Kritsky, and C. Simon. 1983. A simple Mendelian model for the 13- and 17-year life cycle of periodical cicadas, with historical evidence for hybridization between them. Evolution 37: 1162-1180.

Maier, C. T. 1982. Abundance and distribution of the seventeen-year periodical cicada, *Magicicada septendecim* (Linnaeus) (Hemiptera: Cicadidae - Brood II), in Connecticut. Proc. Entomol. Soc. Wash. 84: 430-439.

Maier, C. T. 1985. Brood VI of 17-year periodical cicadas, *Magicicada* spp. (Hemiptera: Homoptera: Cicadidae): New evidence from Connecticut (USA), the hypothetical 4-year deceleration, and the status of the brood. J. N. Y. Entomol. Soc. 93: 1019-1026.

Manter, J. A. 1974. Brood XI of the periodical cicada seems doomed. Mem. Conn. Entomol. Soc. 1974: 10.

Marlatt, C. L. 1898a. The periodical cicada. USDA Div. Entomol. Bull. 14: 1-148.

Marlatt, C. L. 1898b. A consideration of the validity of the old records bearing on the distribution of the broods of the periodical cicada, with particular reference to the occurrence of broods VI and XXIII in 1898. Bull. U.S. Bur. Entomol. 18: 59-78.

Marlatt, C. L. 1907. The periodical cicada. USDA Bur. Entomol. Bull. 71: 1-181.

Marlatt, C. L. 1919. The “17-year locust” in 1919. USDA Cir. 127: 1-10.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with a description of a new 13-year species, *Magicicada neotredecim.* Evolution in press (slated for August 2000).

Martin, A., and C. Simon. 1988. Anomalous distribution of mitochondrial and nuclear gene markers in periodical cicadas. Nature 336: 237-239.

Martin, A., and C. Simon. 1990a. Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography. Evolution 44: 1066-1080.

Martin, A., and C. Simon. 1990b. Temporal variation in insect life cycles: lessons from periodical cicadas. BioScience 40: 359-367.

Moore, T. E. 1993. Acoustic signals and speciation in cicadas (Insecta: Homoptera: Cicadidae), pp. 269-284. *In* D. R. Lees and D. Edwards [eds.], Evolutionary patterns and processes. Academic Press, London.

Riley, C. V. 1885. The periodical cicada. An account of *Cicada septendecim* and its *tredecim* race, with a chronology of all broods known. USDA Div. Entomol. Bull. 8 :1-46.

Simon, C. 1979. Evolution of periodical cicadas: phylogenetic inferences based on allozymic data. Syst. Zool. 28: 22-39.

Simon, C. 1983. Morphological differentiation in wing venation among broods of 13- and 17-year periodical cicadas. Evolution 37: 104-115.

Simon, C. 1988. Evolution of 13- and 17-year periodical cicadas (Homoptera: Cicadidae). Bull. Entomol. Soc. Am. 34: 163-176.

Simon, C. 1990. Discriminant analysis of year classes of periodical cicadas based on wing-morphometric data enhanced by molecular information, pp. 309-323. *In* J. T. Sorensen and R. G. Footit [eds]. Ordinations in the study of morphology, evolution, and systematics: applications and quantitative genetic rationales. Elsevier, New York.

Simon, C., and M. Lloyd. 1982. Disjunct synchronic population of 17-year periodical cicadas: Relicts or evidence of polyphyly? J. N. Y. Entomol. Soc. 110: 275-301.

Simon, C., J. Tang, S. Dalwadi, G. Staley, J. Deniega, and T. R. Unnasch. 2000. Genetic evidence for assortative mating between 13-year cicadas and sympatric "17-year cicadas with 13-year life cycles" provides support for allochronic speciation. Evolution in press (slated for August 2000).

Stannard, L. J. 1975. The distribution of periodical cicadas in Illinois. Ill. Nat. Hist. Surv. Biol. Notes 91: 3-12.

Walsh, B. D., and C. V. Riley. 1868. The periodical cicada. Am. Entomol. 1(4): 63-72.

Williams, K. S., and C. Simon. 1995. The ecology, behavior, and evolution of periodical cicadas. Annu. Rev. Entomol. 40: 269-295.

Williams, K. S., K. G. Smith, and F. M. Stephen. 1993. Emergence of 13-yr periodical cicadas (Cicadidae: *Magicicada*): phenology, mortality, and predator satiation. Ecology 74: 1143-1152.

Young, F. N. 1958. Some facts and theories about the broods and periodicity of the periodical cicadas. Proc. Indiana Acad. Sci. 68: 164-170.

Young, F. N. 1974. Observations on periodical cicadas (Brood XIV) in Indiana in 1974 (Homoptera - Cicadidae). Proc. Indiana Acad. Sci. 84: 289-293.

Table 6.1. The periodical cicada complex

Species	Life Cycle
Magicicada septendecim (L.)	17
Magicicada neotredecim Marshall and Cooley	13
Magicicada tredecim (Walsh and Riley)	13
Magicicada cassini (Fisher)	17
Magicicada tredecassini Alexander and Moore	13
Magicicada septendecula Alexander and Moore	17
Magicicada tredecula Alexander and Moore	13

Table 6.2. Schedule of *Magicicada* broods

Year	17	13	Year	17	13	Year	17	13	Year	17	13	Year	17	13
1825	I		1863	V		1901	IX		1939	XIII		1977		
1826	II		1864	VI		1902	X		1940	XIV		1978	I	
1827	III		1865	VII		1903			1941			1979	II	
1828	IV		1866	VIII		1904			1942			1980	III	
1829	V	XIX	1867	IX		1905	XIII		1943			1981	IV	
1830	VI		1868	X	XIX	1906	XIV		1944	I		1982	V	
1831	VII		1869			1907		XIX	1945	II		1983	VI	
1832	VIII	XXII	1870			1908			1946	III	XIX	1984	VII	
1833	IX	XXIII	1871	XIII	XXII	1909			1947	IV		1985	VIII	XIX
1834	X		1872	XIV	XXIII	1910	I	XXII	1948	V		1986	IX	
1835			1873			1911	II	XXIII	1949	VI	XXII	1987	X	
1836			1874			1912	III		1950	VII	XXIII	1988		XXII
1837	XIII		1875			1913	IV		1951	VIII		1989		XXIII
1838	XIV		1876	I		1914	V		1952	IX		1990	XIII	
1839			1877	II		1915	VI		1953	X		1991	XIV	
1840			1878	III		1916	VII		1954			1992		
1841			1879	IV		1917	VIII		1955			1993		
1842	I	XIX	1880	V		1918	IX		1956	XIII		1994		
1843	II		1881	VI	XIX	1919	X		1957	XIV		1995	I	
1844	III		1882	VII		1920		XIX	1958			1996	II	
1845	IV	XXII	1883	VIII		1921			1959		XIX	1997	III	
1846	V	XXIII	1884	IX	XXII	1922	XIII		1960			1998	IV	XIX
1847	VI		1885	X	XXIII	1923	XIV	XXII	1961	I		1999	V	
1848	VII		1886			1924		XXIII	1962	II	XXII	2000	VI	
1849	VIII		1887			1925			1963	III	XXIII	2001	VII	XXII
1850	IX		1888	XIII		1926			1964	IV		2002	VIII	XXIII
1851	X		1889	XIV		1927	I		1965	V		2003	IX	
1852			1890			1928	II		1966	VI		2004	X	
1853			1891			1929	III		1967	VII		2005		
1854	XIII		1892			1930	IV		1968	VIII		2006		
1855	XIV	XIX	1893	I		1931	V		1969	IX		2007	XIII	
1856			1894	II	XIX	1932	VI		1970	X		2008	XIV	
1857			1895	III		1933	VII	XIX	1971			2009		
1858		XXII	1896	IV		1934	VIII		1972		XIX	2010		
1859	I	XXIII	1897	V	XXII	1935	IX		1973	XIII		2011		XIX
1860	II		1898	VI	XXIII	1936	X	XXII	1974	XIV		2012	I	
1861	III		1899	VII		1937		XXIII	1975		XXII	2013	II	
1862	IV		1900	VIII		1938			1976		XXIII	2014	III	XXII

Table 6.3. Tabulation showing association between 1898 (apparent 13-year Brood XXIII) and 1902 (apparent 17-year Brood X) Missouri *Magicicada* emergence records by county (from Fig. 3), showing that cicadas appearing in 1902 tended to be found in the same counties that reported apparent Brood XXIII cicadas in 1898 (Fisher Exact P=0.001). This pattern is expected if the 1902 records were a combination of (1) four-year stragglers from Brood XXIII populations, which appeared in 1898, and (2) eight-year delayed stragglers from Brood XIX populations, assuming that Brood XIX populations producing eight-year stragglers (appearing in 1902) would be likely to produce four-year stragglers (appearing in 1898) as well. Statistical tabulation calculated using 90 as the base number of Missouri counties containing one or both 13-year broods (all but the northeast corner of the state; Fig. 1).

	Brood X Recorded (1902)	Brood X Not Recorded
Brood XXIII Recorded (1898)	25	24
Brood XXIII Not Recorded	7	34

Figure 6.1. Distributions of *Magicicada* broods, summarized from county-level maps in Simon (1988). I-XIV are 17-year broods; XIX-XXIII are 13-year broods. The remaining year-classes are not known to contain sustaining populations.

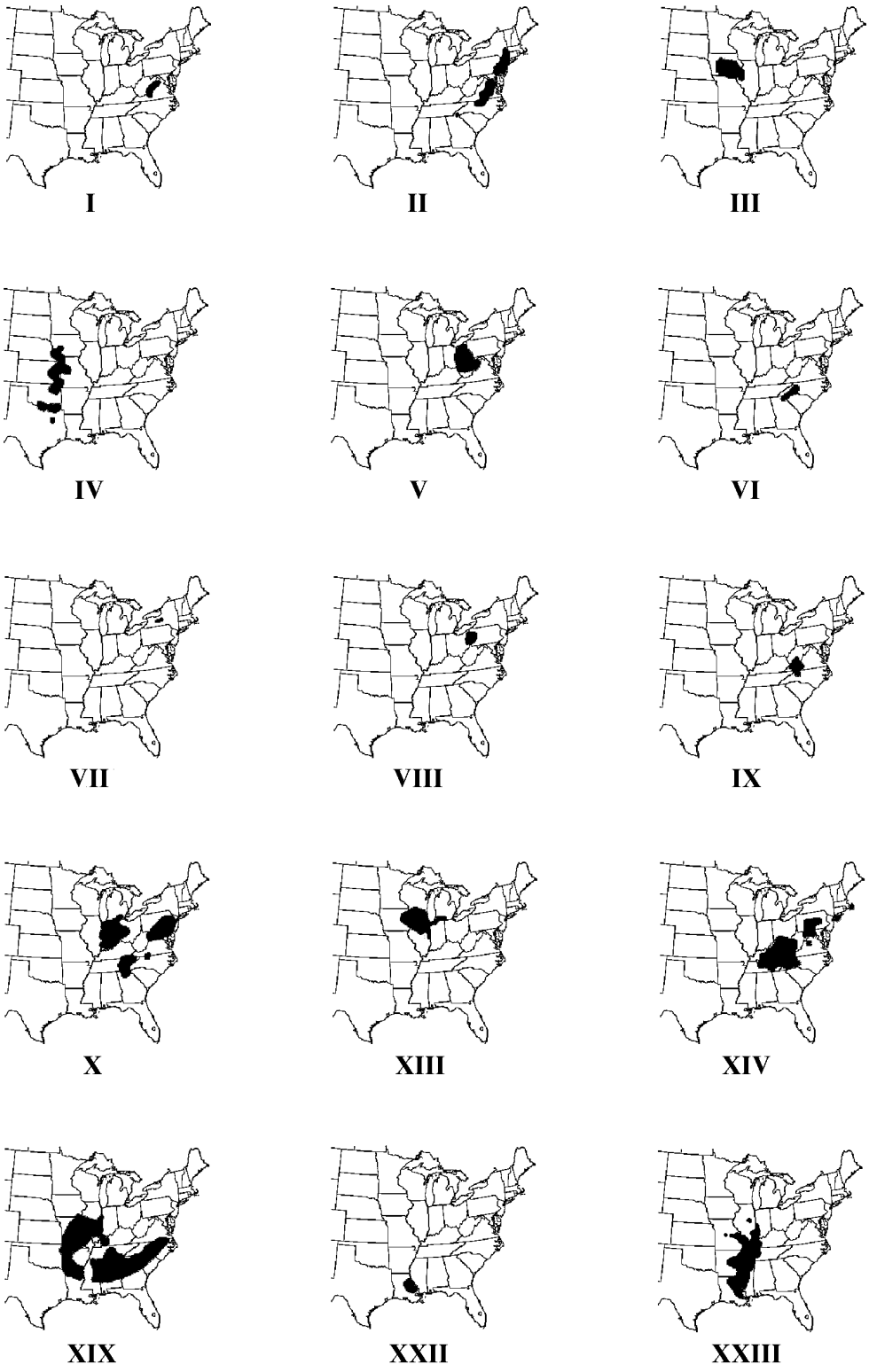


Figure 6.2. *Magicicada* Illinois emergence records from 1898 (Marlatt 1898b), listed by county with available emergence density data. Assignment of records to either 17-year Brood VI or 13-year Brood XXIII was done by Marlatt using *a priori* range estimates. The sparse populations were probably stragglers from 13-year Brood XIX (see Section II). Brood VI is not found in Illinois today, and the 1898 distribution of dense populations approximates the modern distribution of Brood XXIII (Stannard 1975).

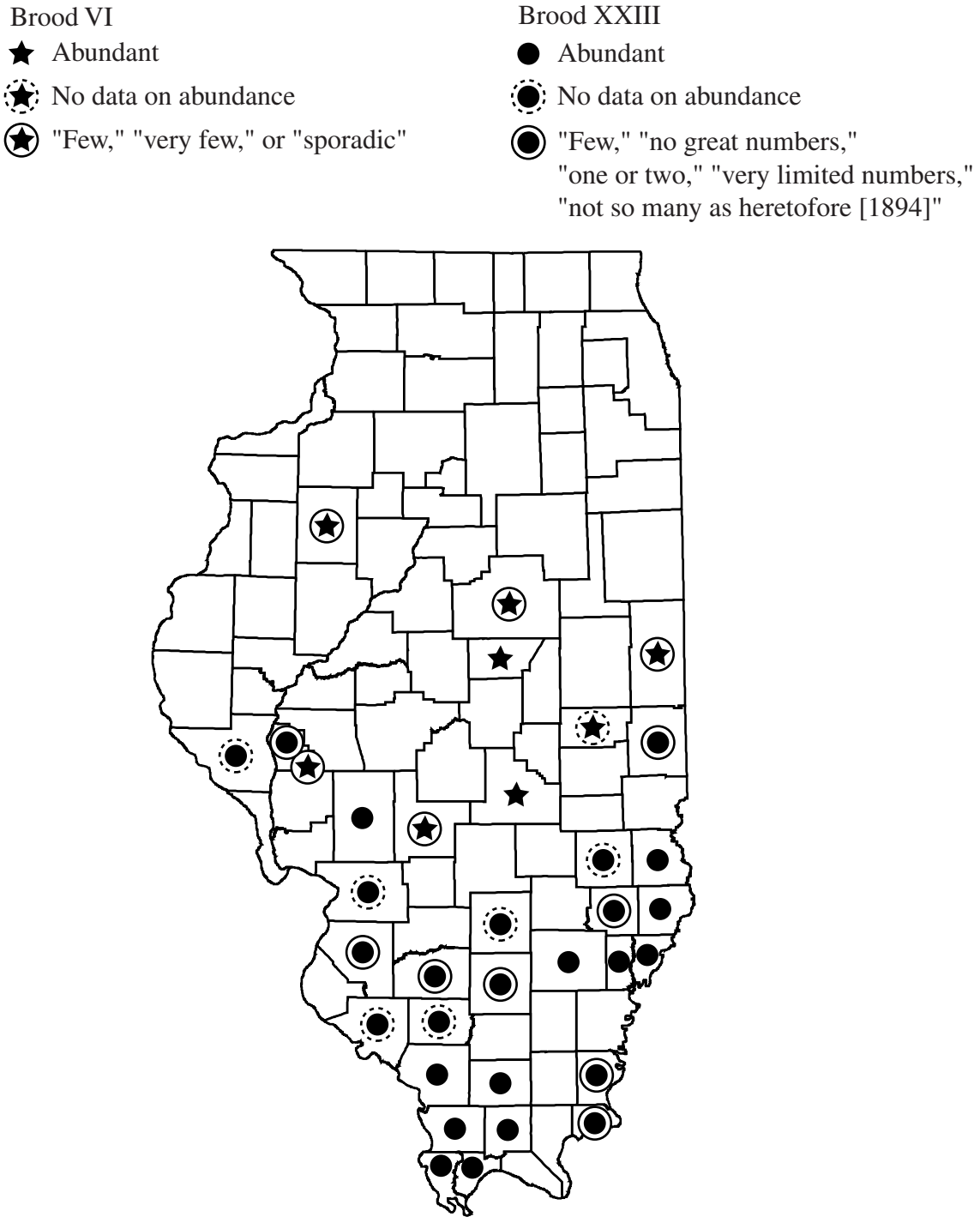


Figure 6.3. Missouri *Magicicada* records plotted by county. A-D: Brood XXIII emergence years 1872-1911. E: Modern distribution of Brood XXIII from Simon (1988). F: Brood X emergence year 1902. Records from 1898 (C) include emergence density information when available (from Marlatt 1898b, 1907): Star = dense populations reported; circled dot = sparse populations reported; dot = emergences reported without abundance data. Records obtained from Froeschner (1952) - 1911 St. Genevieve Co. only, Haseman (1915), Hyslop (1935) - 1885 Barry Co. only, Marlatt (1898b, 1907), Simon (1988), and USDA (1937) - 1898 Scotland and Lewis Cos. only.

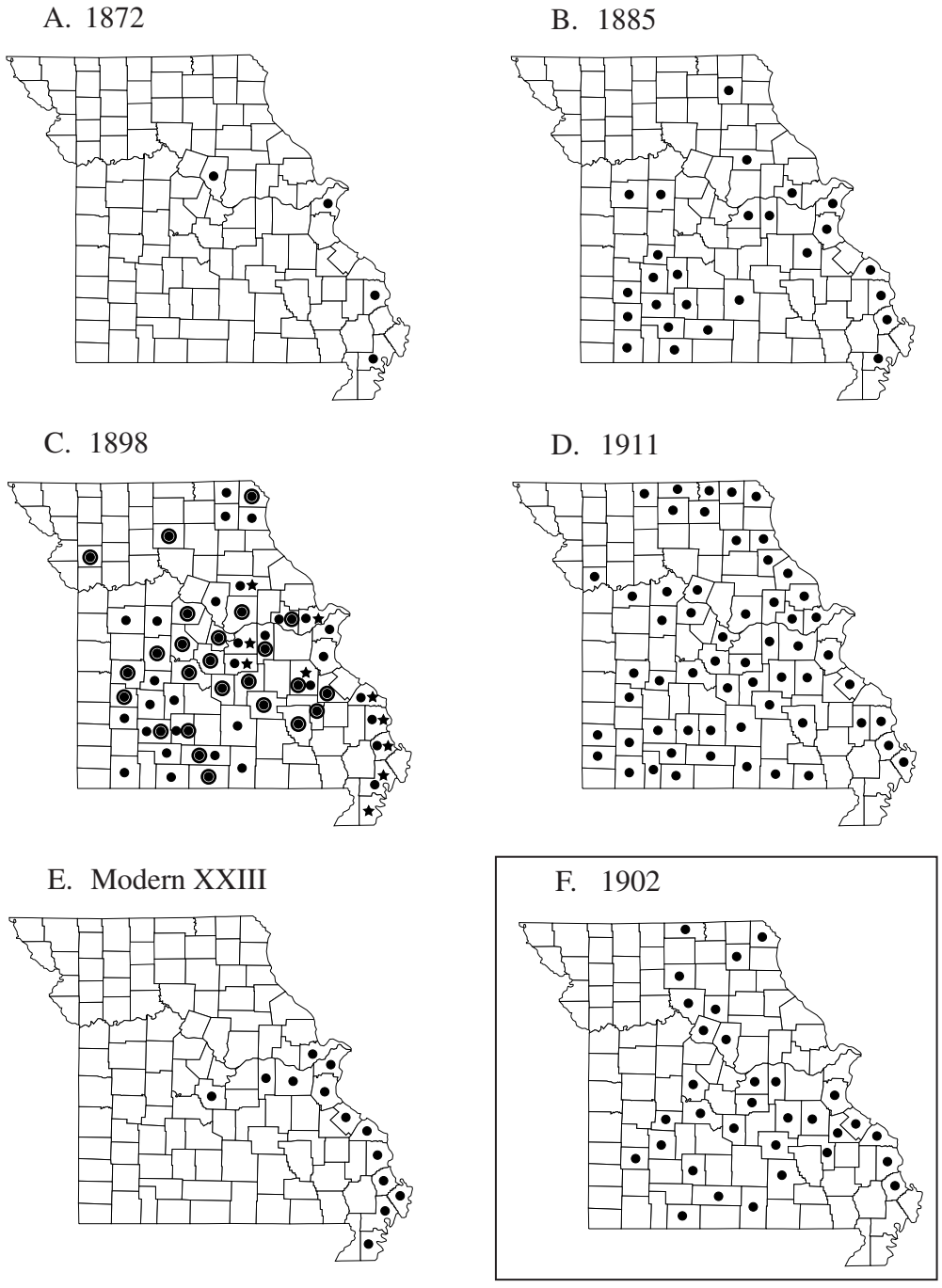
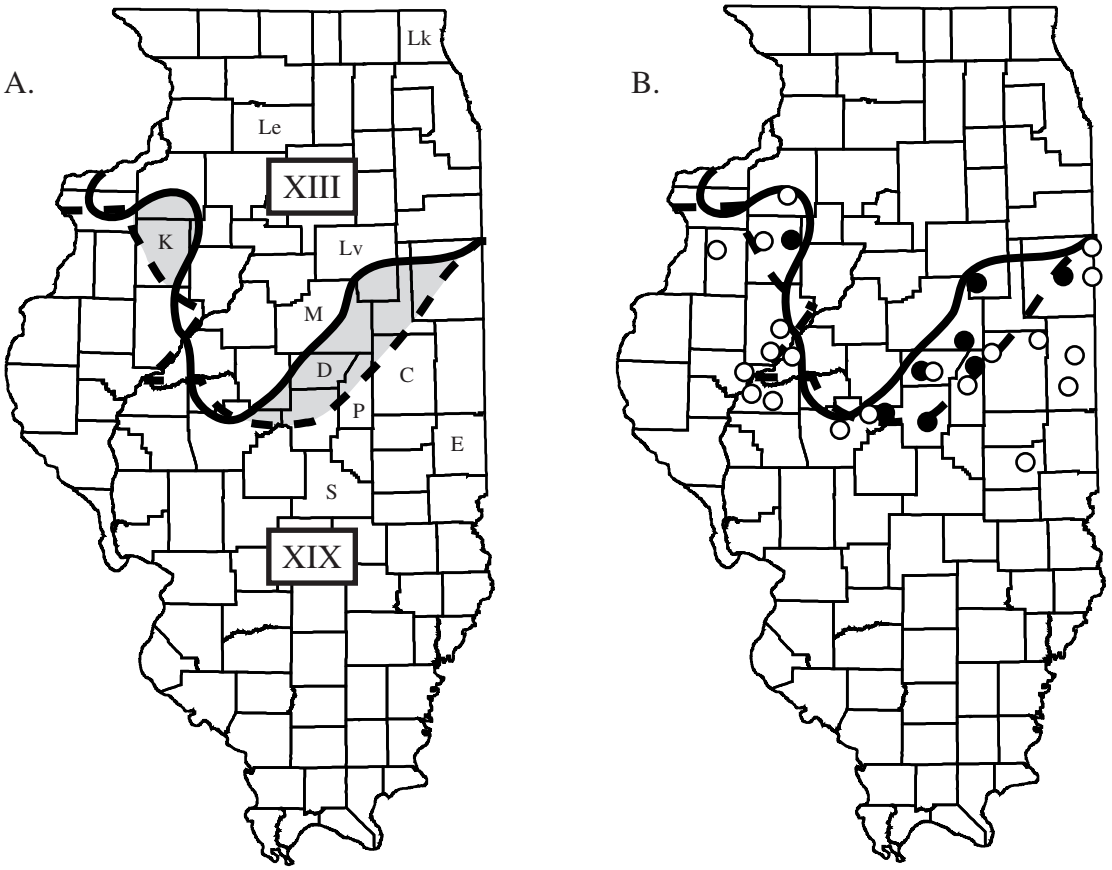


Figure 6.4. a) Apparent retreat (shaded region) of 17-year Brood XIII in central Illinois, adapted from Lloyd et al. (1983). Dashed line is estimated southern boundary of Brood XIII in 1871 (LeBaron 1872). Solid line is estimated limit in 1973 (Stannard 1975). Counties mentioned in discussion are Champaign (C), Dewitt (D), Edgar (E), Knox (K), Lake (Lk), Lee (Le), Livingston (Lv), McLean (M), and Shelby (S). Other Illinois broods not shown. b) Negative Brood XIII records (open circles) used to infer retreat of Brood XIII and positive Brood XIX records (filled circles) used to infer presence (and implied advance) of Brood XIX, from Stannard (1975).



APPENDICES

APPENDIX A

These two programs simulate random mating conditions in a model population of males and females, calculate a variance statistic describing the degree of male mating skew, and estimate the frequency distribution of that statistic by repeating the simulation many times. They are described in Experiment E of Chapter 2.

Simulation using both mated and unmated males

```
program allmales;

{Simulates level of incidental polygyny, the variance in male mating success}
{expected if all males are equally likely to remate on average}

    var
        variancedistribution: array[0..10000] of integer;
        newmales, newmatings, newdeaths: array[0..10] of integer;
        malematingarray: array[0..101] of integer;
        maledeatharray: array[0..101] of integer;
        malesample, malestodate, days, iteration, iterations, index, variance, sum, flag1,
    flag2: integer;
        randomnumber, mean, dummyvar: real;


    {* Seeds Random Number Generator}
    procedure timeseed;

    begin
        GetDateTime(randseed);
    end;


    procedure inputs;

        var
            x: integer;

    begin
        write('Number of simulations desired (maximum 10,000): ');
        readln(iterations);
        write('Total male sample size (maximum 100): ');
        readln(malesample);
```

```
    write('Number of days in simulation (maximum 10): ');
    readln(days);
    for x := 1 to days do
        begin
            writeln('Now getting data for Day: ');
            writeln(x);
            {writeln('Number of new males: ');}
            {readln(newmales[x]);}
            writeln('Number of new deaths: ');
            readln(newdeaths[x]);
            writeln('Number of new matings: ');
            readln(newmatings[x]);
        end;
end;


{Fill arrays}
procedure zeromainvariables;

    var
        counter: integer;

begin
    for counter := 1 to iterations do
        variancedistribution[counter] := 0;
end;


procedure zeroiterationvariables;

    var
        counter: integer;

begin
    for counter := 1 to malesample do
        begin
            malematingarray[counter] := 0;
            maledeatharray[counter] := 0;
        end;
    malestodate := 0;
    mean := 0;
    variance := 0;
    sum := 0;
    dummyvar := 0;
end;


procedure checkchoice;

begin
    flag1 := 0;
    if randomnumber = 0 then
        flag1 := 1;
    if randomnumber < 0 then
```

```
        flag1 := 1;
    if randomnumber = 1 then
        flag1 := 1;
    if randomnumber > 1 then
        flag1 := 1;
    index := trunc((randomnumber * malesample) + 1);
    if index > malesample then
        flag1 := 1;
    if index < 1 then
        flag1 := 1;
    if (maledeatharray[index] = 1) then
        flag1 := 1;
    if (maledeatharray[index] > 1) then
        begin
            writeln('Houston: we have a problem: Males are dying more than once!');
            writeln('Values of index and maledeatharray[index] are ');
            writeln(index, maledeatharray[index]);
            HALT;
        end;
    if (maledeatharray[index] < 0) then
        begin
            writeln('Houston: we have a problem: Males have negative death!');
            HALT;
        end;
end;


{Picks a random number, scales it to the male array, checks for death}
procedure pickmale;

begin
    flag1 := 1;
    while flag1 = 1 do
        begin
            randomnumber := ((ABS(Random)) / 32768);
            checkchoice;
        end;
end;


procedure simulate;
    var
        x, currentday: integer;

begin
    zeroiterationvariables;
    for currentday := 1 to days do
        begin
    {Next subroutine randomly assigns newly dead males (from yesterday)}
            if currentday > 1 then
                if newdeaths[currentday] > 0 then
                    for x := 1 to newdeaths[currentday] do
                        begin
                            pickmale;
```

```
                        maledeatharray[index] := maledeatharray[index] + 1;
                    end;
    {Next subroutine handles first day only, when all new males are mated by
definition}
    {if currentday = 1 then}
    {for x := 1 to newmales[currentday] do}
    {malematingarray[x] := malematingarray[x] + 1;}
    {Next subroutine handles all other days, when two kinds of males must be
managed}
        if newmatings[currentday] > 0 then
            {Next part handles assignment of random matings, among already
        mated males}
            for x := 1 to newmatings[currentday] do
                begin
                    pickmale;
                    malematingarray[index] := malematingarray[index] + 1;
                end;
    {Next part handles assignment of automatic matings to first-mating males}
    {if newmales[currentday] > 0 then}
    {for x := (malestodate + 1) to (malestodate + newmales[currentday]) do}
    {malematingarray[x] := malematingarray[x] + 1;}
    {Last part adds today's males to cumulative mated list for next days random
matings}
    {malestodate := malestodate + newmales[currentday];}
        end;
end;


{Calculates variance of mating totals across male array}
procedure calculatepolygyny;

    var
        x: integer;

begin
    for x := 1 to malesample do
        sum := sum + malematingarray[x];
    mean := sum / malesample;
    for x := 1 to malesample do
        dummyvar := dummyvar + ((abs(malematingarray[x] - mean)) *
    (abs(malematingarray[x] - mean)));
    variance := trunc((dummyvar / malesample) * 1000);
    variancedistribution[iteration] := variance;
end;


procedure iterate;
    var
        iterationcount: integer;

begin
    for iterationcount := 1 to iterations do
        begin
            writeln(iterationcount);
```

```
            iteration := iterationcount;
            simulate;
            calculatepolygyny;
        end;
end;


{For printing out the male mating array when only one test simulation round done}
procedure outputmaledistribution;

    var
        x: integer;

begin
    for x := 1 to malesample do
        writeln(malematingarray[x]);
    writeln('Variance =', variancedistribution[1]);
end;


{Sorts distribution array to prepare for generating percentiles}
procedure bubblesort;
    var
        i, j, k: integer;
begin
    for i := iterations downto 2 do
        begin
            writeln('Bubble: ', i);
            for j := 1 to i - 1 do
                if (variancedistribution[j] > variancedistribution[j + 1]) then
                    begin
                        k := variancedistribution[j];
                        variancedistribution[j] := variancedistribution[j + 1];
                        variancedistribution[j + 1] := k;
                    end;
        end;
end;


procedure printdistribution;

    var
        k: real;
        m: integer;

begin
    k := 0.01;
    writeln('0.01 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
    {writeln(meandistribution[0], variancedistribution[0]);}
    {writeln ( meandistribution [ 1 ] , variancedistribution [ 1 ] );}
    k := 0.05;
    writeln('0.05 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
    k := 0.10;
    writeln('0.10 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
```

```
        k := 0.20;
        writeln('0.20 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.30;
        writeln('0.30 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.40;
        writeln('0.40 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.50;
        writeln('0.50 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.60;
        writeln('0.60 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.70;
        writeln('0.70 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.80;
        writeln('0.80 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.90;
        writeln('0.90 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.95;
        writeln('0.95 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.99;
        writeln('0.99 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        {writeln(variancedistribution[100]);}
        {writeln variancedistribution [ 101 ] );}
        {for m := 1 to iterations do}
        {writeln(variancedistribution[m]);}
    end;


{Main program loop.}
begin
    timeseed;
    inputs;
    zeromainvariables;
    iterate;
    outputmaledistribution;
    bubblesort;
    printdistribution;
    writeln('Do not forget to divide the variance values by 1,000 ');
end.
```

Simulation using mated males only

```
program matedmalesonly;

{Simulates level of incidental polygyny, the variance in male mating success}
{expected even if all males are equally likely to remate on average}

    var
        variancedistribution: array[0..10000] of integer;
        newmales, newmatings, newdeaths: array[0..10] of integer;
        malematingarray: array[0..101] of integer;
        maledeatharray: array[0..101] of integer;
        malesample, malestodate, days, iteration, iterations, index, variance, sum, flag1,
    flag2: integer;
        randomnumber, mean, dummyvar: real;


    {* Seeds Random Number Generator}
    procedure timeseed;

    begin
        GetDateTime(randseed);
    end;


    procedure inputs;

        var
            x: integer;

    begin
        write('Number of simulations desired (maximum 10,000): ');
        readln(iterations);
        write('Total male sample size (maximum 100): ');
        readln(malesample);
        write('Number of days in simulation (maximum 10): ');
        readln(days);
        for x := 1 to days do
            begin
                writeln('Now getting data for Day: ');
                writeln(x);
                writeln('Number of new males: ');
                readln(newmales[x]);
                writeln('Number of new deaths: ');
                readln(newdeaths[x]);
                writeln('Number of new matings: ');
                readln(newmatings[x]);
            end;
    end;


    {Fill arrays}
```

```
procedure zeromainvariables;

    var
        counter: integer;

begin
    for counter := 1 to iterations do
        variancedistribution[counter] := 0;
end;


procedure zeroiterationvariables;

    var
        counter: integer;

begin
    for counter := 1 to malesample do
        begin
            malematingarray[counter] := 0;
            maledeatharray[counter] := 0;
        end;
    malestodate := 0;
    mean := 0;
    variance := 0;
    sum := 0;
    dummyvar := 0;
end;


procedure checkchoice;

begin
    flag1 := 0;
    if randomnumber = 0 then
        flag1 := 1;
    if randomnumber < 0 then
        flag1 := 1;
    if randomnumber = 1 then
        flag1 := 1;
    if randomnumber > 1 then
        flag1 := 1;
    index := trunc((randomnumber * malestodate) + 1);
    if index > malestodate then
        flag1 := 1;
    if index < 1 then
        flag1 := 1;
    if (maledeatharray[index] = 1) then
        flag1 := 1;
    if (maledeatharray[index] > 1) then
        begin
            writeln('Houston: we have a problem: Males are dying more than once!');
            writeln('Values of index and maledeatharray[index] are ');
            writeln(index, maledeatharray[index]);
```

```
            HALT;
        end;
    if (maledeatharray[index] < 0) then
        begin
            writeln('Houston: we have a problem: Males have negative death!');
            HALT;
        end;
end;


{Picks a random number, scales it to the male array, checks for death}
procedure pickmale;

begin
    flag1 := 1;
    while flag1 = 1 do
        begin
            randomnumber := ((ABS(Random)) / 32768);
            checkchoice;
        end;
end;


procedure simulate;
    var
        x, currentday: integer;

begin
    zeroiterationvariables;
    for currentday := 1 to days do
        begin
            {Next subroutine randomly assigns newly dead males (from yesterday)}
            if currentday > 1 then
                if newdeaths[currentday] > 0 then
                    for x := 1 to newdeaths[currentday] do
                        begin
                            pickmale;
                            maledeatharray[index] := maledeatharray[index] + 1;
                        end;
            {Next subroutine handles first day only, when all new males are mated by
        definition}
            if currentday = 1 then
                for x := 1 to newmales[currentday] do
                    malematingarray[x] := malematingarray[x] + 1;
            {Next subroutine handles all other days, when two kinds of males must be
        managed}
            if currentday > 1 then
                begin
                    if newmatings[currentday] > 0 then
                        {Next part handles assignment of random matings, among
                    already mated males}
                        for x := 1 to newmatings[currentday] do
                            begin
                                pickmale;
```

```
                                malematingarray[index] := malematingarray[index] +
                                1;
                        end;
                    {Next part handles assignment of automatic matings to first-mating
                males}
                    if newmales[currentday] > 0 then
                        for x := (malestodate + 1) to (malestodate +
                    newmales[currentday]) do
                            malematingarray[x] := malematingarray[x] + 1;
                    end;
                {Last part adds today's males to cumulative mated list for next days random
                matings}
                malestodate := malestodate + newmales[currentday];
            end;
end;


{Calculates variance of mating totals across male array}
procedure calculatepolygyny;

    var
        x: integer;

begin
    for x := 1 to malesample do
        sum := sum + malematingarray[x];
    mean := sum / malesample;
    for x := 1 to malesample do
        dummyvar := dummyvar + ((abs(malematingarray[x] - mean)) *
    (abs(malematingarray[x] - mean)));
    variance := trunc((dummyvar / malesample) * 1000);
    variancedistribution[iteration] := variance;
end;


procedure iterate;
    var
        iterationcount: integer;

begin
    for iterationcount := 1 to iterations do
        begin
            {writeln(iterationcount);}
            iteration := iterationcount;
            simulate;
            calculatepolygyny;
        end;
end;


{For printing out the male mating array when only one test simulation round done}
procedure outputmaledistribution;

    var
```

```
            x: integer;

    begin
        for x := 1 to malesample do
            writeln(malematingarray[x]);
        writeln('Variance =', variancedistribution[1]);
    end;


    {Sorts distribution array to prepare for generating percentiles}
    procedure bubblesort;
        var
            i, j, k: integer;

begin
        for i := iterations downto 2 do
            begin
                {writeln('Bubble: ', i);}
                for j := 1 to i - 1 do
                    if (variancedistribution[j] > variancedistribution[j + 1]) then
                        begin
                            k := variancedistribution[j];
                            variancedistribution[j] := variancedistribution[j + 1];
                            variancedistribution[j + 1] := k;
                        end;
            end;
    end;


    procedure printdistribution;

        var
            k: real;
            m: integer;

    begin
        k := 0.01;
        writeln('0.01 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        {writeln(meandistribution[0], variancedistribution[0]);}
        {writeln ( meandistribution [ 1 ] , variancedistribution [ 1 ] );}
        k := 0.05;
        writeln('0.05 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.10;
        writeln('0.10 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.20;
        writeln('0.20 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.30;
        writeln('0.30 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.40;
        writeln('0.40 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.50;
        writeln('0.50 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.60;
        writeln('0.60 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
```

```
        k := 0.70;
        writeln('0.70 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.80;
        writeln('0.80 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.90;
        writeln('0.90 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.95;
        writeln('0.95 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        k := 0.99;
        writeln('0.99 Percentile variance = ', variancedistribution[(trunc(iterations * k))]);
        {writeln(variancedistribution[100]);}
        {writeln variancedistribution [ 101 ] );}
        {for m := 1 to iterations do}
        {writeln(variancedistribution[m]);}
    end;


{Main program loop.}
begin
    timeseed;
    inputs;
    zeromainvariables;
    iterate;
    outputmaledistribution;}
    bubblesort;
    printdistribution;
    writeln('Do not forget to divide the variance values by 1,000 ');
end.
```

APPENDIX B

These programs simulate random mating conditions in small model groups of males and females that were mated and then re-mated to each other, calculates a statistic describing the degree of repeatability of male (or female) mating order (see Experiment F of Chapter 2), and then estimates the frequency distribution of that statistic by repeating the simulation many times. Several modified versions of these programs were used in Experiment F; two are included here as examples. The first program calculates the FHLH statistic for Group A in Trial 1 (1996). The second program calculates the SR statistic for the single large Trial 2 group (1997). Each program contains the additional code necessary for the remaining test groups (in brackets); note that the second program contains the code (in brackets) for calculating the SR statistic of most Trial 1 groups.

FHLH simulation - Trial 1

```
program Maleordera;
{This program simulates male group A at once and calculates the FHLH statistic,}
{assuming that the groups are}
{a=8-even number}

    const
        cicadanumber = 8;
        cicadanumber2 = cicadanumber + 1;
    var
        zeroarray, tabarraya, orderarraya, simarraya, arraya, tabarrayb, simarrayb,
        orderarrayb, arrayb, tabarrayc, simarrayc, orderarrayc, arrayc:
        array[1..cicadanumber] of integer;
        zeroarray2, tabarrayd, simarrayd, orderarrayd, arrayd: array[1..cicadanumber2] of
integer;
        iteration, testvalue, arraycounter, iterations, randomnumber: integer;
        Distribution: array[0..10000] of integer;

    procedure setuparrays;

        var
            counteraa, counterbb: integer;

    begin
        for counteraa := 1 to cicadanumber do
            begin
                arraya[counteraa] := counteraa;
```

```
                arrayb[counteraa] := counteraa;
                arrayc[counteraa] := counteraa;
                arrayd[counteraa] := counteraa;
            end;
        arrayd[9] := 9;
        for counterbb := 1 to cicadanumber do
            begin
                zeroarray[counterbb] := 0;
                zeroarray2[counterbb] := 0;
            end;
        zeroarray2[9] := 0;
    end;


{* Seeds Random Number Generator}
    procedure TIMESEED;
    begin
        GetDateTime(randseed);
    end;

    procedure Welcome;
    begin
        writeln('Welcome to Mating Order. You can run up to 10,000 resamplings');
        writeln(' of female cicada Mating Order data. ');

    end;


{*Picks a random number, scales it to the appropriate size}
    procedure PICKRAND;
    begin
        randomnumber := (Trunc(((ABS(Random)) / 32768) * cicadanumber) + 1);
    end;

{*Picks a random number, scales it to the appropriate size}
    procedure PICKRAND2;
    begin
        randomnumber := (Trunc(((ABS(Random)) / 32768) * cicadanumber2) + 1);
    end;


{Gets Input parameters}
    procedure INPUTS;
    begin
        writeln('Please provide the number of iterations');
        writeln('of the simulation , maximum 10,000 : ');
        read(iterations);
    end;


{Checks Input parameters}
    procedure CHECKVAR;
    begin
        if (iterations > 10000) or (iterations < 1) then
```

```
            begin
                writeln('Sorry, but iterations must be an integer greater than zero and less
                than 10,000');
                writeln('program has been terminated');
                HALT;
            end;
    end;

{Fill arrays}
    procedure FILLARRAYa;
        var
            counter: integer;
    begin
        for counter := 1 to cicadanumber do
            begin
                orderarraya[counter] := counter;
                tabarraya[counter] := 0;
                {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
            end;
    end;

    procedure FILLARRAYb;
        var
            counter: integer;
    begin
        for counter := 1 to cicadanumber do
            begin
                orderarrayb[counter] := counter;
                tabarrayb[counter] := 0;
                {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
            end;
    end;

    procedure FILLARRAYc;
        var
            counter: integer;
    begin
        for counter := 1 to cicadanumber do
            begin
                orderarrayc[counter] := counter;
                tabarrayc[counter] := 0;
                {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
            end;
        orderarrayc[2] := 1;
        orderarrayc[6] := 7;
        orderarrayc[7] := 7;
        orderarrayc[8] := 7;
    end;

    procedure FILLARRAYd;
        var
            counter: integer;
    begin
        for counter := 1 to cicadanumber2 do
```

```
        begin
            orderarrayd[counter] := counter;
            tabarrayd[counter] := 0;
            {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
        end;
    orderarrayd[4] := 3;
    orderarrayd[6] := 5;
end;


procedure MAKESIMARRAYa;
begin
    pickrand;
    if tabarraya[randomnumber] = 50 then
        begin
            MAKESIMARRAYa;
        end;
    if tabarraya[randomnumber] = 0 then
        begin
            simarraya[arraycounter] := orderarraya[randomnumber];
            tabarraya[randomnumber] := 50;
        end;
end;

procedure MAKESIMARRAYb;
begin
    pickrand;
    if tabarrayb[randomnumber] = 50 then
        begin
            MAKESIMARRAYb;
        end;
    if tabarrayb[randomnumber] = 0 then
        begin
            simarrayb[arraycounter] := orderarrayb[randomnumber];
            tabarrayb[randomnumber] := 50;
        end;
end;

procedure MAKESIMARRAYc;
begin
    pickrand;
    if tabarrayc[randomnumber] = 50 then
        begin
            MAKESIMARRAYc;
        end;
    if tabarrayc[randomnumber] = 0 then
        begin
            simarrayc[arraycounter] := orderarrayc[randomnumber];
            tabarrayc[randomnumber] := 50;
        end;
end;

procedure MAKESIMARRAYd;
begin
```

```
        pickrand2;
        if tabarrayd[randomnumber] = 50 then
            begin
                MAKESIMARRAYd;
            end;
        if tabarrayd[randomnumber] = 0 then
            begin
                simarrayd[arraycounter] := orderarrayd[randomnumber];
                tabarrayd[randomnumber] := 50;
            end;
    end;


    procedure calculatesumtestvaluea;
        var
            cicadanumbera, countera, counterb, counterc, countercc, firsthalf, secondhalf,
suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumbera := cicadanumber;
        if (cicadanumbera / 2) > (trunc(cicadanumbera / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumbera / 2)) do
                    begin
                        suma := suma + simarraya[countera];
                    end;
                for counterb := (cicadanumbera - ((trunc(cicadanumbera / 2)) - 1)) to
                cicadanumbera do
                    begin
                        sumb := sumb + simarraya[counterb];
                    end;
                testvalue := suma - sumb;
                {writeln('testvaluea=', testvalue);}
                distribution[iteration] := testvalue;
                {writeln('This is simarraya');}
                {for countercc := 1 to cicadanumbera do}
                {begin}
                {writeln(orderarraya[countercc], simarraya[countercc]);}
                {end;}
                {writeln(suma, sumb, testvalue);}
            end;
        if (cicadanumbera / 2) = (trunc(cicadanumbera / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumbera / 2)) do
                    begin
                        suma := suma + simarraya[countera];
                    end;
                for counterb := ((trunc(cicadanumbera / 2)) + 1) to cicadanumbera do
                    begin
                        sumb := sumb + simarraya[counterb];
                    end;
                testvalue := suma - sumb;
```

```
{writeln('testvaluea=', testvalue);}
                distribution[iteration] := testvalue;
{writeln('Here is simarraya');}
{for countercc := 1 to cicadanumbera do}
{begin}
{writeln(orderarraya[countercc], simarraya[countercc]);}
{end;}
{writeln(suma, sumb, testvalue);}
            end;
    end;

    procedure calculatesumtestvalueb;
        var
            cicadanumberb, countera, counterb, counterc, countercc, firsthalf, secondhalf,
            suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumberb := cicadanumber;
        if (cicadanumberb / 2) > (trunc(cicadanumberb / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumberb / 2)) do
                    begin
                        suma := suma + simarrayb[countera];
                    end;
                for counterb := (cicadanumberb - ((trunc(cicadanumberb / 2)) - 1)) to
                cicadanumberb do
                    begin
                        sumb := sumb + simarrayb[counterb];
                    end;
                testvalue := suma - sumb;
                {writeln('testvalueb=', testvalue);}
                distribution[iteration] := distribution[iteration] + testvalue;
                {writeln('This is simarrayb');}
                {for countercc := 1 to cicadanumberb do}
                {begin}
                {writeln(orderarrayb[countercc], simarrayb[countercc]);}
                {end;}
                {writeln(suma, sumb, testvalue);}
            end;
        if (cicadanumberb / 2) = (trunc(cicadanumberb / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumberb / 2)) do
                    begin
                        suma := suma + simarrayb[countera];
                    end;
                for counterb := ((trunc(cicadanumberb / 2)) + 1) to cicadanumberb do
                    begin
                        sumb := sumb + simarrayb[counterb];
                    end;
                testvalue := suma - sumb;
                {writeln('testvalueb=', testvalue);}
                distribution[iteration] := distribution[iteration] + testvalue;
```

```
                {writeln('This is simarrayb');}
                {for countercc := 1 to cicadanumberb do}
                {begin}
                {writeln(orderarrayb[countercc], simarrayb[countercc]);}
                {end;}
                {writeln(suma, sumb, testvalue);}
            end;
    end;

    procedure calculatesumtestvaluec;
        var
            cicadanumberc, countera, counterb, counterc, countercc, firsthalf, secondhalf,
suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumberc := cicadanumber;
        if (cicadanumberc / 2) > (trunc(cicadanumberc / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumberc / 2)) do
                    begin
                        suma := suma + simarrayc[countera];
                    end;
                for counterb := (cicadanumberc - ((trunc(cicadanumberc / 2)) - 1)) to
                cicadanumberc do
                    begin
                        sumb := sumb + simarrayc[counterb];
                    end;
                testvalue := suma - sumb;
                {writeln('testvaluec=', testvalue);}
                distribution[iteration] := distribution[iteration] + testvalue;
                {writeln('This is simarrayc');}
                {for countercc := 1 to cicadanumberc do}
                {begin}
                {writeln(orderarrayc[countercc], simarrayc[countercc]);}
                {end;}
                {writeln(suma, sumb, testvalue);}
            end;
        if (cicadanumberc / 2) = (trunc(cicadanumberc / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumberc / 2)) do
                    begin
                        suma := suma + simarrayc[countera];
                    end;
                for counterb := ((trunc(cicadanumberc / 2)) + 1) to cicadanumberc do
                    begin
                        sumb := sumb + simarrayc[counterb];
                    end;
                testvalue := suma - sumb;
                {writeln('testvaluec=', testvalue);}
                distribution[iteration] := distribution[iteration] + testvalue;
                {writeln('This is simarrayc');}
                {for countercc := 1 to cicadanumberc do}
```

```
                {begin}
                {writeln(orderarrayc[countercc], simarrayc[countercc]);}
                {end;}
                {writeln(suma, sumb, testvalue);}
            end;
    end;

    procedure calculatesumtestvalued;
        var
            cicadanumberd, countera, counterb, counterc, countercc, firsthalf, secondhalf,
suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumberd := cicadanumber2;
        if (cicadanumberd / 2) > (trunc(cicadanumberd / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumberd / 2)) do
                    begin
                        suma := suma + simarrayd[countera];
                    end;
                for counterb := (cicadanumberd - ((trunc(cicadanumberd / 2)) - 1)) to
                cicadanumberd do
                    begin
                        sumb := sumb + simarrayd[counterb];
                    end;
                testvalue := suma - sumb;
                {writeln('testvalued=', testvalue);}
                distribution[iteration] := distribution[iteration] + testvalue;
                {writeln('This is simarrayd');}
                {for countercc := 1 to cicadanumberd do}
                {begin}
                {writeln(orderarrayd[countercc], simarrayd[countercc]);}
                {end;}
                {writeln(suma, sumb, testvalue);}
            end;
        if (cicadanumberd / 2) = (trunc(cicadanumberd / 2)) then
            begin
                for countera := 1 to (trunc(cicadanumberd / 2)) do
                    begin
                        suma := suma + simarrayd[countera];
                    end;
                for counterb := ((trunc(cicadanumberd / 2)) + 1) to cicadanumberd do
                    begin
                        sumb := sumb + simarrayd[counterb];
                    end;
                testvalue := suma - sumb;
                {writeln('testvalued=', testvalue);}
                distribution[iteration] := distribution[iteration] + testvalue;
                {writeln('This is simarrayd');}
                {for countercc := 1 to cicadanumberd do}
                {begin}
                {writeln(orderarrayd[countercc], simarrayd[countercc]);}
```

```
                {end;}
                {writeln(suma, sumb, testvalue);}
            end;
    end;


    procedure iterate;
        var
            countera, counterb: integer;
    begin
        for countera := 1 to iterations do
            begin
                fillarraya;
                {fillarrayb;}
                {fillarrayc;}
                {fillarrayd;}

                for counterb := 1 to cicadanumber do
                    begin
                        arraycounter := counterb;
                        MAKESIMARRAYa;
                        {MAKESIMARRAYb;}
                        {MAKESIMARRAYc;}
                        {MAKESIMARRAYd;}
                    end;
                {arraycounter := cicadanumber2;}
                {makesimarrayd;}
                iteration := countera;
                CALCULATEsumTESTVALUEa;
                {CALCULATEsumTESTVALUEb;}
                {CALCULATEsumTESTVALUEc;}
                {CALCULATEsumTESTVALUEd;}
                {writeln(distribution[iteration]);}
            end;
    end;


{clears this array}
    procedure ClearDistribution;
        var
            counter: integer;
    begin
        for counter := 0 to 10000 do
            Distribution[counter] := 0;
    end;



{Sorts distribution array to prepare for generating percentiles}
    procedure bubblesort;
        var
            i, j, k: integer;
    begin
```

```
        for i := iterations downto 2 do
            for j := 1 to i - 1 do
                if (distribution[j] > distribution[j + 1]) then
                    begin
                        k := distribution[j];
                        distribution[j] := distribution[j + 1];
                        distribution[j + 1] := k;
                    end;
    end;

    procedure makedist2;

        var
            interval, count, countz: integer;

    begin
        bubblesort;
{for countz := 1 to iterations do}
{begin}
{writeln(distribution[countz]);}
{end;}
        interval := (trunc(iterations * (1 / 100)));
        count := interval;
        while count < iterations do
            begin
                writeln(((count / iterations * 100)), 'percentile value is ',
                distribution[trunc(iterations * (count / iterations))]);
                count := count + interval;
            end;
    end;


{The shell of the program}
    procedure MAKEDECISION;
        var
            choicer: integer;
    begin
        writeln('     ');
        writeln('Would you like to:');
        writeln('     (1) Simulate The Remating Order Experiment ?');
        writeln('     (2) End this session?');
        writeln('   ');
        writeln('Choose (1) or (2) please');
        read(choicer);
        if choicer = 1 then
            begin
                Timeseed;
                ClearDistribution;
                Welcome;
                inputs;
                checkvar;
                iterate;
                Makedist2;
            end;
```

```
        if choicer = 2 then
            HALT;
        if (choicer < 1) or (choicer > 2) then
            begin
                writeln('Hey there are only two choices here!');
                Makedecision;
            end;
        writeln('end of program');
    end;


begin
    MakeDecision;
end.
```

SR simulation - Trial 2

```
program Exactmaleorder1997;
{This program adopts the 1996 code for a simulation of mating order in }
{the large 1997 test group (16 cicadas)}
{Note that only the code for group C has been modified and used}

    const
        cicadanumber = 16;
        cicadanumber2 = cicadanumber + 1;
    var
        zeroarray, tabarraya, orderarraya, simarraya, arraya, tabarrayb, simarrayb,
        orderarrayb, arrayb, tabarrayc, simarrayc, orderarrayc, arrayc:
        array[1..cicadanumber] of integer;
        zeroarray2, tabarrayd, simarrayd, orderarrayd, arrayd: array[1..cicadanumber2] of
        integer;
        iteration, testvalue, arraycounter, iterations, randomnumber: integer;
        Distribution: array[0..10000] of integer;

    procedure setuparrays;

        var
            counteraa, counterbb: integer;

    begin
        for counteraa := 1 to cicadanumber do
            begin
                arraya[counteraa] := counteraa;
                arrayb[counteraa] := counteraa;
                arrayc[counteraa] := counteraa;
                arrayd[counteraa] := counteraa;
            end;
        arrayd[9] := 9;
        for counterbb := 1 to cicadanumber do
            begin
                zeroarray[counterbb] := 0;
                zeroarray2[counterbb] := 0;
            end;
        zeroarray2[9] := 0;
    end;


{* Seeds Random Number Generator}
    procedure TIMESEED;
    begin
        GetDateTime(randseed);
    end;

    procedure Welcome;
    begin
        writeln('Welcome to Mating Order. You can run up to 10,000 resamplings');
        writeln(' of female cicada Mating Order data. ');
```

```
    end;


{*Picks a random number, scales it to the appropriate size}
    procedure PICKRAND;
    begin
        randomnumber := (Trunc(((ABS(Random)) / 32768) * cicadanumber) + 1);
    end;

{*Picks a random number, scales it to the appropriate size}
    procedure PICKRAND2;
    begin
        randomnumber := (Trunc(((ABS(Random)) / 32768) * cicadanumber2) + 1);
    end;


{Gets Input parameters}
    procedure INPUTS;
    begin
        writeln('Please provide the number of iterations');
        writeln('of the simulation , maximum 10,000 : ');
        read(iterations);
    end;


{Checks Input parameters}
    procedure CHECKVAR;
    begin
        if (iterations > 10000) or (iterations < 1) then
            begin
                writeln('Sorry, but iterations must be an integer greater than zero and less
                than 10,000');
                writeln('program has been terminated');
                HALT;
            end;
    end;

{Fill arrays}
    procedure FILLARRAYa;
        var
            counter: integer;
    begin
        for counter := 1 to cicadanumber do
            begin
                orderarraya[counter] := counter;
                tabarraya[counter] := 0;
                {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
            end;
    end;

    procedure FILLARRAYb;
        var
            counter: integer;
```

```
begin
    for counter := 1 to cicadanumber do
        begin
            orderarrayb[counter] := counter;
            tabarrayb[counter] := 0;
            {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
        end;
end;

procedure FILLARRAYc;
    var
        counter: integer;
begin
    for counter := 1 to cicadanumber do
        begin
            orderarrayc[counter] := counter;
            tabarrayc[counter] := 0;
            {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
        end;
    orderarrayc[13] := 13;
    orderarrayc[14] := 13;
    orderarrayc[15] := 13;
    orderarrayc[16] := 13;
end;

procedure FILLARRAYd;
    var
        counter: integer;
begin
    for counter := 1 to cicadanumber2 do
        begin
            orderarrayd[counter] := counter;
            tabarrayd[counter] := 0;
            {Writeln('orderarray', orderarray[counter], ' tabarray', tabarray[counter]);}
        end;
    orderarrayd[4] := 3;
    orderarrayd[6] := 5;
end;


procedure MAKESIMARRAYa;
begin
    pickrand;
    if tabarraya[randomnumber] = 50 then
        begin
            MAKESIMARRAYa;
        end;
    if tabarraya[randomnumber] = 0 then
        begin
            simarraya[arraycounter] := orderarraya[randomnumber];
            tabarraya[randomnumber] := 50;
        end;
end;
```

```
    procedure MAKESIMARRAYb;
    begin
        pickrand;
        if tabarrayb[randomnumber] = 50 then
            begin
                MAKESIMARRAYb;
            end;
        if tabarrayb[randomnumber] = 0 then
            begin
                simarrayb[arraycounter] := orderarrayb[randomnumber];
                tabarrayb[randomnumber] := 50;
            end;
    end;

    procedure MAKESIMARRAYc;
    begin
        pickrand;
        if tabarrayc[randomnumber] = 50 then
            begin
                MAKESIMARRAYc;
            end;
        if tabarrayc[randomnumber] = 0 then
            begin
                simarrayc[arraycounter] := orderarrayc[randomnumber];
                tabarrayc[randomnumber] := 50;
            end;
    end;

    procedure MAKESIMARRAYd;
    begin
        pickrand2;
        if tabarrayd[randomnumber] = 50 then
            begin
                MAKESIMARRAYd;
            end;
        if tabarrayd[randomnumber] = 0 then
            begin
                simarrayd[arraycounter] := orderarrayd[randomnumber];
                tabarrayd[randomnumber] := 50;
            end;
    end;


    procedure calculatesumtestvaluea;
        var
            cicadanumbera, countera, counterb, counterc, countercc, firsthalf, secondhalf,
suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumbera := cicadanumber;
        for countera := 1 to cicadanumbera do
            begin
```

```
                testvalue := testvalue + (abs(simarraya[countera] - orderarraya[countera]));
                {writeln(simarraya[countera], orderarraya[countera], testvalue);}
            end;
            {writeln('testvaluea=', testvalue);}
        distribution[iteration] := distribution[iteration] + testvalue;
        {writeln('This is simarraya');}
        {for countercc := 1 to cicadanumbera do}
        {begin}
        {writeln(orderarraya[countercc], simarraya[countercc]);}
        {end;}
        {writeln(suma, sumb, testvalue);}
    end;


    procedure calculatesumtestvalueb;
        var
            cicadanumberb, countera, counterb, counterc, countercc, firsthalf, secondhalf,
suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumberb := cicadanumber;
        for countera := 1 to cicadanumberb do
            begin
                testvalue := testvalue + (abs(simarrayb[countera] - orderarrayb[countera]));
                {writeln(simarrayb[countera], orderarrayb[countera], testvalue);}
            end;
            {writeln('testvalueb=', testvalue);}
        distribution[iteration] := distribution[iteration] + testvalue;
        {writeln('This is simarrayb');}
        {for countercc := 1 to cicadanumberb do}
        {begin}
        {writeln(orderarrayb[countercc], simarrayb[countercc]);}
        {end;}
        {writeln(suma, sumb, testvalue);}
    end;


    procedure calculatesumtestvaluec;
        var
            cicadanumberc, countera, counterb, counterc, countercc, firsthalf, secondhalf,
suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumberc := cicadanumber;
        for countera := 1 to cicadanumberc do
            begin
                testvalue := testvalue + (abs(simarrayc[countera] - orderarrayc[countera]));
                {writeln(simarrayc[countera], orderarrayc[countera], testvalue);}
            end;
            {writeln('testvaluec=', testvalue);}
```

```
        distribution[iteration] := distribution[iteration] + testvalue;
        {writeln('This is simarrayc');}
        {for countercc := 1 to cicadanumberc do}
        {begin}
        {writeln(orderarrayc[countercc], simarrayc[countercc]);}
        {end;}
        {writeln(suma, sumb, testvalue);}
    end;

    procedure calculatesumtestvalued;
        var
            cicadanumberd, countera, counterb, counterc, countercc, firsthalf, secondhalf,
suma, sumb: integer;
    begin
        suma := 0;
        sumb := 0;
        testvalue := 0;
        cicadanumberd := cicadanumber2;
        for countera := 1 to cicadanumberd do
            begin
                testvalue := testvalue + (abs(simarrayd[countera] - orderarrayd[countera]));
                {writeln(simarrayd[countera], orderarrayd[countera], testvalue);}
            end;
            {writeln('testvalued=', testvalue);}
        distribution[iteration] := distribution[iteration] + testvalue;
        {writeln('This is simarrayd');}
        {for countercc := 1 to cicadanumberd do}
        {begin}
        {writeln(orderarrayd[countercc], simarrayd[countercc]);}
        {end;}
        {writeln(suma, sumb, testvalue);}
    end;



    procedure iterate;
        var
            countera, counterb: integer;
    begin
        for countera := 1 to iterations do
            begin
                {fillarraya;}
                {fillarrayb;}
                fillarrayc;
                {fillarrayd;}

                for counterb := 1 to cicadanumber do
                    begin
                        arraycounter := counterb;
                        {MAKESIMARRAYa;}
                        {MAKESIMARRAYb;}
                        MAKESIMARRAYc;
                        {MAKESIMARRAYd;}
                    end;
```

```
                arraycounter := cicadanumber2;
                {makesimarrayd;}
                iteration := countera;
                {CALCULATEsumTESTVALUEa;}
                {CALCULATEsumTESTVALUEb;}
                CALCULATEsumTESTVALUEc;
                {CALCULATEsumTESTVALUEd;}
                {writeln(distribution[iteration]);}
            end;
    end;


{clears this array}
    procedure ClearDistribution;
        var
            counter: integer;
    begin
        for counter := 0 to 10000 do
            Distribution[counter] := 0;
    end;



{Sorts distribution array to prepare for generating percentiles}
    procedure bubblesort;
        var
            i, j, k: integer;
    begin
        for i := iterations downto 2 do
            for j := 1 to i - 1 do
                if (distribution[j] > distribution[j + 1]) then
                    begin
                        k := distribution[j];
                        distribution[j] := distribution[j + 1];
                        distribution[j + 1] := k;
                    end;
    end;

    procedure makedist2;

        var
            interval, count, countz: integer;

    begin
        bubblesort;
        {for countz := 1 to iterations do}
        {begin}
        {writeln(distribution[countz]);}
        {end;}
        interval := (trunc(iterations * (1 / 100)));
        count := interval;
        while count < iterations do
            begin
```

```
                writeln(((count / iterations * 100)), 'percentile value is ',
                distribution[trunc(iterations * (count / iterations))]);
                count := count + interval;
            end;
    end;


{The shell of the program}
    procedure MAKEDECISION;
        var
            choicer: integer;
    begin
        writeln('     ');
        writeln('Would you like to:');
        writeln('     (1) Simulate The Remating Order Experiment ?');
        writeln('     (2) End this session?');
        writeln('   ');
        writeln('Choose (1) or (2) please');
        read(choicer);
        if choicer = 1 then
            begin
                Timeseed;
                ClearDistribution;
                Welcome;
                inputs;
                checkvar;
                iterate;
                Makedist2;
            end;
        if choicer = 2 then
            HALT;
        if (choicer < 1) or (choicer > 2) then
            begin
                writeln('Hey there are only two choices here!');
                Makedecision;
            end;
        writeln('end of program');
    end;


begin
    MakeDecision;
end.
```

APPENDIX C

This program estimates the cumulative frequency distribution of the variance in abdomen color found in mixed *M. neotredecim/ M. tredecim* samples drawn at random (10,000 times) with replacement from existing data, which are fed into the algorithm along with the proportions of each species desired in the mixed-species sample (see Chapter 4). The code runs in THINK Pascal 4.0 for the Macintosh.

```
program Hybrid;

{"neo" is Magicicada neotredecim, "tre" is Magicicada tredecim}

   const
      neosample = 76;   {Number of "allopatric" neotredecim in museum sample, from
   which simulated values will be drawn}
      tresample = 145; {Number of "allopatric" tredecim in museum sample, from which
   simulated values will be drawn}
      samplesize = 300; {Actual sample size of the measured dataset from sympatry, the
   population being simulated}

   var
      neodataarray: array[1..neosample] of real;
      tredataarray: array[1..tresample] of real;
      randomindex, iteration, testvalue, arraycounter, iterations: integer;
      randomnumber, neopercentage, trepercentage, neosimnumber, tresimnumber: integer;
      VarianceDistribution: array[0..10001] of integer;
      neosimmean, tresimmean, neosimvar, tresimvar, globalsimmean: real;
      globalsimvar: integer;
      globalsimarray: array[1..samplesize] of real;
      neosimarray: array[1..samplesize] of real;
      tresimarray: array[1..samplesize] of real;

{Seeds Random Number Generator}
   procedure TIMESEED;
   begin
      GetDateTime(randseed);
   end;

   procedure Welcome;
   begin
      writeln('Welcome to the Hybrid Zone.');
      writeln('You can run up to 10,000 resamplings');
      writeln('of M. neotredecim and M. tredecim abdomen color data. ');
      writeln;
   end;
```

```
{Gets empirical data  - museum specimen data from allopatry - for neo and tre from user}
   procedure fillneotrearrays;
      var
         counter: integer;
   begin
      writeln;
      writeln;
      writeln('Empirical dataset: 76 neo, 145 tre');
      writeln('Enter neo emp. values one at a time');
      for counter := 1 to neosample do
         readln(neodataarray[counter]);
      writeln('Enter tre emp. values one at a time');
      for counter := 1 to tresample do
         readln(tredataarray[counter]);
   end;

{Picks a random number, scales it to a value fitting the neotredecim data array}
{Must output a value called "randomindex"}
   procedure PICKNEORAND;
   begin
      randomindex := (Trunc(((ABS(Random)) / 32768) * neosample)) + 1;
   end;

{Picks a random number, scales it to a value fitting the tredecim data array}
{Must output a value called "randomindex"}
   procedure PICKTRERAND;
   begin
      randomindex := (Trunc(((ABS(Random)) / 32768) * tresample)) + 1;
   end;

{Gets Input parameters}
   procedure INPUTS;
   begin
      writeln('Please enter the number of iterations');
      writeln('of the simulation , maximum 10,000 : ');
      readln(iterations);
      write('Sample size is set to ');
      writeln(samplesize);
      writeln('Enter neo sim. sample size: ');
      {Number of M. neotredecim needed to create a mixed neo/tre sample}
      {that has the same population mean for abdomen color as the *real* measured}
   {population from sympatry, and assuming all neo and tre have the average}
      {abdomen color observed in allopatry for that species.  This value is easily}
   {determined with simple algebra}
      readln(neosimnumber);
      writeln('Enter tre sim. sample size: ');
      {Number of M. tredecim needed to create a mixed neo/tre sample}
      {that has the same population mean for abdomen color as the *real* measured}
   {population from sympatry, and assuming all neo and tre have the average}
      {abdomen color observed in allopatry for that species.  This value is easily}
   {determined with simple algebra}
      readln(tresimnumber);
   end;
```

```
{Checks Input parameters}
   procedure CHECKVAR;
   begin
      if (iterations > 10001) or (iterations < 1) then
         begin
            writeln('Sorry, but iterations must be an integer greater than zero and less than
         10001');
            writeln('program has been terminated');
            HALT;
         end;
   end;

{Fill neo and tre and global data simulation arrays for each iteration}
   procedure FILLARRAYS;
      var
         counter: integer;
   begin
      for counter := 1 to neosimnumber do
         neosimarray[counter] := 0;
      for counter := 1 to tresimnumber do
         tresimarray[counter] := 0;
      for counter := 1 to samplesize do
         globalsimarray[counter] := 0;
   end;

{Makes new simulated data samples for neotredecim and tredecim, then global simulation
array, from global source arrays}
   procedure MAKESIMARRAYS;
      var
         counter: integer;
   begin
      for counter := 1 to neosimnumber do
         begin
            pickneorand;
            neosimarray[counter] := neodataarray[randomindex];
            globalsimarray[counter] := neodataarray[randomindex];
         end;
      for counter := 1 to tresimnumber do
         begin
            picktrerand;
            tresimarray[counter] := tredataarray[randomindex];
            globalsimarray[counter + neosimnumber] := tredataarray[randomindex];
         end;
{Below are test subroutines for writing out simulated data arrays to error-check them}
{writeln('Neo sim array:');}
{for counter := 1 to neosimnumber do}
{writeln ( neosimarray [ counter ] );}
{writeln ( 'Tre sim array:' );}
{for counter := 1 to tresimnumber do}
{writeln ( tresimarray [ counter ] );}
{writeln ( 'Global sim array:' );}
{for counter := 1 to samplesize do}
{writeln ( globalsimarray [ counter ] );}
```

```
{halt;}
   end;

{Calculates variance statistic for simulated mixed population}
   procedure CALCULATETESTVALUES;
      var
         counter, counterb, counterc: integer;
         neosum, tresum, globalsum, neovar, trevar, globalvar: real;
   begin
      neosum := 0;
      tresum := 0;
      neovar := 0;
      trevar := 0;
      globalsum := 0;
      globalvar := 0;
      neosimvar := 0;
      tresimvar := 0;
      neosimmean := 0;
      tresimmean := 0;
      globalsimmean := 0;
      globalsimvar := 0;
      for counter := 1 to neosimnumber do
         neosum := neosum + neosimarray[counter];
      neosimmean := neosum / neosimnumber;
      for counter := 1 to tresimnumber do
         tresum := tresum + tresimarray[counter];
      tresimmean := tresum / tresimnumber;
      for counter := 1 to neosimnumber do
         neovar := neovar + ((abs(neosimarray[counter] - neosimmean)) *
      (abs(neosimarray[counter] - neosimmean)));
      neosimvar := neovar / neosimnumber;
      for counter := 1 to tresimnumber do
         trevar := trevar + ((abs(tresimarray[counter] - tresimmean)) *
      (abs(tresimarray[counter] - tresimmean)));
      tresimvar := trevar / tresimnumber;
      for counter := 1 to samplesize do
         globalsum := globalsum + globalsimarray[counter];
      globalsimmean := globalsum / samplesize;
      for counter := 1 to samplesize do
         globalvar := globalvar + ((abs(globalsimarray[counter] - globalsimmean)) *
      (abs(globalsimarray[counter] - globalsimmean)));
      globalsimvar := trunc((globalvar / samplesize) * 100);
   end;

{Establishes and fills simulation data arrays, calculates test values, deposits them, and
repeats until iterations done}
   procedure ITERATE;
      var
         countera, counterb: integer;
   begin
      for countera := 1 to iterations do
         begin
            iteration := countera;
            FILLARRAYS;
```

```
            MAKESIMARRAYS;
            CALCULATETESTVALUES;
            variancedistribution[countera] := globalsimvar;
        end;
    end;

{clears statistical data arrays}
    procedure ClearDistribution;
        var
            counter: integer;
    begin
        for counter := 0 to 10001 do
            VarianceDistribution[counter] := 0;
    end;

{Sorts distribution array of test statistics to prepare for generating cumulative frequency
distribution}
    procedure bubblesort;
        var
            i, j: integer;
            k: integer;
    begin
        for i := iterations downto 2 do
            for j := 1 to i - 1 do
                if (variancedistribution[j] > variancedistribution[j + 1]) then
                    begin
                        k := variancedistribution[j];
                        variancedistribution[j] := variancedistribution[j + 1];
                        variancedistribution[j + 1] := k;
                    end;
    end;

{Generates cumulative frequency distribution for test statistic}
    procedure MakeDistribution;
        var
            DistributionTab, count, countz, cumucount, countera, counterb: integer;
            counter: integer;
            k: real;
    begin
        bubblesort;
        k := 0.01;
        writeln('0.01 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.05;
        writeln('0.05 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.10;
        writeln('0.10 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.20;
        writeln('0.20 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.30;
```

```
        writeln('0.30 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.40;
        writeln('0.40 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.50;
        writeln('0.50 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.60;
        writeln('0.60 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.70;
        writeln('0.70 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.80;
        writeln('0.80 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.90;
        writeln('0.90 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.95;
        writeln('0.95 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
        k := 0.99;
        writeln('0.99 Percentile:   ', 'Variance = ', variancedistribution[(trunc(iterations * k))
        + 1]);
    end;

{The shell of the program}
    procedure MAKEDECISION;
        var
            choicer: integer;
    begin
        writeln('    ');
        writeln('Would you like to:');
        writeln('    (1) Simulate The Hybrid Zone?');
        writeln('    (2) End this session?');
        writeln('  ');
        writeln('Choose (1) or (2) please');
        read(choicer);
        if choicer = 1 then
            begin
                Timeseed;
                ClearDistribution;
                Welcome;
                inputs;
                checkvar;
                fillneotrearrays;
                iterate;
                MakeDistribution;
            end;
        if choicer = 2 then
            HALT;
        if (choicer < 1) or (choicer > 2) then
```

```
            begin
                writeln('Hey there are only two choices here!');
                Makedecision;
            end;
    end;

begin
    MakeDecision;
end.
```