Effect of Mating and Presence of Males on Reproductive Success and Lifespan of the Tribolium castaneum Female Beetles

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Article information

Key words
lifespan, polyandry, reproduction, Tribolium.

Received 20 November 2022, Accepted 01 February 2023, Available online 10 February 2023

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Abstract

Abstract

Tribolium castaneum is a polyandrous species, in which females mate multiply with the same male or different males. The frequency of mating in insects, including this species, is common and often beneficial to female reproductive output and longevity but also often costly behaviour. In this study, we investigated the effect of multiple mating on reproduction and longevity of this species by different mating protocols from using virgin females, once mated females, monogamy (each female kept with one male) to polyandry (each female kept with 5 males). This study found no evidence for the effects of multiple mating on the fecundity and egg hatching success of female T. castaneum at least over the first six days of oviposition. The number of mates to which females were exposed significantly affected female lifespan. Females kept with 5 males lived shorter than the other groups, while virgin females lived for longer than the groups. Our results suggest that the presence of many males with a female of T. castaneum is costly to the female since females which were kept with 5 males died earlier and produced less offspring than females were kept with one male each.

I. INTRODUCTION

Polyandry is when a female mates with two or more different males (Boulton and Shuker, 2013). Repeated mating could be costly to females that mate with more than one male. In addition to wasting time and energy in looking for mating and mating itself, and the risk of sexually transmitted diseases, there may be increased predation risks while searching for and mating with males (Jennions and Petrie, 2000).

Flour beetles of the genus Tribolium have been utilized as informative study system for over a century and contributed to major advances across many fields (Pointer et.al., 2021). Tribolium castaneum mate multiply and readily, this might be because there are no costs of mating multiply, but it seems unlikely that costs could be zero, if only because matings interrupt other activities. Possibly females mate multiply due to male coercion as mating resistance may have costs that are greater than simply submitting to mating. Alternatively, females may gain benefits from matings that are more than the costs

In addition to sperm, seminal fluid contains many secretory products from the male accessory gland (Simmons, 2001), and it is clear that female insects often use male seminal products to increase their reproductive success (Eberhard, 1996). The main function of male accessory glands of insects is the production of the spermatophore for the transfer of sperm from male to female (Chen, 1984). However, accessory glands also produce a diverse range of products that are transferred to females, where they may have beneficial or manipulative effects. The ejaculate of the fruit fly Drosophila melanogaster is estimated to contain at least 85 different proteins (Chen, 1988). In Drosophila melanogaster, males with larger accessory glands re-mate more than those with smaller accessory gland (Bangham et. al., 2002). The two major responses to male accessory secretions in the female are elevation of oviposition rate and repression of female sexual receptivity (Chen, 1984). In butterflies, Bissoondath and Wiklund (1995) studied the effect of polyandry on ejaculate protein content in 11 species of butterfly, they found the first spermatophore produced by polyandrous species contained significantly more protein than those of relatively monandrous species.

There is evidence that male accessory gland secretions enter the hemolymph of the females following mating and affect the nervous and/or endocrine system (Chen, 1984). Roony and Lewis (1999) studied the function of spermatophore in two lampyrid beetles (Ellychnia corrusa and Photinus ignitus). They found that 62% of spermatophre-derived proteins were incorporated by females of P. ignitus into maturing oocytes within 2 days. In contrast in E. corrusa 46% appeared in the female fat body 6 days after mating, supporting the idea that short-lived, non feeding females allocated a greater proportion of male-derived nutrients to reproduction (P. ignitus) while longer lived feeding females store a greater proportion in their somatic reserves (E. corrusa). Hayashi and Kamimura (2002) examined the possibility of incorporation of male-derived substrates into female reproduction of the leafhopper Bothrogonia ferruginea (Cicadellidae) suggesting that females could incorporate proteinaceous material derived from male spermatophores and/or sperm-binding material into their oocytes.

Osikowski and Rafinski (2001) found that multiply inseminated females of Montandon's newt, Triturus montandoni, laid more eggs and had a lower percentage of non-developing eggs than singly mated females. They suggested that multiply mated females increased their reproductive success by replenishing sperm resources in their spermatheca. Wagner et. al., (2001) examined the effect of male ejaculates on female lifespan and fecundity of field cricket Gryllus lineaticeps. They found that females mated either repeatedly (with one male) or multiply (with more than one male) lived longer than singly mated females and multiply mated females laid more eggs than singly mated ones suggesting that these benefits may have resulted from beneficial seminal products that males transfer to females during mating. In Drosophila arizonae, polyandry caused females to produce significantly more offspring for a longer period of their lives than monogamous females mated at similar rate and polyandrous females lived significantly longer than both virgin and serially monogamous females (Croshaw and Gomez, 2018). Li et. al. (2021) concluded that mating can shorten the longevity of Coccophagus japonicus females and can significantly improve the number of eggs laid by females. Waker and Allen (2010) found that mating significantly increased the longevity of females of Mnesampela private moth, suggesting that females acquire essential resources from male ejaculate while multiple mated females showed a decreasing rather than increasing reproductive output.

In some other polyandrous species, females may gain indirect benefits (genetic benefits) for their offspring. In their study, Tregenza and Wedell (1998) examined the effects of multiple mating on the field cricket Gryllus bimaculatus by allocating females identical numbers of matings but different numbers of mates. They found that the hatching success of eggs increased significantly with the number of mates. In a similar experiment, Konior et. al., (2001) found that polyandry increased the offspring fecundity of bulb mites, Rhizoglyphus robini. They studied the effect of multiple male mating on progeny fitness (longevity and reproduction), they found that daughters of females mated to six males had significantly higher fecundity than daughters of females mated to one male whereas the longevity of both were unaffected. Lewis, et. al. (2020) found that extinction rates were significantly lower in populations of the red flour beetles, T. castaneum, founded by females given polyandrous opportunities to mate with two males compared to populations founded by monogamous females. Recently, Vasudeva, et. al. (2021) found that polyandry protected females of T. castaneum against reduced male fertility.

Matings are sometimes very costly to females; by keeping females of Drosophila melanogaster in two groups of high and low mating rate, Fowler and Partridge (1989) showed that mating significantly reduced the lifespan of female D. melanogaster. Chapman et. al. (1995) demonstrated that exposure of female Drosophila melanogaster to seminal fluid products derived from male accessory glands is costly as these products reduced female lifespan. Castrezana et. al. (2017) found that a single copulation enhanced female survival compared to survival of lifelong virgins, and multiple copulations enhanced the number of offspring for both monogamous and polyandrous females in Drosophila melanogaster. Females of spider mite Tetranychus urticae paid a cost of mating as multiply mated females laid fewer eggs than once mated females (Rodrigues, et. al. 2020).

There could be other non-mating costs of exposure to males rather than seminal fluid. This was reported by Partridge and Fowler (1990) who investigated the effects of exposure to non-mating males on females of Drosophila melanogaster in the absence of female remating. Their results showed that females exposed to 2 males who were rendered incapable of mating, survived less well than females exposed to no males suggesting that at least part of the reason for this non-mating cost may be due to various non-sexual activities such as competition, disturbance or contamination of the food medium.

In Tribolium castaneum, Lewis and Austad (1994) examined the effects of multiple mating on female progeny production and found that total adult progeny produced was significantly higher in the multiple mating treatments. Nilsson et. al. (2002) showed that female T. castaneum exposed to a lifetime of high mating frequency had a shorter lifespan than females with low mating frequency and female fecundity was higher at high mating frequency compared to low mating frequency. Pai and Yan (2003) did not detect an increase in fecundity or adult offspring production and survivorship of females of T. castaneum that mated with multiple mates for 24hr, suggesting that there was no effect, if any, of nutrient or toxins in the ejaculate.

The aims of this study were to examine the effects of multiple mating on fecundity and egg hatching success in the red flour beetle Tribolium castaneum, which are highly promiscuous. We also aimed to examine costs of polyandry affecting female lifespan and offspring production by comparing multiply mated females (with one male or more than one male) with virgin or singly mated females.

Methods:

In the following experiments, the wild type Ga1 strain of the red flour beetle (Tribolium castaneum) was used. Pupae were sexed and kept individually to ensure the virginity of both males and females. Pupae were kept individually with excess food (a mixture of 95% flour and 5% yeast) in an incubator at 30°C and approximately 67% Rh. The eclosion dates of each pupa were recorded. All experimental females were between 7 to 13 days post-eclosion.

1- Multiple mating and egg hatching success:

In this experiment, we followed a procedure based on that used by Lewis and Austad (1994). Females were divided into three groups; in the first group; a virgin female and a virgin male were kept together in a pot for 24hr to allow them to mate repeatedly (avoiding the risk of sperm transfer failure that accompanies single mating). In the second group; a virgin female was kept together with two virgin males in a pot for 24hr. In the third group; a virgin female was kept with four virgin males for 24hr.

After the 24hr mating period, each female was removed to a 100ml pot containing 30ml of a mixture of flour and yeast to lay eggs for 72hr. After the oviposition period the female was removed and these pots were kept in a dark incubator at 30oC and about 67% Rh for 45 days, after which the number of adult beetles were counted from each female in each group.

After the first 72hr oviposition period, the females were transferred to another 100ml pot containing very fine flour, to allow us to collect eggs easily, for another period of 72hr.

Eggs collected from each female were counted and transferred to a 100ml pot containing 30ml of food (a mixture of flour and yeast). After 45 days the number of adult beetles emerging from these eggs were counted and the hatching success for each female in each group was calculated.

2- Effect of multiple mating on female lifespan and offspring production:

This was divided into 4 groups:

Group (1): in which virgin females (n=20) were kept individually in a 100ml pot containing about 30ml flour. Beetles were checked weekly where the dead beetles were removed and the date of death was recorded for each one. Every 3 weeks the female was transferred to a new pot with fresh flour.

Group (2): this group comprised virgin females (n=20) which were allocated a single mating each with a virgin male, and then treated the same as the females in group 1. 5 females did not produce offspring, (presumably because of sperm transfer failure in the singe mating they had received and were excluded from the analysis).

Group (3): each virgin female was kept with a virgin male in a 100ml pot containing about 45ml flour (n=20), and otherwise treated as in group 1, with both the male and female being transferred to a new pot of flour every 3 weeks.

Group (4): in the last group, each virgin female was kept with 5 virgin males in a 100ml pot containing about 50ml flour (n=20), and moved every 3 weeks as in previous groups.

The number of offspring produced by each female in these groups was recorded for the period of 18 weeks. The lifespan of each female was recorded

6.3 Results:

1- Multiple mating and egg hatching success:

Data were normally distributed, thus parametric tests were used in the analysis. There was no significant difference between the three groups (fig.1) in their offspring production for the first 3 days after the mating period of 24hr (F2,68=0.87, p=0.42).

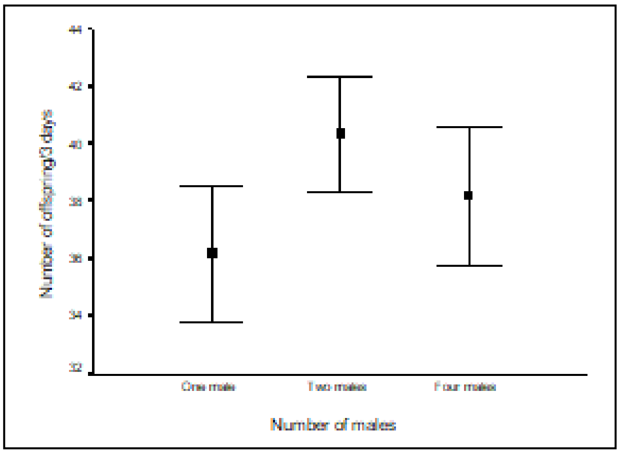

Figure (1): Mean number of offspring (± standard error) produced by 3 groups of virgin females for 3 days after 24hr of mating period.

Eggs collected for the second 3 day period of offspring production from the three groups (fig.2) also failed to show a significant difference in their hatching success (One-way ANOVA: F2,65=0.76, p=0.47).

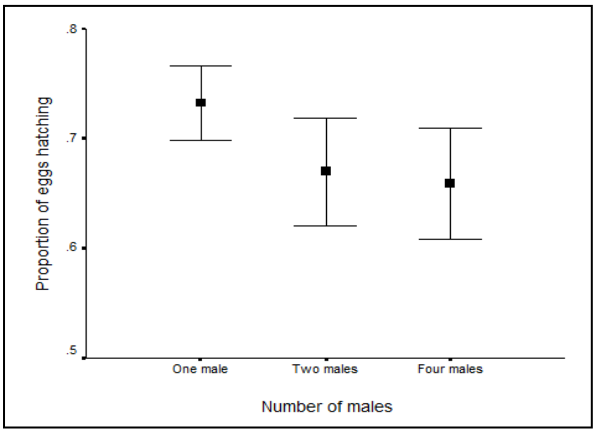

Figure (2): The proportion of the eggs hatching from the three groups of females for three days (Means ± standard error).

2- Effects of multiple mating and presence of males on female lifespan and offspring production:

a) Effect of polyandry on female lifespan:

Number of mates significantly affected female lifespan (Kruskal-Wallis test was used as the data were not normally distributed: □2=8.6, d.f.=2, p=0.01). From figure (3) group 1 - virgin females, lived longer than any other group of singly or multiply mated females, while the group of females kept with 5 males lived for a shorter period of time than any other group (Median=354.5 days for the virgin females and Median=181.5 days for the females kept with 5 males).

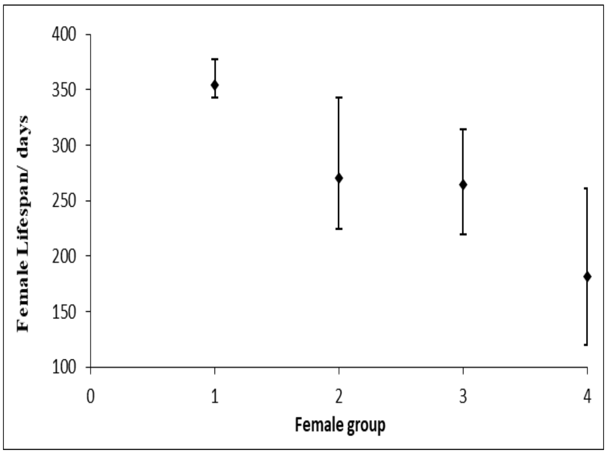

Figure (3): The lifespan (median ±quartiles) of the females of four groups, virgin females (1), singly mated female (2), females kept with one male (3) and females kept with five males (4).

b) Offspring production:

There was a significant difference between the three groups of females (singly mated, females with one male and females with 5 males groups) in the period of offspring production (Kruskal-Wallis test: □2=34.1, d.f.=2, p<0.001). Figure (4) shows that singly mated females produced progeny for (Median=9) weeks. However, the maximum period of offspring production was by the second group in which the females were kept with only one male (Median=30 weeks), while the third group of females (each with 5 males) died earlier than the other groups and laid eggs for (Median=22.5) weeks.

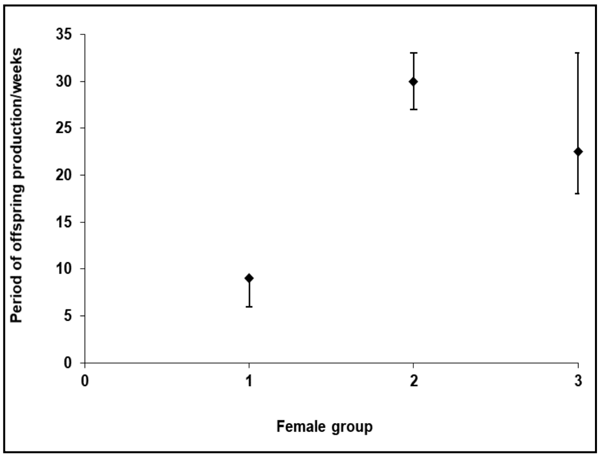

Figure (4):The period of offspring production (Median±Quartiles)of the three groups of females, singly mated females (1), females with one male (2) and females with 5 males

The mean number of offspring produced by the three groups of females over the period of 18 weeks was significantly different between the 3 groups (One-way ANOVA: F2,53=11.1, p<0.001). Females that were kept with only one male produced the maximum average of progeny (Mean=1015±59 S.E.). Although singly mated females produced the minimum average of progeny, there was no significant difference between this group and the group in which females were kept each with 5 males (t-test: t=1.8, d.f.=33, p=0.083), see figure (5). Mean offspring produced by singly mated females was (505.2±84) and was (716±81) for the females that were kept with 5 males. The females kept with 5 males produced the minimum offspring average almost every week for the 18 weeks (Figure,6).

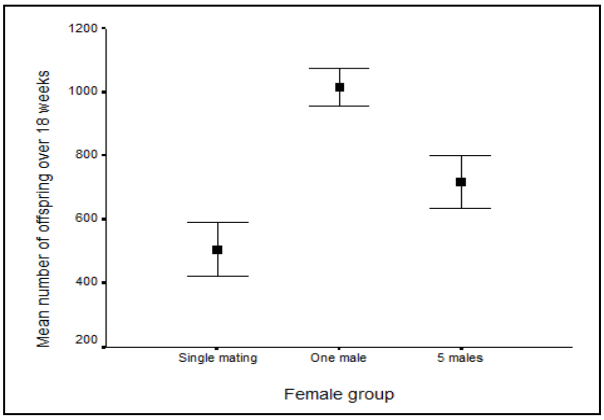

Figure (5): The mean number of offspring (± standard error) produced by the three groups of females mated to 3 different number of males over the period of 18 weeks

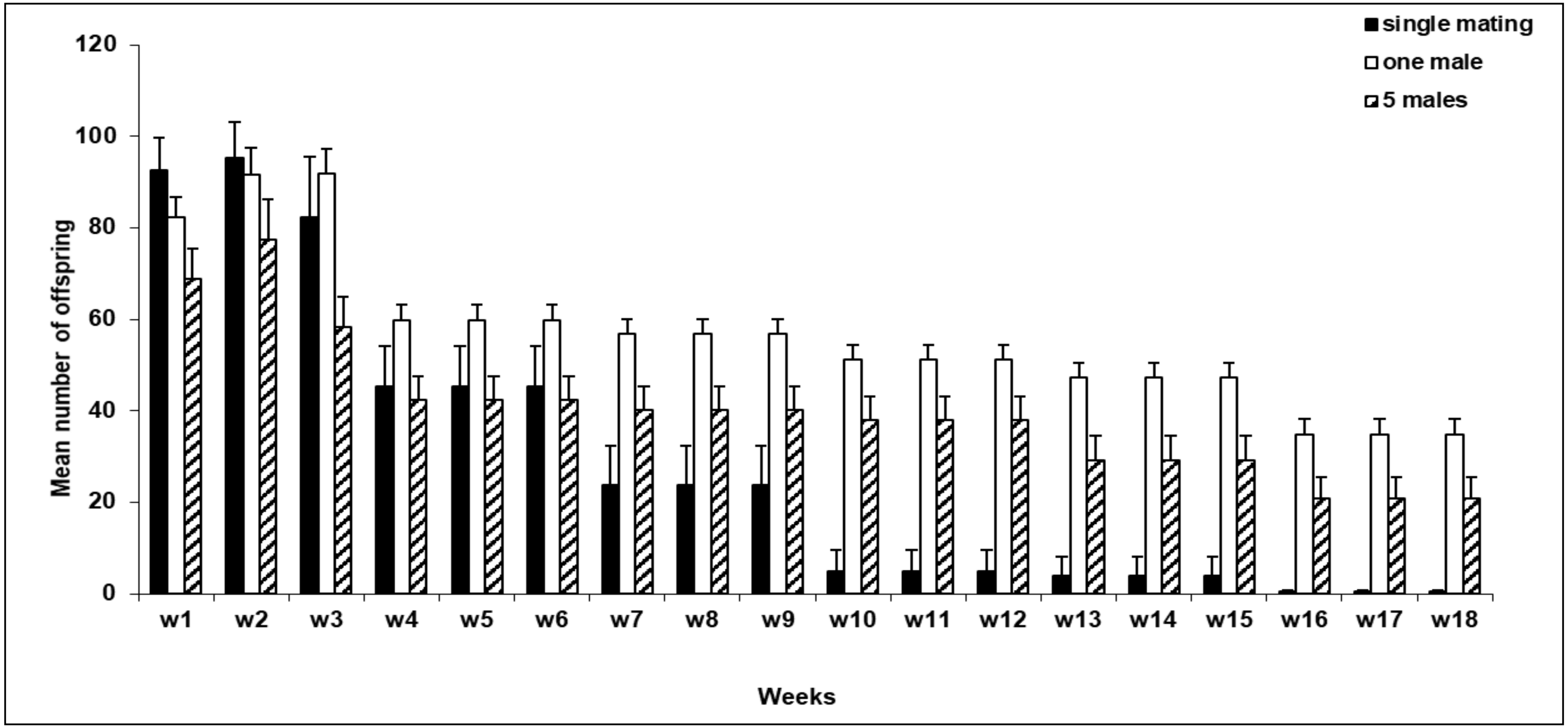

Figure (6): Mean number of offspring (± S.E.) emerging from flour provided to females in the three treatments each week for 18 weeks

Discussion:

1- Multiple mating and egg hatching success:

This study found no evidence for an effect of multiple matings on the fecundity and egg hatching success of female T. castaneum at least over the first 6 days of oviposition. Similarly, in experiments of Lewis, (2004), T. castaneum males were found to engage in two to six repeated copulations with the same female and did not increase short-term female fecundity. However, in in different species, Drosophila arizonae, in which female remate frequently, females with greater access to males laid significantly more eggs than those mated just once (Croshaw and Gomez, 2018).

In an earlier experiment, which is similar to this one, Lewis and Austad (1994) reported that female fecundity increased significantly with multiple mating. They found a significant increase in adult progeny production for female T. castaneum allowed to mate with multiple males. In their experiment, they collected the offspring produced for 72hr after a 24hr mating period for those females mated with one male, after a 48hr mating period for the females allowed to mate with 2 males for 2 sequential 24hr periods, and after 4 days of mating where females mated to 4 males over 4 sequential 24hr of mating periods.

In our study, offspring were also collected from eggs laid over a 3 days period, but the mating period for the three groups was 24hr where males and females in all groups were kept together for 24hr only. Thus, the difference between our results and Lewis and Austad's results may be due either to differences in the time for which females were allowed to mate which covaried with number of mates in the previous study, or to differences in the time after mating during which eggs were collected. It has been reported that in T. castaneum egg laying rate increased gradually and the maximum rate was reached 2-3 days after the female started laying eggs (Sokoloff, 1974). Lewis and Austad (1994) collected offspring after different intervals following the start of the mating period (1, 2 and 4 days for single, double and quadruple mated females respectively). Accordingly, female egg laying may have increased from 1 to 2 to 4 days after mating and the differences between the three groups should be expected. Attia, (2004) reported that, the mean number of offspring produced by double mated Ga1 females increased over the first 3 days of oviposition and the difference between days was highly significant (mean number of offspring (±S.E.) was 3.55 ±0.52, 8.3 ±0.8 and 10.42 ±0.9 for day 1, day 2 and day 3 respectively ($F_{2,92}$= 20.2, $p<0.001$)).

This does not mean that there is no stimulation of egg laying after mating. The fertility of Tribolium species increased in the presence of males and T. castaneum females mated to sterile males on average produced fewer eggs every 24hr than females mated to fertile males, suggesting the existence of male stimulating effects on oviposition (Sokoloff, 1974).

All Tribolium species are long-lived and produce eggs continuously over a long period (Sokoloff, 1974). Rooney and Lewis (1999) predicted that in short-lived species females should allocate spermatophore-derived proteins primarily to current reproductive output, in contrast, they expected long-lived species females to show greater allocation of these proteins towards their own somatic maintenance. Hence, if males of T. castaneum transfer any proteins within their spermatophore ejaculates, then females may perhaps be expected to use most of these proteins in their somatic maintenance.

2- Effect of multiple mating and presence of males on female lifespan and offspring production:

The number of mates to which females were exposed significantly affected female lifespan. Females kept with 5 males lived for a shorter time than the other groups while virgin females lived for longer than the other groups. The effect of seminal fluid products on females varies between species and can have completely opposite effects on female lifespan. The ejaculate of Drosophila melanogaster is costly to females due to the toxicity of seminal products, which reduce the lifespan of females (Chapman et. al., 1995). In contrast, Croshaw and Gomez (2018) found that polyandrous females of Drosophila arizonae lived significantly longer than both virgin and serially monogamous females. Longevity of Mnesampela private moth females was increased by mating suggesting that females benefit from ejaculate resources (Waker, 2010). However, in some species of butterfly, it has been shown that females gain benefits from their mates (Wedell, 1996, Wedell et. al., 2002). In the comma butterfly, Polygonia c-album, females mated to high quality males lived significantly longer than females mated to males providing them with smaller donations, which suggests that females can use male nutrients for somatic maintenance (Wedell, 1996). Wedell et. al., (2002) found that in the green-veined white butterfly (Pieris napi) female longevity was positively correlated with degree of polyandry, indicating that polyandrous females allowed to mate freely can utilize male donations more efficiently than females mating a fewer number of times. Pai and Yan (2003) did not find any effect of multiple mating (with multiple males) on survivorship of female T. castaneum. In their experiment Pai and Yan did not keep males and females together for the period of study. They enabled virgin females to mate with 1, 2, 4, 8 or 16 virgin males simultaneously for 24hr. Males were removed from the vial after 24hr and females were transferred to vials with fresh flour medium every 6 days until females stopped producing viable eggs for 2 consecutive weeks. However, adult offspring production and egg to adult viability were not affected by the number of mates. Moreover, there was no significant difference between the 5 treatments in female fertility retention and female survivorship.

This suggests that the difference between their results and those presented here may indicate that the effects we observed may be due either to the much greater difference in the number of matings females were likely to receive in our treatments compared to theirs, or because direct interference by males, rather than ejaculate effects are influencing female lifespan.

There are several possible explanations for the effect of the presence of males with females on female lifespan. The first explanation is that multiple mating leads to transfer of larger quantities of seminal fluids to females. These seminal fluids might stimulate oviposition rate and reduce the lifespan of females. In D. melanogaster, seminal fluids are known to have several functions such as increasing oviposition rate and delaying remating of females, reducing fertilisation success of sperm from previous mates and reducing the lifespan of females (Kalb et. al., 1993, Harshman and Prout, 1994, Chapman et. al.,1995, Castrezana et. al., 2017). The second possible explanation for female short lifespan from multiple mates in this species is female harassment and cost of mating attempts by males. The third cause of early death of multiply mated females is competition between beetles either for food or mating, both of which are unlikely in this experiment because there was excess food available in each treatment and there have been no reports that red flour beetles engage in fights over matings.

Despite our finding that singly mated females produced offspring for a shorter period than females in the other treatments, which is likely to be due to sperm depletion from the female reproductive tract, the group of females with five males each produced offspring for shorter period of time than females with one male each. Also females with five males produced less offspring than females with one male. This effect is partly because females kept with 5 males had shorter lifespan, but this alone is not enough to explain the difference. The small number of adult offspring produced by females kept with five males may in fact be unrelated to the number of eggs laid by females, but may be the result of cannibalism by the adult male beetles present. The flour beetle’s active stages (adult and larvae) are cannibalistic on the inactive ones (eggs and pupae) (Sokoloff, 1974). Similar results were recorded from multiple mating of T. confusum where cultures containing one male and one female produced the same number of eggs as cultures containing two males and one female because of cannibalism, which counteracted the stimulation of increased copulation, but in cultures contained three males and one female there was a decrease in egg production because of increase of egg consumption by adults (Sokoloff ,1974). We tried to overcome this problem by adding more food (fresh flour medium) to the pots that contained more adults. And tried also to keep adults with immature stages as long as possible which may be important as in natural population where different stages of the life cycle live and interact together.

Gillott (2003) suggests that the second male’s sperm could displace and incapacitate sperm remaining from the first mating. Thus, if T. castaneum males have evolved their seminal fluid via sperm competition to compete with other males’ sperm by destroying or incapacitating them, then the group of females mated multiply with five males may produce less offspring due to sperm competition between sperm of the five males destroying and inhibiting each other. However, Tregenza et. al. (2009) found no evidence for sperm competition heritability.

From this study it seems that multiple mating and the presence of multiple males are costly to females of this species, which obtain no direct benefit from males such as nutrients in the seminal fluid for oviposition. Polyandrous females of this species might gain some genetic benefit from additional mating by promoting sperm competition between males and these benefits may be more than the costs of multiple mating. In a quantitative genetic model, Yasui (1997) showed that multiply mated females can receive good genes from the male with greater sperm competitive ability because such males possess genes for greater general viability, however the present study cannot test for this possibility.

It has been reported that females of some species may gain indirect benefits from multiple mating with many males. Wedell and Tregenza (1999) studied the mating success of male field crickets, Gryllus bimaculatus, and their offspring. They found that sons of successful males obtained significantly more copulations than sons of unsuccessful males. In T. castaneum, Pai and Yan (2002) found that offspring males (F1) from multiply mated females inseminated (produced viable offspring from their mates) more than (F1) males from mothers with a single mating.

Finally, more work is needed to investigate whether these costs to females were caused by male's ejaculates or other reasons such non-mating costs such as competition or coercion

REFERENCES

Attia FA. (2004) costs and benefits of multiple mating in the red flour beetle *Tribolium castaneum.* Leeds university press, Leeds.

Bangham, J., Chapman, T. & Partridge, L. (2002) Effects of body size, accessory gland and testis size on pre and post-copulatory success in *Drosophila melanogaster*. Animal Behaviour, 64, 915-921.

Bissoondath, C. J. & Wiklund, C. (1995) Protein content of spermatophores in relation to monandry/polyandry in butterflies. Behavioral Ecology and Sociobiology, 37, 365-371.

Boulton, R. A. and Shuker, D. M. (2013) Polyandry. Current biology, 23 (24), 1080-1081.

Castrezanz, S., Faircloth, B. C., Bridges, W. C. and Gowaty, P. A. (2017) Polyandry enhances offspring viability withsurvival costs to mothers only when mating exclusively with virgin males in *Drosophila melanogaster*. Ecology and Evolution, 7, 7515-7526.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature, 373, 241-144.

Chen, P. S. (1984) The functional morphology and biochemistry of insect male accessory gland and their secretions. Annual Review of Entomology, 29, 233-255.

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M. & Bohlen, P. (1988) A male accessory gland peptide that regulates reproductive behaviour of female *Drosophila melanogaster*. Cell, 54, 291-298.

Croshaw, D. A. and Gomez, M. (2018) Polyandrous mating increases offspring production and lifespan in male *Drosophila arizonae*. Behavioral Ecology and Sociobiology, 72 (11), 1-10

Eberhard, W. G. (1996) Female control: sexual selection by cryptic female choice. Princeton University press.

Fowler, K. & Partridge, L. (1989) A cost of mating in female fruitflies. Nature, 338, 760-761.

Gillott, C. (2003) Male accessory gland secretions: modulators female reproductive physiology and behavior. Annual Reviews of Entomology, 48, 163-184.

Harshman, L. G. & Prout, T. (1994) Sperm displacement without sperm transfer in *Drosophila melanogaster*. Evolution, 48, 758-766.

Hayashi, F. & Kamimura, Y. (2002) The potential for incorporation of male derived proteins into developing eggs in the leafhopper *Bothrogonia ferruginea*. Journal of Insect Physiology, 48, 153-159.

Jennions, M. D. & Petrie, M. (2000) Why do females mate multiply? A review of the genetic benefits. Biological Review, 75, 21-64.

Kalb, J. M., DiBenedetto, A. J. & Wolfner, M. F. (1993) Probing the function of *Drosophila melanogaster* accessory glands by directed cell ablation. Proceedings of the National Academy of Sciences the United States of America, 90, 8093-8097.

Konior, M., Radwan, J. & Kobdziejczyk, M. (2001) Polyandry increases offspring fecundity in the bulb mite. Evolution, 55, 1893-1896.

Lewis, R. C., Pointr, M. D., Friend, L. A., Vasudeva, R., Bemrose, J., Sutter, A., Gage, M. J. G. and Spurgin, L. G. (2020) Polyandry provides reproductive and genetic benefits in colonising populations. Ecology and Evolution, 10 (19), 10851-10857.

Lewis, S. (2004) multiple mating and repeated copulations: effects on male reproductive success in red flour beetles. Animal Behaviour, 67, 799-804.

Lewis, S. M. & Austad, S. N. (1994) Sexual selection in flour beetles: the relationship between sperm precedence and male olfactory attractiveness. Behavioral Ecology, 5, 219-224.

Li, X., Shen, S., Fu, Y., Chen, J., Li, L., Itan, D., Zhu, J. and Zhan, F. (2021) Effects of mating on reproductive performance of *Coccophagus japonicus* Compere (Hymenoptera: Aphelinidae). Scientific Reports, 11 (1), 1-12

Nilsson, T., Fricke, C. & Arnqvist, G. (2002). Patterns of divergence in the effects of mating on female reproductive performance in flour beetles. Evolution, 56, 111-120.

Osikowski, A. & Rafinski, J. (2001) Multiple insemination increases reproductive success of female montandon's newt (*Triturus montandoni, caudata, salamandridae*). Behavioral Ecology and Sociobiology, 49, 145-149.

Pai, A. & Yan, G. (2002) Polyandry produces sexy sons at the cost of daughters in red flour beetles. Proceedings of the Royal Society of London (B), 269, 361-368.

Pai, A. & Yan, G. (2003) Rapid female mating in red flour beetles (*Tribolium castaneum*). Canadian Journal of Zoology, 81, 888-896.

Partridge, L. & Fowler, K. (1990) Non-mating costs of exposure to males in female *Drosophila melanogaster*. Journal of Insect Physiology. 36, 419-425.

Pointer, M. D., Gage, M. J. G. and Spurgin, L. G. (2021) Tribolium beetles as a model system in evolution and ecology. Heredity, 126, 869- 883.

Rodrigues, L. R., Figueiredo, A. R. T., Van Leeuwen, T., Olivieri, I. and Magalhaes, S. (2020) Costs and benefits of multiple mating in a species with first-male sperm precedence. Journal of Animal Ecology, 89, 1045-1054.

Rooney, J. & Lewis, S. M. (1999) Differential allocation of male-derived nutrients in two lampyrid beetles with contrasting life-history characteristics. Behavioral Ecology, 10, 97-104.

Simmons, L. (2001) Sperm competition and its evolutionary consequences in the insects. Princeton University Press.

Sokoloff, A. (1974) The biology of *Tribolium*. Vol. II. Oxford University Press.

Tregenza, T. Attia, F. A. and Bushaiba, S. S. (2009) Repeatability and heritability of sperm competition outcomes in males and females of *Tribolium castaneum*. Behavioral Ecology and Sociobiology, 63, 817-823

Tregenza, T. & Wedell, N. (1998) Benefits of multiple mates in the cricket *Gryllus bimaculatus.* Evolution, 52, 1726-1730.

Vasudeva, R. C., Dickinson, M. Sutter, A., Powell, S., Sales, K. and Gage, M. J. G. (2021) Facultative polyandry protects females from compromised male fertility caused by heatwaves condition. Animal Behaviour, 178, 37-48.

Wagner, W. E. Jr., Kelley, R. J., Tucker, K.R. & Harper, C. J. (2001) Females receive a life-span benefit from male ejaculates in a field cricket. Evolution, 55, 994-1001.

Waker, P. W. and Allen, G. R. (2010) Mating frequency and reproductive success in an income breeding moth, *Mnesampela private*. Entomologia Experimentalis et Applicanta, 136, 290-300.

Wedell, N. & Tregenza, T. (1999) Successful fathers sire successful sons. Evolution, 53, 620-625.

Wedell, N. (1996) Mate quality affects reproductive effort in a paternity investing species. Behavioral Ecology and Sociobiology, 148, 1075-1088.

Wedell, N., Wiklund, C. & Cook, P. A. (2002) Monandry and polyandry as alternative lifestyles in a butterfly. Behavioral Ecology, 13, 450-455.

Yasui, Y. (1997) A 'good sperm' model can explain the evolution of costly multiple mating by females. Behavioral Ecology and Sociobiology, 149, 573-584.