International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 07-09-2021, Accepted: 24-09-2021, Published: 08-10-2021
Volume 6, Issue 5, 2021, Page No. 145-148

Paraquat effects on accessory gland traits and sperms transferred to mated females in *D. melanogaster*

Krishna M S
Department of Studies in Zoology, Drosophila Stock Centre, Stress Biology Lab, University of Mysore, Manasagangotri, Mysore, Karnataka, India

Abstract
The majority of chemicals utilized in the field were insecticides, which have substantial consequences for reproduction and fitness. Proteins generated from the accessory gland cause post-mating physiological changes in mated females and have an impact on fitness in many insects. As a result, insects are an excellent model organism for studying the effects of environmental chemical contaminants like paraquat on male accessory gland and sperm characteristics.In the present investigation male diet effects on accessory gland traits and sperm has been studied in *D. melanogaster*. Oregon K strain of *D.melanogaster* raised on either control diet or paraquat treated diet were used to examined the outcome of paraquat effects on accessory gland and sperm traits to understand its effect of male reproductive success in *D. melanogaster*. It was noticed that male fly raised on control diet had more small main cells in their accessory gland and produced more accessory gland protein; whereas males raised on paraquat treated diet had fewer large main cells and produced much less accessory gland protein. Furthermore, males raised on a control diet copulated longer, transferred more accessory gland protein and sperm to mated females than males raised on a paraquat-treated diet. Thus paraquat treatment in *D. melanogaster* has a significant negative impact on accessory gland protein, sperm characteristics and male fitness.

Keywords: *D. melanogaster*, paraquat, accessory gland proteins, sperms transferred

Introduction

Humans utilized pesticides of various types (insecticides, fungicides, and herbicides) to regulate their environment and preserve agricultural products grown for food supply from pests. (Desneux *et al.*, 2007) [7]. As a result, it's critical to understand how species interact with their surroundings and respond to current and emerging environmental stressors including rising temperatures, biological invasions, habitat degradation, and chemical pollution. Proteins generated from the accessory gland, the male reproductive system's secretary tissue, are essential for fertility in many insects. As a result, insects are an excellent model organism for studying the effects of environmental chemical contaminants like paraquat on male accessory gland and sperm characteristics. The effects of paraquat on the male reproductive system (which includes the testes, epididymis, seminal vesicles, and prostate) included a reduction in the number of sperms, cell division, and spermatogonial cells (Shukla *et. al.*,2014) [11]. Paraquat decreases longevity (Bonilla-Ramirez *et. al.*, 2013; Chaudhuri *et. al.*, 2007; Weber *et. al.*, 2012) [1, 3, 13] decreases dopaminergic neuron number (Shukla *et. al.*, 2014) [11].

Through the process of copulation, accessory gland product and sperms enter the female vaginal system, affecting the reproductive physiology and behaviour of the mated female (Herndon and Wolfner, 1995; Chen, 1996; Tram and Wolfner, 1999; Chapman *et al.*, 2000; Heifetz *et al.*, 2000) [6, 4, 12, 2, 5]. Increased egg production, decreased readiness to remate, storage/utilization of sperm, and other alterations are together referred to as post-mating reactions (Wolfner, 1997) [15]. Many factors, including genotype, biotic and abiotic influences, have been shown to influence accessory gland anatomy and secretion quantity (Santhosh and Krishna, 2013) [10]. However, no research on the effects of paraquat on accessory gland characteristics and sperm transfer to mated females in *D. melanogaster* has been done. As a result, the current study was conducted in *D.melanogaster* with the following goals:

1. The amount of accessory gland protein production changes depending on whether or not the male is treated with paraquat. What impact does this have on the size and number of major cells in the accessory gland, as well as the size of the accessory gland and the amount of accessory gland proteins produced?
2. To determine whether there is a link between male paraquat exposure, copulation duration, and the amount of accessory gland secretion/sperm transmitted to a mated female.

Materials and Procedures

The Drosophila Stock Centre, University of Mysore, Mysore provided the experimental stock Oregon-K strain of *D. melanogaster*. The flies were cultured using twenty flies per culture bottle (150ml) containing 30 ml of standard wheat cream agar media with yeast. These culture bottles were kept at a temperature of 221°F and a relative humidity of 70–80%.

Treatment with paraquat

The wheat cream agar medium (WCA) diet is a high-quality food that closely reflects the nutritional content of the wild diet of *D. melanogaster*. Food was allowed to cool to 35° C before being put into standard Drosophila rearing vials with a paraquat concentration of 10/ 20/ 30 mM to wheat cream agar medium. Each vial contained a total of 15 flies. The wheat cream agar medium diet was used to keep the control flies.

The influence of paraquat on accessory gland size, number of cells (per lobe), and cell size

The accessory glands of paraquat-treated and control males were dissected out separately using Medium A, fixed in 1N HCl for 5 minutes, and stained with 2 percent Lactoacetoorcein stain for 20 minutes. These glands were used to measure accessory gland size, the number and size of the main cells in the accessory glands were measured separately following the procedure of Santhosh and Krishna, (2013). For control and paraquat-treated flies, a total of 50 replicates per diet were used to compute accessory gland size, number of main cells, and accessory gland size. Using the SPSS 10.1 programme, a one-way ANOVA followed by Tukey’s test was performed on the above data.

Effect of paraquat on total protein content in unmated men Unmated male sample preparation.

Unmated (etherized) males (paraquat treated and control males) had their accessory glands removed using insect saline and entomological needles. These glands were preserved in 95% ethanol. Ethanol-fixed glands were rinsed in a 1:1 solution of methanol and chloroform and dried in an incubator at 37° C for 15 minutes. To dissolve the glands and secretions, 100 l of sample buffer (0.625 M tris-HCL pH 6.8, 1 percent SDS, 1 percent b-mercaptoethanol, and 10% glycerol) was added to each sample. Ten pairs of accessory glands collected separately from each of the control and paraquat treated flies were used for quantitative estimation of accessory gland protein using Bradford's method.

Bradford's technique is used to estimate protein quantitatively.

About 50 mL of accessory gland protein was separately combined with 5 mL of Bradford reagent from each of the unmated /mated males (paraquat treated and control males) samples as stated previously (100 mg CBB G-250 in 50 mL of 95 percent ethanol and 100 mL of 85 percent phosphoric acid, diluted to 1 L). A spectrophotometer set to 595 nm was used to determine the optical density of the solution. Extrapolation was used to determine the amount of protein present in the sample, using bovine serum albumin as the standard. The optical density of the sample was read against the blank at 595 nm. A total 50 trials were run separately for unmated /mated males of paraquat treated and control males. One way ANOVA followed by Tukey's post hoc test was carried out on the data of the quantity of protein of unmated males.

Paraquat effect on copulation duration, quantity of protein in mated males, and transferred quantity of protein and sperm

Sample preparation of mated males

To obtain mated males, (paraquat treated /control males) a five- to six-day-old virgin female and an unmated (paraquat treated and control males) were individually placed into an Elens-Wattiaux mating chamber and observed for 1 hr. Pairs that remained unmated within 1 hr were discarded. If mating occurred, the duration of copulation was recorded (time between initiations to termination of copulation of each pair). Soon after copulation (within 5 min), mated males were etherized and dissected to obtain Acps and fixed in 95% ethanol. These glands were used to estimate accessory gland proteins using the Bradford method, as described above.

The mated females were individually dissected out using a 20 µL of Beadle-Ephrussi saline solution (128.3 mMNaCl, 4.7 mMKCl, and 23 mM CaC12). The spermatheca organs were then stained with lacto aceto orcein for 10 min, and were used to count sperm using a light compound microscope at 100x. A total of 50 trials were run separately for each of the control and paraquat treated males for copulation duration, quantitative estimation of Acps of mated males, and transferred quantity of Acps and sperm to mated females. One-way ANOVA followed by Tukey's post hoc test was carried out on the above data. The difference in the mean value of total accessory gland proteins from unmated to mated males was considered as the transferred quantity of accessory gland proteins to the mated female.

Effect of paraquat on copulation duration, protein transmitted, fecundity, and fertility

Every 24 hours, mated females acquired as described above were individually transferred into a new vial containing wheat cream agar medium until death. The number of eggs laid and progeny produced were counted. Fifty trials were conducted independently for each of the control and paraquat-treated flies. On the above data, SPSS 10.0 was used to perform a one-way ANOVA followed by a Tukey's test.

Results and Discussion

Size of the accessory gland, number and size of main cells in accessory gland are known to influence the quantity of accessory gland protein generated (Chen *et al.*, 1996; Wolfner 1997) [4, 15], and these structures also vary with fly size, age, and food (Chen *et al.,* 1996; Wolfner 1997) [4, 15]. The effect of an insecticide, paraquat, on accessory gland traits in *D.melanogaster* was investigated in this study. Control males, with many smaller main cells in their accessory glands, generated more protein, while paraquat exposure males, with fewer larger main cells in their accessory glands, produced the least protein (Table 1). Thus, our investigations in *D. melanogaster* imply that the number and size of the main cells in the accessory gland, as well as the size of the accessory gland, play an essential role in accessory gland protein production, and that the paraquat exposure of experimental flies had an effect on these variables. The amount of accessory gland protein in control males was higher than in paraquat exposure males (Table 1). This suggests that male diet has significant influence on accessory gland structure and secretion in *D.melanogaster*. This confirms the earlier works related to factors affecting accessory gland structure and secretion in *D.melanogaster* (Chen *et al.*, 1996; Wolfner 1997) [4, 15]. According to Ravi Ram and Ramesh (2002) [9], the amount of protein produced may be influenced by the secretary activity of cells in the auxiliary gland. In this investigation, the same pair of flies that were involved in copulation were allowed to finish copulation before being utilized to measure the amount of protein and sperm delivered to the female to learn more about the link between paraquat exposure, copulation time, and the amount of protein and sperm transmitted (Table 1 and 2).Males in the control group copulated for longer than paraquat exposure males. It's unclear why control males copulated for longer. There are theoretical grounds to believe that control males who haven't seen a female in a long time will put greater effort into the first female they see (Parker, 1970; Wedell *et al*., 2002) [8, 14]. Three other ideas could possibly account for this outcome. For starters, control males may be unable to deliver sperm quickly, necessitating longer copulations. Second, control males are more likely to transfer bigger amounts of sperm, necessitating more time. Third, during extended copulations, control males may transport more accessory fluid in their ejaculates. According to the first explanation, control males are less effective at transporting sperm than paraquat exposure ones. The second and third theories propose that control males invest more resources per mating and demonstrate that in *D. melanogaster*, control males invest more resources per mating. Control males who copulated for longer periods of time transported more protein and sperm to the females, supporting the second and third hypothesized explanations. Because of their shorter copulation times, paraquat exposure males transported considerably less protein and sperm to females. Females who mated with control males had considerably higher fecundity than females who mated with paraquat exposure males because control males with longer copulation time transported a bigger amount of protein. Our findings back up the theory that accessory gland proteins play a role in egg production (Wolfner 1997) [15]. The more protein that is delivered to the females, the more eggs they will produce. The findings also support the significance of accessory gland secretion in females' post-mating physiological changes, including as receptivity, fecundity, and fertility (Chen *et al.,* 1996; Wolfner, 1997) [4, 15]. Female *D.melanogaster* who mated with control males received more protein and sperm than females who mated with paraquat exposure flies, allowing them to have a higher fecundity and generate more progeny (Table 3). Thus these results suggests that Paraquat treatment has a significant negative impact on accessory gland protein, sperm and male fitness in *D. melanogaster*.

Table 1: Paraquat effects on accessory gland traits in *D.melanogaster*

Parameters	Main cell size in Accessory gland	No. of main cells in Accessory gland	Accessory gland size(mm)	Quantity of accessory gland protein in unmated males(μg/male)	Quantity of accessory gland protein in mated male	Quantity of accessory gland protein transferred to mated females
Control	0.0068±0.006^a	1556±0.21^a	0.375±0.003^a	15.91±0.15^a	11.32±0.16^a	4.59± 0.24
10mM PQ	0.0062±0.004^b	1432±0.31^b	0.342±0.005^b	15.10±0.18^a	11.30±0.19^b	3.80± 0.31
20mM PQ	0.0056±0.007^c	1401±0.35^c	0.331±0.004^c	14.10±0.25^b	10.17±0.22^c	3.93± 0.62
30mM PQ	0.0052±0.006^d	1386±0.26^d	0.330±0.003^d	13.11±0.29^c	10.10±0.25^d	3.01± 0.59
40mM PQ	0.0041±0.008^e	1274±0.19^e	0.321±0.004^e	12.10±0.31^d	8.68±0.29^e	3.42± 0.42
F- value	15.324**	12.334**	14.334**	12.16**	2348.2***	453.2***

P<0.001;*P<0.0001 level [PQ=paraquat treatment]
Different letters in superscript indicate significant at 0.05 level by Tukey's post hoc test

Table 2: Paraquat effects on sperms traits in *D. melanogaster*

parameter	Sperms in Spermthecae	Sperms in Seminal receptacle	Total sperm transferred to mated female
Control	34.12±0.15^a	176.12±0.05^a	6300±0.15^a
10mM PQ	24.11±0.25^b	110.11±0.06^b	4800±0.71^b
20mM PQ	20.15±0.31^c	96.11±0.07^c	3800±0.62^c
30mM PQ	17.15±0.39^d	94.10±0.08^d	3500±0.81^d
40mM PQ	15.21±0.42^e	84.10±0.06^e	3300±0.46^e
F- value	2433.93***	43638.742***	4533.903***

***P<0.0001 level [PQ=paraquat treatment]
Different letters in superscript indicate significant at 0.05 level by Tukey's post hoc test

Table 3: Paraquat effects on fecundity and progeny in *D. melanogaster*

parameter	Copulation duration	Fecundity (In no.)	Progeny (In no.)
Control	17.14±0.15^a	376±0.35^a	250±0.34^a
10mM PQ	12.34±0.25^b	255±0.49^b	241±0.56^b
20mM PQ	11.35±0.31^c	160±0.56^c	200±0.71c
30mM PQ	9.32±0.41^d	130±0.91^d	135±0.64^d
40mM PQ	7.12±0.35^e	120±0.84^e	110±0.74^e
F- value	43.3**	452628.906***	342.02***

P<0.001;*P<0.0001 level [PQ=paraquat treatment]
Different letters in superscript indicate significant at 0.05 level by Tukey's post hoc test

Acknowledgments

I am grateful to the University of Mysore for sanctioning minor research project and providing financial help. I am also grateful to Chairperson, Department of Studies in Zoology, University of Mysore, for providing facilities to carry out this work.

References

1. Bonilla-Ramirez L, Jimenez-Del-Rio M, Velez-Pardo C. Low doses of paraquat and polyphenols prolong life span and locomotor activity in knock-down parkin Drosophila melanogaster exposed to oxidative stress stimuli: Implication in autosomal recessive juvenile Parkinsonism. Gene,2013:512:355-363.
2. Chapman T, Neubaum DM, Wolfner MF, Partridge L. The role of male accessory gland protein Acp36DE in sperm competition in Drosophila melanogaster. Proceedings of the Royal Soceity B: Biological Sciences,2000:267:1097-1105.
3. Chaudhuri A, Bowling K, Funderburk C, Lawal H, Inamdar A, Wang Z *et al*. Interaction of genetic and environmental factors in a Drosophila Parkinsonism model. J. Neurosci,2007: 27:2457-2467.
4. Chen PS. The accessory gland proteins in male Drosophila: structural, reproductive, and evolutionary aspects. Experientia,1996:52:503-510.
5. Heifetz Y, Lung O, Frongillo EA, Wolfner MF. The Drosophila seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. Current Biology,2000:10:99-102.
6. Herndon LA, Wolfner MF. A Drosophila seminal fluid

protein, Acp26Aa, stimulates egg laying in females for 1 day after mating. Proceedings of the National Academy of Sciences,1995:92:10114-10118.
7. Nicolas Desneux, Axel Decourtye, Jean-Marie Delpuech. The sublethal effects of pesticides on beneficial arthropods. Ann. Rev. Entomol,2007:52:81-106
8. Parker GA. Sperm competition and its evolutionary consequences in the insects. Biological Reviews,1970:45:525-567.
9. Ravi Ram K, Ramesh SR. Male accessory gland secretory proteins in nasuta subgroup of Drosophila: Synthetic Activity of Acp. Zoological Science,2002:19:513-518.
10. Santhosh HT, Krishna MS. Relationship between male age, accessory gland, sperm transferred, and fitness traits in *Drosophila bipectinata*. Journal of Insect Science,2013:13:159.
11. Shukla AK, Pragya P, Chaouhan HS, Tiwari AK, Patel DK, Abdin MZ *et al*. Heat shock protein-70 (Hsp-70) suppresses paraquat-induced neurodegeneration by inhibiting JNK and caspase-3 activation in Drosophila model of Parkinson’s disease. PLoS one,2014:9:98886.
12. Tram U, Wolfner MF. Male seminal fluid proteins are essential for sperm storage in Drosophila melanogaster. Genetics,1999:153:837-844.
13. Weber AL, Khan GF, Magwire MM, Tabor CL, Mackay TFC, Anholt RRH. Genome-wide association analysis of oxidative stress resistance in Drosophila melanogaster. PLoS one,2012:7:34745.
14. Wedell N, Gage MJG, Parker GA. Sperm competition, male prudence and sperm limited females. Trends in Ecology and Evolution,2002:17:313-320.
15. Wolfner MF. Tokens of love: functions and regulation of Drosophila male accessory gland products. Insect Biochemistry and Molecular Biology,1997:27:339-346.