



Paraquat effects on mating success and courtship behavior in *D. melanogaster*

Krishna M S

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

Abstract

In the agricultural setting, herbicide is widely used to manage weeds. However, in a variety of vertebrate species, it can have a deleterious impact on male reproductive performance. Its impacts on invertebrate reproductive performance, on the other hand, are less common. The effects of varying doses of paraquat on mating and courtship behaviors in *Drosophila melanogaster* were investigated in this study. In *D. melanogaster*, paraquat exposure had a substantial impact on mating success, mating latency, courting activity, and copulation duration. This study suggests that paraquat exposure affects mating and courtship activities in *D. melanogaster*. Paraquat exposure reduces mating success, takes longer for mating, shows less courtship activities, females showed greater rejection responses to mating males, and copulated shorter than crosses involving control male and female. These results thus indicate that paraquat has negative effects on mating success and courtship activities in *D. melanogaster*.

Keywords: copulation duration, *D. melanogaster*, paraquat mating activities

Introduction

Global changes are occurring as a result of human activity (Pace *et al.*, 2015) [15]. We need a better understanding of how species interact with their habitats and adapt to existing and emerging environmental stresses including rising temperatures, biological invasions, habitat destruction, and chemical pollution to better predict their consequences (Crain *et al.*, 2008; Jackson *et al.*, 2016) [6,8]. Pesticides and medicines have been created to cause parasite and pest populations to die rapidly. This puts these species under a lot of selection pressure, which could lead to the evolution of resistance to xenobiotics. Xenobiotics may influence the morphology, life history, or behavior of organisms without killing them, in addition to causing lethality. Many pesticides, for example, diminish organism fertility and/or longevity, while others produce paralysis, affecting organisms' capacity to find food or mates, or to flee from potential predators (Desneux *et al.*, 2007) [14]. (Arnaud and Haubruge, 2002) [12]. Despite being usually disregarded, these sub lethal impacts can have a considerable impact on an organism's reproductive biology and fitness. As a result, natural selection is likely to work in favor of minimizing the negative effects of pesticides. A well-known inducer of mitochondrial oxidative stress is paraquat, a bipyridinium pesticide (Przedborski and Ischiropoulos, 2005) [16]. Paraquat exposure in the *D. melanogaster* reduces longevity (Bonilla-Ramirez *et al.*, 2013; Chaudhuri *et al.*, 2007; Weber *et al.*, 2012) [3,4,19], decreases dopaminergic neuron number (Shukla *et al.*, 2014), changes dopamine and dopamine metabolite levels (Shukla *et al.*, 2014) [17], and changes motor functions (Jimenez *et al.*, 2010) [19]. However, the effects of paraquat on reproductive biology and their implications for organism fitness have not been investigated. *Drosophila* has contributed significantly to the development of sexual selection theory, and much is known about the patterns and fitness implications of female partner choice (Partridge, 1980, Hegde and Krishna, 1997) [11,7]. The

goal of this study is to use the *D. melanogaster* as a model genetic system to investigate the effects of paraquat on Mating and courtship behaviors.

Materials and Methods

Experimental stock Oregon-K strain of *D. melanogaster* obtained from the Drosophila Stock Centre, University of Mysore, Mysore. Twenty flies per culture bottle (150ml) containing 30 ml of standard wheat cream agar medium (wca) with yeast. The wheat cream agar media (WCA) diet is a high quality diet that closely resembles the nutritional content of the diet to which *D. melanogaster* is adapted in the wild. For the WCA method, food was allowed to cool to 35 C and poured into standard Drosophila rearing vials with a PQ concentration of 10, 20, or 30 mM and control flies were also culture using wheat cream agar media. A total of 15 flies were placed in each vial. These culture bottles were maintained at 22±1 °C and 70–80% relative humidity. Flies obtained from these culture bottles were used for the present experiments

Effect of paraquat on mating behaviour and courting patterns

Females and males aged 5-6 days were employed to evaluate mating activities and courtship patterns in control and paraquat-exposure females and males (see Table I). Mating was done in pairs. For each of the control and paraquat exposure flies, 50 pair-wise matings were made independently. Latency in mating (introduction of male and female into the mating chamber until initiation of copulation), Male courtship activity (tapping, scissoring, and vibration, licking, circling,) and female rejection responses (ignoring, extruding, and decamping) were all measured using the Hegde and Krishna technique (1997).

Results and Discussion

Herbicides were employed to manage weeds in agricultural

Settings, according to compelling studies (Vogel *et al.*, 2015) [1]. However, in a variety of vertebrate species, they have a deleterious impact on male reproductive performance. The effects of paraquat, an herbicide used to control weeds in agricultural fields, on mating and wooing behaviors in *D.melanogaster* were investigated in this study. The table1 strongly suggests that ecologically appropriate dosages of paraquat exposure alter *D. melanogaster* mating and courting behaviors. The mating success of paraquat-exposure flies was significantly lower than that of control flies (paraquat unexposure). As the concentration of paraquat utilized increased, the proportion of mating success reduced even more (Table1). This shows that paraquat exposure has an effect on *D.melanogaster* mating success. The current study also backs up the findings of Vogel *et al.*, (2015) [1], who discovered that atrazine, a herbicide, has detrimental impacts on male reproductive function in *D.melanogaster* while examining the species. They discovered that males that have been exposed to atrazine took longer to mate previously mated females. They also discovered that females that mated with atrazine-exposed males produced fewer eggs and progeny. In *Drosophila* species, many parameters such as size, age, nutrition, and rearing conditions have been recognized to influence mating success (Hegde and Krishna, 1997) [7]. The size differences between control and paraquat exposure flies were not significant in this experiment, indicating that the increased mating success of control flies over paraquat exposure flies was not due to a size difference. Furthermore, because the age of control and paraquat exposure flies was the same, the observed higher mating success of control flies compared to paraquat exposure flies was not related to age differences. Because the environmental conditions for culture of control and paraquat exposure flies were the same, the greater mating success of control flies over paraquat exposure flies was not due to a difference in rearing temperatures of control and paraquat exposure flies. The only difference in this study was paraquat exposure, which suggests that paraquat exposure decreased mating success in *D. melanogaster*. Because mating success is connected to the rate of encounter and courtship time, comparing the behaviors of control and paraquat exposure flies during courtship can help explain why control flies have a greater mating success than paraquat exposure flies. Table 1 shows that control flies took less time to commence copulation than paraquat exposure flies, implying that paraquat exposure had an effect on mating latency. Flies that took more time were slow maters, while those that took less time

were fast maters, because speed is the inverse of time. This indicates that control flies in *D. melanogaster* were rapid maters, whereas paraquat exposure insects were slow maters. This suggests that paraquat exposure affects *D. melanogaster* mating behavior. In this study, it was also discovered that control males performed several courtship acts faster and more frequently than paraquat-exposure flies. The control Male's use these courtship activities to not only provide sexual signals to the females, but also to stimulate them (Partridge 1980) [11]. Control males have a faster ability to communicate sexual signals to and arouse courting females. Paraquat-exposure flies, on the other hand, displayed higher rejection responses than control females. Control males, on the other hand, can repeatedly and swiftly stimulate females to surpass the copulation threshold because they are more active. Copulations lasted the longest when both the control male and female were present, implying a role for paraquat exposure in *D. melanogaster* courtship activities (Table1). This backs up previous research indicating age and other environmental factors influence courtship behavior in various *Drosophila* species (Hegde and Krishna, 1997) [7]. *Drosophila* males use these courtship practices to better communicate chemical, auditory, and visual signals to females and to try to persuade females to mate faster than paraquat-exposure flies. This is consistent with previous *Drosophila* research, which found that males that engage in more courtship activities are better mates and have higher mating success than males who do not engage in as many courtship activities (Hegde and Krishna, 1997) [7]. Male and female courtship activities culminate in the beginning of copulation during mating behaviour (Partridge, 1980) [11]. Control flies copulated for longer than paraquat-exposure flies, as seen in Table 1. This shows that paraquat exposure has an effect on the length of copulation in *D.melanogaster*. As the number of ejaculations increases with increased copulation length, control females appear to receive more sperm than paraquat exposure females. These results thus indicate that paraquat exposure has negative effects on mating success and courtship activities in *D.melanogaster*

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Table 1: Paraquat effects on mating success and courtship activities in *D. melanogaster*.

Parameters		Paraquat Treatment				F-Value
		Control	10mMPQ	20mMPQ	30mMPQ	
Mating success (%)		85	70	62	54	
Male courtship activities (in. numbers)	Tapping	13.72±0.411 ^a	5.48±0.32 ^b	7.08±0.25 ^c	8.80±0.171 ^d	135.692**
	Scissoring	18.92±0.37 ^a	12.40±0.35 ^b	15.00±0.23 ^c	17.40±0.27 ^d	81.477*
	Vibration	11.12±0.26 ^a	8.92±0.20 ^b	9.80±0.18 ^b	9.68±0.23 ^c	16.715*
	Licking	7.56±0.192 ^a	4.28±0.17 ^b	6.44±0.216 ^c	7.04±0.15 ^{ac}	59.123*
Female rejection responses (in. numbers)	Circling	18.24±0.20 ^a	14.40±0.23 ^b	16.08±0.25 ^c	17.32±0.20 ^d	55.314*
	Ignoring	5.00±0.17 ^a	7.52±0.183 ^b	6.44±0.15 ^c	5.60±0.182 ^a	39.620*
	Extruding	15.44±0.20 ^a	23.12±0.38 ^b	19.08±0.24 ^c	19.00±0.23 ^c	132.342**
	Decamping	14.80±0.23 ^a	23.60±0.35 ^b	18.64±0.25 ^c	16.72±0.18 ^d	200.813**
Mating latency (in min)		4.75±0.21 ^a	10.89±0.31 ^b	8.85±0.11 ^c	5.57±0.12 ^d	187.727**
Copulation duration (in min)		17.15±0.35 ^a	12.02±0.41 ^b	10.47±0.51 ^c	8.21±0.71 ^d	43.3**

*Significant at 0.00 level;** P<0.0001 level; Same letter in the superscript indicate insignificant by Turkey's test [Different letters in superscript indicate significant at 0.05 level by Tukey's post hoc test]

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