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Effect of developmental nutrition on morphometric variations and phenotypic plasticity in fruit fly, *Drosophila malerkotliana*

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***Abstract:*Dietary macronutrient composition is one of the key ecological factors that influence development, behaviour, physiology and fitness in any organism. In the present study, we have studied the effect of high yeast: low sugar and low yeast: high sugar diet on sexual and non-sexual morphological traits in fruit fly, *Drosophila malerkotliana* using isofemale lines. The present study also analyzes, how genetic and phenotypic variation influences dietary environments at the time of development in *D. malerkotliana.* The genetic basis of plasticity has been tested for the concerned traits. The traits showed varied response to different diet in a sex-dependent manner. Phenotypic variability in the form of coefficient of variation was estimated for each trait and diet, revealing greater phenotypic variation in both sexes for traits in low yeast: high sugar. Genetic variation as intraclass correlation, between line variance, evolvability was estimated. The estimates showed increasing trendin low yeast: high sugar in females for all the traits while, in males decreasing values were found for low yeast: high sugar diet when compared to high yeast: low sugar and standard diet. The norms of reaction graphs plotted for different traits revealed significant genotype-by-diet interaction for each trait, thus showing varied phenotypic plasticity among genotypes.**

Index Terms:*environmental nutrition, morphological traits, phenotypic plasticity,*Drosophilamalerkotliana

I. INTRODUCTION

In the natural environment, organisms are subjected to profound spatial and temporal variations in dietary resources. Diet undoubtedly is recognized as one of the crucial ecological factors affecting life span, reproductive fitness and various other life-history parameters in holometabolous insects. *Drosophila* has been employed as a model organism in several diet related studies, due to the presence of highly conserved physiological pathways between humans and *Drosophila*(Rubin and Lewis 2000).Many studies performed in *Drosophila* validate the fact that quantitative and qualitative changes in the dietary composition has a large impact on its morphology, physiology, behavior and various fitness traits. Experimental approaches in nutrition related studies in *Drosophila* involve either manipulations of the ratio of nutrients for which the effect wants to be ascertained, while keeping the other dietary compositions constant or by dilution of nutrients in food medium(Min and Tatar2006). Such type of dietary manipulations utilizing food as an environmental variable has been a focus of a large number of studies, especially in the area of gerontology (Nusbaum and Rose 1999; Maklakov *et al.* 2008; Skorupa *et al.*2008).

Diet during ontogenesis plays an important role in effecting phenotypic variations in adult traits. The adult body size and structures in *Drosophila* are essentially regulated by nutrient availability during the premetamorphic larval stages. Metamorphosis in *Drosophila* isinitiated through several hormonal cascades after the attainment of critical size or critical weight in final stage of larva. This critical size in *Drosophila* is controlled by insulin/insulin-like growth factor signaling (IIS) pathway. The IIS pathway in turn is regulated by nutritional dependent release of Insulin- like peptides which are produced

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by insulin-producing cells in the brain and various endocrine tissues (Stern 2003).In addition to body size, nutritional stresses like dietary restriction and starvation resistance in *D. melanogaster* also effects lifespan, developmental time, adult body mass, and rate of ageing and reproductive capacity in *Drosophila* (Chippindale *et al.* 1993; Partridge *et al.* 2005; Rion and Kawecki 2007).

In natural environment, organisms inhabiting different niches encounter heterogeneous nutritional environment and may show different phenotypic responses in response to varied nutrition (Bhumika and Singh 2018, 2019). The ability of a particular genotype to produce different phenotype in response to environmental conditions is termed as phenotypic plasticity (Pigliucci*et al*. 2006;Bhumika and Singh 2018, 2019). Canalization, in contrast to phenotypic plasticity depicts the ability of genotypes to withstand the genetic or environmental perturbations (Hornstein and Shomron 2006). Phenotypic plasticity facilitates a genotype to achieve phenotypic optima under different environments, therefore, conferring it an adaptive advantage(Debat and David 2001). Differential effect of a particular environment on phenotypic expression in distinct genotypes gives rise to genotype-by-environment interaction (G x E). In nutrition related studies and studies related to complex diseases the word 'environment' which could be any non-genetic factor, has been replaced by the term 'diet' and is termed as genotype -by-diet interaction (G x D interaction).G x E interactions can be represented as the norms of reaction graph which are two - dimensional graphs. The norms of reaction graphs consist of several curves or lines called reaction norms each of which represents a particular genotype showing responses to experimental environmental variations. Inferences regarding G x E interactions can be drawn by studying shapes of reaction norms whether they are parallel or intersecting (Fuller *et al.* 2005; Kim *et al.* 2014).

The fruit fly, *Drosophila malerkotliana* used in the present study as a model organism belongs to *D. bipectinata* species complex (Parshad and Paika 1964). It was first identified from Malerkotla, Chandigarh and Pinjore in India. It is commonly found throughout Southeast Asia, extending into Northeastern Australia, the Indian subcontinent and South Pacific (Singh and Banerjee 2016). Markow and O'Grady (2005) referred *D. malerkotliana* as a sub-cosmopolitan species. The present study was undertaken to assess the role of dietary carbohydrate: protein ratio on morphometric traits in isofemale lines of *D. malerkotliana.* Phenotypic plasticity through isofemale line technique is studied by subjecting different genotypes to varying environmental gradients (David *et al.* 2005). Overall the purpose of the following study was twofold. 1. To assess the effect of dietary perturbations in the form of high protein: low carbohydrate and low protein: high carbohydrate diet on morphometric traits in both males and females of *D. malerkotliana.* 2. To estimate the phenotypic variability and genetic variability of different traits for each isofemale line in varied dietary regimes as well as to assess genetic variation in phenotypic plasticity. The estimates of genetic variation may or may not be consistent with the changing environmental conditions. Changes in quantitative genetic parameters in stressful or novel environment is related to the evolutionary consequences and thus finally with the evolutionary potential of the concerned species. An increase in genetic variance corresponds to increase in additive and non-additive components thereby increasing the adaptive potential (Bubliy and Loeschcke 2000). However, different environments may have unpredictable effects on heritable variations due to plasticity in traits (Hoffmann and Merila 1999).

II. Materials and methods

Six isofemale lines of *D. malerkotliana* utilized in the present investigation were derived from natural population of Bilaspur, Chhattisgarh state of India (22.0797°N, 82.1409° E) which was collected in the year 2015. The lines were maintained in the normal laboratory condition at 24 ± 1°C with a 12 hour of photoperiod in standard yeast-agar medium.

A. Experimental diet and culture procedure

Two different diets were prepared as experimental diet: High Yeast: Low Sugar (HY: LS) and Low Yeast: High Sugar (LY: HS). Standard medium (SM) and experimental diets were having following constituents: yellow cornmeal, brown sugar, yeast powder (mixture of active yeast and yeast extract), agar, nipagin and propionic acid. Experimental food has changes in the ratio of yeast and sugar while all other constituents were kept constant. The overall composition of the SM, HY: LS and LY: HS is provided in Table I.

From each isofemale line, 20 pairs of 7-day-old virgin males and females were taken and allowed to mate in culture bottles with standard food medium for 48 hours. The flies were then allowed to oviposit at 25°C for 24 hours. Eggs were collected for each isofemale lines and transferred to vials having standard and experimental diets (HY: LS and LY: HS).

Table I. Concentrations (in g/l) of different components of standard and experimental dietary medium

Materials	SM	HY:LS	LY: HS
Maize powder	45	45	45
Brown sugar	40	5	50
Yeast powder	15	50	5
Agar-Agar	15	15	15
Nipagin	3.33	3.33	3.33
Propionic acid	3.33 ml/l	3.33 ml/l	3.33 ml/l

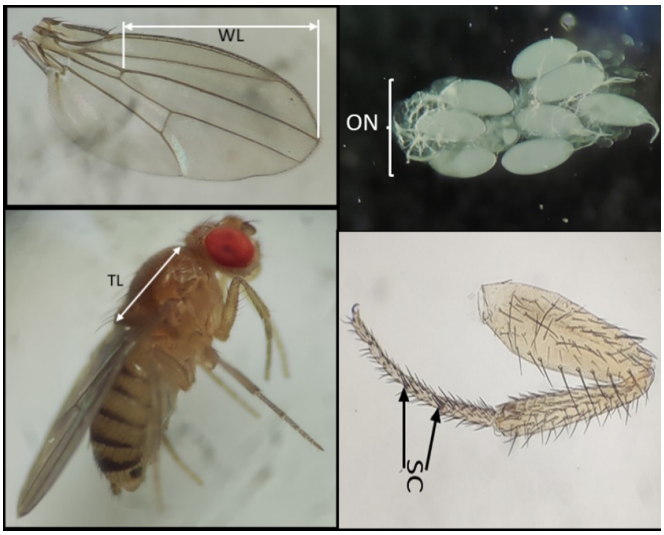

Fig. 1.Wing length (WL), thorax length (TL), ovary showing ovariole and sex comb in *D. malerkotliana*

B. Measurement of morphological traits

Emerging adult virgin flies were collected (males and females separately) and were aged for 5-7 days for maturity. 15 males and 15 females per isofemale lines, per diet were chosen randomly and were analyzed for the following morphometric traits–Wing length (WL), Thorax length (TL), Wing by Thorax ratio (W/T), Sex comb tooth number (SCTN) in males and Ovariole number (ON) in females (fig.1). WL was measured as an absolute distance between the anterior crossvein and the distal tip of the third longitudinal vein under a microscope at 50 X magnification using an ocular micrometer (1 unit = 15.00µ). For TL, the absolute distance was measured from the anterior end of the thorax to the posterior end of the scutellum. W/T ratio was calculated from the data of WL and TL. For ON in females, ovaries were dissected out in insect saline. They were stained with a drop of 2% aceto-carmine for 2 minutes, washed and mounted in 45% acetic acid and visualized under microscope at 50X magnification. *D. malerkotliana* possesses four sex combs, two each on its first and second tarsomeres. Each of the

Fig. 1.Wing length (WL), thorax length (TL), ovary showing ovariole and sex comb in *D. malerkotliana*

tarsomere has a lower number of teeth in the (1-2) proximal comb than in the distal comb (3-4). Forelegs of males were dissected and mounted in insect's saline and the number of teeth of all segments was counted under a microscope at 50 X magnification. Total number of SCTN per leg includes the teeth on first and second tarsal segments.

C. Statistical Analysis

Genetic variation was estimated as coefficient of intra-class correlation (r_I) in the corresponding one-way ANOVA (Hoffmann & Parsons 1991). Evolvability (I_A) was estimated as V_B/M where V_B denotes among line variance and M denotes square of the trait mean (Houle, 1992,Imasheva and Bubliy 2003). Phenotypic variance was calculated as Coefficient of variation (CV) for different traits and dietary regimes. One-way analysis of variance (ANOVA) followed by Bonferroni Post-hoc test was used to compare differences in mean for traits as well as two-way ANOVA was utilized to assess genotype-by-diet interactions for different traits in different food media. ANOVA's were performed using Sigma Stat (version 2.0) software.

III. Results

Table II represents the mean values (mean ± SE) for different morphological traits of *D. malerkotliana* raised on the different dietary food media (data for isofemale lines are combined). The values showed decrease in TL in both males and females for HY: LS and LY: HS when compared to SM and the differences were significant. For WL in females, there was decrease in mean value for LY: HS diet as compared to SM, though the differences were non-significant. In males, WL showed no strait in female, i.e., ON, a decrease in number was found for bothHY: LS diet and LY: HS diet, while SCTN in males did not show any significant difference. W/T ratio in both males and females also failed to show a significant response to dietary variation, although there was increase in W/T ratio for both the

Table II. Trait means (Mean ± SE) for different morphological traits of *D. malerkotliana* reared on standard and experimental food media

		Females		
Traits	**SM**	**HY:LS**	**LY:HS**	**F**
WL	86.27±0.575a	86.10±0.488a	84.06±1.137a	2.444
TL	65.05±0.496a	63.88±0.444a	61.90±0.563b	10.03*
ON	22.96±0.772a	21.66±0.651ab	19.16±0.944b	5.871*
W/T	1.328±0.011a	1.350±0.011a	1.357±0.019a	1.039
		Males		
Traits	**SM**	**HY:LS**	**LY:HS**	**F**
WL	74.51±0.561a	74.58±0.462a	74.22±0.742a	0.0984
TL	57.48±0.423a	55.97±0.655ab	54.98±0.431b	5.993*
SCTN	7.660±0.372a	7.525±0.128a	7.302±0.249a	0.334
W/T	1.298±0.013a	1.335±0.013ab	1.353±0.016b	3.999*

Means within a row followed by the same letter are not significantly different at the 5% level by Post-hoc Bonferroni test, *F* Variance ratio, *P<0.

experimental treatments when compared to SM.

One-way ANOVA (Tables IIIa, IIIb, IIIc) was performed for both males and females to determine whether there is difference in morphometric traits among isofemale lines when reared in

Table IIIa. Analysis of Variance for morphometric traits in both sexes of isofemale lines of *D. malerkotliana* for standard medium

Traits	Source of variation	DF	SS	MS	F
Male (WL)	Total	89	500.489		
	Between treatment	5	141.556	28.311	6.626**
	Within treatment	84	358.933	4.273	
Female (WL)	Total	89	554.900		
	Between treatment	5	133.833	26.767	5.340**
	Within treatment	84	421.067	5.013	
Male (TL)	Total	89	409.789		
	Between treatment	5	58.589	11.718	2.803
	Within treatment	84	351.200	4.181	
Female (TL)	Total	89	582.722		
	Between treatment	5	110.856	22.171	3.947*
	Within treatment	84	471.867	5.617	
Male (SCTN)	Total	89	165.600		
	Between treatment	5	62.267	12.453	10.123**
	Within treatment	84	103.333	1.230	
Female (ON)	Total	89	1084.722		
	Between treatment	5	269.656	53.931	5.558**
	Within treatment	84	815.067	9.703	
Male (W/T)	Total	89	0.229		
	Between treatment	5	0.0800	0.01600	8.994**
	Within treatment	84	0.149	0.00178	
Female (W/T)	Total	89	0.325		
	Between treatment	5	0.0607	0.0121	3.853*
	Within treatment	84	0.265	0.00315	

DF degree of freedom, *SS* Sum of Square, *MS* Mean Square, *F* Variance ratio, *P<0.05, **P<0.001.

different food media. The test revealed high F value in both males and females for multiple traits when raised in standard medium except for TL in males (F=2.803). For HY: LS diet, highly significant F value (p <0.001) was found for male TL and W/T and female ON and W/T, whereas, for female WL.

Table IIIb. Analysis of variance for morphometric traits in both sexes of isofemale lines of *D. malerkotliana* for High Yeast: Low Sugar diet

Traits	Source of variation	DF	SS	MS	F
Male (WL)	Total	89	565.956		
	Between treatment	5	96.089	19.218	3.436*
	Within treatment	84	469.867	5.594	
Female (WL)	Total	89	712.100		
	Between treatment	5	107.167	21.433	2.976
	Within treatment	84	604.933	7.202	
Male (TL)	Total	89	572.900		
	Between treatment	5	192.633	38.527	8.510**
	Within treatment	84	380.267	4.527	
Female (TL)	Total	89	823.656		
	Between treatment	5	88.589	170718	2.025
	Within treatment	84	735.067	8.751	
Male (SCTN)	Total	89	138.456		
	Between treatment	5	7.389	1.478	0.947
	Within treatment	84	131.067	1.560	
Female (ON)	Total	89	962.322		
	Between treatment	5	190.989	38.198	4.160**
	Within treatment	84	771.333	9.183	
Male (W/T)	Total	89	0.260		
	Between treatment	5	0.0730	0.0146	6.563**
	Within treatment	84	0.187	0.00222	
Female (W/T)	Total	89	0.277		
	Between treatment	5	0.0573	0.0115	4.389**
	Within treatment	84	0.219	0.00261	

DF degree of freedom, *SS* Sum of Square, *MS* Mean Square, *F* Variance ratio, *P<0.05 **P<0.001.

Table IIIc. Analysis of variance for morphometric traits in both sexes of isofemale lines of *D. malerkotliana* for Low Yeast: High Sugar diet

Traits	Source of variation	DF	SS	MS	F
Male (WL)	Total	89	653.556		
	Between treatment	5	248.089	49.618	10.279**
	Within treatment	84	405.467	4.827	
Female (WL)	Total	89	1948.722		
	Between treatment	5	580.989	116.198	7.136**
	Within treatment	84	1348.722	16.283	
Male (TL)	Total	89	839.956		
	Between treatment	5	83.556	16.711	1.856
	Within treatment	84	756.400	9.005	
Female (TL)	Total	89	668.100		
	Between treatment	5	142.633	28.527	4.560**
	Within treatment	84	525.467	6.256	
Male (SCTN)	Total	89	126.900		
	Between treatment	5	27.833	50567	4.720**
	Within treatment	84	99.067	1.179	
Female (ON)	Total	89	937.822		
	Between treatment	5	400.756	80.151	12.536**
	Within treatment	84	537.067	6.394	
Male (W/T)	Total	89	0.447		
	Between treatment	5	0.106	0.0212	5.233**
	Within treatment	84	0.341	0.00406	
Female (W/T)	Total	89	0.495		
	Between treatment	5	0.149	0.0297	7.196**
	Within treatment	84	0.347	0.00413	

Table IV. Two-way ANOVA for the effect of genotype and diet on the four morphometric traits of *D. malerkotlianain* males and females

			Female		Male	
Trait	**Source of variation**	**DF**	**MS**	**F**	**MS**	**F**
WL	Genotype	5	64.95481	6.83811**	62.43259	12.74685**
TL		5	20.28222	2.950312	40.37037	6.83753**
W/T		5	0.022632	6.866494**	0.036862	13.71381**
SCTN		5	79.28593	9.409167**	14.40593	10.88653**
WL	Diet	2	138.8593	14.61839**	3.214815	0.656368
TL		2	228.8444	33.28839**	134.7815	22.82794**
W/T		2	0.022182	6.730227**	0.067707	25.18936**
SCTN		2	334.0037	39.63751**	2.181481	1.648541
WL	Interaction (G x D)	10	49.72148	5.234423**	17.35704	3.543783*
TL		10	24.06667	3.500808*	13.29259	2.251367*
W/T		10	0.015331	4.651498**	0.007491	2.786839*
SCTN		10	46.49704	5.517983**	2.545926	1.92395*
WL	Residual	252	9.498942		4.897884	
TL		252	6.874603		5.904233	
W/T		252	0.003296		0.002688	
SCTN		252	8.426455		1.32328	

DF degree of freedom, *MS* Mean Square, *F* Variance ratio,*P<0.05, **P<0.001

significant F values were found. Test for LY: HS diet revealed highly significant F values ($p<0.001$) for each of the traits in both males and females except for TL ($F=1.856$) in males.

The result of two-way ANOVA (Table IV) reveals significant genotype-by-diet interaction ($p<0.05$) for all the morphometric traits in males. Females show highly significant genotype-by-diet interaction ($p<0.001$) for all the traits except for TL ($p<0.05$). Fig. 2 and Fig. 3 show the norms of reaction graphs in females and males, respectively. The extensive crossing over of norms of reaction for different morphometric traits reveals a significant G x D, i.e., each genotype responds to the environmental manipulations in different way.The parameters related to phenotypic and genetic variation are tabulated in Table V. The value shows increasing trend for all the traits in LY:HS when compared to SM in females, while in males, only WL showed an increasing value of all the parameters for LY:HS diet.

IV. Discussion

The present study examines the effect of dietary perturbations (HY:LS and LY: HS) on morphological traits (WL, TL, W/T, SCTN and ON) in six isofemale lines of *D. malerkotliana*. The assessment of genetic and phenotypic variation depicts the evolutionary potential of the species in response to novel environmental condition. The investigated traits showed variable responses to different diets in a sex dependent manner. The sexual trait in females (ON) showed plasticity for the experimental diets.We found that in females, ON showed significant decrease in mean values when reared on LY: HS food compared to HY: LS and SM. In contrast, SCTN in males showed non-significant changes when reared on different diets. Ovary size in *Drosophila* measured as ovariole number showed strong phenotypic plasticity in response to varying larval

nutrition (Hodin and Riddiford, 2000). Flies when reared on low protein or less yeast possess fewer ovarioles as compared to flies reared on high yeast. The results of the present experiments clearly reveal that protein is a major macronutrient and contributes chiefly to reproduction. The requirement of high protein in female diet is strongly needed for the synthesis of the egg-yolk protein vitellin (Adams and Gerst, 1991). Secondary sexual trait in male (SCTN) showed developmental stability to nutritional variations, i.e., they were found to be environmentally canalized. Pavkovic-Lucic et al. (2013) found similar results for SCTN in *D. melanogaster*. Growth in *Drosophila* by nutrition during development is mainly regulated by *Drosophila* Insulin-like Peptides which acts via insulin /insulin-like growth factor signaling pathway (Mirth et al., 2007). Nutritional plasticity of different morphological traits suggests the sensitivity to changes in signaling to these pathways. Our finding confirms that SCTN is relatively insensitive to insulin-signaling thereby showing insignificant change to nutrition, hence showing nutritional canalization.

The non-sexual trait WL, in both males and females was not affected significantly, though a decrease was found for the experimental diets when compared to SM. While TL in both males and females showed a significant decrease in mean value for the experimental media. The non-sexualtraits showed varied response to variable dietary conditions. It has been well documented by several studies in *D.melanogaster* that wing measurement is affected by larval nutrition (Vijendraverma et al., 2011, Guler et al., 2015). However, we did not find any such result in *D. malerkotliana.* The result reflects the insensitivity of *D. malerkotliana* wing structure to nutrition dependent IIS pathway thus depicting canalization. TL in both males and females showed decrease in mean value for LY: HS food. HY: LS diet did not have much effect on WL and TL as compared to LY: HS diet. This could be due to the fact that nutritional deficiency could be generally overcome by compensatory feeding activity (Carvalho et al., 2005). From the result, we can say that carbohydrate deficiency in diet might be balanced by compensatory feeding but protein deficit could not be. We also analyzed W/T ratio in both males and females from the data of WL and TL. Ratio of wing length to thorax length has been inversely related to wing loading capacity. We found significant difference in W/T ratio in males while in females no significant difference was found. A higher W/T ratio has been related to lower wing loading capacity and thus higher flight adaptability (Azevedo et al.,1998). phenotypic variability estimated as coefficient of variation has been found to increase for all the traits in LY: HS dietary medium. The reason for such increase in phenotypic variation for LY: HS diet could be attributed to the fact that low dietary protein medium creates a more stressful environment than a low carbohydrate medium. Increased phenotypic variability in stressful environment permits rapid adaptation, thereby enhancing fitness in heterogeneous environments (Geiler-Samerotte et al., 2013).Quantitative genetic variation in fluctuating environmental condition has been found to be either increasing or decreasing and is highly trait dependent (Hoffmann & Parsons 1991, Hoffmann &Merila 1999, Charmantier&Garant 2005). The effect of developmental nutrition on life span, metabolism

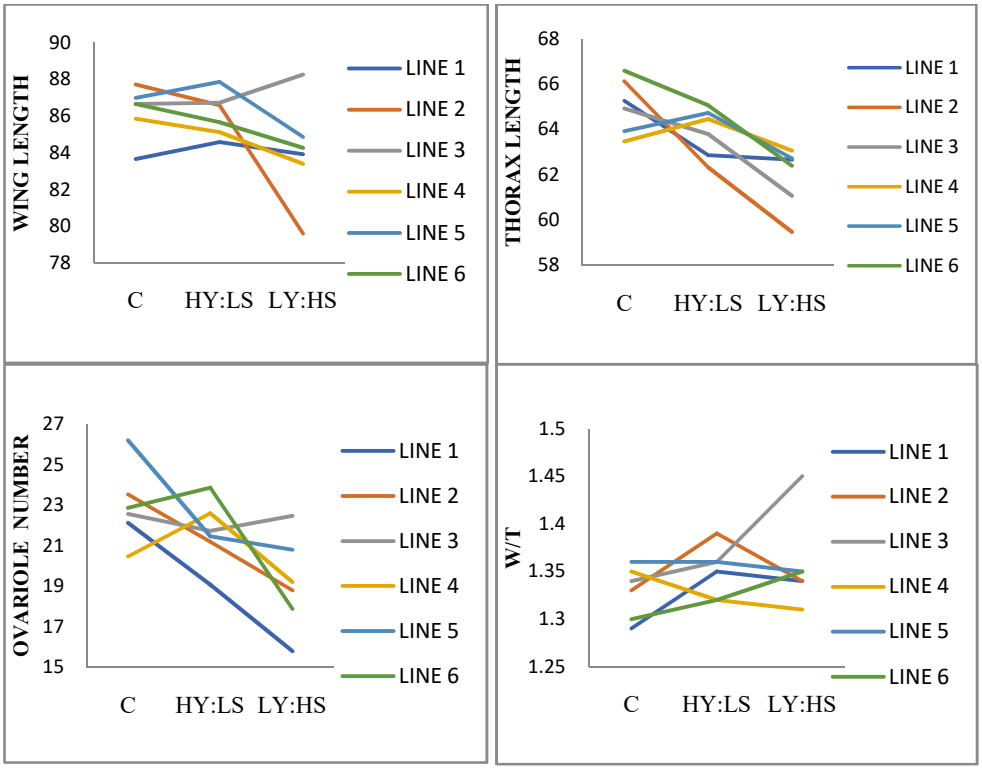

Fig. 2.Reaction norms for Genotype x Diet interaction in females of *D.malerkotliana*for WL, TL, ON and W/T. Each point represents the mean of morphometric traits for a particular isofemale line in each dietary environment. Six isofemale lines are denoted by different line formats. X-axis: food media and Y-axis: morphometric traits

Table V. Coefficient of variation (CV), between the line component of variance ($V_{B)}$, Coefficient of intraclass correlation (r_I) and Evolvability (I_A x 100) for *Drosophila malerkotliana* raised in standard and experimental media

		Females					Males		
Trait	**Variable**	**SM**	**HY:LS**	**LY:HS**	**Trait**	**Variable**	**SM**	**HY:LS**	**LY:HS**
WL	CV	1.63	1.38	3.313	**WL**	CV	1.843	1.516	2.449
	V_B	1.487	1.191	6.455		V_B	1.572	1.067	2.756
	r_I	0.224	0.1164	0.290		r1	0.272	0.139	0.3821
	I_A	0.0199	0.0161	0.0913		I_A	0.028	0.019	0.050
TL	CV	1.867	1.703	2.226	**TL**	CV	1.804	2.863	1.920
	V_B	1.232	0.984	1.585		V_B	0.651	2.14	0.928
	r_I	0.164	0.064	0.191		r_I	0.107	0.33	0.053
	I_A	0.0291	0.0241	0.0413		I_A	0.019	0.068	0.030
W/T	CV	2.100	1.985	3.530	**W/T**	CV	2.503	2.314	2.867
	V_B	0.0006	0.0006	0.0016		V_B	0.0118	0.0008	0.001
	r_I	0.159	0.184	0.292		r_I	0.877	0.0008	0.220
	I_A	0.034	0.044	0.086		I_A	0.700	0.0448	0.055
ON	CV	8.23	7.37	12.06	**SCTN**	CV	11.98	4.153	8.353
	V_B	2.996	2.122	4.453		V_B	0.691	0.0821	0.309
	r_I	0.233	0.174	0.434		r_I	0.378	-0.003	0.198
	I_A	0.568	0.452	0.949		I_A	1.177	0.1449	0.579

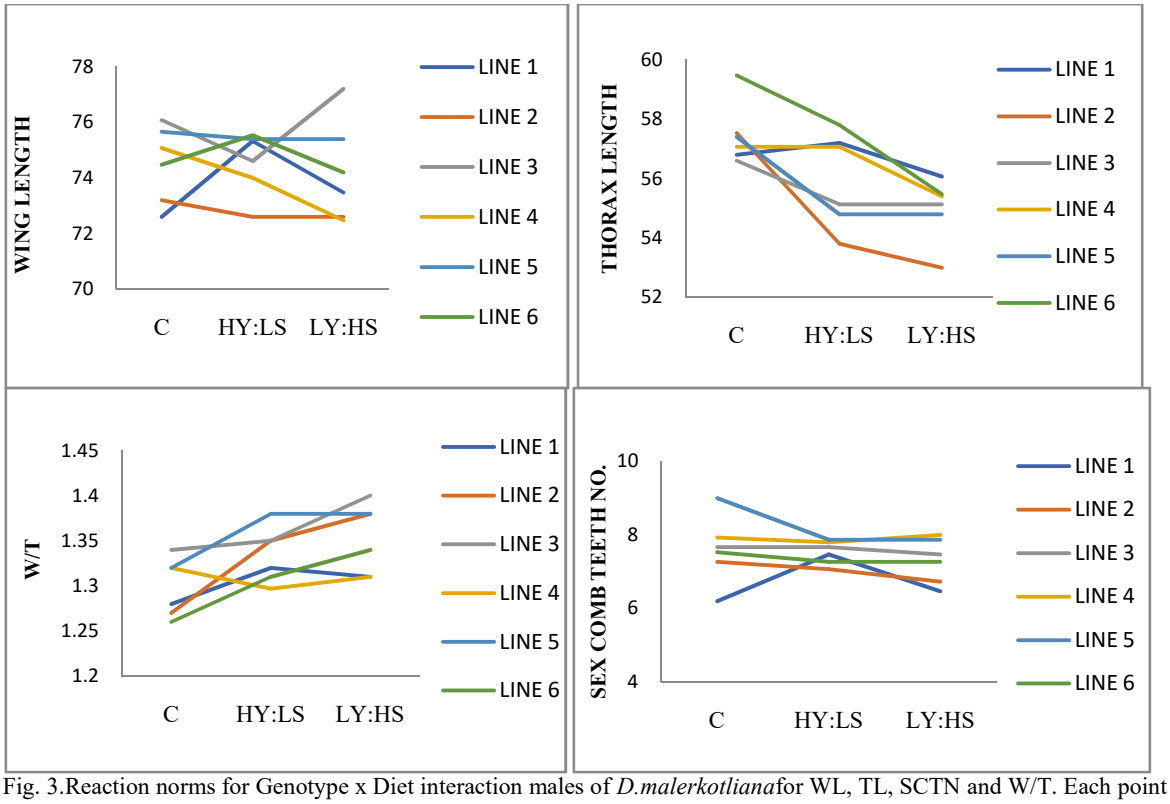

Fig. 3.Reaction norms for Genotype x Diet interaction males of *D.malerkotliana*for WL, TL, SCTN and W/T. Each point represents the mean of morphometric traits for a particular isofemale line in each dietary environment. Six isofemale lines are denoted by different line formats. X-axis: food media and Y-axis: morphometric traits

and reproductive efficiency including fecundity has been well documented in *Drosophila* (May et al 2015, Klepsatel et al 2020).

Intraclass correlation provides measure of genetic variability among isofemale lines (Capy et al., 1994).In *D. malerkotliana,* we found higher intraclass correlation in females for all the traits examined for LY: HS as compared to HY: LS and standard diet. The plausible reason for such result is that stressful nutritional environment increases additive and non-additive genetic variation, thereby ultimately increasing the genetic variation. Another accountable reason for increased genetic variation, according to 'selection history' hypothesis might be that during stressful situations, selection always favors buffering mechanism that increases canalization, but under novel nutritional stress, earlier selection for increased canalization has not occurred thereby increasing genetic variation (Waddington, 1960). The work of Imasheva et al., (1999) also shows increase in genetic variation for some morphological traits when *D. melanogaster* flies are subjected to nutritional stress. I_A which suggests better than heritability, the ability of population to respond to selection (Houle, 1992) was observed to increase for all the traits in females in LY: HS and HY: LS media as compared to SM. In males, it only increased for WL and TL.Genotype-by-diet interaction occurs when different genotypes respond to dietary variation in different ways, i.e. when there is genetic variation for it. Studies previously done in *D. melanogaster* inbred lines have revealed highly significant genotype-by-diet interaction for metabolic phenotypes when raised in different experimental diet (Reeded al. 2010). The presence of significant G x D interaction in both males and females for sexual and non- sexual traits suggest that diet plays a substantial role in shaping the phenotypes but in a highly genotype dependent manner. In males, we found that genotype is contributing more significantly to the traits in contrast to diet while G x D is constant for all the traits. In females, genotype (except for TL), diet and G x D playsignificant role in shaping up the traits. The results of the present study have also been

confirmed from NoR Graphs where extensive crossing over of the reaction norms were seen for traits, as each genotype responds to nutritional variation in a different way, thus showing phenotypic plasticity and G x D in *D. malerkotliana.*

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