Remating Behavior and Polyandry of Female Ring-legged Earwigs, *Euborellia annulipes*

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Abstract

Remating preferences of female ring-legged earwigs, *Euborellia annulipes*, were investigated by observing the number and duration of rematings when females were paired with the same male and a novel male. We hypothesized that females are more likely to remate with novel males than with males they had mated with initially. Polyandry, female mating with multiple males, has benefits and costs, but we hypothesized that the benefits outweigh the costs. Potential benefits of polyandry include genetic diversity and maternal fecundity. The costs of polyandry are the input of time and energy, risk of sexually transmitted disease, and increased predation. While observing remating preferences, we also compared the duration of the first and second matings. We hypothesized that the second mating would be shorter due to female sperm storage organs approaching maximum capacity and exhaustion due to the time and energy put into the first mating. Our experimental design included a first mating, then a subsequent mating in which half of the females were paired with the same male from the first mating while the other half were paired to a new male who had already mated with other females. The duration of mating was significantly shorter for the second mating, and females showed a slight preference for polyandry rather than monogamy. From our experiments, we concluded that earwigs favor shorter subsequent matings and the benefits of polyandry.

Introduction

Mating is essential for the production of offspring. Although an organism will not perish without mating, unsuccessful reproductive efforts will result in none of the organism's genes being passed to another generation. When mating, an organism should choose the most genetically fit partner to ensure a better rate of survival in their offspring since they often have to adapt to environmental changes (Singh et al. 2002). Females, being the choosier sex due to their limited supply of eggs, pursue male partners with copious sperm amounts, genetic advantages for the offspring, and advantages for themselves such as nutrition from seminal fluid or food offerings from their partner (Thornhill and Alcock 1983).

In order to gain the maximum benefits from mating, females have two major methods of mating. For individuals who can receive the necessary mating requirements from one male, the female will mate with her partner once or possibly multiple times to obtain the highest amount of sperm. Multiple matings with the same male are known as repeated mating. On the other hand, females may mate with multiple partners, which is known as polyandry (Jimenez-Perez et al. 2003).

Polyandry is known to occur in insects, fish, reptiles, birds, and mammals. The positive effects of polyandry can be better fertility due to the diversity in the male sperm, sufficient sperm to fill the female's sperm storage organ, genetic diversity, and having the ability to substitute genetically inferior with genetically superior sperm (Thornhill and Alcock 1983; Kamimura 2003). The drawbacks include increased susceptibility to sexually transmitted disease, the inconvenience of the time and energy put into mating, and increased risk predation while mating (Rolland et al. 2003).

Polyandry is often controlled by the capacity of the sperm storage organs, body size of the female, sperm competition, and the efficiency of the sperm transferred (Singh et al. 2002). If the female is larger, her sperm storage organ should also be larger, allowing the female to store more sperm (Jimenez-Perez et al. 2003). A unique advantage for males in at least some species of earwigs is their ability to remove the seminal fluid of previous mates and replace it with their own. The male's sexual organ, the virgae, can enter the female's sexual organ, the spermatheca, and remove or compress another mate's sperm. The sperm competition allows more room for additional sperm from the new mate (Kamimura 2000). The earwig *Diplatys flavicollis* had two virgae, one for sperm removal and one for sperm placement (Kamimura 2004).

In our study, we examined remating behavior and polyandry in the ring-legged earwig, *Euborellia annulipes*. The purpose of our experiments was to determine if *E. annulipes* females would prefer to be monogamous or polyandrous in their mating. We determined the preference by the occurrence and duration of remating with familiar or novel males. We hypothesized that females would prefer to mate with novel males rather than previously encountered mates, and that their duration of mating would be longer with novel mates. Another purpose of our study was to examine the duration of

successive matings of the earwig. We hypothesized that since mating requires time and energy, subsequent mating would be shorter in duration, both because of exhaustion and female sperm storage capacity; although there may be sperm displacement, only so much sperm can enter the sperm storage organs.

Methods

Remating Preference Experiment

Virgin female ring-legged earwigs and males who had been isolated for at least a week were used in all experiments. A male and virgin female earwig were obtained and introduced to one another inside of a mating arena, a 90-mm petri dish lined with moist filter paper. A total of 24 mating pairs were given up to 15 minutes to mate, but if mating did not occur, the female was not used for the second mating. Once mating was complete, the pair of earwigs was separated by removing the male from the arena. The duration of each mating was recorded. Once secluded from one another, the *E. annulipes* were given a 15-minute resting period.

After the hiatus, a male would be reintroduced to the arena. Of the 20 females who mated initially, 10 were reunited with their original male, while 10 were introduced to an unfamiliar male who had mated successful with a different female during the first round of mating. Once again, the duration of the mating was recorded along with the number of earwigs that mated.

Data Analysis

The duration of each mating and occurrence of a second mating were graphed using Microsoft Excel. A statistical t-test was performed using Microsoft Excel to determine if our results were in accord with our hypotheses.

Results

Duration of mating was affected by the number of times the earwigs had previously mated (Fig. 1). The first mating was significantly longer (average of 320 seconds) than the subsequent mating (average of 165 seconds: Fig. 1). The large difference in mating duration between the first and second matings was statistically significant (P= .00088).

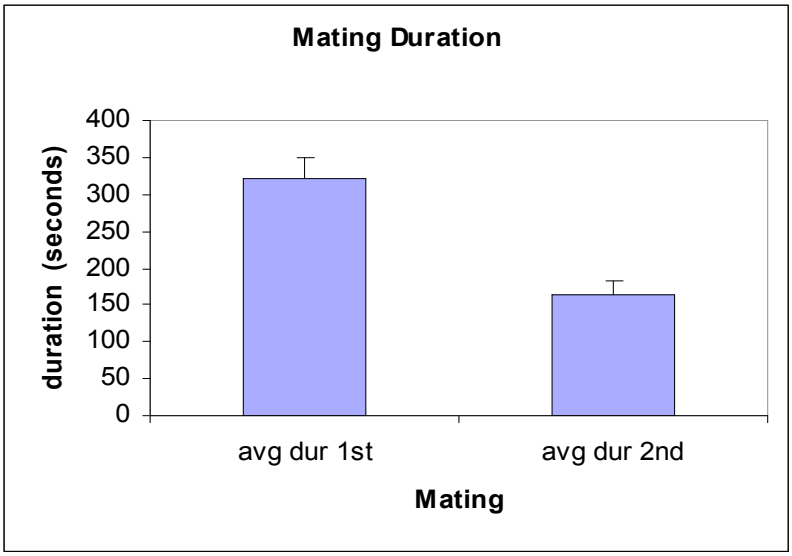

Figure 1: Duration of mating: the average duration of copulation for the first mating compared to the average duration of copulation for the second mating. Error bars represent the standard error.

When given the opportunity to mate with the same male from the first mating, only 40% of females mated with the male during the second mating (Fig. 2). When novel males were presented to the female for the second mating, 60% of the females mated (Fig. 2). Not only was there a higher occurrence of mating with novel males, the duration of mating with the novel male increased as well (Fig. 3). On average, mating with a novel male (172 seconds) was longer than mating with the same male (151 seconds; Fig. 3).

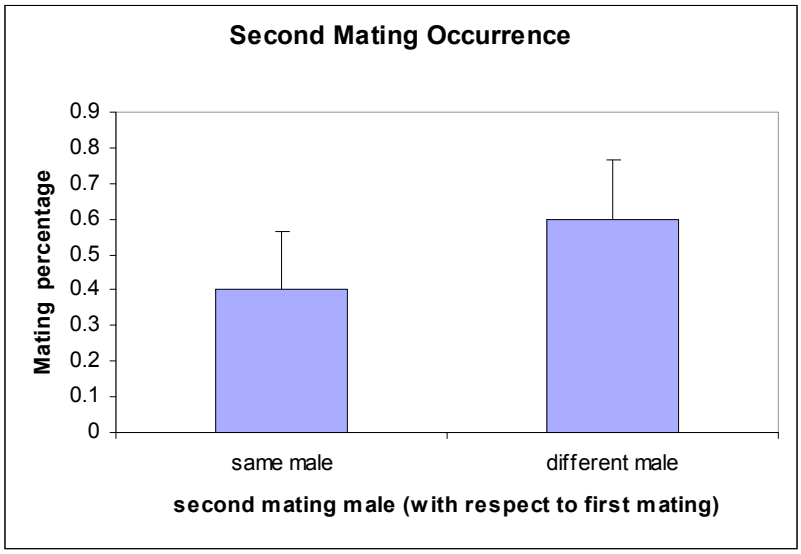

Figure 2: Second mating occurrence: percentage of females who preferred a novel mate or a previous mate. Error bars represent the standard error.

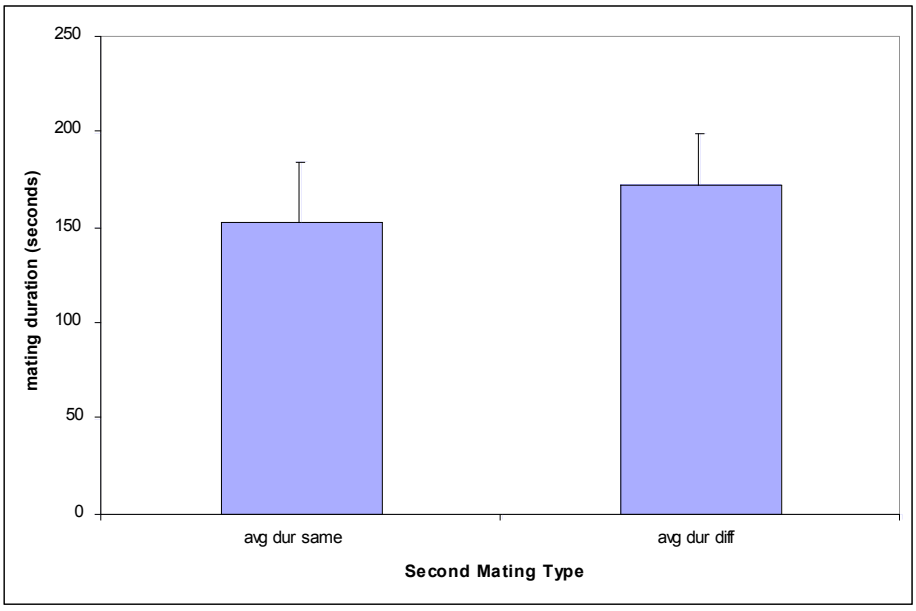

Figure 3: Duration of second mating: comparison of the average duration of mating for a second mating between the same male from the first mating and a novel mate.

Discussion

Polyandrous mating has been observed in earwigs (Kamimura 2003). Through comparison of the benefits and costs of polyandry, we hypothesized that the ring-legged earwig would prefer to mate with multiple partners. We thought that earwigs would favor this type of mating because of the resulting genetic diversity of offspring. Genetic diversity is advantageous for organisms because offspring will have a better chance of survival due to their ability to successfully adapt to environmental changes (Thornhill and Alcock 1983). Since earwigs are found throughout the world, we thought that they would need genetic diversity, which comes from polyandry, in order to survive.

To determine if earwigs preferred to be monogamous or polyandrous, we observed the mating occurrence and duration of second mating. Our results suggest that females may prefer to mate with a novel male rather than a previously encountered male (Fig. 2, 3). Not only was there a higher occurrence of mating, the mating durations were also longer (Fig. 2, 3). In other insects, it is known that the longer the duration of mating, the more sperm is transferred (Singh and Singh 2000). Similar results were found in experiments with hide beetles; females preferred to mate with novel males, and the matings with the novel mates were longer than matings with previously encountered mates (Archer and Elgar 1999). This study suggested that females mate with multiple partners because of increased genetic diversity and fitness benefits (Archer and Elgar 1999). Another study also observed better fitness due to polyandry. Females involved in polyandrous mating laid more clutches than monogamous earwigs (Kamimura 2003). Our results, although consistent with these studies, were not statistically significant most likely due to a small sample size.

While observing polyandry, we also compared the duration of the first and second matings. The mating duration of the second mating was considerably shorter than the first mating (Fig.1). The results were statistically significant and supported our hypothesis (Fig. 1). Similar observations were found in a study of *Drosophila* (Singh and Singh 2000). For *Drosophila*, the shorter mating duration was attributed to the male having less sperm since most was lost in the first mating (Singh and Singh 2000). The same thing could have occurred in our experiment. Since males of some species of earwigs have two virgae,

which suppress or remove other male's sperm from the female's sperm storage organ, it is likely that the shorter duration was not due to a full sperm storage organ (Kamimura 2000). Another possibility for the reduced time of mating could be due to exhaustion. The earwigs only had a fifteen-minute hiatus, which may not have been enough time for them to recuperate.

To obtain more research and explore reasoning behind our results there are follow up experiments we would like to perform. An increase of sample size would provide a stronger test of our hypotheses. Another interesting experiment would be to examine how females recognize and discriminate against males they have previously mated with, whether it be through pheromones or other identification methods. One last experiment could be to compare the offspring of a female who mated with one mate versus a female who mated with multiple partners to determine if there is an increase in fitness for polyandrous earwigs.

The results of our experiment suggest that female ring-legged earwigs have a preference for polyandry, but second matings are significantly shorter than initial matings. Our results further suggest that the benefits of multiple partners may outweigh the potential costs. Although some of our results were not statistically significant, each graph showed trends that supported our hypotheses. In summary, female earwigs had shorter subsequent matings and appeared to prefer polyandry over monogamy, which may result in increased genetic diversity of their offspring.

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