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Effects of male attractiveness on prostaglandin E2 production levels

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Effects of male attractiveness on prostaglandin E_2 production levels

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Contents

Abstract

Sexual conflict or sexual antagonism can encourage co-evolution between males and females of a given species. In house crickets (Acheta domesticus), unattractive (smaller) and attractive (larger) males seem to use differential allocation of prostaglandin E_2 in their spermatophore to pursue different propagation strategies in influencing female oviposition. This study aims to determine the means by which attractive and less attractive males press their reproductive advantage by focusing on prostaglandin E_2. To test the hypothesis that the difference in prostaglandin E_2 concentration is essential for female reproductive manipulation, spermatophores were retrieved from 21 pairs of mated crickets. Using an ELISA assay, the prostaglandin content of each spermatophore was determined. In support of my hypothesis, the results show a negative relationship between male size and prostaglandin levels. Additionally, unattractive males on average spent a higher percent of their time chirping in comparison to attractive males. While both results did not show significance, their promising trends indicate that further research may confirm the negative relationship between male size and prostaglandin levels as well as chirping duration consistency.

Introduction

Mate choice is a perpetual source of intraspecific competition and sexual conflict. One of these selective pressures is an individual's ability to invest in reproductive costs. In essence, the goal of each sex is to maximize their reproductive success while minimizing their costs. For males, one of the first forms of competition to overcome is attracting a mate through calling. Male courtship calls are composed of a series of ticks and chirps with chirps being more complex (by containing pulses) and harder to produce than ticks (Adeola et al., 2022; Barquin et al., 2015; Tanis et al., 2018). By modulating their chirps and ticks (ie. syllable periods), males produce three distinct call types: aggression which allows them to compete with other males for good territory, advertisement which alerts females to their presence, and courtship which encourages mating after locating a female (Adeola et al., 2022; Gray, 1997; Nelson & Nolen, 1997; Stout et al., n.d.). Since in house crickets size and immunity are both heritable and positively correlated, females prefer calls with more pulses per chirp (a more complex song) and they differentially allocate their resources in response to hearing such a call, notably by laying more eggs after mating (Adeola et al., 2022; Gray, 1997; Head et al., 2006; Ryder & Siva-Jothy, 2001; Stoffer & Walker, 2012). Therefore, smaller males or males weakened by immunity or muscular causes will produce less complex songs than larger or stronger males and receive a smaller egg allocation from females (Adeola et al., 2022; Barquin et al., 2015).

This action leads to the second form of competition that males must overcome, as successful attraction and spermatophore delivery does not equate to a fair chance at inseminating a female's eggs. To compensate for the gap between male ability and female

regulated opportunity, smaller, less attractive males may try to influence or change females physiological or behavioral responses or boost the probability that their sperm will be utilized. Guarding behavior is one of these methods. Males guard females after mating which limits a female's ability to remate, minimizing competition (Stanley & Kim, 2011). If a female mates more than once, the male's spermatozoa mix within the female's spermatheca providing another opportunity for male competition (Murtaugh & Denlinger, 1985; Worthington et al., 2015).

Sperm competition within the spermatheca happens in many forms. The first is sperm dilution. As all sperm are stored together in the spermatheca, how much sperm a male has transferred in comparison to how much she is already storing impacts the male's odds of fertilizing her eggs. For females, newer sperm is preferred over saved sperm and better-quality sperm, derived from a larger male, is also preferred (Klaus Reinhardt, 2005; Sirot et al., 2015). Furthermore, larger males may be better able to handle the energy expenditure of producing young sperm, which further increases this preference imbalance. In conjunction with general deterioration as expected with time, sperm deterioration from seminal fluid proteins (SFPs) of competing sperm also affects the vitality of a male's sperm (Sirot et al., 2015). In Australian field crickets (Teleogryllus oceanicus) it was previously shown that male crickets differentially allocate their sperm based on the mating experience of their partnering female cricket presumably to offset energy expenditure in response to competition risk and intensity (Melissa L. Thomas, 2007).

In addition to affecting the sperm of other male's, SFPs also affect the female crickets. Research into SFPs has positioned them as sources for signaling cascade events within females from initiating physiological reproductive processes to manipulating post-

mating behavior across species (Destephano & Brady, 1977; Sirot et al., 2015). In female Mediterranean fruit flies (*Ceratitis capitata*), females exposed to SFPs change their olfactory preference from that of male pheromones to the smell of ripe guava, a host fruit, (Jang, 1995). In common fruit flies (*Drosophila melanogaster*) mated females show a preference for yeast, a critical protein source for egg production, a behavior not seen in virginal flies that have been deprived yeast for three days (Ribeiro & Dickson, 2010). In mice, seminal fluid has been shown to influence T cell tolerance to paternal antigens through activating Treg cell populations in the mated females via environmental and antigenic signaling (Robertson et al., 2009).

First discovered in human seminal fluid, prostaglandins have since been identified as a conserved hormone-like signaling compound derived from arachidonic acid that functions in reproduction, immunity, smooth muscle contraction, ion transport and in mucosal protection across species (Stanley & Kim, 2011). In beet armyworms (*Spodoptera exigua*), biosynthesis inhibition of prostaglandins led to the loss of oogenesis and the reintroduction of prostaglandins, resulting in the return of oogenesis and choriogenesis (Abdullah Al Baki & Kim, 2019). Similar results were also found in mice (Hayashi et al., 1988). In cows, luteinizing hormones initiate a cascade that induces prostaglandin secretions which acts as a protective shield for sperm against polymorphonuclear neutrophils, an innate immune response mediator (Marey et al., 2014).

In house crickets, a single mating is enough to induce oviposition for the duration of a female's lifespan (Murtaugh & Denlinger, 1985). Female crickets require about 55 min to completely transfer sperm from the spermatophore, a proteinaceous container that transfers sperm, SPFs and other fluids from the male to the female, it is usually removed

prematurely by wiping it off in as little as 3 minutes after attachment, which still provides enough material for the initial induction of oviposition (Klaus Reinhardt, 2005; Mautz & Sakaluk, 2008; Murtaugh & Denlinger, 1985). Studies have shown that prostaglandin E_2 (PGE2) is delivered to females in the spermatophore during mating and this hormone is what stimulates oviposition in female crickets (Destephano & Brady, 1977; Murtaugh & Denlinger, 1985).

Recent research by Wilson and Walker in house crickets (*Acheta domesticus)* has highlighted a potential form of sexual conflict between male and female crickets. Through investigating how female crickets' age affects their survival-reproduction cost trade off, potential male manipulation of female reproductive allocation was discovered but the mechanism of manipulation remains unclear (Wilson & Walker, 2019). They postulated that spermatophore contents or behavioral interactions could play a role in the seen effects (2019). This postulation, in conjunction with PGE2s regulatory role in oogenesis, positions PGE2 as a determining factor for the results seen by Wilson and Walker (2019). PGE2s role in innate immunity and sperm protection may also inadvertently downregulate a female's immune system, leading to the shorter lifespans seen by Wilson (Sirot et al., 2015; 2019). Based on these possibilities, it is of general interest to determine the potential effect of PGE2 in connection to female manipulation via male spermatophores. Here I will determine if PGE2s are differentially allocated in the sperm of "attractive" and "unattractive" crickets.

Materials and Methods

Experimental Animals

Commercially bred house crickets (*Acheta domesticus*) were acquired from Fluker's Cricket Farm (Port Allen, LA, USA). Their strain, the "American Mix", is a product of breeding between different US based farm strains. Two mixed sex groups were reared in large, opaque waste bins (32 gal) containing egg cartons for shelter and to increase rearing surface area until they reached their adult stage. The bins were checked daily, and all adult crickets were removed and housed individually in clear, plastic containers (739 mL). The colony and individual crickets were kept in the same environmentally controlled room (72ºF, shifted 12h:12h light:dark cycle) and were provisioned with water gels (Fluker's Cricket Quencher) in petri dishes and dry cat food (Purina) in their respective containers ad libitum throughout the study. Crickets were weighed during transfer to individual containers. No oviposition substrate was provided.

Mating Trials and Spermatophore Retrieval

Only virgin crickets were paired for experimental data to account for different sperm viability distribution in female mating status (no mating vs one vs multiple matings) found by Thomas (2007). Before the trial, the males were assigned attractive or unattractive based on their mass relative to the group average mass ($0.27\ \text{g} \pm 0.077$). The females were then randomly assigned to the attractive or unattractive group and paired with a male. During trials, the temperature of the environmentally controlled room was raised to between 76-81ºF. The males were placed in clear containers (36 × 23 × 17 cm) for a

minimum of five minutes to acclimate to the new environment, after which the females were then placed in the container at the farthest location from the males. During each trial, an instantaneous ethogram was completed[1]. Latency to initiate courtship was also recorded. When the spermatophore was transferred or visible, the crickets were placed on dry ice and the spermatophore was removed and placed into an Eppendorf tube. The spermatophores were stored at -80ºC. Of the 21 mating trials, 16 spermatophores were successfully collected.

ELISA Assay

Thawed spermatophores were homogenized using the using the Tissue Tearer Model 985370 in 150 µl of phosphate homogenization buffer (0.1 M, pH 7.4) to release their contents and then centrifuged at 8000 g for 30 seconds. The supernatant was then collected for the Cayman Chemical Prostaglandin E_2 ELISA assay (Item No. 514010) where each sample was run in duplicate. Assays were performed per the manufacture's instructions. The plate was read at 410 nm. Prior to the ELISA reading, a spill occurred where the displaced liquid was not able to be returned to the wells. Additionally, the retest was unable to reach sufficient maximum binding (B_0) levels for plate reading. Prostaglandin levels were determined using a standard curve.

Statistical Analysis

To examine whether males' size impacted premating behavior, a Mann-Whitney U Test was used to compare the chirping and latency to mount data (defined by Mautz as the time from when the male initiated courtship to when the female mounted the male). This test was used because the data did not follow a normal distribution, making a nonparametric test

appropriate. The effect of male weight on spermatophore PGE was analyzed using a linear mixed-effect model with male weight as a random effect.

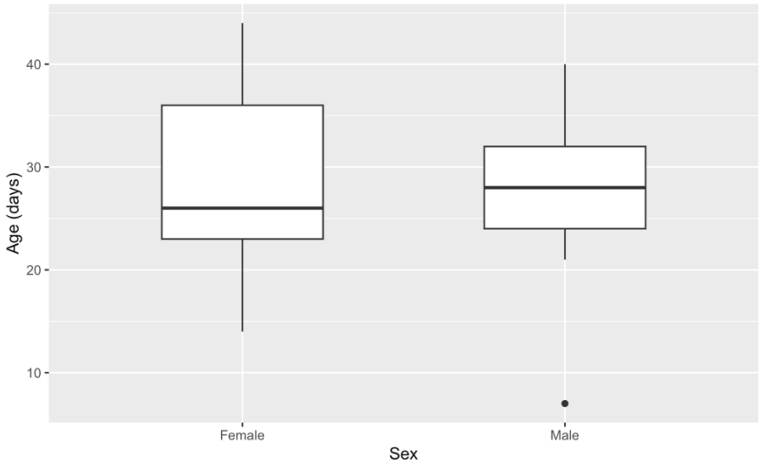
Results

Two preliminary t-tests were performed to confirm the assumptions of the experimental conditions before prostaglandin testing and analysis as a precautionary measure due to staggered trial timing. There was no significant difference found between the ages of males and females used in the study ($p = 0.8776$) (Figure 1-A). Conversely, there was a significant difference between the treatment group masses ($p = 0.0002834$) (Figure 1-B). Both results confirmed the experimental assumptions to be true.

The role of mating behavior, particularly chirping, was established as a determinant for female interest. These observations revealed that while attractive and unattractive males did differ in the number of times they called (Figure 2), the difference was not significant ($w = 50$, $p = 0.7999$) (Figure 3 -A) as is also true for latency to mount ($w = 10$, $p = 0.3917$) (Figure 3-B).

Finally, I investigated prostaglandin levels in the males' spermatophores. While there was no significant difference ($p = 0.0652$, $X^2 = 3.4$) (Table 1), there was a trend toward significance whereby the unattractive males have more prostaglandin in their spermatophore than attractive males. The random effect of male mass did not contribute to the results of the model.

A



B

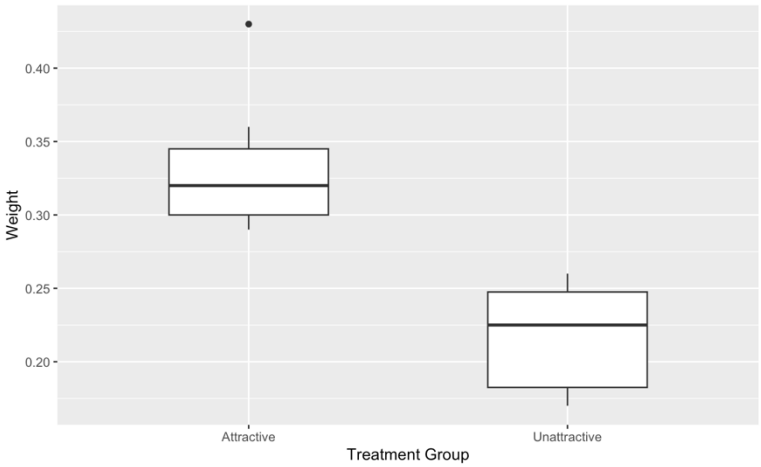


Figure 1 *Demographics of house crickets t-test results. (A) Age of crickets at time of mating trial (n = 21, p = 0.8776, df = 42.84). (B) The weight of the males in each group on the day of transfer to isolated housing (p = 0.0002834, df = 11.35). Box plots are mean ± SD.*

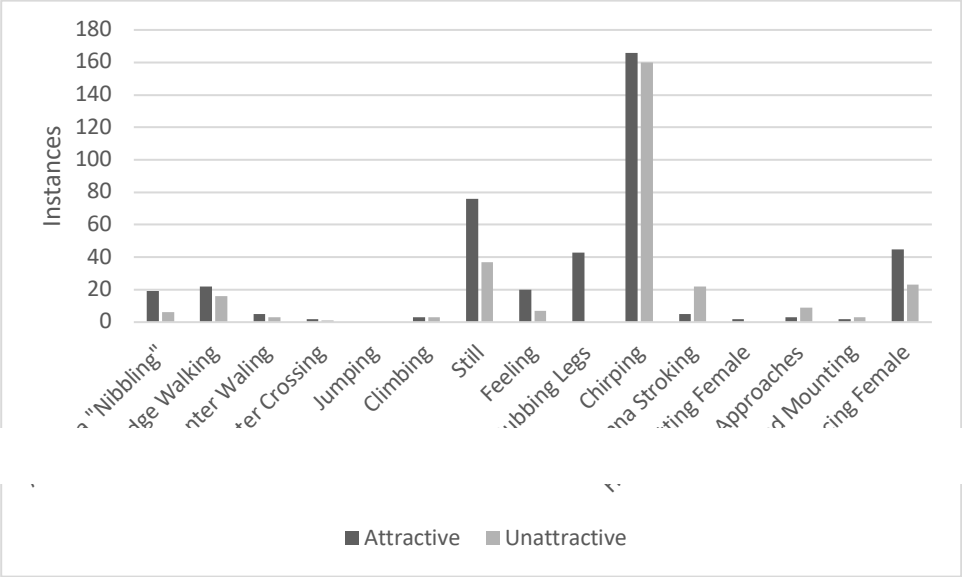


Figure 2 *Mating trial ethogram observations. In the attractive treatment, 5 out of the 9 males' most recorded behavior was chirping. In the unattractive group it is 9 out of 12. As an aggregate, 40% of total attractive ethogram minutes was spent chirping in comparison to 55% by the unattractive group.*

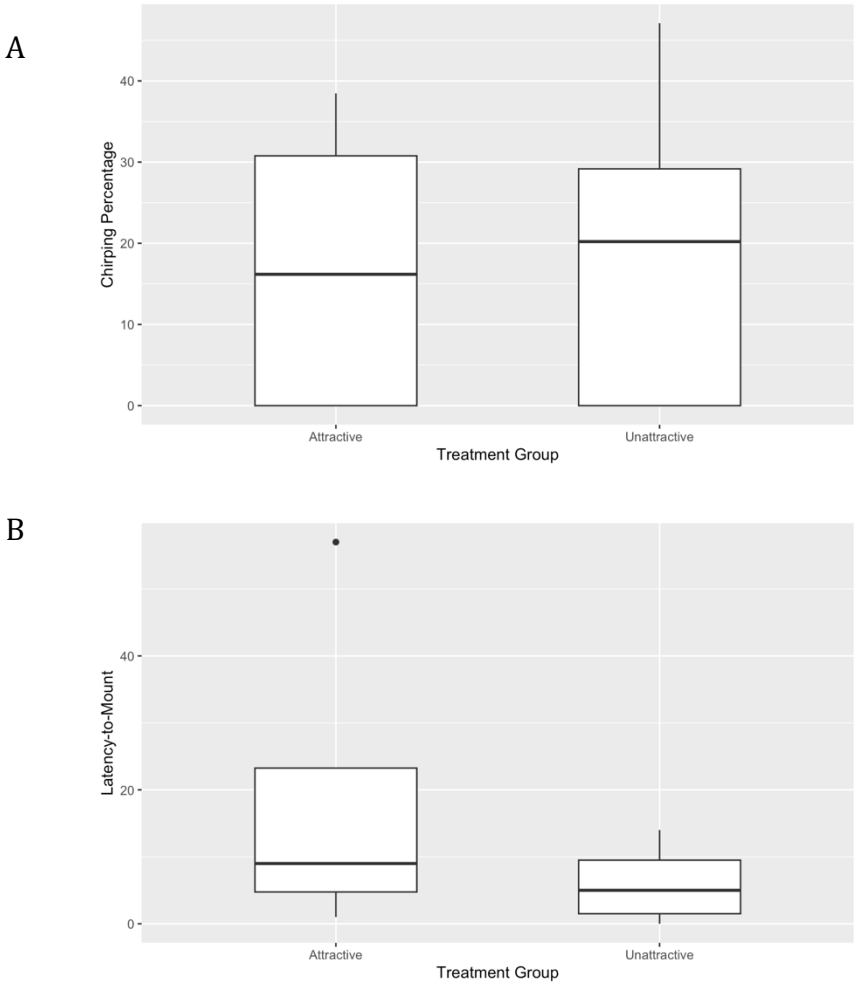


Figure 3 *Ethogram Man-Whitney U test results comparing two behaviors. (A) percentage of time spent chirping (w = 50, p = 0.7999, means = 17.64, 20.1). (B) latency-to-mount (w = 10, p= 0.6857, means = 19,6), between the two treatments. Box plots are mean ± SD.*

Table 1

	Wald Chi^2	p-value		
Prostaglandin Content	3.4	0.0652		
	Average		**95% Conf. Low**	**95% Conf. High**
Unttractive	3.67		3	4.36
Attractive	2.77		2.09	3.45
	W	**p-value**		
Latency to Mount	10	0.6857		
Chirping Percentage	50	0.7999		

Wald Chi Squared test results to compare prostaglandin spermatophore content to male weight. The results were calculated by taking the prostaglandin content found through the ELISA assay and comparing them with the weight of the males that contributed the sample (n=21).

Discussion

Male manipulation of female house cricket oviposition is an accepted concept yet the mechanism by which it takes places remains to be known (Wilson & Walker, 2019). Here I investigated the potential for differential allocation of prostaglandin content in the spermatophores of males of differing sizes to build a case for this to be an important component of male manipulation. I also explored the potential for behavioral variations between large and small males.

Male secondary sexual traits and behaviors, particularly calling in crickets, are understood to influence their ability to attract mates (Gray, 1997; Ohkubo et al., 2018; Ting et al., 2017). Therefore, I assumed that in conjunction with different prostaglandin levels, the males would exhibit different degrees of mating behavior. Instead, I found that the differences in mating behavior were not significant. Future research may decide to record the mating trials so that software can more precisely calculate chirping behavior between the two groups.

Using an ELISA assay, I showed that there is a trend of unattractive males allocating more prostaglandin to their spermatophore than their attractive counterparts. The inability to be more conclusive with my results may be a result of the spillage that took place during the ELISA test and the consequent under development of the retrial. Future trials might also consider a larger sample size to increase points of reference for comparison.

These results are promising and are in line with my reasoning and hypothesis that the difference in prostaglandin E_2 concentration is essential for the female reproductive

manipulation we see from large and small males. This novel means of manipulation would be particularly beneficial to smaller males as they are at a disadvantage when competing with larger males. This may be an indication of divergently beneficial reproductive dynamics whereby large male experience female favored manipulation (through female mate choice and preferential allocation) while small males experience this male favored manipulation (Head et al., 2006; Ting et al., 2017). This female manipulation could also hint towards a stabilizing effect in crickets as seen in other species. In the common fly (*Drosophila melanogaster*), medium-size males were found to be more fecund than smaller or larger males, producing a stabilizing effect (Lefranc & Bundgaard, 2004). As a medium-size treatment was not utilized in this study, future research could investigate the prostaglandin E_2 content for similar results in medium-size crickets.

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1. Ethogram Description

Category	Sub category	Behaviors	Abbreviations	Definitions
Male	Non-Mating	Antenna "Nibbling"	AN	a grooming behavior where the crickets antenna is placed in their mouth + other grooming behavior such as scrathcing
		Edge Walking	EW	walking along the edge of the mating container
		Center Walking	CW	walking atleast 2 inches from the edge
		Center Crossing	CC	walking through the center of the mating container
		Jumping	J	jumping
		Climbing	C	attempts to climb the edge of the mating container
		Still	NM	no movement
		Feeling	F	appears to be investigating the container
	Mating	Rubbing Legs	RL	rubbing hind legs
		Chirping	S	striking wings to produce sound
		Antenna Stroking	AS	stroking the female with their antenna
		Headbutting female	HF	headbutting female
		Approaches	AF	approaches female cricket
		Forced Mounting	MFM	the male positions himself underneath the female and attempts to lift her on top of him (as opposed to her lifting herself onto him)
		Facing Female	FF	facing the female cricket with awaremess (ie. knows she's there not just looking in her direction coincidentally)
Female	Non-Mating	Antenna "Nibbling"	AN	a grooming behavior where the crickets antenna is placed in their mouth
		Edge Walking	EW	walking along the edge of the mating container
		Center Walking	CW	walking atleast 2 inches from the edge
		Center Crossing	CC	walking through the center of the mating container
		Jumping	J	jumping
		Climbing	C	attempts to climb the edge of the mating container
		Still	NM	no movement
		Still & Facing Away	SA	no movement and avoiding the direction of the male cricket
		Feeling	F	appears to be investigating the container
	Mating	Facing Male	FM	facing the male cricket with awaremess
		Mounts	M	mounts male cricket
		Approaches	AM	approaches male cricket