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# Evaluating Hypotheses That Explain Non-continuous Responses to Syllable Periods ("Skipping") During Phonotaxis by Female Acheta Domesticus

Tori Joy Steely
*Andrews University*, steely@andrews.edu

ABSTRACT

EVALUATING HYPOTHESES THAT EXPLAIN NON-CONTINUOUS RESPONSES TO SYLLABLE PERIODS (“SKIPPING”) DURING PHONOTAXIS BY FEMALE *ACHETA DOMESTICUS*

by

Tori Joy Steely

Chair: Gordon Atkins

ABSTRACT OF GRAUATE STUDENT RESEARCH

Thesis

Andrews University

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Title: EVALUATING HYPOTHESES THAT EXPLAIN NON-CONTINUOUS RESPONSES TO SYLLABLE PERIODS (“SKIPPING”) DURING PHONOTAXIS BY FEMALE *ACHETA DOMESTICUS*

Name of researcher: Tori Joy Steely

Name and degree of faulty chair: Gordon Atkins, Ph.D.

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One way to classify female cricket phonotactic response is as “non-skipping” or “skipping.” “Skipping” is defined as crickets that respond to a non-continuous range of calling songs. This investigation evaluates the temporal aspects of typical phonotactic protocols and attempts to determine if “skipping” is due to a filtering mechanism or if “skipping” is an artifact of testing

protocol.  Rather than a notch filter or testing parameters inhibiting a phonotactic response to a syllable period within the range of a band pass filter, I hypothesize that “skipping” occurs as a result of the probabilistic nature of phonotaxis.
Keywords: *Acheta domesticus,* phonotaxis, “skipping”

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A Thesis

Presented in Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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APPROVAL BY THE COMMITTEE:

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Gordon Atkins, Ph.D.

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Dennis Woodland, Ph.D.

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David MBungu, Ph.D. Date approved

For my parents

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# CHAPTER 1

## INTRODUCTION

Many animals, including insects, respond to a variety of stimuli such as chemical signals, sights, and sounds. Many of these stimuli are used in mating behaviors (Nottebohm, 1970; Sebeok, 1977; Andersson, 1986; Nolen & Hoy, 1986; Searcy & Andersson, 1986; Bailey, 1991; Webster *et al.,* 1992; Romer, 1993; Grammer *et al*., 2003). Female crickets recognize and then either walk or fly towards the calling song of conspecific males (positive phonotaxis; Popov & Shuvalov, 1977; Moiseff *et al.*, 1978; Pollack & Hoy, 1981b; Pollack & Plourde, 1981; Schmitz *et al.*, 1982; Thorson *et al*., 1982; Stout *et al.,* 1983; Nolen & Hoy, 1986; Jeffrey *et al*., 2005). Phonotactic behavior was first described as a fixed, automatic, species-specific behavior that precedes mating (Pierce, 1948; Walker, 1957; Popov & Shuvalov, 1977; Pollack & Hoy, 1981a; Thorson *et al.,* 1982; Stout *et al.*, 1983; Doherty, 1985; Stout & McGhee, 1988; Huber & Gerhardt, 2002). However, in 1977, Popov and Shuvalov suggested that the phonotactic response of female crickets is a plastic, complex behavior that is modified by external and/or internal conditions. More recent studies (Shuvalov *et al.*, 1990; Navia, 2005; Atkins *et al*., 2008; Stout *et al.,* 2010) have agreed with Popov and Shuvalov (1977). The conditions causing this plasticity include environmental factors, previous experience, and hormonal actions (Doherty, 1985; Stout *et* al., 1987; Stout *et al.*, 1991; Pires & Hoy, 1992; Atkins & Stout, 1994; Gray, 1999; Wagner *et al.*, 2001; Stout *et al.*, 2002; Navia *et al.*, 2010).

In 1991, Walikonis *et al.* supported plasticity by demonstrating that aging female crickets became less selective for syllable periods. In 1999, Gray confirmed this age-related change in phonotactic selectivity. Plasticity in phonotactic selectivity has been demonstrated in at least four species of crickets (Stout *et al.,* 2010).

Female phonotactic responses are categorized in different ways. One categorization is based on the degree of selectiveness to syllable periods of the male’s calling song. “Selective crickets” respond to a narrow range of syllable periods (between one and five out of the seven syllable periods tested; Stout *et al*., 1987; Walikonis *et* *al*., 1991; Henley *et al.*, 1992; Atkins & Stout, 1994; Stout *et al.*, 1998a; Stout *et al.*, 2002; Stout *et al*., 2010; Navia *et al.*, 2010). "Unselective crickets" respond to six or seven of the seven calling songs tested (Stout *et al*., 2010). Young crickets (4-7 days after final molt) tend to be more selective, while older crickets (21+ days after final molt) tend to be more unselective (Stout *et al.,* 1987; Walikonis *et al*., 1991; Henley *et al.*, 1992; Atkins & Stout, 1994; Stout *et al.,* 1998a; Stout *et al.*, 2002; Stout *et al.*, 2010; Navia *et al.*, 2010).

Schildberger (1984; see also Schildberger & Horner 1988) proposed that female phonotaxis was determined by a band-pass filter. They hypothesized that both a high-pass and a low-pass filter were present in female crickets. When a male's calling song falls within these two filters, they activated a band-pass filter that results in band-selective phonotaxis. This type of filtering could cause the selective contiguous behavior described by Stout *et al*. (2010). However, "Shildberger's model" does not account for the plasticity observed in several species of crickets (Popov & Shuvalov, 1977; Doherty, 1985; Shuvalov *et al.*, 1990; Wagner *et al.*, 2001; Stout *et al.,* 2010). Neural processing that occurs in the prothoracic ganglion is also involved in auditory recognition and may explain this plasticity (Atkins *et al*., 1992; Stout *et al.,* 1997; Atkins *et al.*, 2008; Stout *et al*., 2010).

Navia *et al.* (2003) evaluated the specific role the L3 neuron plays in phonotaxis. When exposed to a male's calling song, this neuron has two responses: an immediate response and a prolonged response (Navia *et al.*, 2003). The immediate response is phonotactically selective for specific syllable periods (Navia, 2005). Also, the L3's selective response is significantly correlated with the female cricket's response to specific syllable periods (Navia, 2005; Samuel, 2008).

Another classification of female crickets' phonotactic responses is that they can be designated as either "non-skipping" or "skipping" crickets. "Non-skipping" crickets respond to a continuous range of calling songs, whereas "skipping" crickets respond to a non-continuous range of calling songs (Stout *et al.,* 2010).

Stout *et al.* (2010) hypothesized the presence of a notch filter to explain the skipping observed in several species of crickets. He suggested that female crickets, in addition to having a band-pass filter that recognizes a range of attractive syllable periods, have an additional filter that eliminates their response to certain syllables within the range of attractiveness.  For example, the band-pass filter would recognize calling songs with syllable periods between 40-60 ms.  However, the proposed notch filter would eliminate a female's normal attractive response to a 50 ms calling song within the band-pass filter's recognition.  This means that instead of exhibiting positive phonotaxis to this syllable, the female would demonstrate negative phonotaxis, even though she had positively responded to both 40 and 60 ms syllable periods. She "skipped" the 50 ms syllable period.

In 1991, Doherty demonstrated that in order for female phonotaxis to occur, the calling song must be recognized and localized.  The neural correlates behind this recognition and localization have been tested in several species of crickets (Wohlers & Huber, 1982; Atkins *et al.*, 1984; Schildberger, 1984; Pollack, 1986; Henning, 1988; Atkins *et al.*, 1992; Stout *et al*., 1997; Bronsert *et al.*, 2003).  An alternative explanation to "skipping" is that the testing parameters are hindering the ability for females to either recognize or localize the calling song. It is an artifact of this testing protocol that is being interpreted as "skipping."  For example, the length of time female crickets are given to recognize and localize the conspecific male's calling songs may be either not long enough or too long. Also the number of tests or the silent interval between tests might interfere with the phonotactic choices made.

This investigation evaluates the temporal aspects of typical phonotactic protocols and attempts to determine if "skipping" is due to a filtering mechanism or if "skipping" is an artifact of the testing protocol.

# CHAPTER 2

# METHODS

## Animal Care

Four-week-old nymphal *Acheta domesticus* were purchased from Flukers’ Cricket Farm (Baton Rouge, Louisiana). The crickets were placed in 100-L plastic containers under a LD 12:12 hr photoperiod (lights on at 06.00 hr) and raised to adults. The temperature was kept at 21-22°C. Cricket chow (Flukers’ Cricket Farm, Baton Rouge, Louisiana), water, and egg cartons (for shelter) were provided in each container. The containers were checked daily and newly molted adults were removed. Adult females were transferred to 16-L containers, where fresh cricket chow, water, and egg cartons were provided daily. Adult males were discarded. This ensured that virgin, adult females ranging from 1-40 days-old, who have never previously heard a male’s calling song, were available for testing.

## Behavioral Testing

### Sound Stimuli

Computer-generated model calling songs were produced using SoundEdit 16, version 2 (Computer: Macbook Pro, Apple OS X 10.8.2, Apple Inc., Cupertino, California; Software: Adobe Corp., San Jose, California). Each calling song had three syllables with a duration of 25 ms, a chirp period of 667 ms, and a sinusoidal envelope with a carrier frequency of 5 kHz, which is within the natural range of the conspecific male’s calling songs (Desutter-Grancolas & Robillard, 2003). The intensity and syllable period (30-90 ms) of each calling song could be varied. Songs were played through an amplifier (Technics VC-4; Panasonic Corp., Secaucus, New Jersey) and broadcasted from a loud speaker (model 40-1221; Radio Shack, Fort Worth, Texas). When a range of syllable periods was being tested, calling songs were presented in a standard non-sequential order (50, 90, 70, 40, 60, 30, 80 ms).

### Orientation Arena

Phonotaxis was evaluated in a circular, sand-covered arena (diameter 152 cm), which was contained inside a square chamber lined with dense fiberglass material developed for absorbing sound and reducing echoes (Atkins *et al.,* 1984). The edge of the arena was bordered by a plastic strip 10 cm high and inclined inward at 45°. An omnidirectional speaker was (Radio Shack 40-1221) isolated from the floor (to eliminate vibrations) and placed in the center of the arena. Dense acoustic absorbing material (thickness 10 cm, diameter 20 cm) was placed above the speaker to absorb any upward projecting sound. Sound did not vary more than ±2 dB around the edge of the arena. White cloth covered the speaker, preventing the cricket from reaching the speaker and eliminating any visual response (Stout *et al.,* 1987). The temperature of the arena was kept between 22-24°C.

**Test of Phonotaxis**

Females (one to four at a time) were placed along the edge of the orientation arena. All female crickets used were virgin and untested. After a 5-min period of silence for acclimation, model calling songs were played from the center speaker. Each syllable period was presented, one at a time, for a total of 5 min (unless otherwise indicated). Songs were played at 85 dB (unless otherwise indicated). If all the crickets being tested reached the center speaker before the 5 min were up, then the test was terminated. Between each test a silent/no sound period of 3 min (unless otherwise indicated) was given before the presentation of the next song. Crickets usually returned to the edge of the arena within 30 sec of each sound termination. In the rare cases that they didn’t return, they were gently oriented towards the edge using a yardstick.

Cricket orientation was observed using a video camera that was mounted directly above the arena. The camera was then connected to a computer where its video feed was viewed using Apple Photo Booth (Apple Corp., Cupertino, California). A transparency was placed on the computer screen and each female cricket’s movement was traced using permanent markers that were color coded for each calling song. This setup kept the experimenter out of site of the arena while still providing orientation tracks for analysis.

Positive phonotaxis was identified when the cricket reached the speaker using a path that continuously approached the speaker (i.e., no turning away from the speaker) and stayed within one quadrant of the arena.

## Statistics

ANOVA and *t*-tests (two-sample assuming equal variances) were performed (Microsoft Excel) to determine if there was a significant difference between data sets.

# CHAPTER 3

# RESULTS

## Effects of Repeating Calling Songs

### Repeating Attractive Songs

A calling song with a 65 ms syllable period was presented 7 times in a row to each female. Only crickets that responded to the first presentation were used for further testing. Of the 33 crickets tested, 14 responded positively to all seven repetitions, whereas the remaining 19 crickets responded to 6 or fewer calling songs (Fig. 1A, B). Only about 70% of the crickets responded to the seventh presentation of the calling song (Fig. 1A, B).

### Repeating Unattractive Songs

A calling song with a 35 ms syllable period was presented 7 times in a row to each female. Only crickets that did not respond to the first presentation were used for subsequent testing. Of the 30 crickets tested, 17 didn’t respond to any of the calling songs played whereas 23 responded to between 1 and 4 calling songs (Fig. 1C, D).

Positive responses peaked at the third repetition and then declined to no response to the last calling song presented (Fig. 1C, D).

**Alternating Attractive and Unattractive Songs**

Two calling songs, one with an unattractive (35 ms) syllable period and one with an attractive (65 ms) syllable period, were alternated starting with the unattractive (35 ms) calling song. Only crickets that did not respond to the first (35 ms) syllable period played and responded to the second (65 ms) syllable period played were used. Subsequent responses to the previously unattractive (35 ms) syllable period increased to as much as 30%, whereas responses to previously attractive (65 ms) syllable period decreased by 30% (Fig. 1E, F). These results are similar to the results obtained when only the attractive or unattractive calling song was played 7 times in a row.

**Changing the Duration of the Calling Song**

For these tests, calling song durations of 2.5, 5, or 10 min were evaluated. The syllable periods played, silent period length, and intensity level followed the standard test of phonotaxis protocol. Of the 30 crickets tested, with a calling song duration of 2.5 mins (Fig. 2A, D), 23% of them demonstrated “skipping” behavior. When tested with a calling song duration of 5 mins, 40% demonstrated “skipping” behavior (Fig. 2B, D). With a calling song duration of 10 mins, 47% demonstrated “skipping” behavior (Fig. 2C, D). A significant difference exists between the number of syllable periods responded to for the different calling song durations (ANOVA, $p = 0.002528$). A significant difference also exists between the number of syllable periods “skipped” in each of the different calling song durations (ANOVA, $p = 0.039571$).

**Changing the Duration of the Silent Period**

For these tests, silent period durations of 1, 3, or 6 min were evaluated. The syllable periods played, calling song duration, and intensity level followed the standard test of phonotaxis protocol. With a silent period of 1 min, 40% demonstrated “skipping” behavior (Fig. 3A, D). With a standard silent period of 3 mins, 40% demonstrated “skipping” behavior (Fig. 3B, D). Finally, a silent period of 6 min resulted in 33% demonstrating “skipping” behavior (Fig. 3C, D). There was no significant difference in the number of syllable periods responded to for these tests

(ANOVA, $p = 0.118769$). There was also not a significant difference between the number of syllable periods "skipped" in each of the different silent period durations (ANOVA, $p = 0.840292$).

## Changing the Intensity

For these experiments, calling songs were tested at either 65 or 85 dB. The syllable periods played, calling song duration, and silent period duration followed the standard test of phonotaxis protocol. With an intensity of 65 dB, 74% demonstrated "skipping" behavior (Fig. 4A, C). With an intensity of 85 dB, 40% of the females demonstrated "skipping" behavior (Fig. 4B, C). There was no significant difference in the number of syllable periods responded to ($t$-test, $p = 0.553555$). However, there was a significant difference in the number of syllable periods "skipped" between the different test intensities ($t$-test, $p = 0.050507$).

## Effects of Repeated Testing

The standard test of phonotaxis protocol was followed. However, after one testing set (including all 7 syllable periods, 30-90 ms) was completed, another testing set was immediately started. Three min of silence were given between each testing set. This procedure was repeated until each cricket had been tested for 5-6 testing sets.

Some crickets (both young and old) did not show a likelihood to “skip” and were rather consistent in responding to a particular range of syllable periods. Young females, in this group, were more likely to respond selectively and older females, in this group, were more likely to respond unselectively (Figs. 5 & 6).

Other crickets (both young and old) “skipped” more often, including many that “skipped” syllable periods in the first set of testing as well as “skipping” in subsequent testing sets. These crickets were less likely to be very selective to syllable periods and were more variable in their responses from one repetition to the next (Fig. 7).

**Figure 1**

The effects of repeated testing of attractive versus unattractive songs. All female crickets tested were between 4-7 following their final molt. Each calling song was played for 5 min with a 3 min period of rest in between calling songs. Each test was played at 85 dB. Darkened boxes indicate positive phonotaxis. Each row represents the phonotactic responses of one female cricket. **A.** A calling song with a syllable period of 65 ms (an attractive song) was played 7 times in a row. **B.** Line graph showing the percentage of responses from A. **C.** A calling song with a syllable period of 35 ms (an unattractive song) was played 7 times in a row. **D.** Line graph showing the percentage of responses from C. **E.** 7 calling songs were played, alternating between unattractive (35 ms) and attractive (65 ms) calling songs. **F.** Line graph showing the percentage of responses from E.

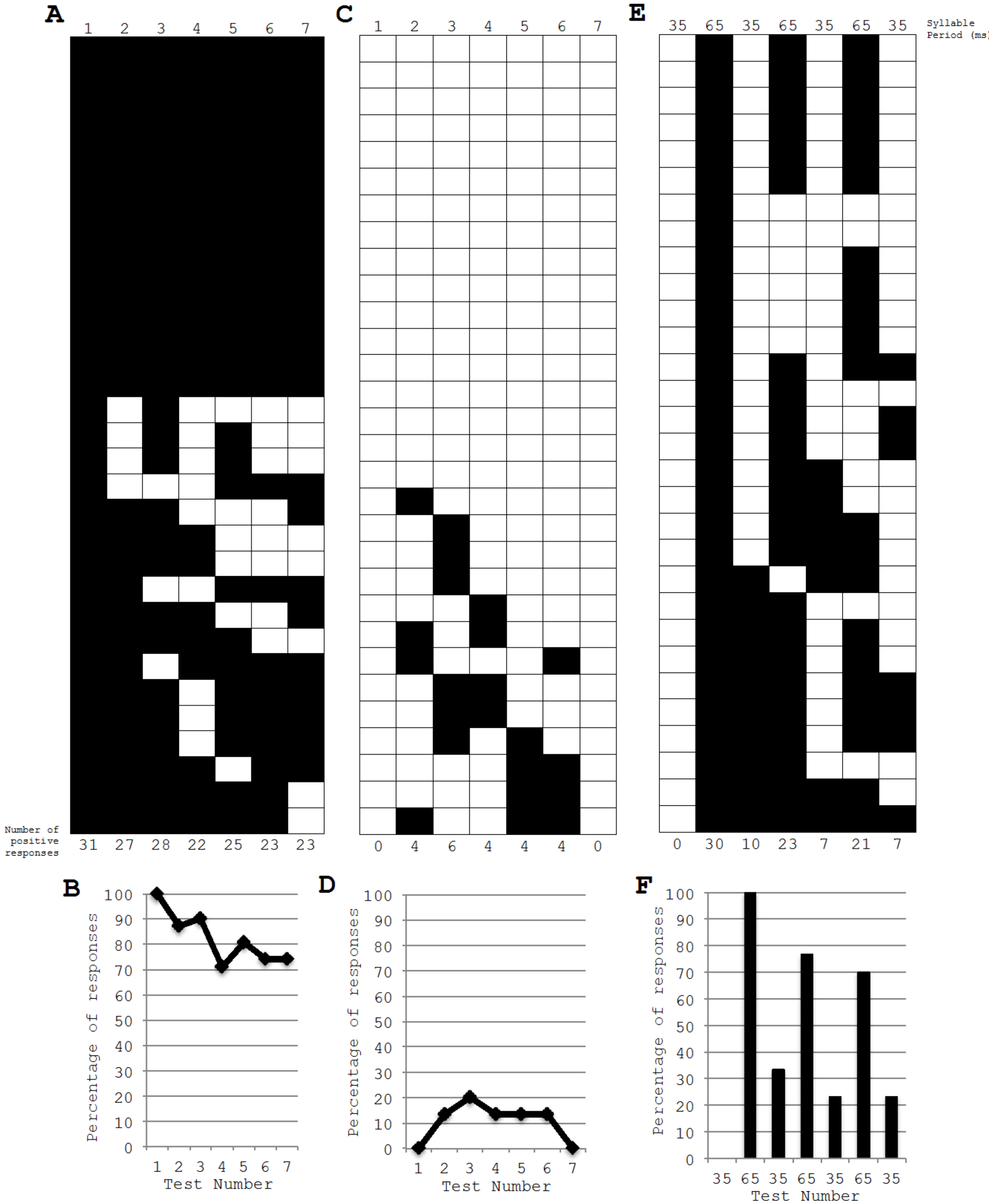

A
1 2 3 4 5 6 7
Number of positive responses
31 27 28 22 25 23 23
C
1 2 3 4 5 6 7
0 4 6 4 4 4 0
E
35 65 35 65 35 65 35
Syllable Period (ms)
0 30 10 23 7 21 7
B
Percentage of responses
Test Number
D
Percentage of responses
Test Number
F
Percentage of responses
35 65 35 65 35 65 35
Test Number

**Figure 2**
The effects of changing the duration of the calling song. All female crickets tested were between 4-7 following final molt. Calling songs were composed of syllable periods between 30-90 ms, in 10 ms increments. Songs were presented in a standard non-sequential order (50, 90, 70, 40, 60, 30, 80 ms). Each cricket was given 3 min of rest between each test. Each calling song was played at 85 dB. **A.** All calling songs in this set were played for 2.5 min each. **B.** All calling songs in this set were played for 5 min each. **C.** All calling songs in this set were played for 10 min each. **D.** Line graph showing the percentages of responses in A-C.

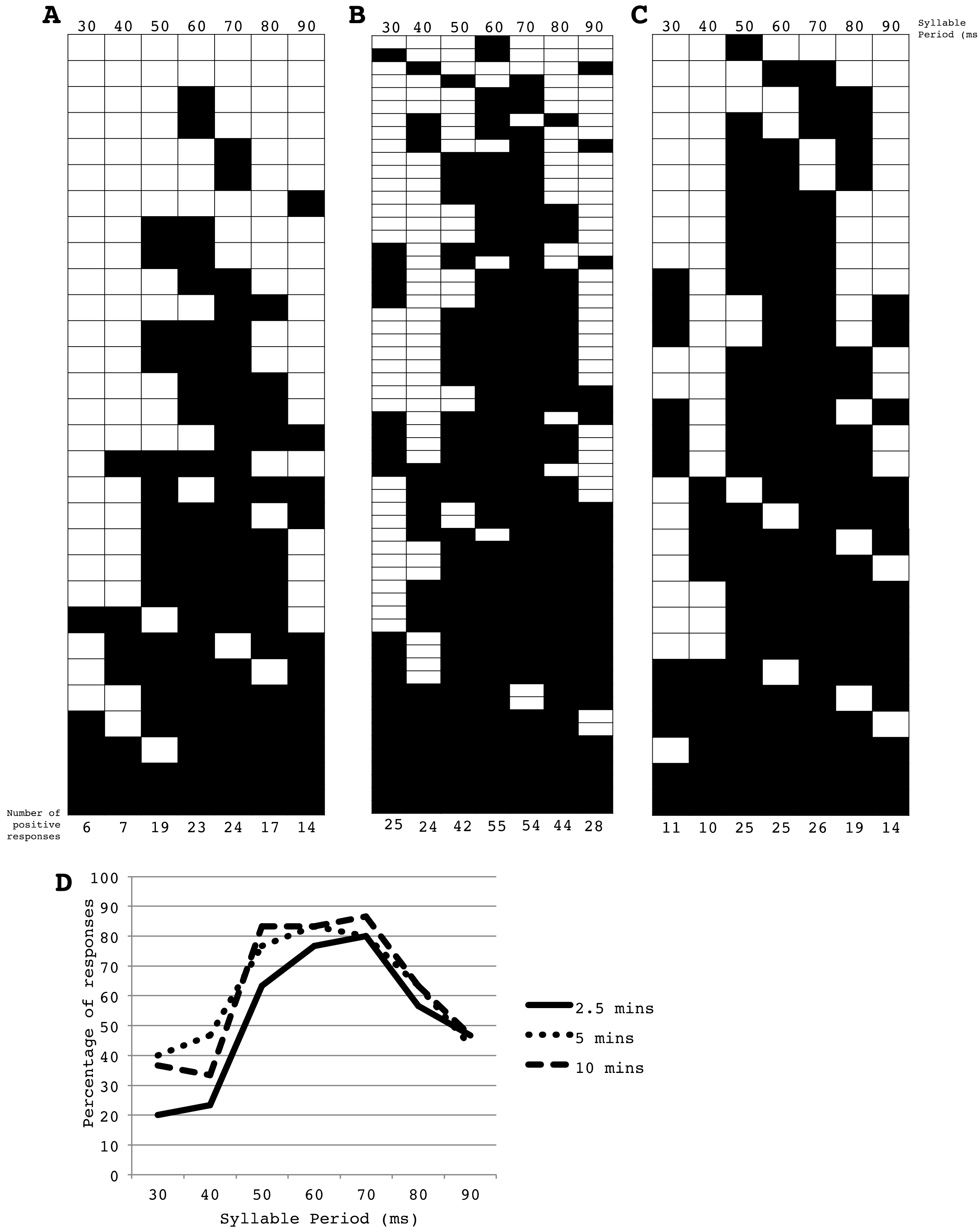

A
B
C
30 40 50 60 70 80 90
Syllable Period (ms)
Number of positive responses
6 7 19 23 24 17 14
25 24 42 55 54 44 28
11 10 25 25 26 19 14
D
Percentage of responses
100
90
80
70
60
50
40
30
20
10
0
30 40 50 60 70 80 90
Syllable Period (ms)
2.5 mins
5 mins
10 mins

**Figure 3**
The effects of changing the duration of the silent period between tests. All female crickets tested were between 4-7 days following their final molt. Calling songs were composed of syllable periods between 30-90 ms, in 10 ms increments. Songs were presented in a standard non-sequential order (50, 90, 70, 40, 60, 30, 80 ms). Each calling song was played for 5 min at 85 dB. **A.** 1 min of silence was given between each calling song played in this set. **B.** 3 min of silence were given between each calling song played in this set. **C.** 6 min of silence were given between each calling song played in this set. **D.** Line graph showing the percentage of responses in A-C.

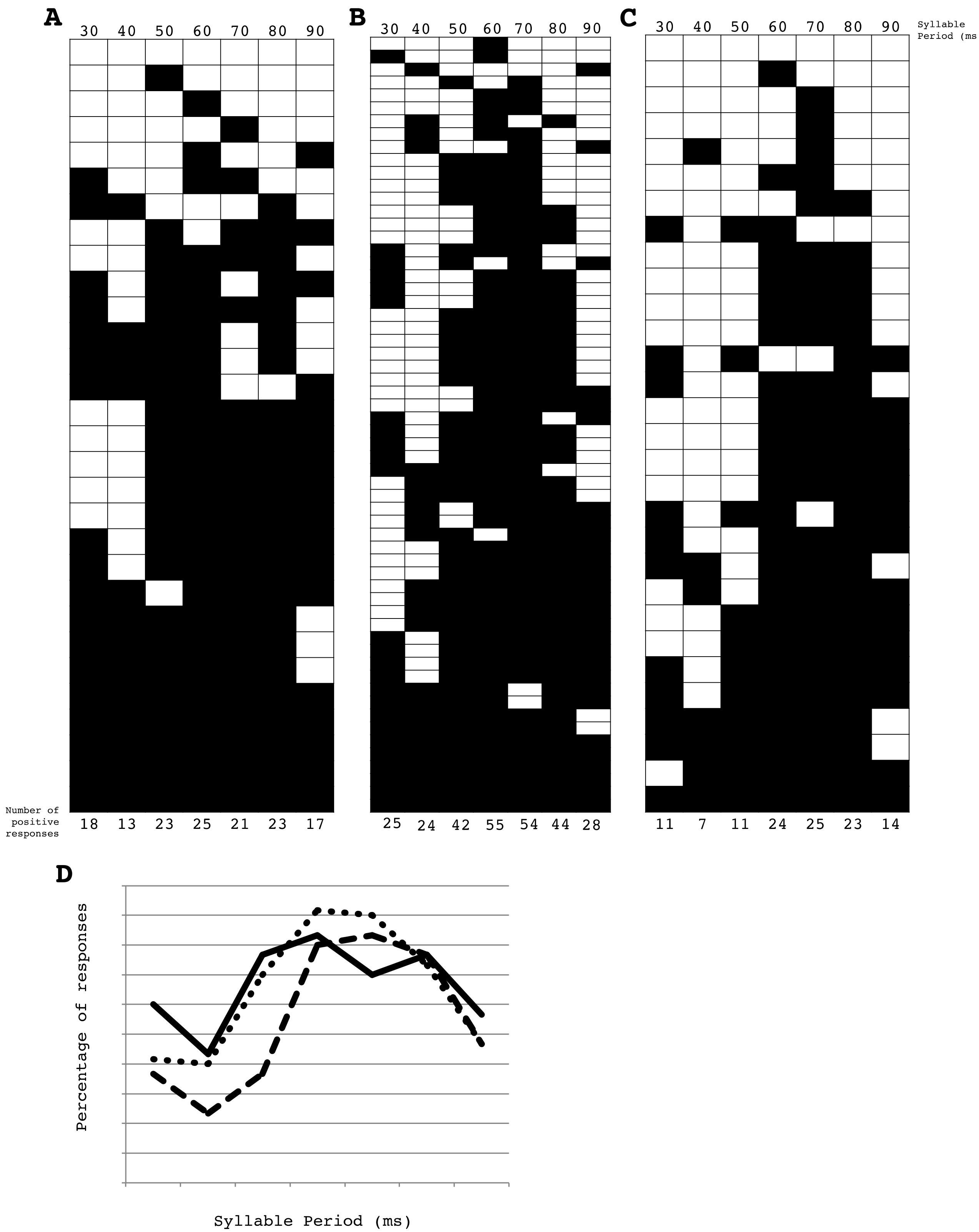

A
B
C
30 40 50 60 70 80 90
Syllable Period (ms)
Number of positive responses
18 13 23 25 21 23 17
25 24 42 55 54 44 28
11 7 11 24 25 23 14
D
Percentage of responses
Syllable Period (ms)

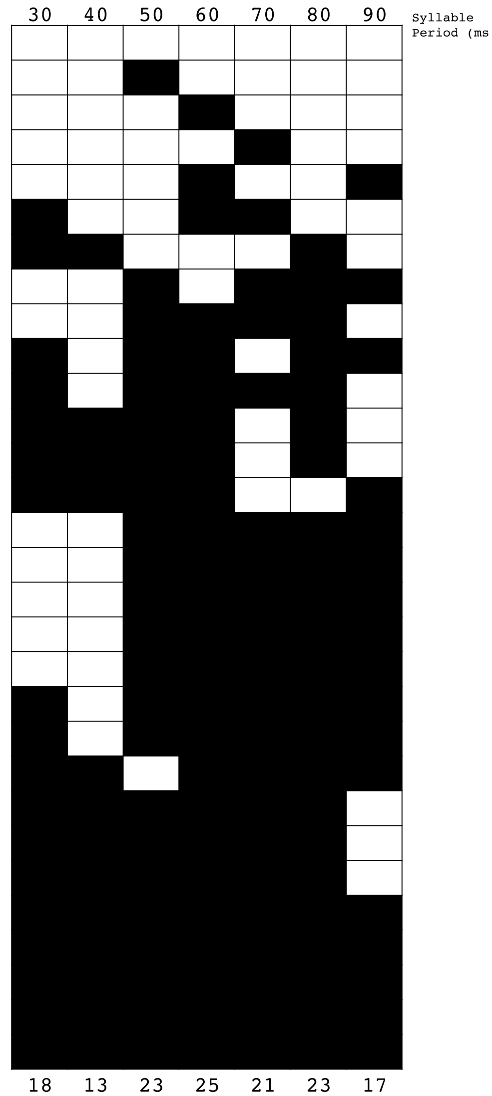
**Figure 4**
The effects of changing the intensity level. All female crickets tested were between 4-7 days following their final molt. Calling songs were composed of syllable periods between 30-90 ms, 10 ms increments. Songs were presented in a standard non-sequential order (50, 90, 70, 40, 60, 30, 80 ms). Each calling song was played for 5 min with a 1 min silent period between songs. **A.** All tests in this set were done at 65 dB. **B.** All tests in this set were done at 85 dB. **C.** Line graph showing percentages of A & B.

**A**

0 60 70 80 90

Number of positive responses 14 14 27 19 19 21 22

**B**




**C**

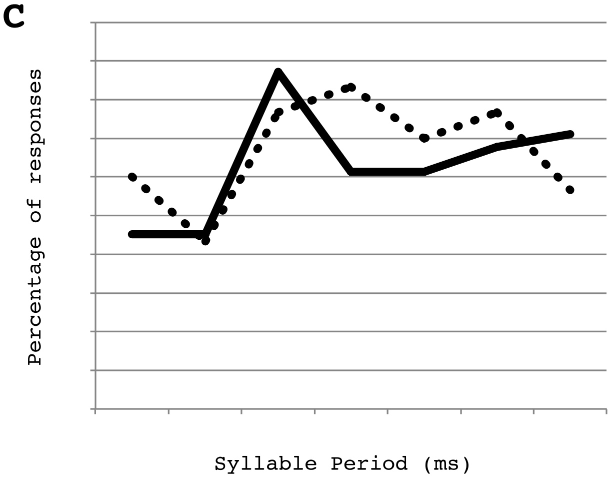


**Figure 5**
Effects of repeated testing; 1 or fewer syllable periods "skipped" per testing bout. Each song was played for 5 min with a 3 min silent period in between tests. All songs were played at 85 dB. After the initial test (7 calling songs) were completed, another set was immediately started using the same 7 calling songs in the same order as the previous test. 3 min of silence were given between testing set. All crickets were tested for 5-6 testing sets. Each set (A-F) represents the responses of an individual cricket. Horizontal lines represent the testing set (1-6) and the vertical line represents the syllable periods (30-90 ms). Darkened boxes indicate positive phonotaxis. **A-C** are young (4-7 days following their final molt) females, whereas **D-F** are old (21-28 days following their final molt) females.

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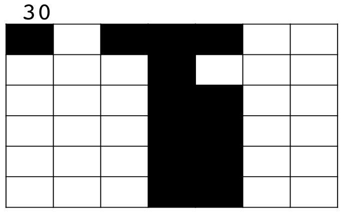

**Figure 6**

The effects of repeated testing; 2 syllable periods "skipped." Calling songs were composed of syllable periods between 30-90 ms, in 10 ms increments. Songs were presented in a standard non-sequential order (50, 90, 70, 40, 60, 30, 80 ms). Each song was played for 5 min with a 3 min silent period in between tests. All songs were played at 85 dB. After the initial test (7 calling songs) was completed, another set was immediately started using the same 7 calling songs in the same order as the previous test. 3 min of silence were given between testing sets. All crickets were tested for 5-6 testing sets. Each set (A-E) represents the responses of an individual cricket. Horizontal lines represent the testing set (1-6) and the vertical lines represent the syllable periods (30-90 ms). Darkened boxes indicate positive phonotaxis. **A, B** are young (4-7 days following their final molt) females, whereas **C-E** are old (21-28 days following their final molt) females.

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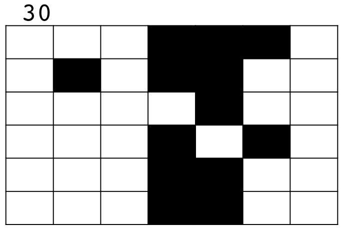

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Test number

Test number

**Figure 7**
The effects of repeated testing; 3+ syllable periods "skipped." Calling songs were composed of syllable periods between 30-90 ms, in 10 ms increments. Songs were presented in a standard non-sequential order (50, 90, 70, 40, 60, 30, 80 ms). Each song was played for 5 min with a 3 min silent period in between tests. All songs were played at 85 dB. After the initial test (7 calling songs) was completed, another set was immediately started using the same 7 calling songs in the same order as the previous test. 3 min of silence were given between testing sets. All crickets were tested for 5-6 testing sets. Each set (A-I) represents the responses of an individual cricket. Horizontal lines represent the testing set (1-6) and the vertical lines represent the syllable periods (30-90 ms). Darkened boxes indicate positive phonotaxis. **A-F** are young (4-7 days following their final molt) females, whereas **G-I** are old (21-28 days following their final molt) females.

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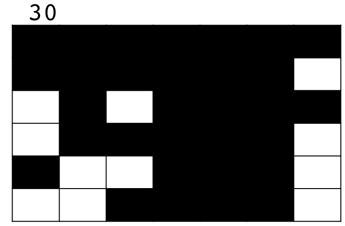

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# CHAPTER 4

# DISCUSSION

## Repeated Testing Effects

The results in Figure 1A, B demonstrated that when an attractive calling song was repeated, the probability of positive phonotactic responses by female crickets diminishes over time. Conversely, calling songs with an unattractive syllable period became attractive some of the time when tested in a repeated sequence (Fig. 1C, D). To rule out possible habituation to repeated identical calling songs, unattractive and attractive songs were tested in alternation. The sequence started with the unattractive syllable period because Wagner *et al.* (2001) showed that previous calling songs could influence a cricket's response to later calling songs. Alternating previously unattractive and attractive calling songs did not always result in positive phonotaxis to the previously attractive syllable period nor did it always result in no phonotaxis to the previously unattractive syllable periods (Fig. 1E, F). The results of these experiments were consistent with the idea that the likelihood of phonotaxis occurring to an attractive stimulus was a probabilistic event–the call was attractive but the response to that call was not absolutely certain. Similarly, not responding to unattractive signals was also probabilistic.

The results in Figures 8-10 were also consistent with the idea that phonotaxis was probabilistic. Unselective crickets remained generally unselective to subsequent sets of tests (Fig. 8). However, the number of syllable periods females “skipped” varied (Figs. 9 & 10).

Schildberger (1984; Schildberger & Horner, 1988) suggested that a band-pass filter in the brain was responsible for the range of attractive syllable periods during phonotaxis. According to this model, females should respond to a range of syllable periods dictated by the filter and should not “skip” syllable periods. The occurrence of a high degree of “skipping” (over half the females tested) prompted Stout *et al.* (2010) to hypothesize that a notch filter reduced the number of syllable periods responded to within the range determined by the band-pass filter.

If this proposed notch filter were present, we would expect that a “skipped” syllable period would continue to be “skipped” in subsequent rounds of testing. In addition, attractive syllable periods should remain attractive and syllable periods outside the attractive range should remain unattractive. The diminishing responses of female crickets to attractive syllable periods over time (Fig. 1A, B, D, E), the occurrence of positive responses to repeated previously unattractive syllable periods (Fig. 1C, D, E, F), and the irregular pattern of “skipping” in the same females from one test to the other (Figs. 9, 10) did not support this type of notch filter. Rather, these data suggested that phonotaxis to specific syllable periods was a probabilistic event. Whether this probability was caused by an internal source or was an artifact of the testing procedures was not determined by these tests.

## Changing Testing Parameters

I evaluated some of the parameters that define the protocols of phonotaxis testing to see if the probability of phonotaxis was an artifact of the testing procedures. Changing the calling song duration did demonstrate a significant difference in number of syllable periods females responded to (Fig. 2). There was also a significant difference in the amount of “skipping” that occurred. If the test period was too short, phonotaxis occurs to fewer syllable periods and skipping was decreased. By shortening the calling song duration, I created an artifact of testing protocol. A shorter duration meant the crickets didn’t respond to as many calling songs. Therefore, they didn’t have the opportunity to “skip” as many syllable periods. Fortunately, this change in responsiveness was affected only by test durations that were less than what has been typically used in the lab at Andrews University, Berrien Springs, MI (Stout *et al.*, 1983; Atkins *et al.,* 1984; Stout & McGhee, 1988; Kohne *et al.,* 1992; Stout *et al.,* 1997; Atkins *et al.,* 2008; Stout *et al.,* 2010).

Changing the length of the silent period did not demonstrate a significant difference between tests, within the time ranges we tested (Fig. 3). This range includes the lengths of silent periods typically used in phonotaxis tests (Stout & McGhee, 1988; Atkins *et al.,* 2008; Stout *et al.,* 2010). Although there was no significant difference between the tests, about 14% less “skipping” occurred to the longer silent period (6 min) compared to the shorter silent period (1 min). Further testing with longer silent periods should be performed. By lengthening the silent period beyond what was tested, one could test whether it is possible to eliminate “skipping” completely if this factor is effective.

Changing the intensity level did not produce a significant difference in the number of syllable periods tested (Fig. 4). However, it did show a significant difference in the amount of “skipping” that occurred. This range of intensities included intensities that were typically used in phonotaxis tests (Atkins *et al.,* 1984; Stout & McGhee, 1988; Kohne *et al.,* 1992; Stout *et al.,* 1997; Bronsert *et al., 2003;* Atkins *et al.,* 2008; Stout *et al., 2010*). My results demonstrated that at higher intensity levels, females are less likely to demonstrate “skipping” behavior. These results not only support the theory that phonotaxis is a probabilistic event but they also stress the importance of consistent intensities between test sets.

In addition to using an arena to test phonotaxis, some investigators have used a Kramer treadmill (Weber *et al.*, 1981; Thorson *et al.*, 1982; Stout *et al.*, 1987; Weber *et al.*, 1987; Wendler, 1989; Walikonis *et al.*, 1991; Kohne *et* *al.,* 1992; Stout *et al.,* 1998b; Jeffery *et al.,* 2005; Atkins *et al.,* 2008; Verburgt *et al.,* 2008; Stout *et al.*, 2010). Even though this method of testing uses very different protocols, "skipping" was observed in several species (Stout *et al.,* 2010). The fact that "skipping" was seen in two different types of testing methods argues against the idea that "skipping" is an artifact of the testing protocol.

**What Then Is "Skipping"?**

Recognition of syllable period has been shown to occur in the prothoracic ganglion (Atkins *et al.,* 1992; Stout *et al.,* 1997; Atkins *et al.,* 2008; Stout *et al.,* 2010). The phonotactic response and the prothoracic auditory neurons (L3, AN2, and ON1) exhibit plasticity. Although the data described above best support the idea that "skipping" is a result of phonotaxis being a probabilistic event, they do not completely rule out a notch filter. However, this notch filter would have to exhibit extremely plastic behavior in order to account for all the variation seen in Figures 1, 5-7.

influence the level of "skipping" (Figs. 2-4). However, increases in "skipping" were seen only in extreme parameters outside the range that is typically used for phonotaxis testing (Stout *et al.,* 1983; Atkins *et al.*, 1984; Stout & McGhee, 1988; Kohne *et al.*, 1992; Stout *et al.*, 1997; Bronsert *et al.,* 2003; Atkins *et al*., 2008; Stout *et al.*, 2010). Rather than a notch filter or testing parameters inhibiting a phonotactic response to a syllable period within the range of a band-pass filter, I hypothesize that “skipping” occured as a result of the probabilistic nature of female phonotaxis. In other words, just because the syllable periods of the calling song fell within the attractive range of the band-pass filters (recognition), and can be localized, it doesn’t mean that females will do phonotaxis at that moment. Factors, such as hormones, neurons, health and physiology, etc., that are responsible for this probability of response to attractive syllable periods could be a subject of further investigation.

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