



## Effect of Rolled Oats on Starvation Resistance of *Drosophila melanogaster*

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### Abstract

Stress is any alteration in the environment that decreases an organism's fitness. Diet and other external environmental factors can have an impact on environmental stress. For creatures to grow, develop, stay healthy, reproduce, and survive, they need food. The variation in starvation resistance is influenced by the type and amount of nutrients found in various diets. The current study shows effect of rolled oats on starvation withstanding ability in *Drosophila melanogaster* when it is cultivated in wheat cream agar media and mixed oats media of different concentrations. According to the findings, flies fed with wheat cream agar media were more resistant to starvation than those fed with 25%, 50% and 75% oats media. This demonstrated that increased oats concentration has negative effects to resist starvation compared to wheat cream agar media that provided better energy and nutrition to withstand famine. This experiment also showed that, female flies were more resistant to starvation than male flies. In all the diets, this study also depicted that, mated flies were significantly more resistant to starvation compared to unmated flies. The study suggested that the supplementation of oats along with the sooji in different proportions would not provide the sufficient energy and food storage to withstand the starvation for longer periods. Thus, the ability to resist starvation decreased in the *D. melanogaster* that fed with oats.

**Keywords:** Diet, Starvation resistance, *D. melanogaster*, Mated, Unmated (Virgins).

### Introduction

Starvation refers to an animal's ability to survive a lack of food. Most of the time, starvation might occur suddenly or gradually. For example, seasonal variations and a shortage of food supply can cause extended starvation, which can lead to animal death (McCue, 2010) <sup>[1]</sup>. Finding food is a challenge for many animal species, and an animal's reproductive fitness is closely linked to its foraging skills (Chippindale *et al.*, 1996; Wayne *et al.*, 2006) <sup>[2, 3]</sup>. Food scarcity is the most common environmental problem that animals face, and the length of time an animal can survive starvation depends on its diet and nutritional status. The nutritional basis of this resistance has not been thoroughly investigated, even though a great deal of research has been done on the ecology and evolutionary aspects of starvation resistance using fruit flies from the *Drosophila* genus (Hoffmann and Harshman, 1999; Rion and Kawecki, 2007) <sup>[4, 5]</sup>. Natural populations frequently experience a decrease in the availability of food resources (Zwaan *et al.*, 1991) <sup>[6]</sup>, and further research into evolution and its possible effects on human health is particularly interested in the physiological adaptations to starvation (Kapahi *et al.*, 2017; Piper *et al.*, 2014) <sup>[7, 8]</sup>. Since an animal's physiological ability to endure times without food is influenced by both its current nutritional state and previous feeding experiences, the nutritional quality of its diet has a significant impact on its resistance to starvation (Lee & Jang, 2014) <sup>[9]</sup>. According to Lee and Jang (2014) <sup>[9]</sup>, the main

factor affecting survival in *Drosophila* during starvation is the quantity of lipid reserves maintained in the body; however, the mechanisms underlying the different ratios of body components (proteins, carbs, and lipids) are still largely unidentified. The amount and quality of food consumed has a substantial impact on several life history traits, such as stress tolerance, reproductive success, and disease susceptibility (Kiran & Krishna, 2023) <sup>[10]</sup>. Studies in *Drosophila* flies cultured in different culture media showed varying resistance to starvation due to the quantity and quality of nutrient. According to Anusree *et al.*, 2024 <sup>[11]</sup>, Manaswini *et al.*, 2024 <sup>[12]</sup>, and Kiran K, Krishna M S, 2023 <sup>[10]</sup>, *Drosophila* flies showed increased resistance to starvation in mass gainer, whey protein and Jeeni millet mixed media respectively. In the following experiment since all the flies were cultured in the same environmental conditions except the diet, the variation to starvation was solely based on the difference in culture media.

Males and female flies respond differently to starvation conditions. Age, strain, mating status, and test conditions all affect the pattern of sexual dimorphism in starvation resistance in *D. melanogaster* (Service, 1989; Huey *et al.*, 2004; Vermeulen *et al.*, 2006; Matzkin *et al.*, 2009) <sup>[13-16]</sup>. Males consume body lipids as an energy source during starvation, but females use glycogen and body lipids, which are stored in large quantities, as an energy source. Because of their higher lipid reserves and lower metabolic rates, female

flies are often more resistant to famine than males. This allows them to endure longer periods of time when deprived of nutrients (Maklakov *et al.*, 2009; Schwasinger-Schmidt *et al.*, 2012) [17, 18]. Additionally, studies using artificial selection reveal that although both sexes can develop greater resistance to starvation, females frequently exhibit a more noticeable adaptation response, which is probably due to their higher energy requirements for reproduction (Chippindale *et al.*, 1996) [2].

*Drosophila* also exhibit distinct resistance to starvation with the context of mating. According to studies, mated female *D. melanogaster* are more resistant to hunger than unmated females. Mating-induced behavioural and physiological changes, such as improved metabolic efficiency and increased food intake. During mating, accessory gland proteins (ACPs), seminal fluid proteins (SFPs), especially sex peptide (SP), are transported to the female, causing modifications that increase food absorption and eating behaviour (Carvalho *et al.*, 2006; Ribeiro & Dickson, 2010) [19, 20]. In males, compared to mated flies, unmated flies are typically more tolerant to starvation. The energy and physiological costs of mating and courting activities are the main cause of this variation. Males require a lot of energy during mating, including the generation and transport of sperm and seminal fluid proteins (SFPs), both of which are energy-intensive (Wigby *et al.*, 2009) [21]. Furthermore, mated males frequently display higher levels of activity, including courtship behaviour and mating effort, which can further exhaust energy stores.

One of the ancient grains and food crops grown and consumed globally is oats. Oats (*Avena sativa* L.) are special among cereal crops because they include a variety of nutrients that are useful for human consumption, animal feed, medicine, and cosmetics (Butt *et al.*, 2008; Varma *et al.*, 2016) [22, 23]. It is one of the oldest crops known to human civilization (Lasztity, R. 1998) [24] and has been grown annually for over 2000 years in many parts of the world (Sang, S. and Chu, Y. 2017) [25]. Carbohydrates, dietary soluble fiber, balanced protein, lipids, various phenolic compounds, vitamins, and minerals are all abundant in this cereal (Joyce *et al.*, 2019) [26].

However, there is no published information on how eating oats affects an organism's capacity to withstand starvation or other environmental stresses. Thus, the purpose of this study was to determine how oats affect *D. melanogaster* ability to resist starvations

### Materials and Methods:

Rollled Oats (Gluten free) was purchased through Swiggy instamart app, Mysuru. This oat product was used to prepare the experiment media.

### Establishment of Stock

*D. melanogaster* flies (O K Strain) were collected from the

*Drosophila* stock centre, Dept. of studies in Zoology, University of Mysore, Manasagangotri, Mysuru. The flies were cultured in glass bottles with Wheat cream agar media. Flies were maintained in laboratory conditions with 22±1°C temperature, 12:12 light and dark cycle and humidity level of approximately 70% RH.

### Establishment of Experimental Stock

Flies obtained from the above-mentioned stocks were used to establish experimental cultures in various media.

**Wheat Cream Agar Media (Control Media):** 100g of jaggery, 100g of Rava powder (sooji) and 10g of agar in 1L of boiling distilled water and 7.5ml of propionic acid.

### Mixed Oats Media

**25% Oats Media:** 100g of jaggery, 75g of sooji, 25g of Rolled Oats powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

**50% Oats Media:** 100g of jaggery, 50g of sooji, 50g of Rolled Oats powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

**75% Oats Media:** 100g of jaggery, 25g of sooji, 75g of Rolled Oats powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

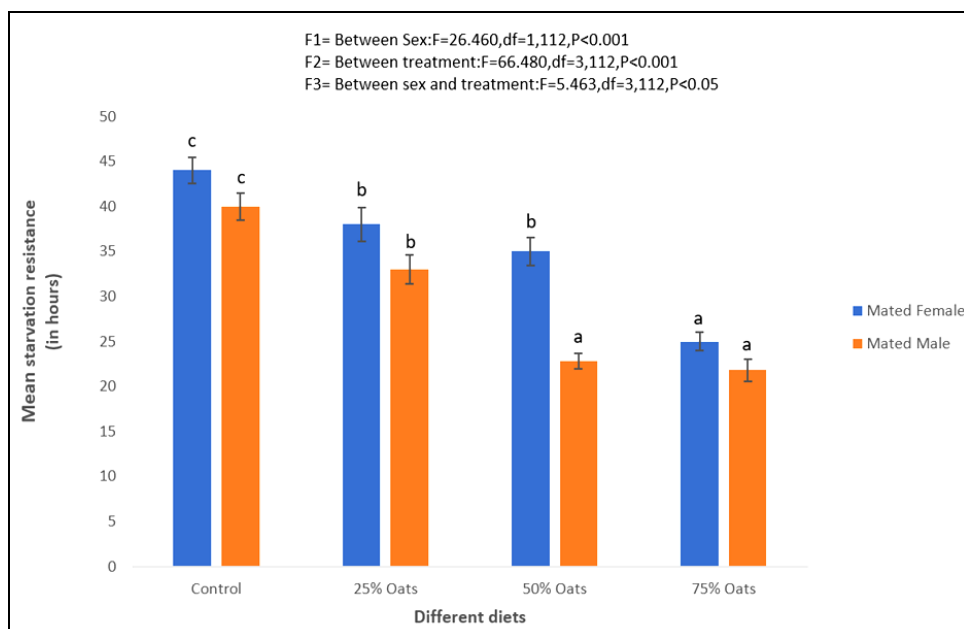
These flies were maintained under the laboratory condition mentioned above and used to study cold resistance in *D. melanogaster*.

### Starvation Resistance

Both unmated and mated five-day-old flies were taken from the control and mixed oats media to investigate starvation resistance. Fifteen male and female flies from each of the control, and mixed media were placed in empty vials, each containing five flies, and sealed with cotton. These vials were maintained in a laboratory setting, and each fly's resistance to starvation was noted every hour until its death. The tests for mated and unmated flies were conducted separately.

### Results

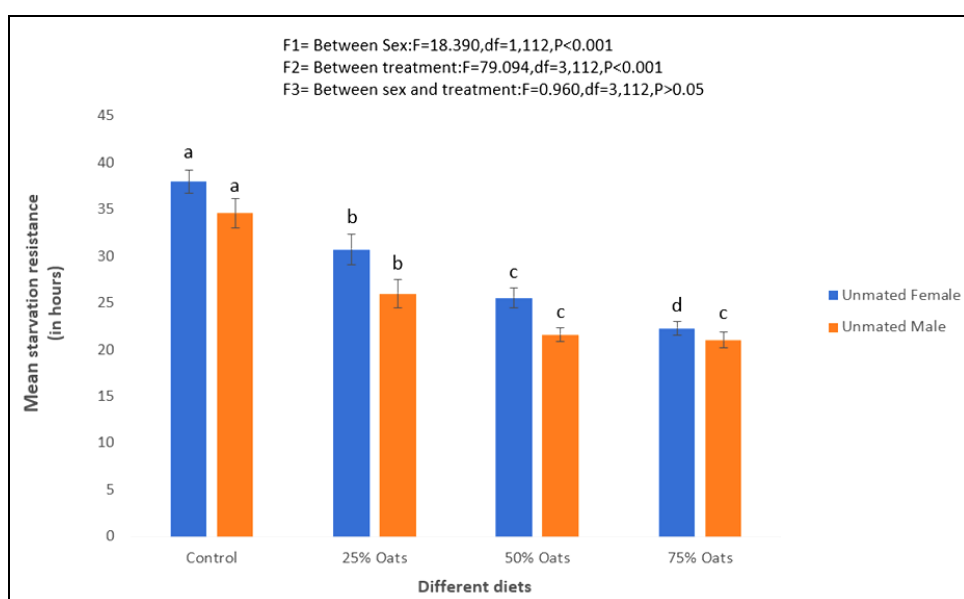
Figure 1 shows the mean and standard error value of starvation resistance in mated female and male flies raised in Wheat cream agar media and mixed oats media. According to this results, female flies exhibited higher levels of starvation resistance across all given diets than males. When compared to flies reared in mixed oats media, wheat cream agar medium had a higher level of starvation resistance. The mean starvation duration of mated male and female flies increased on a control media and gradually decrease across 25%, 50% and 75% oats media. The starvation resistance data that was subjected to a two-way ANOVA and then Tukey's Post hoc test. Significant difference in starvation resistance was observed between sex, treatment and sex\*treatment using Tukey's post hoc test.



**Fig 1:** Effect of oats on starvation resistance in mated female and mated male of *Drosophila melanogaster*. (The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

Figure 2 shows the mean and standard error value of starvation resistance for unmated female and unmated male flies that were raised in mixed oats media and wheat cream agar media (control). According to the study, unmated female flies were more resistant to starvation than unmated male flies in all diets. When compared to control media, flies raised in mixed oats media demonstrated lesser resistance to starvation. The starvation duration of unmated male and female flies

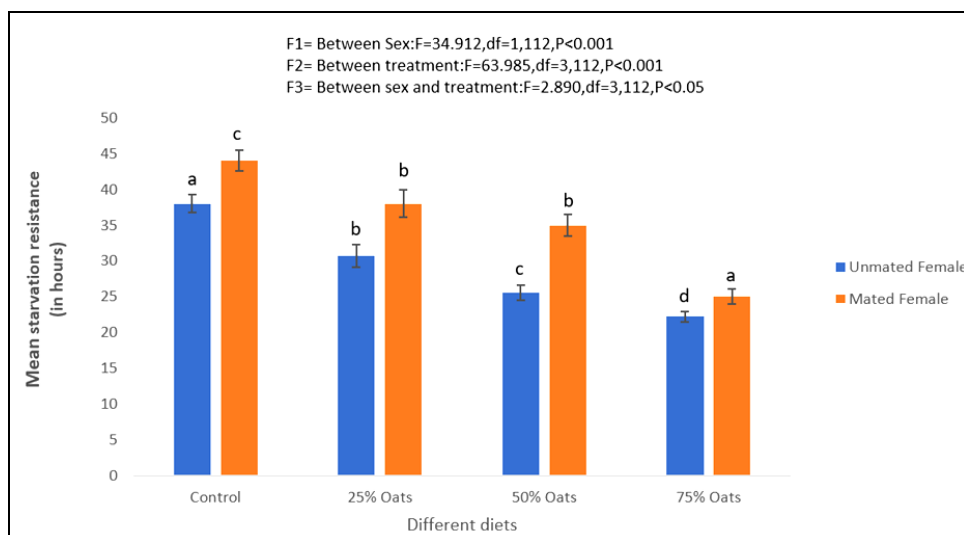
raised on control and mixed oats media varied significantly, according to the above starvation resistance data that was subjected to a two-way ANOVA and then Tukey's Post hoc test. Significant differences in starvation resistance were observed in the sex and treatment interaction. Significant differences in sex-specific starvation resistance were found using Tukey's post hoc test.



**Fig 2:** Effect of oats on starvation resistance in unmated female and unmated male of *Drosophila melanogaster*. (The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

Figure 3 shows the mean starvation resistance of unmated and mated female flies cultured in wheat cream agar and mixed oats media. Flies cultured in control media showed higher resistance to starvation compared to mixed oats media. Starvation resistance decreased with increasing concentration of oats in media. According to this study, unmated female flies raised in wheat cream agar media and mixed oats media showed poor tolerance to starvation than mated females. The above starvation resistance data given to two-way ANOVA

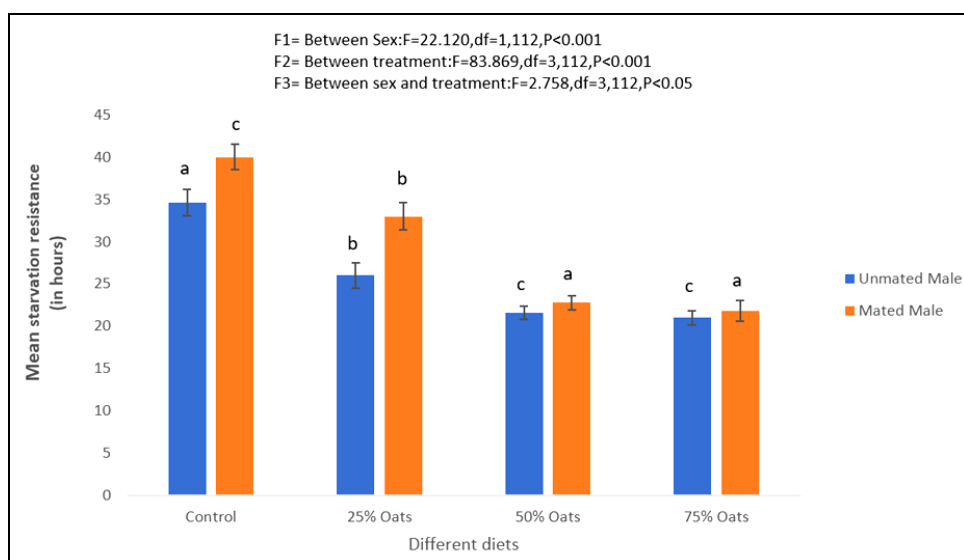
followed by Tukey's Post hoc test demonstrated significant variation in the starvation time it took for mated male and female flies grown in control and mixed oats media. Significant difference in starvation resistance was also observed when sex and treatment were combined. The starvation resistance of the Unmated and mated flies grown in control, mixed, and whey protein media varied significantly, according to Tukey's post hoc analysis.



**Fig 3:** Effect of oats on starvation resistance in unmated female and mated female of *Drosophila melanogaster*. (The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

Figure 4 shows the mean and standard error of starvation resistance in mated and unmated males, that were grown in wheat cream agar and mixed oats media. These results demonstrated that mated male flies grown in wheat cream agar and different concentrations of oats media had a higher level of starvation resistance than unmated male flies. The time it took for mated male and unmated male flies raised on control and mixed oats media to thrive in a food-deprived

condition varied significantly, according to the above starvation resistance data that subjected to a two-way ANOVA and then Tukey's Post hoc test. Significant differences in starvation resistance were also observed in the sex\*treatment interaction. The control and mixed oats media starvation resistance differed significantly, according to Tukey's post hoc analysis.



**Fig 4:** Effect of oats on starvation resistance in unmated male and mated male of *Drosophila melanogaster*. (The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

## Discussion

The current study found that flies raised in oat-mixed media had lower starvation resistance compared to those raised in wheat cream agar (control) media (Fig. 1–4). This implies that starvation resistance in *D. melanogaster* decreases gradually with increasing concentration of oat in culture media. Quality and nutrition of the diet play a significant role in determining starvation resistance (Clark and Keith, 2018; Zanco *et al.*, 2021) [27-28]. Since insects cannot obtain additional energy while starving, they must rely on previously stored energy reserves (Wong *et al.*, 2009) [29]. *Drosophila* can enhance tolerance to starvation in three primary ways: by tolerating the loss of a significant portion of their initial energy reserves, by storing more energy in the form of lipids, carbohydrates, and

proteins, or by regulating energy expenditure in accordance with metabolic rates and activity levels (Thimman *et al.*, 2010; Arrese and Soulages, 2010; Hoffmann *et al.*, 2001) [30-32]. According to studies, *D. melanogaster* shows enhanced lipid buildup in the form of TAGs is the cause of starvation resistance (Chippindale *et al.*, 1996; Harshman *et al.*, 1998) [2, 33]. Increased oat content decreases cholesterol levels in mammals (Anderson, *et al.* 1990; Davidson, *et al.*, 1991; Kirby, *et al.*, 1981; Shinnick, *et al.*, 1990; Guo, *et al.*, 2014; Tong, *et al.*, 2015; Braaten, *et al.*, 1994; Hicks, *et al.*, 1995) [34-41] and lowered lipid levels in nematode model-*C. elegans* (Gao C, *et al.*, 2015) [42]. Solubility of OBG, rather than amount is a driving component to decrease cholesterol (Beer *et al.* 1995) [43]. These might be the probable reason for



decreasing lipid content in oat fed flies that resulted in reduced resistance to starvation.

By adjusting the temperature between 18 and 23 degrees Celsius, Jang and Lee (2018) [44] investigated the impact of temperature on *D. melanogaster*'s resistance to starvation and discovered that exposure to high temperatures during starvation decreased resistance. This is brought on by a higher metabolic rate and quicker lipid level reduction. In *D. melanogaster*, Prakash *et al.* (2014) [45] showed that higher humidity (85% RH) boosts starvation resistance while lower humidity (35% RH) decreases both starvation resistance and body lipid levels. Since the flies were kept at a constant temperature (22±1°C) and humidity level (about 70%), the results of this study indicate that neither temperature nor humidity significantly affects the flies' ability to withstand starvation.

The current study findings, Fig. 1 & 2 demonstrated that female flies were more resistant to starvation than male flies in control and mixed oats diets and average starvation resistance decreased significantly ( $P < 0.001$ ) with increase in the oats concentration. Variations in energy metabolism during starvation may be the cause of this. Males and females require distinct diets in many creatures. This results in sex-specific differences in starvation resistance (Hoyenga and Hoyenga, 1982) [46]. Age, strain, mating status, and test conditions all affect the pattern of sexual dimorphism in starvation resistance in *D. melanogaster* (Service, 1989; Huey *et al.*, 2004; Vermeulen *et al.*, 2006; Matzkin *et al.*, 2009) [47-50]. Males consume body lipids as an energy source during starvation, but females utilize both glycogen and body lipids, which are typically stored in larger quantities, to meet energy demands under nutrient-deprived conditions (Zwaan *et al.*, 1991; Karpac and Jasper, 2009) [6, 51]. Increased energy storage or a slower rate of decomposition of these reserves are associated with improved resistance to starvation in females. (Hoffmann and Harshman, 1999; Rion and Kawecki, 2007; Gibbs and Reynolds, 2012) [4, 5, 52]. In addition to variations in the composition of a particular tissue/compound that facilitate energy acquisition, sex-specific starvation resistance may also arise from differences in how that different sexes are capable of using specific tissue/compounds for energy acquisition (Aggarwal, 2014) [53].

The results of this study (Fig. 3) demonstrate that mated female flies raised in control media and oat mixed media exhibited greater resilience to starvation than unmated female flies. Because they consume more food, mated females accumulate more lipids than unmated females (Carvalho *et al.*, 2006) [19]. This is because mating causes females to consume more food, become less sexually receptive, and produce more eggs. Sex-peptides from male seminal fluid are the cause of these effects (Chen *et al.*, 1988; Herndon and Wolfner, 1995) [53, 54]. In mated females, accessory gland proteins (Acps) also alter behaviour and physiology (Gillot, 2003) [55]. The physiological linkages linked with starvation are also influenced by a wide range of internal and external factors, including temperature, genetic variety, age, social interactions, diet, and others (Vermeulen *et al.*, 2006; Pijpe *et al.*, 2007; Rush *et al.*, 2007; Lee and Jang, 2014) [56-58, 9].

The results of the study (Fig. 4) showed that mated male flies in oat mixed media and wheat cream agar media were more resistant to starvation than mated males. Mating also affects *D. melanogaster* resilience to starvation (Service, 1989; Rush *et al.*, 2007; Goenaga *et al.*, 2012) [47, 58, 59]. The seminal fluid, a complex protein mixture that the accessory gland produces and delivers to the female, is secreted following copulation

(Wolfner, 2002) [60]. Some studies have found that mating can enhance starvation resistance in males, possibly due to hormonal or behavioral priming that prepares them for post-mating resource scarcity (McKean & Nunnery, 2005) [61]. Another study supports that pheromones produced by female flies during copulation may expose mated male flies to various stimuli continuously, altering their physiology and maybe increasing their resistance to starvation compared to virgin males. Zajitschek *et al.* (2016) [63] found that exposure to females or mating opportunities can trigger changes in males that improve stress resistance, possibly through neuroendocrine signalling pathways that are activated during courtship and copulation.

## Conclusion

Thus, we can infer from this study that rolled oats reduced starvation resistance in *D. melanogaster*. Also, compared to mated male flies, female mated flies were more resistance to starvation. Additionally, in every diet examined, mated flies exhibited noticeably higher levels of starvation than unmated flies.

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