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Differential climbing patterns based on gender, mating status, age, and Lithium Chloride (LiCl) Treated *Drosophila melanogaster*

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Abstract
The climbing assay (negative geotaxis) evaluates locomotor behavior in *Drosophila melanogaster*. The study examines the effects of age, mating status, and lithium chloride (LiCl) treatment on climbing ability. Flies were grouped according to gender, mating status and different ages with varying concentrations of LiCl (15 mM, 45 mM, and 75 mM). The climbing performance of flies was measured at different intervals of distances ranging from 0-5 cm to 15-20 cm and the time was noted. Results showed that younger flies exhibited better climbing ability than old ones. Flies treated with 15 mM LiCl performed better than higher concentrations and control. Virgin flies consistently outperformed mated ones. MANOVA confirmed significant differences across age, groups, treatment, and distance, highlighting the influence of both age and LiCl treatment on locomotion. These findings provide insights into the effects of aging, mating, and chemical treatments on locomotor performance, presenting a valuable method for studying behavioral physiology found in different animals.

Keywords: Negative geotaxis, chemical treatment, gender, distances, time, age, mating.

Introduction

Climbing activity or Negative geotaxis behavior is the ability of flies to move vertically upward and serves as a widely used indicator of locomotor behavior (Zhong *et al.*, 2022) [47]. It was first described in 1970s (Ganetzky and Flanagan, 1978) [17]. The climbing or locomotor behavioral assay has studied in different organisms including fruit fly, *Drosophila melanogaster* (Bartholomew *et al.*, 2015) [4], rice weevil, *Sitophilus oryzae*, and cigarette beetle, *Lasioderma serricorne* (Matsuda *et al.*, 2020) [32], asian honey bee, *Apis cerana cerana* (Gao *et al.*, 2020) [19], spotted lanternfly, *Lycorma delicatula* (Nixon *et al.*, 2021) [34], caribbean fruit fly, *Anastrepha suspensa* (Teets *et al.*, 2019)[41], parasitoid wasp, *Nasonia vitripennis* (King *et al.*, 2000) [27]. Among these organisms, *Drosophila melanogaster* is widely used to study negative geotaxis and to measure the deterioration of locomotor performance and endurance in flies (Rhodenizer *et al.*, 2008 [35]; Yadav *et al.*, 2016 [44]). The climbing activity evaluates the energetically demanding process of induced climbing, which reflects the impact of genetic mutations, neurological conditions, and environmental factors on behavior (Barone and Bohmann, 2013) [3]. This makes it an essential tool for understanding age-related diseases like Parkinson's and Alzheimer's that exhibit motor impairment in organisms (Aggarwal *et al.*, 2019) [1]. Aging is a slow and irreversible process characterised by a progressive decline in tissue and cellular functions. It is accompanied by a risk of various age-related conditions, such as neurodegenerative disorders, cardiovascular diseases, metabolic issues, musculoskeletal disorders, and immune system dysfunctions (Guo *et al.*, 2022) [20].

In addition to age, mating plays a crucial role in the evolutionary process and can influence locomotor performance. Mating strategies like polyandry, where females mate with multiple males, and repeated or prolonged mating have significant impacts on female fitness (Choe, 1997) [10]. Polyandry, common in natural insect populations, provides various genetic benefits that enhance offspring fitness (Yasui, 1998) [45]. In addition to age and mating, external factors such as chemical treatments can also affect the climbing ability of *Drosophila* (Lovejoy and Fiumera, 2019) [29]. Lithium chloride (LiCl), a widely used compound for treating bipolar disorder (Jin *et al.*, 2022) [24], has been shown to influence various behavioral parameters in flies, including locomotion and aggression. Studies have revealed that age can have both positive and negative influences on locomotor performance concerning lithium treatment with different concentrations, further emphasizing the importance of treatment duration in behavioral analysis (Wang *et al.*, 2023) [42]. Lithium effects on neurodegeneration are particularly relevant for understanding diseases such as Alzheimer's and Parkinson's, where climbing assays are used to measure motor decline (Singulani *et al.*, 2024) [39].

Many studies regarding climbing assay were done with particular distance, gender, age, time, and mating pattern (Rhodenizer *et al.*, 2008 [35]; Bagu *et al.*, 2020 [2]; Gao *et al.*, 2020 [19]; Wongchum and Dechakhamphu, 2021 [43]; Kharat *et al.*, 2020 [26]; Horn *et al.*, 2019 [22]; Bretman and Fricke, 2019 [6]; Rivera *et al.*, 2019 [36]). The information on the climbing activity of different age groups, multiple mated flies and time to cross the different intervals of distance is not available. In view of this, *D. melanogaster* was taken to study the climbing assay on the above aspects.

Materials and Methods

Drosophila Stock Culture and Maintenance

D. melanogaster (Oregon-K) (Figure 1) was obtained from the *Drosophila* Stock Center, Department of Zoology, University of Mysore, Karnataka, India. The flies were cultured and kept in the laboratory with a constant temperature of 22 ± 1 ºC and a relative humidity ranges between 70-80% (Shivanna *et al.*, 1996) [37].

Experimental Design

Flies were exposed to different concentrations of LiCl as reported by Castillo-Quan *et al.*, (2016) [7]. Based on the

optimum LC_{50} value, the concentrations were determined (15 mM, 45 mM and 75 mM). Flies were grouped into, Virgin Male (VM); Virgin Female (VF); One male and One female – as Mated Male (MM) and Mated Female (MF); Ten Males (TM) and Single Female (SF); Ten Female (TF) and Single Male (SM).

Climbing Activity

Newly eclosed flies were kept separately according to experimental design and transferred to fresh culture bottles every 2-3 days. The flies were treated with yeast (control) and yeast dissolved in LiCl as treatment at a concentration of 15 mM, 45 mM and 75 mM LiCl for 11, 21 and 31 days. Flies of different ages were released into marked glass tube of 3.7 cm diameter (0-5, 5-10, 10-15 and 15-20 cm, Figure 2) and tapped thrice to gather the flies at the bottom. The number of flies and time taken to climb the marked distances were recorded separately from 10 replicates. The activity was repeated thrice with an interval of 30 minutes for each group, the experiment was video recorded for counting the number of flies and the time taken by each fly. The data was subjected to Multivariate Analysis of Variance (MANOVA) to analyse the influence of age, groups, and treatments on the mean number of flies crossing the distance and the mean time taken for climbing different distances using the SPSS 21.0 version.

Results

The mean number and time taken to climb different dista nces by control and treated flies was measured. The climbing activity was analysed with different concentrations of LiCl (15, 45, and 75 mM) across four distances (I= 0–5 cm, II= 5–10 cm, III= 10–15 cm, IV= 15–20 cm) and of three age groups (11, 21, and 31 days old) (Figures 3 and 4).

Figure 3 revealed more number of flies (9.86) for 15 mM treated 11-day-old VM flies climbing the distance (0-5 cm) at a lesser time of 2.05 seconds (s), whereas 31-day SM flies (6.73) took more time (12.82 s) to cross 0-5 cm mark. A Similar trend for VM and SM was observed for 5-10 cm distance (9.83 at 3.54 s and 6.36 at 13.02 s), 10-15 cm (9.19 at 9.84 s and 5.69 at 26.47 s) and 15-20 cm distances (8.89 at 10.93 s and 4.96 at 28.19 s). The TM and MM groups are intermediates, among which TM flies took less time (9.73 at 2.80 s), (9.69 at 4.94 s), (9.13 at 12.45 s) and (8.66 at 14.11 s) compared to MM (8.19 at 6.63), (8.06 at 9.32 s), (7.53 at 18.39 s) and (7.16 at 19.64 s) of 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm respectively.

Climbing activity in female flies also showed a similar result as of male flies. Figure 4 revealed that 15 mM treated flies with a maximum number of flies (9.63) took less time (3.82 s) compared to SF having fewer flies (7.06) took more time (15.93 s) to cross 0-5 cm distance. This observation also seen at 5-10 cm, 10-15 cm, and 15-20 cm distances. TF and MF flies were intermediates with TF flies climbing activity is better than SF. Overall, male flies showed better climbing activity than female flies. 11-day-old flies performed better than 31-day-old flies. Virgin flies performed better than mated flies. 15 mM treated flies showed better climbing activity than control, 45 mM and 75 mM LiCl treated flies.

The statistical analysis of males and females revealed that factors such as age, groups, and treatment have a significant influence on the number of flies and the time required to cross the distances. Among the combined factors a significant effect was observed for the influence of parameters on Age × Groups, Age × Distances, and Groups × Distances at time in both males and females. The effect of Groups × Treatment on the number of flies was significant in both males and females. Significance was found in Distances × Treatment on time only in males (Table 1).

Table 2 revealed that the overall effect of age, groups and treatment in both male and female flies was significant. The combined effect of Age × Groups was significant in males only, whereas the combined effect of Age × Distances, Group × Distances and Groups × Treatments was found significant in both male and female flies. However, the combined effect of Age × Treatment, and Distances × Treatment was not significant in both males and females.

Discussion

The present results revealed that age significantly affects climbing speed, particularly the time taken to cover longer distances (III and IV) at higher LiCl concentrations. As flies age, the number of flies (both and female) climbing showed age-related reductions (Figures 3 and 4) and also a decrease in climbing speed, ultimately impacting their overall ability to move vertically. Negative geotaxis measured when flies were young, was reduced significantly by older age (Gargano *et al*. 2005) [18]. Additionally, climbing speed decreases and response latency increases with age in flies (Rhodenizer *et al.,* 2008) [35]. All the factors such as age, groups, and treatment significantly affected the climbing activity with different distances (mean number of flies and time) of both sexes. Whereas combined effects of these factors had a differential influence. When compared between the two dependent factors, time was influenced by the interaction of more factors than the number of flies (Table 1). When the overall climbing effect (dependent groups combined) was analysed among sexes, males and females were equally influenced by all the factors such as age, groups, and treatments. This infers that the climbing activity does not influence the sex (Table 2). Male and female flies equally show good motor activity in younger flies than older flies to climb the maximum distance. Similarly, the effect of age and lifetime behavior on flight capacity in *D. melanogaster* revealed that flight capacity was affected by age and lifetime flight behavior, and had a combined effect on flight. Flight impairment reduced as age increased (Rhodenizer *et al.,* 2008 [35]; Yusuf *et al.,* 2024 [46]).

While most of the previous studies often focused on specific ages and distances, different ages concerning different intervals of distances were not taken into consideration, whereas the present study highlights different age and distance-specific patterns in locomotor behavior. These results align with earlier studies highlighting the gradual decline in motor abilities due to age-related neuromuscular function deterioration, which is essential for climbing (Cobb *et al.,* 2020 [12]; Wang, 2023 [42]).

Among the groups, virgin flies showed more climbing performance than mated groups. Males and females in all treatments showed significant variation within and between groups (Table 2). The effects of mating status with more time and individuals revealed that virgin males and females

exhibited more climbing ability compared to their mated counterparts, highlighting the physiological costs of reproduction on motor function (Flatt *et al.,* 2008) [16]. This was particularly evident in unequal mating (TM with SF and TF with SM) (Figures 3 and 4), where mated flies showed reduced climbing performance, likely due to the energy allocation toward reproduction rather than neuromuscular maintenance. Polyandry and multiple mating strategies have been shown to influence female fitness in reproduction rather than their physical performance across various insect species (Kvarnemo and Simmons, 2013 [28]; Shuster *et al.*, 2013 [38]). Males of *D. melanogaster* can mate 5 to 7 times per day (Douglas *et al.,* 2020) [14], maximizing their reproductive success by mating with multiple females, provided they have access to sufficient energy and resources (Dukas and Mooers, 2003 [15]; Lynn *et al.,* 2024 [30]). However, females mate only once or twice per day, largely due to the transfer of a sex peptide during mating that reduces their receptivity for several hours or days (Chapman *et al.*, 2003 [8]; Mackay *et al.*, 2005 [31]; Keleman *et al.*, 2012 [25]). While high mating frequencies in females can lead to fitness costs, such as reduced longevity (Billeter *et al.*, 2012) [5], their reproductive strategy generally favours fewer mating events unless required by specific environmental factors (Churchill *et al.,* 2019) [11]. Mating induces physiological and behavioral changes that lead to decreased locomotor activity (Harano *et al.*, 2007) [21]. In terms of sexual differences, male flies consistently outperformed female flies in climbing assays, regardless of treatment or age. Male *D. melanogaster* responds to exercise training with improved endurance, climbing speed, and flight ability (Sujkowski *et al.* 2017) [40]. Similar trends were noted in climbing speed across different species (Damschroder *et al.,* 2018 [13]; Cobb *et al.,* 2020 [12]; Moulin *et al.,* 2021 [33]).

Lithium is known for its potential anti-aging properties (Castillo-Quan *et al.,* 2016) [7]. LiCl treatments had significant effects on climbing performance (Table 2). Flies treated with low concentrations (15 mM) of LiCl demonstrated enhanced climbing ability, outperforming both the control group and those exposed to higher concentrations, suggesting that lithium at lower doses may improve motor function. However, higher concentrations (45 mM and 75 mM) were detrimental, emphasizing the dose-dependent effects of lithium on locomotion (Figures 3 and 4). Earlier studies showed contrasting results related to influence of lithium on aggressive behaviour varied based on treatment duration, acute treatments (5 mM to 50 mM for 1 hour) suppressed aggression in males, but did not significantly affect walking speed or climbing ability (Wang *et al.,* 2023) [42]. Apart from this, levodopa, used in Parkinson's treatment, impaired forced-climbing locomotion in both sexes of *D. melanogaster* (Moulin *et al.,* 2021) [33]. Castillo-Quan *et al.,* (2016) [7] and Jans, (2024) [23] studied the Glycogen synthase kinase-3 (GSK-3), Neurotrophic Growth Factor (DNGF), Dopamine transporter (DAT), Ras/mitogen-activated protein kinase (Ras/MAPK) and heat shock proteins to know the neuronal function and behavior on *D. melanogaster* and reported that lithium may serve as a therapeutic agent for improving motor functions including climbing activity.

Table 1: Effect of age, groups, distance and treatment on climbing efficiency of male and female flies corresponding to their time.

	Flies		**Time**		
	Male	**Female**	**Male**	**Female**	**df**
Age	184.623*	91.966*	355.595*	385.878*	2, 1919
Groups	277.657*	200.064*	455.884*	452.449*	3, 1919
Distances	162.490*	120.814*	3384.337*	3020.019*	3, 1919
Treatment	135.084*	68.112*	207.823*	250.140*	3, 1919
Age*Groups	1.129	0.467	2.445*	2.817*	6, 1919
Age* Distances	0.177	0.183	6.757*	4.192*	6, 1919
Age*Treatment	1.518	0.605	0.613	0.160	6, 1919
Groups * Distances	1.219	0.706	29.225*	4.958*	9, 1919
Groups * Treatment	2.957*	2.646*	2.074*	1.014	9, 1919
Distances * Treatment	0.690	0.205	2.017*	0.409	9, 1919
*Significant at p<0.05					

Table 2: Effect of age, groups, distance and treatment on overall climbing activity.

	Males groups		**Female groups**		
	F	**Wilk's Λ**	**F**	**Wilk's Λ**	**df**
Age	232.173	0.621*	214.343	0.642*	4, 3454
Groups	290.606	0.442*	269.246	0.464*	6, 3454
Distances	970.199	0.139*	890.364	0.154*	6, 3454
Treatment	149.339	0.630*	142.820	0.642*	6, 3454
Age*Groups	1.767	0.988*	1.633	0.989	12, 3454
Age* Distances	3.439	0.977*	2.182	0.985*	12, 3454
Age*Treatment	1.079	0.993	0.382	0.997	12, 3454
Groups * Distances	14.731	0.862*	2.819	0.971*	18, 3454
Groups * Treatment	2.517	0.974*	1.823	0.981*	18, 3454
Distances * Treatment	1.341	0.986	0.308	0.997	18, 3454

*Significant at *p<0.05*

Fig 1: Male and Female flies of *Drosophila melanogaster*.

Fig 2: Distance marked glass tube

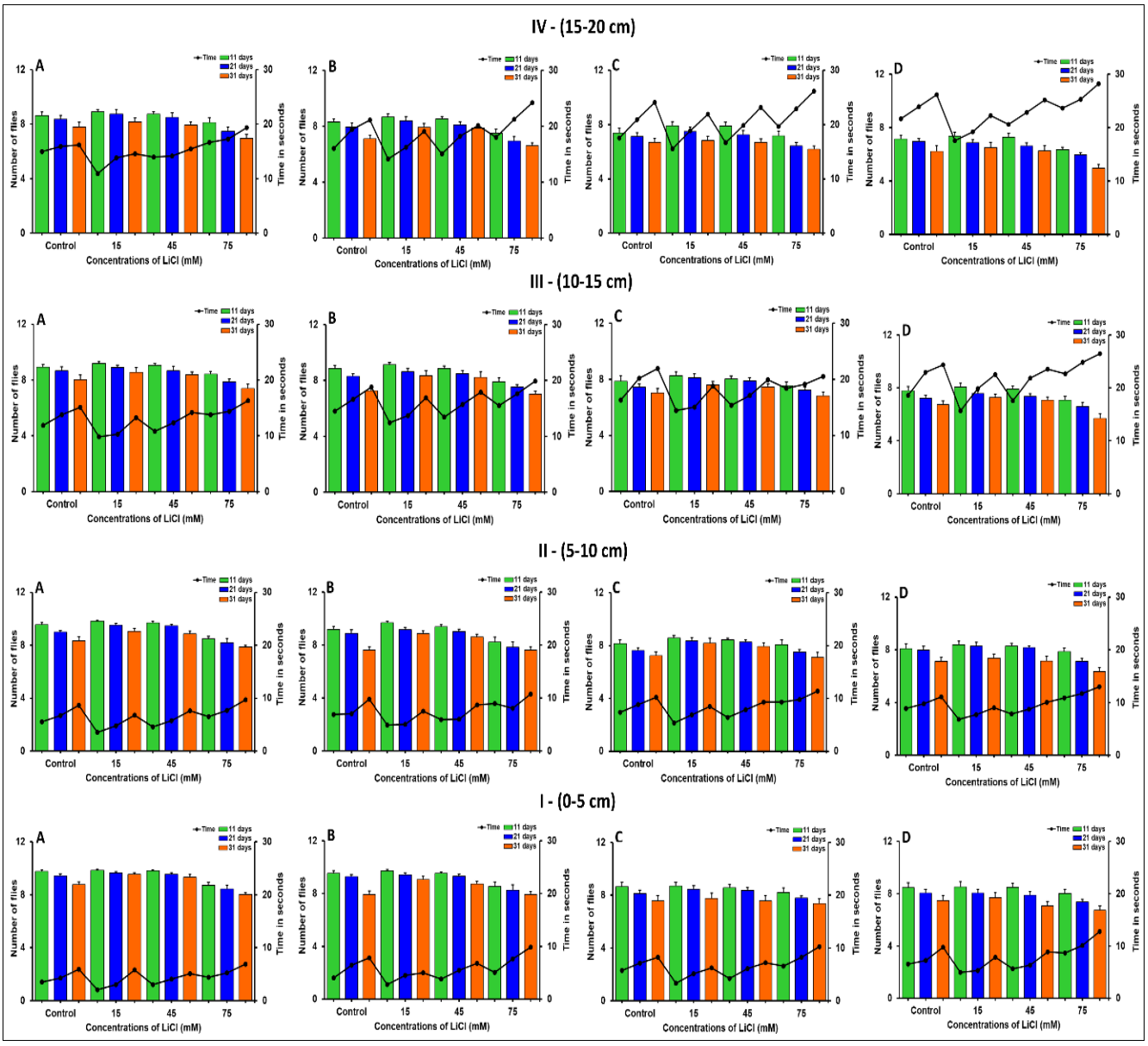

Fig 3: Climbing activity (number and time) of control and with different concentrations of LiCl treated male flies, A- VM, B- TM, C- MM, D- SM of all ages and distances (I- IV).

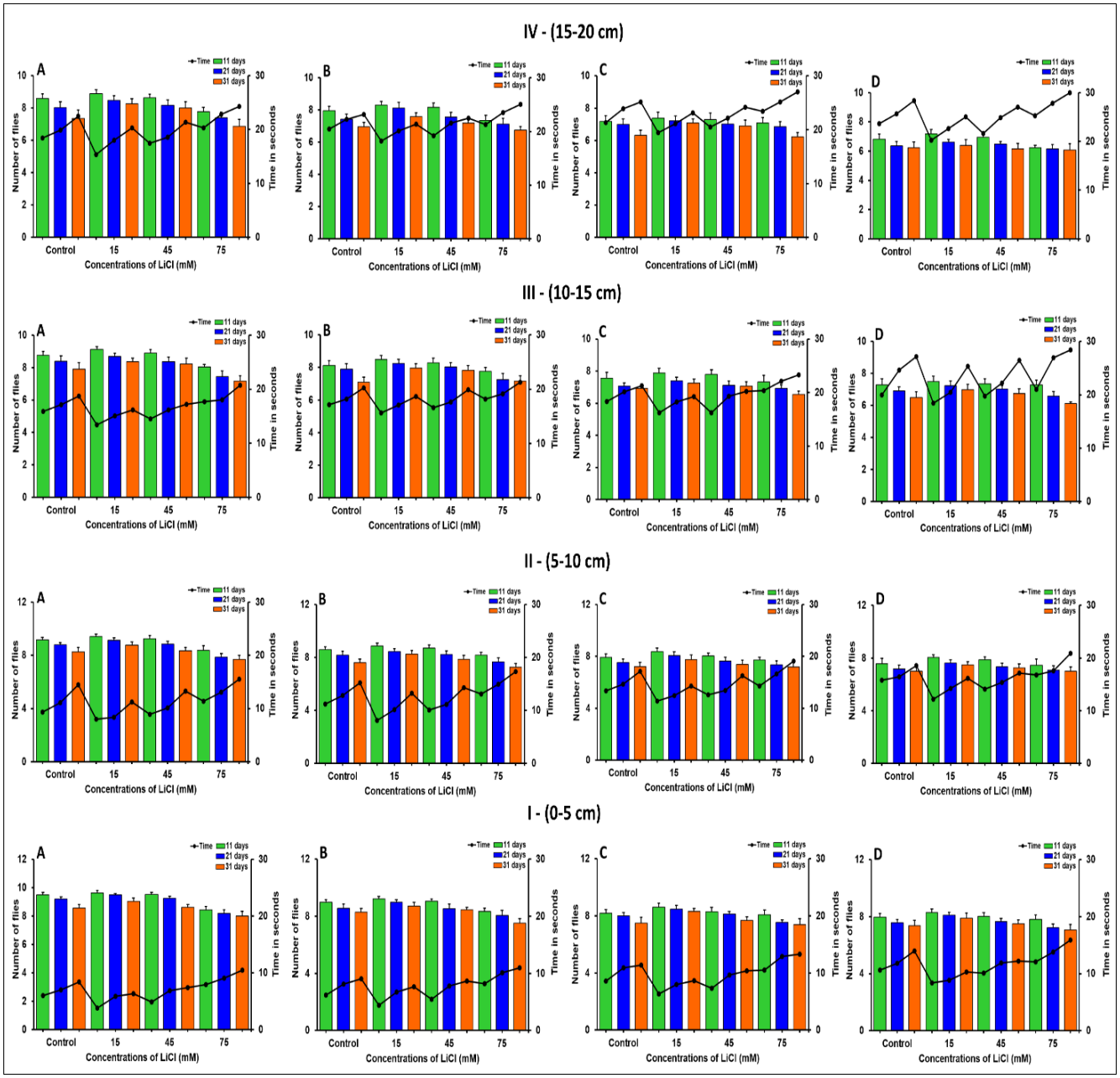

Fig 4: Climbing activity (number and time) of control and with different concentrations of LiCl treated female flies, A- VF, B- TF, C- MF, D- SF of all ages and distances (I- IV).

Conclusion

The present study revealed that, the climbing ability of *D. melanogaster* has influenced by age, Groups, and lithium chloride treatment. Virgin flies, especially males, showed better locomotor performance at low-dose lithium, while higher doses were detrimental. Apart from age and mating effect- related reductions, the low dose of lithium chloride treatment showed that more number of flies climbed specific distances within less time compared to control indicates that lithium chloride has influence on physical fitness of the flies. These results are important for understanding the effect of lithium on trade-offs in other studies.

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