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Fear and Aggression in the Desert Spider *Agelenopsis aperta*

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**Appendix D - UNIVERSITY HONORS PROGRAM
SENIOR PROJECT - APPROVAL**

Name: Laura Robertson
College: Arts + Sciences Department: Ecology + Evolutionary Bio.
Faculty Mentor: Susan E. Riechert
PROJECT TITLE: Fear and Aggression in the Desert Spider
Agelenopsis aperta

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: Susan E. Riechert, Faculty Mentor

Date: May 6, 99

Comments (Optional):

Fear and Aggression in the Desert Spider *Agelenopsis aperta*

Laura Robertson

Senior Project

Susan E. Riechert, mentor

May 7, 1999

Fear and Aggression in the Desert Spider *Agelenopsis aperta*

By Laura Robertson

Susan E. Riechert, mentor

Abstract

Two populations of *Agelenopsis aperta*, one from Arizona and one from New Mexico, show strong behavioral characteristics of fear and aggression, respectively. The population in AZ is under high predation risk while the NM population has a high level of competition; therefore, AZ spiders exhibit more fear while NM spiders are more aggressive. Maynard Smith and Riechert (1984) proposed a model of the genetic basis of fear and aggression that assumes fear to be an autosomally inherited trait and aggression to be sex-linked. In my study I developed two new protocols to test *A. aperta* for fear and aggression. These tests, Habituation and Puff and Prod, were both carried out on a circular track. This spider species can see a distance of two centimeters, so it relies mainly on tactile and vibratory information. For this reason, puffs of air from a camera cleaning brush bulb were used to mimic the attack of predatory birds; prods with a pencil eraser were used to mimic a physical attack. I also completed a test of spider habituation to a predatory cue (i.e. rear puff). Only the data on NM spiders is represented here; the sample sizes of the other genetic classes were too small. *Agelenopsis aperta* responded differently to puffs versus prods. Prods caused a stronger fear response than puffs, as estimated by mode and distance moved away from the stimulus. There was not a significant difference between front and rear stimuli, order of presentation, or the total distance moved by each sex. Overall, both female and male New Mexico spiders showed a significantly stronger response to physical prods than to puffs of air independent of the

direction of the stimulus. The spiders did not habituate to the stimulus of a rear puff offered at two minute intervals.

Introduction

Previous studies of the desert spider *Agelenopsis aperta* (Gertsch) have demonstrated a correlation between its behavior and genetic composition. Testing in the field and lab have shown *A. aperta* to possess numerous traits that are fitness linked (e.g. Maynard Smith and Riechert 1984; Riechert and Maynard Smith 1989; Hedrick and Riechert 1989; Riechert and Hedrick 1990; Riechert and Hedrick 1993). This group of traits includes foraging behavior (Hedrick and Riechert 1989), agonistic behavior and territory size demanded (Maynard Smith and Riechert 1984; Riechert and Maynard Smith 1989), and anti-predatory behavior (Riechert and Hedrick 1990). These traits correspond to two basic, conflicting behavioral responses, fight or flight. These tendencies can also be referred to as aggression and fear (Maynard Smith and Riechert 1984).

The level of fear and aggression demonstrated by two populations of *A. aperta* vary according to their habitat. In the arid New Mexico habitat, web sites and prey are limited resulting in high competition among the spiders (Riechert 1981). In Arizona web sites and prey are abundant but there are many avian predators (Riechert 1981). Consequently, the New Mexico spiders require more territory than their Arizona counterparts (Riechert 1981). New Mexico spiders also are quicker to respond to prey on the web and return to the web faster after a disturbance (Hedrick and Riechert 1989; Riechert and Hedrick 1990). Tests on F1 hybrids and back crosses with parental lines (Maynard Smith and Riechert 1984; Riechert and Maynard Smith 1989) led to the development of a model of spider behavior on a continuum from fearful to aggressive

(Riechert and Maynard Smith 1989). In this model, fear is inherited autosomally and aggression is sex-linked (Riechert and Maynard Smith 1989).

Riechert and Hedrick (1993) showed that levels of fear exhibited in latency to return to prey after a disturbance accurately predicted aggressiveness to prey. Riechert and Maynard Smith (1989) showed a similar correlation between fighting behavior and size of territory demanded. The purpose of my project was to design a group of tests that elicited fear and aggression responses from the spiders. Most of the tests examined fear responses of *A. aperta* to predatory cues (puffs) like those used by Riechert and Hedrick (Hedrick and Riechert 1989; Riechert and Hedrick 1990; and Riechert and Hedrick 1993) and also to physical attacks (prods from a pencil eraser). The second interest of the study was to test the spiders for habituation to a predatory cue. Numerous stimuli have been shown to cause varying degrees of stress responses in other animals suggesting a way to quantify or qualify the “severity of the stress response” (Blanchard et al. 1998).

Blanchard et al. (1998) described a series of gradually decreasing responses that rats exhibited as they habituated to the stimulus of chronic predatory stress. Analysis of behavioral differences between the sexes was also of interest, as Singer and Riechert (1994) found no evidence of differences between the fitness-linked traits of females and males.

Methods

Two protocols were designed to test the spiders for fear and aggression using “puffs” and or “prods” to elicit behavioral responses. *Agelenopsis aperta*’s range of vision is approximately two centimeters. For this reason, it was possible to use the puff of air from a camera bulb brush to mimic the attack of a predatory bird. Similarly, prods

from a pencil eraser were used to mimic a physical attack from a web invader (i.e. another spider). Both tests were completed in a circular track. The track was a plastic tray with a one-centimeter wide lane. The circumference of the track was marked at five-centimeter intervals.

General Protocol

The spider was placed at 0 cm on the track using an open-ended syringe. A stopwatch was started, and the spider's behavior was recorded. A list of actions was made to standardize movement terminology (Table 1). After five minutes, the spider was stimulated. The type and interval of stimulation varied for the two tests, but the distance and mode of movement were recorded each time. All tests were carried out in the lab on individuals from each genetic class.

Puff and prod test

Protocol

Following the general protocol given above, the spider was placed on the track. After five minutes the spider was stimulated with either a front or rear prod or a front or rear puff. The order of presentation of these four stimuli was randomized using a random numbers table. Each of the stimuli was assigned a number to correspond to the table:

1= Front prod

2 = Back prod

3 = Front puff

4 = Back puff

The stimuli were presented to the spider at five minute intervals. Each time, the distance the spider moved and the mode of movement (Table 1) exhibited were recorded. Spiders

that crawled off of the track were re-tested. After the test, the track was wiped down with 70% ethanol and allowed to dry between trials.

Results

The sample size for all genetic classes besides NMxNM were too small and are not presented here. All individuals tested were mature adults. The NM sample size was 57.

The order of presentation of stimuli was not significant in relation to how far the spider moved (one-way ANOVA F test, $P > 0.3945$) (Table 3, Fig. 1). Whether the stimulus was a puff or a prod was significant (two-way ANOVA F test, $P > 0.0000$) (Table 3, Fig. 2). The direction of the stimulus was not significant for either prod class (rear and front) ($P > 0.1131$) or puff class ($P > 0.5776$) (one-way ANOVA F test, Table 3). The sex of the spider was not a significant factor in the total distance moved by the spider (one-way ANOVA F test, $P > 0.8916$) (Table 3, Fig. 3).

Data from incomplete trials due to spider escapes was inconclusive. Only four escapes out of 26 were a direct response to the stimulus (Table 2). The other escapes were not frenzied but occurred during searching in between stimuli. A chi-square test showed no significance between sex of the spider and number of escapes (d.f.=1; $\chi^2 = 0.7580$) (Table 4).

Aggressive behavior (raising of the front legs) only occurred as a response to forward attacks. This happened four times and was elicited by both front prods and front puffs.

Habituation

Protocol

The spider was placed at 0 cm in track as described in the general protocol. After five minutes the spider was given a puff from 7 cm behind. The distance the spider moved and mode of movement were recorded. After 120 seconds, the spider was puffed again and its behavior was recorded. This sequence was continued until the spider did not move through three consecutive puffs or a maximum of ten puffs was given. The last puff that caused movement was recorded (i.e. $X=6$ for a spider that last responded on the sixth puff or $X=10$ for a spider that was still responding to the tenth puff). The track was wiped down with 70% ethanol and allowed to dry between trials.

Results

Mature adults and penultimate spiders were tested. The NMxNM sample size was 72. Out of 72 trials, 62 spiders did not habituate to the stimulus of a rear puff over two minute intervals. The X values for the ten spiders that did habituate showed a range of values (Table 5, Fig. 4).

Discussion

The puff and prod test showed that NM spiders exhibit a stronger fear response to prods (physical attacks) than to puffs of air (predatory bird attacks). The difference between the two is highly significant. A possible explanation for this difference lies in the environment of the NM population. In this desert grassland, the spiders are under low risk from avian predators. There are few birds present in the habitat. Furthermore, the NM site is very windy. If air currents are continually changing and are non-threatening,

perhaps these spiders have not adapted any sort of defense to warn them against changes in air currents.

The statistical analysis showed that the direction of the stimulus was not significant to the response of the spider, emphasizing that the important factor is whether or not the attack is a physical prod or a puff of air. New Mexico spiders must compete for limited web sites (Riechert 1981). The high aggressive, territorial nature of the NM population has been documented previously (Riechert 1979; Riechert 1981).

The insignificance of sex in relation to total distance moved can be explained by the similar selection pressures on the two sexes. *A. aperta* spiders live solitary lives and both sexes have to exhibit similar levels of fear and aggression to survive.

Order of presentation did not affect the distance moved by the spiders. This corresponds to the results of the habituation test. The spiders did not habituate to the same stimulus over two minute intervals. This infers that after a five minute interval, the stimulus would be a new event to the spider. The rate of habituation to a stimulus has been shown to increase when the time interval between stimuli was shortened. The concept for habituation used in the trials of *A. aperta* may have been faulty. Graduated responses toward frequency and amplitude of stimulus (Pilz and Schnitzler 1996 and Blanchard et al. 1998) have been documented and could be present in *A. aperta*.

In conclusion, female and male *A. aperta* spiders from a desert grassland habitat exhibit a significantly stronger fear response to a physical prod than a puff of air. Furthermore, the direction of the stimulus and order of presentation do not affect the strength of the fear response. *Agelenopsis aperta* spiders do not habituate to air puffs offered at two minute intervals.

Future Testing

The puff and prod test will be carried out on Arizona spiders, F1 hybrids, and back crosses to test for differences in fear response among the genetic classes. The habituation protocol is being redeveloped currently to determine what time interval causes the greatest number of spiders to habituate. Once this time interval is found, the habituation test will also be extended to the other genetic classes. Another possibility is to test for differential habituation by the spiders to puffs and prods. Because the NM spiders showed greater fear in response to prods than puffs, there is a possibility that they may habituate at different levels to the two stimuli. The spiders may show a slowed tendency to habituate to a prod or they might not habituate to the stimulus at all.

Acknowledgements

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Table 1. Actions Observed during Track Behavioral Tests

(Habituation and Puff & Prod)

In ascending order of activity

Crouch

Adjust Position –shuffle legs then end up spread

Move

Turn to Side

Turn 360 degrees

Step (walk 1 step)

 Hop –1 step only

 Jump –1 step only

Walk

Laying Silk

Scamper

Run

Attack

 A. Raise front legs

 B. Raise front legs and lunge

Scramble –frenzied run (trying to get out of track)

Scoot –puff of air pushes spider in the direction of the air

Table 2. Puff and Prod Escape Data							
n= 26 incomplete trials (F=11, M=15)							
sex	sequence	dist.@rel.	stimulus 1	stimulus 2	stimulus 3	stimulus 4	stimulus preceding escape
M	X	X					release
M	X	X					release
F	X	X					release
F	1432	5	10	X			back puff
F	X	X					release
F	3412	0	X				back puff
F	3241	4	1	7	95		back prod
F	2431	45	X				back prod
M	4123	10	X				back puff
M	4312	15	0	1	X		front prod
F	4312	0	X				back puff
F	3214	0	X!				front puff
M	1234	1	50	110	X		front puff
M	3412	0	100	8	67	X	back prod
M	X	X					release
M	3124	50	X				front puff
M	1342	0	X				front prod
M	2431	6	4	0	X		front puff
M	X	X					release
M	3124	40	1	1	X		back prod
M	1243	65	X				front prod
M	2413	65	10	45	X		front prod
M	3214	30	5	3	X!		front prod
F	2413	9	10	5	X!		front prod
F	X	X					release
F	2314	1	X!				back prod

Note: X denotes an escape; X! denotes a frenzied escape in direct response to stimulus

Table 3. Statistical Analysis of Significance

Test	P Value
stimulus order	0.3945
puff vs. prod	0.0000
prod direction	0.1131
puff direction	0.5776
sex	0.8916

Table 4. Chi-Square Test

	Males		Females		Totals
	Observed	Expected	Observed	Expected	
Escape	15	13.02	11	12.71	26
No Escape	27	28.98	30	18.29	57
Total	42		41		83

$$X^2 = 0.7580$$

$$d.f. = 1$$

Table 5. Habituation X Values

X Value	Frequency
3	2
5	1
7	2
8	2
9	3
10	62

Fig. 1. Distance Moved for Each Ordered Stimulus

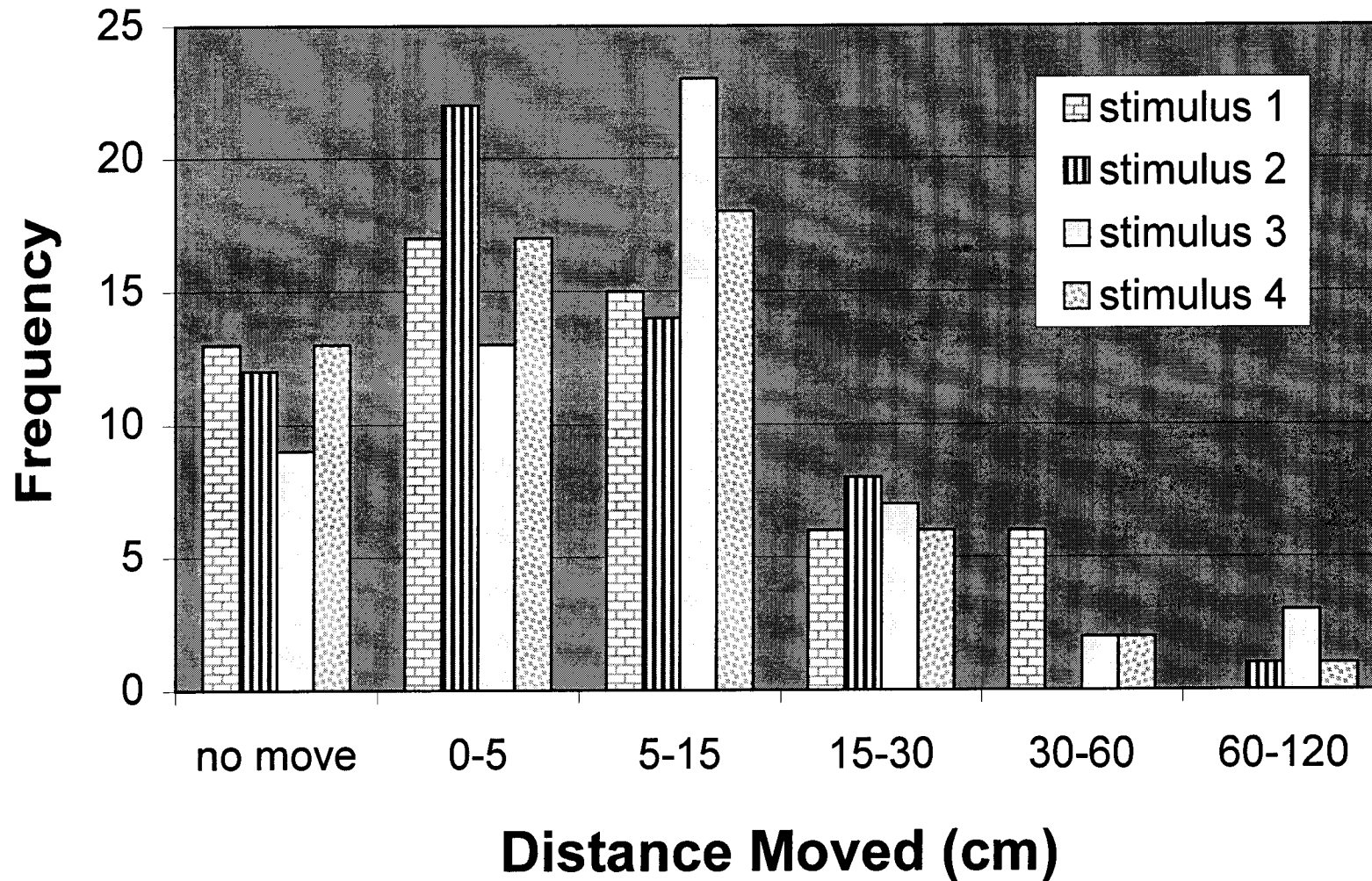


Fig. 2. Distance for Each Stimulus

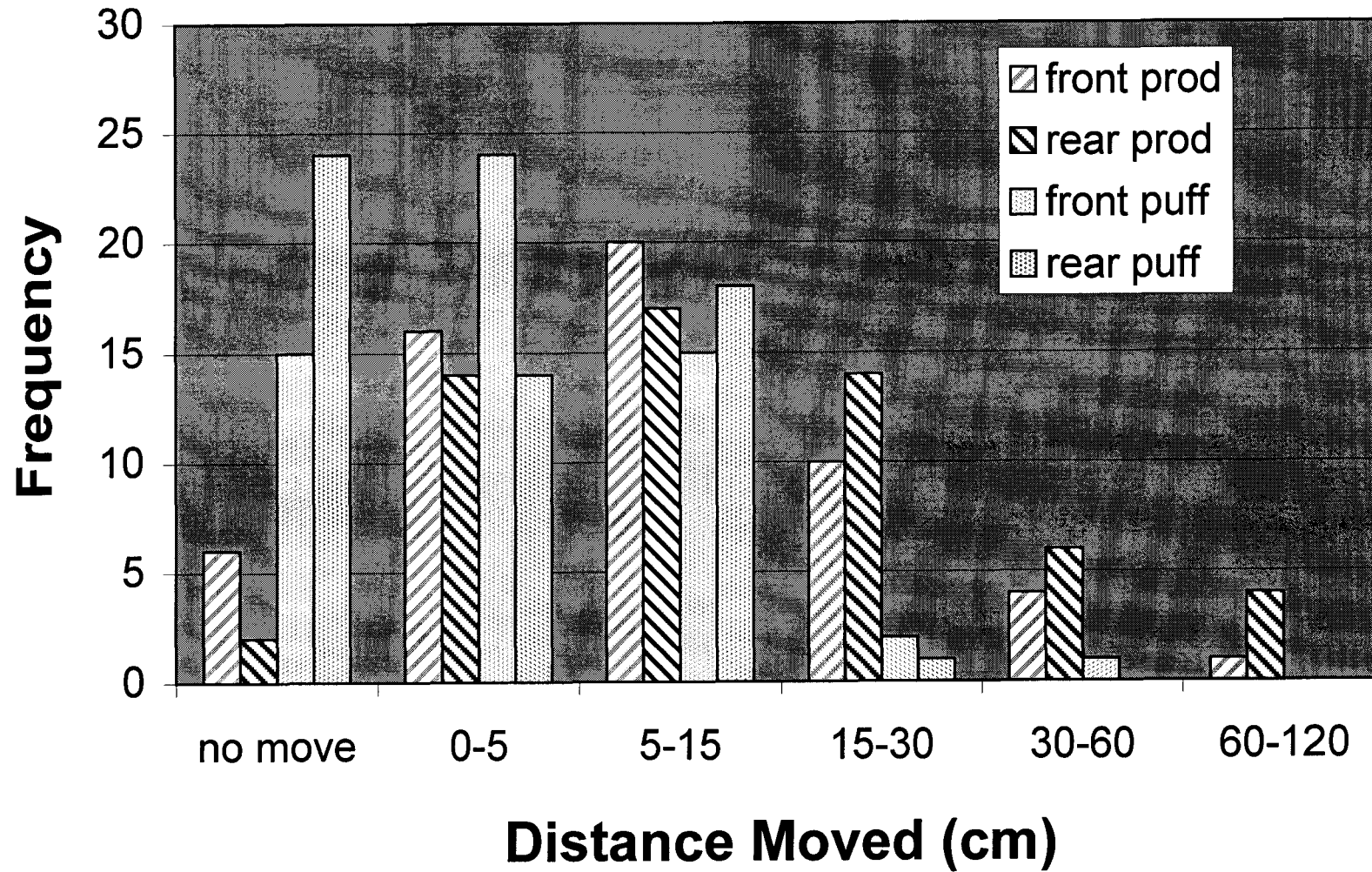


Fig. 3. Total Distance Moved by Each Sex

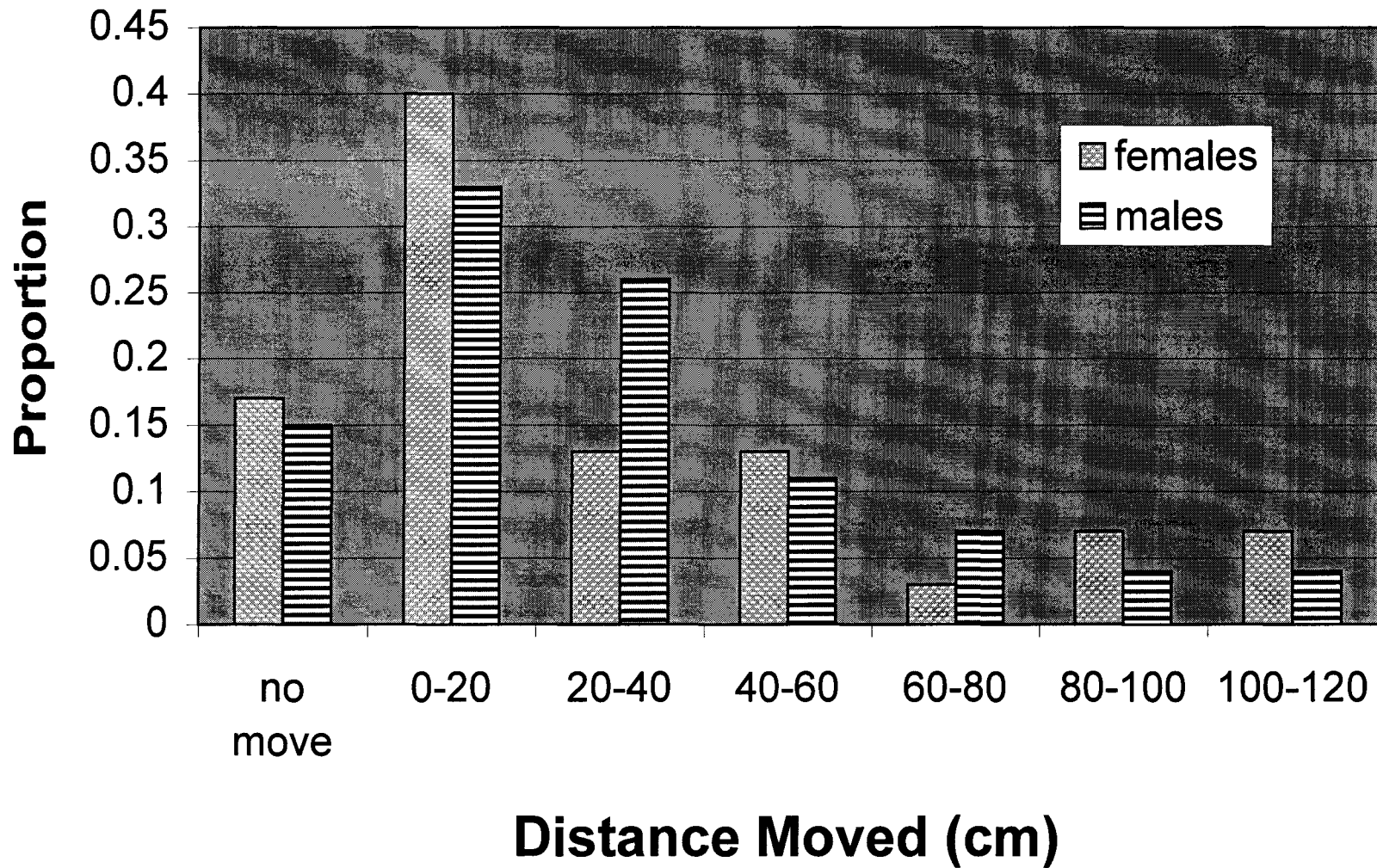
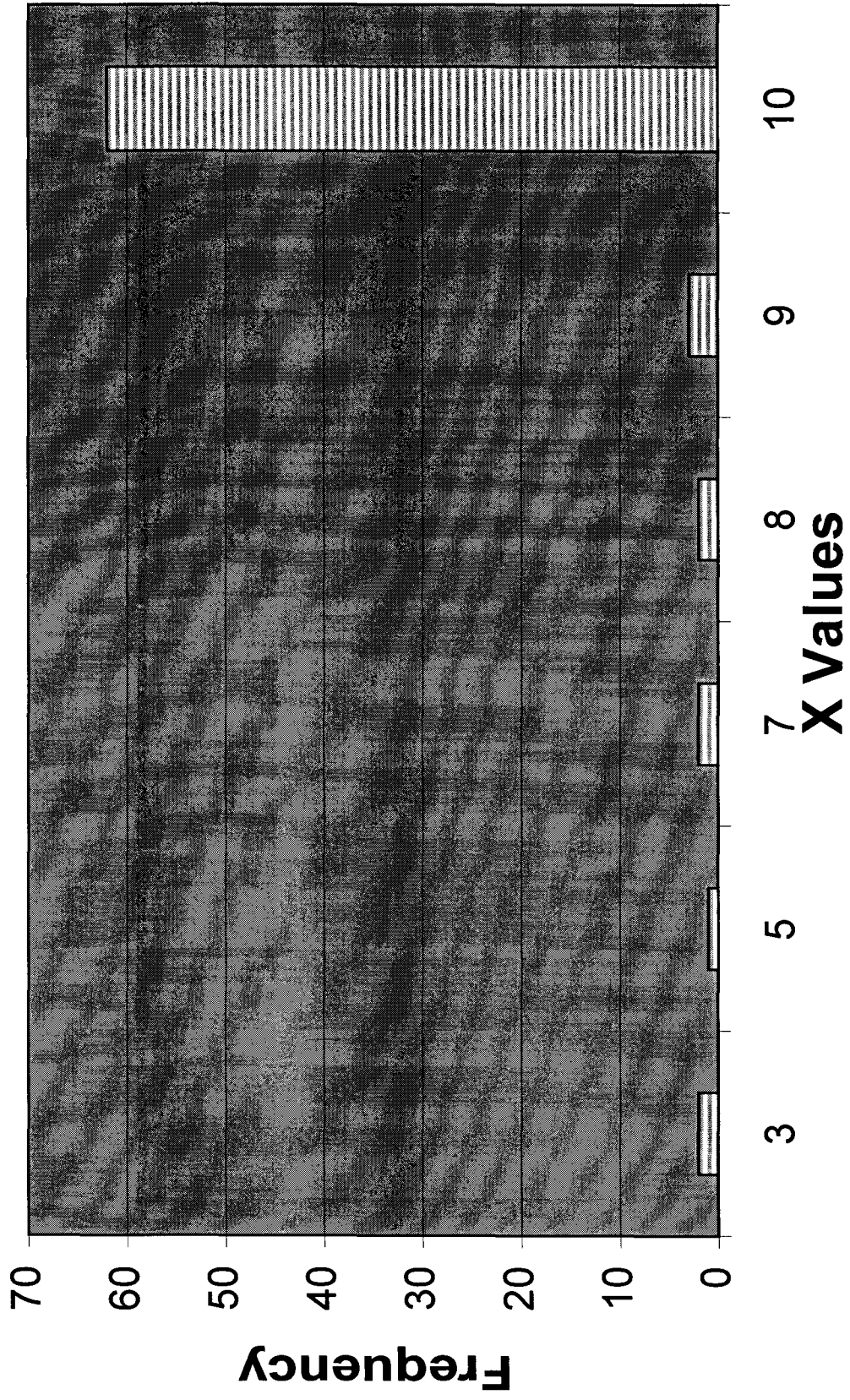


Fig. 4 Habituation X Values



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