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Role of Nutritional and Photoperiodic Factors in Regulating Physiological Activities of Drosophila melanogaster

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Abstract- Drosophila melanogaster serves as an indispensable in vivo model system for mapping how external environmental variations and nutritional inputs dictate physiological adaptations and behavioural choices. This study presents a multi-generational evaluation of how dietary variance (standard cornmeal vs. banana-enriched vs. orange-enriched media) pairs with photoperiodic conditions to modulate ontogeny, locomotor agility, and larval chemotaxis. Across three successive generations (F1–F3), cohorts reared on a nutrient-dense banana medium exhibited accelerated metamorphic transitions and robust pupation rates. Conversely, an orange-supplemented diet delayed developmental milestones and reduced total yield compared to uniform controls. Photoperiodic restrictions (sustained dark phases) consistently decelerated growth metrics and decreased motor output across all dietary groups. Quantifiable behavioural deficits under low-light regimes were verified via negative geotaxis assays, where light-exposed flies displayed markedly superior vertical climbing performance. Furthermore, larval olfactory assays revealed a stark chemotactic bias toward volatile food-derived attractants (ethyl acetate) over aversive ionic stimuli (sodium chloride). Taken together, these data illuminate the complex interplay between systemic metabolic programming and sensory-driven behavioural phenotypes in response to immediate ecosystem shifts.

Keywords- Drosophila melanogaster, diet, photoperiod, locomotor activity, olfactory behaviour, negative geotaxis.

I. INTRODUCTION

The common fruit fly, Drosophila melanogaster, has anchored experimental genetics, neurobiology, and developmental physiology since the seminal chromosomal inheritance studies pioneered by Thomas Hunt Morgan (Morgan, 1910). The utility of this model system stems from a highly optimized biological profile: a compressed 12 to 14 day lifespan, prolific reproductive yields, minimal maintenance logistics, and ethically unencumbered culture scalability (Ashburner et al., 2005). Crucially, approximately 60% to 70% of human disease-linked genetic loci share functional homologs within the Drosophila genome, making the insect a premier surrogate for dissecting foundational metabolic pathways, signalling cascades, and neurodegenerative mechanisms (Rubin & Lewis, 2000; Pandey & Nichols, 2011).

Drosophila is a holometabolous insect, navigating distinct metamorphic shifts across four life stages: the embryo, three distinct larval instars, a sessile pupal phase, and the mature adult (Ashburner et al., 2005). Because it is an ectothermic organism, its internal metabolic rate scales tightly with microclimate temperature, exhibiting optimized reproductive kinetics, (Klepsatel et al., 2023). Within the culture, sexual dimorphism manifests clearly through external anatomical readouts. Mature females display elongated, tapered, and lightly pigmented posterior abdomens to support substantial ovarian volume, whereas males feature a smaller, blunt abdomen capped with fused, dark cuticular segments (Ashburner et al., 2005; Greenspan, 2004). Structurally, males are explicitly characterized by sex combs—a specialized row of rigid, dark bristles on the first tarsal segment of the forelegs used to secure the female during complex courtship rituals (Hall, 1994).

At the molecular level, sex determination operates independently of a dominant Y-chromosome master switch, relying instead on the ratio of X-chromosomes to autosomes (X:A ratio), where a 1.0 ratio directs female differentiation and a 0.5 ratio drives male development (Gilbert, 2014). This chromosomal equilibrium governs downstream courtship

pathways and sensory-motor dynamics (Spieth, 1974; Greenspan & Ferveur, 2000).

Beyond wild-type paradigms, discrete mutations offer clear windows into pigment synthesis and tissue morphogenesis. For example, cuticular colour variants like black (b) and yellow (y) trace back to recessive disruptions in melanin synthesis pathways like the ebony and yellow genes (Wittkopp et al., 2002; True et al., 2005; Massey et al., 2016). Eye colour mutations, notably the classic X-linked recessive white (w), underscore critical ATP-binding cassette transporter defects that block ommatidial pigment deposition (Morgan, 1910; Mackenzie et al., 2000). Similarly, structural mutations like vestigial (vg) and dumpy (dp) reveal severe mechanical signalling defects within wing imaginal discs and cellular matrices (Williams et al., 1991; Wilkin et al., 2000).

Because larval development is energetically taxing, the precise nutritional profile of the culture medium acts as a major upstream driver of fitness, growth trajectories, and longevity (Lee et al., 2008). While standard laboratory protocols rely on consistent cornmeal-yeast-agar recipes (Lüersen & Rimbach, 2019), incorporating varied fruit extracts allows researchers to simulate natural foraging ecosystems, offering key insights into behavioural adaptations and systemic metabolic strain (Markow & O'Grady, 2006).

While existing literature thoroughly documents isolated dietary or photoperiodic impacts, integrated studies evaluating their combined, transgenerational influence on larval chemotaxis, adult motor function, and life-cycle kinetics remain sparse. This investigation addresses that gap by assessing how varied fruit media and photoperiodic shifts concurrently shape developmental timing and multi-generational behavioural profiles in Drosophila melanogaster.

II. MATERIALS AND METHODS

The experimental work for this study was carried out at Drosophila Resource Centre, Department of Zoology, Daulat Ram College, University of Delhi, New Delhi, India.

Strain Maintenance and Medium Composition

All experimental assays used healthy, stable stocks of a yellow-body mutant strain of Drosophila melanogaster. The baseline control media was synthesized by heating distilled water, agar, cornmeal, and sugar to establish a uniform matrix, followed by the addition of active yeast at roughly 55 degree celsius to maintain maximum vitamin and sterol viability.

To evaluate distinct nutritional pressures, three separate dietary tracks were maintained: Control (C): Unsupplemented standard cornmeal matrix.

Banana Diet (B): Standard medium enriched with a liquefied, fresh ripe banana homogenate. Orange Diet (O): Standard medium enriched with a fresh orange juice extract.

Experimental Setup and Environmental Manipulations

The experimental matrix was distributed across clean glass culture vials, organized into pairs of duplicates for each nutritional variable (designated as Ctrl 1/2, BAB 1/2, and ORJ 1/2). Founder generations were established by introducing three virgin females and two mature males into each vial, allowing uninhibited mating and egg-laying. Environmental variations were introduced by wrapping half of the treatment vials in dense black matte paper to create a continuous low-light environment, while the matching halves remained uncovered under the ambient diurnal light cycles of the laboratory.

Ontogenetic Monitoring and Behavioural Assays

Embryonic progression and population counts across distinct life stages (eggs, L1, L2, L3 instars, prepupae, pupae, and emerged adults) were tracked and recorded daily across three distinct generations (F1, F2, F3).

Negative Geotaxis

Adult motor function was measured using a custom-built negative geotaxis climbing chamber. Ten active, unanesthetized flies from the F2 generation were transferred into a clean, vertically oriented glass tube. After a gentle downward tap mechanical disturbance to concentrate the flies at the base, an electronic timer was started. The number of individuals that successfully scaled an 8 cm baseline within a 10-second window was recorded. This protocol was repeated across three separate trials to calculate definitive mean performance values.

Larval Chemotaxis Assay

To evaluate larval sensory processing, 15 active third-instar (L3) larvae were harvested from the F2 generation using a soft brush. Testing took place in clean Petri dishes divided into two distinct chemical zones: one side received an application of volatile ethyl acetate (functioning as a fruit-mimicking

attractant), while the opposite side was treated with a localized deposit of sodium chloride (NaCl, serving as an ionic control). The larvae were placed precisely at the centre of the dish and allowed to move freely for 5 minutes. Afterward, direct physical counts determined the final alignment of the larvae toward each stimulus.

III. RESULTS AND DISCUSSION

Nutritional Modulation of Ontogenetic Progress

Daily monitoring demonstrated that the chemical profile of the culture medium acts as a primary driver of developmental timing. Cohorts reared on the banana extract medium (BAB) consistently showed accelerated growth rates, reaching larval and pupal milestones significantly ahead of the other groups. Conversely, flies reared on the acidic, orange- supplemented medium (ORJ) faced development delays and lower total survival rates. The standard cornmeal control groups (CTRL) maintained a predictable, moderate growth timeline.

The faster growth observed in the banana-enriched cohorts likely stems from its dense, accessible carbohydrate layout and supportive sugars, which provide the high metabolic energy required during active larval feeding stages. This aligns with prior observations by Eickelberg et al. (2022), which noted that well-balanced, sugar-rich natural substrates optimize metabolic homeostasis and enhance reproductive fitness. Conversely, the growth delays seen on the orange diet suggest that high acidity or suboptimal sugar-to-protein ratios can disrupt larval digestion, extending the time needed to reach critical pupariation weight thresholds (Chattopadhyay et al., 2015).

			BAB 1	BAB 2	CTRL 1	CTRL 2	ORJ 1	ORJ 2
Day 1	18.02.2026							
Day 2	19.02.2026	No. of eggs	230	240	215	216	115	120
		L1	35	40	38	36	20	25
Day 3	20.02.2026	No. of eggs	150	145	130	135	80	85
		L1	85	80	70	75	40	45
		L2	40	45	35	38	25	28
		L3 (feeding)	5	8	3	5	2	3
Day 4	21.02.2026	No. of eggs	140	138	140	145	90	92
		L1	30	25	35	30	20	18
		L2	65	60	55	58	35	38
		L3(feeding)	40	45	30	32	25	28
Day 5	22.02.2026							
Day 6	23.02.2026	No. of eggs	45	50	40	42	28	30
		L1	10	12	8	9	5	6
		L2	30	35	28	30	18	20
		L3 (feeding)	65	70	55	60	35	38
		L3 (wandering)	50	55	40	45	25	28
		Prepupa	15	18	12	15	8	10
Day 7	24.02.2026	No. of eggs	25	28	22	25	15	18
		L1	5	6	4	5	2	3
		L2	20	22	18	20	10	12
		L3(feeding)	45	50	40	45	25	28
		L3(wandering)	55	60	45	50	30	32
		Prepupa	25	28	20	22	12	15
		Pupa	8	10	6	8	4	5
Day 8	25.02.2026	No. of eggs	0	5	8	10	3	4
		L1	11	4	5	6	2	3
		L2	12	8	10	12	6	7
		L3(feeding)	6	12	6	20	10	12
		L3(wandering)	21	18	7	12	15	18

		Prepupa	22	22	42	75	25	6
		Pupa	0	0	0	3	0	0
Day 9	26.02.2026	No. of eggs	0	0	0	0	0	0
		L1	14	1	2	3	1	1
		L2	2	4	6	8	3	4
		L3(feeding)	14	6	10	12	5	6
		L3(wandering)	6	10	15	20	8	10
		Prepupa	20	12	42	71	23	9
		Pupa	1	1	4	21	0	1
Day 10	27.02.2026	No. of eggs	6	22	31	30	15	32
		L1	2	3	4	5	2	3
		L2	2	4	6	8	4	5
		L3(feeding)	3	6	10	12	6	8
		L3(wandering)	5	8	12	15	8	10
		Prepupa	7	12	39	79	18	12
		Pupa	13	11	8	21	1	14
		Adults (F1)	1	1	6	14	0	0
Day 11	28.02.2026							
Day 12	01.03.2026							
Day 13	02.03.2026	No. of eggs	5	18	28	26	12	20
		L1	1	2	3	4	2	2
		L2	2	3	5	6	3	4
		L3(feeding)	3	5	8	10	5	6
		L3(wandering)	4	7	10	12	6	8
		Prepupa	9	15	42	82	20	14
		Pupa	15	13	10	24	2	16
		Adults (F1)	2	2	8	16	1	1

Interaction of Nutritional Profiles and Photoperiodic Constraints

When photoperiodic variations were introduced via dark-vial masking, development delayed across all dietary tracks. Flies raised in open, light-accessible vials progressed through metamorphic checkpoints uniformly. In contrast, those under constant dark conditions showed notable development lag and lower overall yields, a trend that persisted across successive generations.

			BAB 1 (with cover)	BAB 2 (without cover)	CTRL 1 (with cover)	CTRL 2 (without cover)	ORJ 1 (with cover)	ORJ 2 (without cover)
Day 1	11.03.2026	No. of eggs	220	235	210	215	110	120
Day 2	12.03.2026	No. of eggs	180	200	170	175	95	100
		L1	40	45	38	40	20	22
		L2	20	25	18	20	10	12
		L3 (feeding)	10	15	8	10	5	6
Day 3	13.03.2026	No. of eggs	120	130	110	115	70	75

		L1	60	65	55	58	30	32
		L2	40	45	35	38	20	22
		L3(feeding)	20	25	18	20	10	12
Day 4	14.03.2026							
Day 5	15.03.2026							
Day 6	16.03.2026	No. of eggs	20	25	18	20	10	12
		L1	10	12	8	10	5	6
		L2	20	25	18	20	10	12
		L3 (feeding)	40	45	38	40	20	22
		L3(wandering)	30	35	28	30	15	18
		Prepupa	18	22	15	18	10	12
Day 7	17.03.2026	No. of eggs	10	15	8	10	5	6
		L1	5	6	4	5	2	3
		L2	10	12	8	10	5	6
		L3(feeding)	25	30	22	25	12	15
		L3(wandering)	28	32	25	28	15	18
		Prepupa	22	26	18	22	12	14
		Pupa	10	12	8	10	4	5
Day 8	18.03.2026	No. of eggs	5	8	4	5	2	3
		L1	2	3	2	3	1	1
		L2	5	6	4	5	2	3
		L3(feeding)	15	18	12	15	8	10
		L3(wandering)	20	22	18	20	10	12
		Prepupa	25	28	22	25	14	16
		Pupa	18	22	15	18	8	10
Day 9	19.03.2026							
Day 10	20.03.2026							
Day 11	21.03.2026							
Day 12	22.03.2026							

Day 13	23.03.2026	No. of eggs	2	4	2	3	1	1
		L1	1	1	1	1	0	1
		L2	2	3	2	2	1	1
		L3 (feeding)	8	10	6	8	4	5
		L3 (wandering)	10	12	8	10	5	6
		Prepupa	15	18	12	15	8	10
		Pupa	22	26	20	24	12	14
		Adults	6	8	5	6	2	3
Day 14	24.03.2026	No. of eggs	1	2	1	1	0	1
		L1	0	1	0	1	0	0
		L2	1	2	1	1	0	1
		L3 (feeding)	5	6	4	5	2	3
		L3 (wandering)	6	8	5	6	3	4
		Prepupa	10	12	8	10	5	6
		Pupa	25	30	22	26	14	16
		Adults	10	12	8	10	4	5
Day 15	25.03.2026	No. of eggs	0	1	0	1	0	0
		L1	0	0	0	0	0	0
		L2	1	1	1	1	0	1
		L3 (feeding)	3	4	3	3	1	2
		L3 (wandering)	4	5	4	4	2	3
		Prepupa	6	8	5	6	3	4
		Pupa	28	32	25	28	16	18
		Adults	15	18	12	14	6	7
Day 16	27.03.2026	No. of eggs	0	0	0	0	0	0
		L1	0	0	0	0	0	0
		L2	0	1	0	1	0	0
		L3 (feeding)	2	3	2	2	1	1
		L3	3	4	3	3	1	2

		(wandering)						
		Prepupa	4	5	3	4	2	3
		Pupa	30	35	28	30	18	20
		Adults	20	25	18	20	8	10
Day 17	28.03.2026							
Day 18	29.03.2026							
Day 19	30.03.2026	No. of eggs	3	5	2	3	1	2
		L1	1	2	1	1	0	1
		L2	1	2	1	1	0	1
		L3 (feeding)	2	3	2	2	1	1
		L3 (wandering)	2	3	2	2	1	1
		Prepupa	2	3	2	2	1	1
		Pupa	15	18	12	14	8	10
		Adults	28	32	24	28	12	15
Day 20	31.03.2026							
Day 21	01.04.2026	No. of eggs	8	12	6	8	3	5
		L1	3	4	2	3	1	2
		L2	2	3	2	2	1	1
		L3 (feeding)	1	2	1	1	0	1
		L3 (wandering)	1	2	1	1	0	1
		Prepupa	1	2	1	1	0	1
		Pupa	10	12	8	10	5	6
		Adults	32	36	28	32	15	18
Day 22	02.04.2026	No. of eggs	12	16	10	12	5	7
		L1	5	6	4	5	2	3
		L2	3	4	3	3	1	2
		L3 (feeding)	2	3	2	2	1	1
		L3 (wandering)	1	2	1	1	0	1
		Prepupa	1	1	1	1	0	0

		Pupa	6	8	5	6	3	4
		Adults	35	40	30	35	18	20
Day 23	03.04.2026							
Day 24	04.04.2026							
Day 25	05.04.2026							
Day 26	06.04.2026	No. of eggs	18	22	15	18	8	10
		L1	8	10	6	8	3	4
		L2	5	6	4	5	2	3
		L3 (feeding)	3	4	3	3	1	2
		L3 (wandering)	2	3	2	2	1	1
		Prepupa	1	2	1	1	0	1
		Pupa	4	5	3	4	2	3
		Adults	34	38	30	34	18	20
Day 27	07.04.2026	No. of eggs	22	26	18	22	10	12
		L1	10	12	8	10	4	5
		L2	6	8	5	6	3	4
		L3 (feeding)	4	5	3	4	2	2
		L3 (wandering)	3	4	2	3	1	2
		Prepupa	1	2	1	1	0	1
		Pupa	3	4	2	3	1	2
		Adults	32	36	28	32	17	19
Day 28	08.04.2026	No. of eggs	25	30	20	25	12	15
		L1	12	15	10	12	5	6
		L2	8	10	6	8	3	4
		L3 (feeding)	5	6	4	5	2	3
		L3 (wandering)	3	4	3	3	1	2
		Prepupa	1	1	1	1	0	0
		Pupa	2	3	2	2	1	1
		Adults	30	34	26	30	15	18

Day 29	09.04.2026	No. of eggs	28	34	24	28	15	18
		L1	15	18	12	15	6	8
		L2	10	12	8	10	4	5
		L3 (feeding)	6	8	5	6	3	4
		L3 (wandering)	4	5	3	4	2	2
		Prepupa	1	1	1	1	0	0
		Pupa	1	2	1	2	0	1
		Adults	28	32	24	28	14	16
Day 30	10.04.2026	No. of eggs	30	36	26	30	18	20
		L1	18	22	15	18	8	10
		L2	12	15	10	12	5	6
		L3 (feeding)	8	10	6	8	4	5
		L3 (wandering)	5	6	4	5	2	3
		Prepupa	1	1	1	1	0	0
		Pupa	1	1	1	1	0	1
		Adults	25	30	22	25	12	15

This multi-generational slowdown highlights the key role photoperiod plays in stabilizing the fruit fly's internal molecular clock. Long-term light restriction likely disrupts downstream metabolic and endocrine signalling, such as ecdysteroid synthesis, slowing growth timelines (Ruchitha et al., 2024).

Behavioural Output: Negative Geotaxis Assays

The negative geotaxis assays demonstrated clear behavioural differences under different light regimes. Across all dietary backgrounds, flies maintained in open, light-accessible vials consistently outpaced their dark-reared counterparts in climbing performance.

These climbing data show that consistent light exposure supports higher locomotor activity and enhances behavioral readiness. Chronic dark rearing appears to dull sensory responsiveness or reduce overall baseline movement, likely due to diminished visual and structural stimulation (Karageorgi et al., 2023). Furthermore, the subtle differences in climbing success between diets highlight how nutrition supports motor function, likely by optimizing cellular energy reserves and muscular performance (Eickelberg et al., 2022).

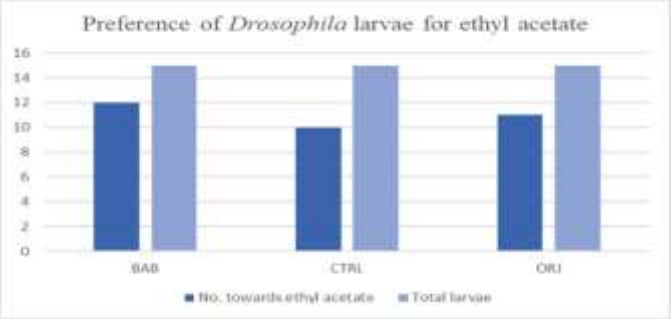
Treatment	Condition	Total Flies	Trial 1	Trial 2	Trial 3	Average
BAB 1	With cover	10	7	5	6	6
BAB 2	Without cover	10	8	7	6	7
CTRL 1	With cover	10	7	6	8	7
CTRL 2	Without cover	10	9	8	6	8
ORJ 1	With cover	10	7	5	6	6
ORJ 2	Without cover	10	8	7	9	7

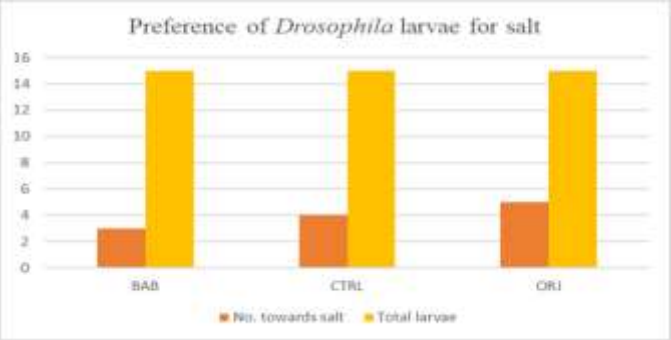
Larval Olfactory Preferences

Chemotactic assays highlighted the advanced sensory discrimination present during early larval stages. Third-instar larvae showed a distinct directional preference for volatile ethyl acetate over sodium chloride.

Banana Culture (BAB): 12 larvae aligned with ethyl acetate, 3 toward salt (n=15) Standard Culture (CTRL): 10 larvae aligned with ethyl acetate, 5 toward salt (n=15) Orange Culture (ORJ): 11 larvae aligned with ethyl acetate, 4 toward salt (n=15)

	No. towards ethyl acetate	No. towards salt	Total larvae
BAB	12	3	15
CTRL	10	5	15
ORJ	11	4	15





This strong attraction to ethyl acetate reflects an innate evolutionary drive to locate fermenting wild fruits, which serve as primary feeding and egg-laying sites (Dweck et al., 2013). Conversely, the neutral or avoidant reaction to high-concentration sodium chloride suggests it lacks appealing chemical signals or functions as a mild behavioural deterrent, confirming that Drosophila larvae rely heavily on volatile chemical cues to navigate their foraging environments (Lihoreau et al., 2016).

IV. CONCLUSION

This study demonstrates how nutritional profiles and photoperiodic conditions interact to shape the development and behaviour of Drosophila melanogaster. Enriched banana media consistently accelerated growth and boosted survival rates, whereas orange-supplemented media introduced noticeable development lag. Ambient light exposure proved vital for maintaining normal metabolic timelines and maximizing locomotor performance, as quantified via negative geotaxis testing. Additionally, larval chemotaxis assays confirmed a clear behavioural bias toward fruit-associated volatile odours over chemical deterrents.

Future work should investigate the underlying neuro-endocrine pathways and gene expression shifts that drive these changes. Such molecular insights will expand our understanding of evolutionary environmental adaptation and metabolic resource management.

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