Trans-generational effect of protein restricted diet on adult body and wing size of *Drosophila melanogaster*

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Running title: Effect of protein restrictions in *Drosophila.*

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Abstract:

Dietary Restriction (DR) via protein restriction (PR) has turned out to be a very inquisitive field as past studies opened about the feasible trade-offs between various fitness and behavioral traits in *Drosophila melanogaster* to understand lifespan or aging in a nutritionally challenged environment. However, the phenotypes of body size, weight and wing length respond according to the factors such as flies' genotype, environmental exposure and parental diet. Hence, understanding the long-term effect of PR on these phenotypes is essential. Here, we demonstrate the effect of PR diet on body size, weight and normal & dry wing length of flies subjected to PR50 & PR70 (50% & 70% protein content present in control food respectively) for 20 generations from the pre-adult stage. We found that the PR fed flies have lower body weight, relative water content (in males), smaller normal & dry body size as compared to its control and generations 1 & 2. Interestingly, the wing size of PR flies and the pupal size of PR70 flies are smaller and also showed significant effects of diet and generation. Thus, these traits are sex and generation dependent along with an interaction of diet, which is capable of modulating these results variably. Our study suggests that the trans-generational effect is more prominent in influencing these traits and moreover wing length might not be a predictor for body size. Taken together, the trans-generational effect of dietary protein restriction on fitness and fitness-related traits might be helpful to understand the underpinning mechanisms pertaining to evolution and aging in fruit flies *D. melanogaster*.

Keywords: *Drosophila*, protein restriction, selection, life-history traits, trans-generational effect.

Introduction:

Organisms vary in body size not only across species, but also within a particular species. The variations in the body composition can influence phenotypic traits like body size, body weight etc., while these trait variations can be attributed to various environmental and genetic factors 1-3]. The environmental factors that can influence organismal body size and weight, including wing length (especially in insects) can be nutrition [4], temperature [5-6], crowding [4, 7], latitudinal clines [8] and certain cases of laboratory selection pressures for faster development [9] etc. Body size, weight and wing length are certain parameters that ensure the overall fitness of organisms including fruit flies. Thus, variations in these phenotypes can be used to understand the genotypic changes that are bound to occur [10].

Fruit flies *Drosophila melanogaster* for the past three decades, has been widely used as a model organism for studying aging via nutritional approaches including diet restriction (DR), food dilution, intermittent feeding, etc., [11-13]. The diet of fruit flies commonly comprises of carbohydrates and proteins as the major source, with lipids, vitamins, minerals present in minor quantities. Restricting protein source (yeast) in the fly food is a type of dietary implementation (Protein Restriction; PR henceforth), and is seen to influence a range of fitness and fitness-related traits such as lifespan, fecundity, stress resistance, activity, development time, etc., [13-16]. Interestingly, PR is known to influence traits like body size, weight and wing length, wherein variations in yeast concentration can significantly alter the body size and weight of the flies, and also have an influence on their wing length in a single generation itself (environment effect; [1, 2, 17]). This might be due to the sudden change in the protein composition; while it is necessary to assay long term (genetic effect) effect of PR. A high protein diet can yield unaffected pupal size [18], while the long duration of high protein increased body mass [19], also decreased body weight and fat levels [20].

PR from pre-adult stage is highly debatable as some studies suggest its negative effect on lifespan, body size, fecundity [1, 21, 22]; while studies claim its positive effect on lifespan and other traits [13, 23, 24]. The adult body size need not necessarily influence the lifespan of the organism raised under varied nutritional conditions [25]. Since, nutrition during pre-adult stage largely determines the size of the adult upon eclosion [1, 2, 25] alongside the influences of juvenile hormones [26], it is essential to study the long-term effect of PR and not just one or two generations [27]. Moreover, assaying for one or two generations might address the immediate effect of parents or grandparents' diet on the off springs and whether it is maternally inherited or not [28, 29]. The current study assays a trans-generational effect of 20 generations (gen 1, 2, and 20) and does not deal with understanding the mode of inheritance (maternal or paternal).

Here we address the effect of 50% and 70% yeast concentrations (as against the control-AL diet) across generations 1, 2 and 20. This study will address the effect of PR on a single generation, its off spring (gen 2) and also long-term effect (gen 20) of the corresponding protein restriction. The assessed traits include body size, weight, relative water content, pupal size and wing length in the normal and dry conditions of the fly body. After 20 generations of PR implementation, the PR males and females have lower body weight as compared to their control in their respective generations and within the PR generations. Interestingly, the relative water content is higher in females and not males in spite of long-term PR diet. Moreover, the flies at generation 20 have lower body size as compared to the generations 1 and 2 and their control, showing that the body size and weight might be positively correlated. Since the body size is an adult trait, we measured the intermediate pupal size. The PR50 flies at gen 1 and 2 showed the highest pupal size as compared to PR70 and control, while their size was similar at the end of 20 generations, which might be reflected as a part of smaller adult body size as well. Lastly, since wing length itself can be an indicator of body size [30], measuring the same revealed that PR had a diet and generation dependent effect on producing flies with shorter wings. Thus, after 20 generations, PR diets produce flies with smaller body and wing, lower weight, unaltered pupal size and relative water content (in females). Thus, this study would benefit to understand the influence of diet and/or the genetic effect (generational study) in mediating variations in the assayed traits.

Results:

Normal and dry body weight:-

To check whether the body size and weight are proportional to each other under the imposed PR, we assayed body weight and size (normal and dry) of the AL and PR stocks at gen 1, 2 and 20 generations of diet imposition. ANOVA followed by post hoc multiple comparisons using Tukey's test on the normal body weight of the freshly eclosed male and female fruit flies showed statistically significant effect of diet (D; $F_{2,72}$=193.04, p<0.0001), generation (G; $F_{2,72}$=99.63, p<0.0001), sex (S; $F_{1,72}$=490.70, p<0.0001) and their interaction (D × G × S;

$F_{4,72}$=9.54, p<0.0001; Table 1). The results show that PR50 (males) and PR70 (males and females) have significantly lower body weight at gen 20 as compared to their previous generations (Fig. 1A); while PR50 females at gen 20 have lower body weight than gen 2, but not gen 1. The PR50 and PR70 (males and females) have lower body weight at all tested generations against their respective control. Thus, with PR and long-term restrictions, the adult body weight is lower. To assess whether this lower body weight is due to the water content, we weighed the dry weight of the flies.

As expected, the ANOVA on the dry body weight of flies revealed statistically significant effect of D ($F_{2,72}$=106.75, p<0.0001), G ($F_{2,72}$=37.78, p<0.0001), sex (S; $F_{1,72}$=289.74, p<0.0001) and their interaction (D × G × S; $F_{4,72}$=10.8, p<0.0001; Table 1). Post hoc multiple comparisons by Tukey's test revealed significantly decreased dry body weight of PR50 (males and females) at gen 20 have lower dry weight as compared to their gen 1, but not gen 2. Interestingly, PR70 males show no effect of generations as their dry weight is not different, while PR70 females at gen 20 have lower weight as compared to gen 1 and 2 (Fig. 1B). The effect of diet shows that at gen 1, the PR50 females are lower in weight, while the others weigh similar to AL. At gen 2, only PR50 females were lower, but surprisingly at gen 20, PR50 and PR70 (males and females) dry weight difference is prominent and weigh lower than the control. Thus, the results confirm that the body weight of PR flies is lower than the AL due to long term diet implementation.

Since there exists a difference between the normal and dry body weights across generations, we assessed the relative water content in the PR flies. ANOVA on the relative water content revealed statistically significant effect of D ($F_{2,72}$=11.4, p<0.0001) and its interaction with generation (D × G; $F_{4,72}$=10.38, p<0.0001; Table 1), but not G ($F_{2,72}$=3.03, p<0.0547), sex (S; $F_{1,72}$=3.87, p<0.0531). The relative water content of the PR50 flies at gen 2 is comparatively higher than gen 1, while PR70 males and females have higher water content at gen 1 and 20 respectively (Fig. 1C). For the effect of diet across generations, at gen 1, the PR50 and PR70 flies are equal to AL, while at generation 2, the PR50 males have similar water content at that of its respective control, while PR70 males and PR50 females exhibit lower and higher water content respectively. But interestingly at gen 20, PR males have lower relative water content, while in PR females' it is higher. Thus, long term PR has facilitated higher water content in PR females and not males.

Normal and dry body size:-

The flies maintained on PR50% and 70% for 20 generations from the pre-adult stage were measured for their normal and dry body size. ANOVA on the normal body size of the freshly eclosed adult males and females showed a statistically significant effect of diet (D; $F_{2,522}$=38.5, p<0.0001), generation (G; $F_{2,522}$=98.2, p<0.0001), sex (S; $F_{1,522}$=611.6, p<0.0001) and their interaction (D × G; $F_{4,522}$=21.8, p<0.0001) (Table 1; Fig. 2A). Further, post hoc multiple comparisons using Tukey's HSD test revealed the generation effect is prominent in the PR flies as their body size at gen 20 is comparatively smaller than the previously tested generations (1 and 2). The effect of diet shows that at gen 1, PR males do not show any difference in body size, while PR females are smaller. But surprisingly at gen 2, the PR fed males are larger than AL, while females are similar in size as that of AL, while at gen 20, PR males and females are smaller than the AL flies. Thus, after 20 generations the PR produces smaller flies as compared to AL even though minor fluctuations in their body size were observed at gen 1 and 2.

Further, ANOVA followed by multiple comparisons by Tukey's HSD test on the dry body size showed statistically significant effect of D ($F_{2,522}$=21.23, p<0.0001), G ($F_{2,522}$=163.13, p<0.0001), S ($F_{1,522}$=363.37, p<0.0001) and their interaction (D × G; $F_{4,522}$=41.12, p<0.0001; Table 1; Fig. 2B). All the PR flies at gen 2 are bigger as compared to gen 1 and 20, except for PR70 males wherein their dry body size is similar to that observed at gen 1. The effect of diet on the dry body size revealed that PR flies are similar in size to AL at gen 1, while at gen 2 the PR70 (males and females) are bigger than AL. Similar to the results of normal body size, the PR fliesare smaller than their control flies at gen 20. Surprisingly, there exist changes in the response of PR diet on the normal and dry body size, showing that the normal body size and dry body size might not be equivalent and the difference between them is not constant, and the reason might be attributed to the various forms of storage reserves.

Pupal size:-

ANOVA followed by Tukey's on the normal wing length showed a statistically significant effect of D ($F_{2,261}$=29.34, p<0.0001), G ($F_{2,261}$=7.96, p<0.0004) and their interaction (D × G; $F_{4,261}$=5.91, p<0.0001; Table 1). Post hoc multiple comparisons by Tukey's test showed that among PR50 flies across generations, gen 1 was the highest, while at gen 2 and 20 they were similar. Across diets, PR50 flies had a higher pupal size in gen 1, 2 and gen 20 as compared to AL and PR70 flies. Thus, showing that the PR50 flies have pupal size higher as compared to the control in all the generations, but within its own generations, the observed highest pupal size at generation 1 might have been a startle response for PR.

Normal and dry wing length:-

Post assaying of body size, we intended to assay the wing length as it is commonly thought to be a measure of body size as mentioned earlier. ANOVA on the normal wing length showed statistically significant effect of D ($F_{2,522}$=72.28, p<0.0001), G ($F_{2,522}$=28.07, p<0.0001), S ($F_{1,522}$=301.92, p<0.0001) and their interaction (D × G; $F_{4,522}$=10.6, p<0.0001; Table 1). Interestingly, multiple comparisons by Tukey's test within diets across generations shows that gen 20 females have wing length similar to gen 2, while the males have lower wing length compared to gen 2. Moreover, PR50 females have smaller wing length as compared to AL in all tested generations; while PR70 females have smaller wings as compared to AL in the first generation alone (Fig. 3A). The effect of diet shows that PR flies (males and females) have shorter wings than AL fliesat gen 20. Thus, the concept of wing length as a measure for body size might not hold true in the presence of dietary parameters influencing them across generations.

ANOVA on dry wing length of fruit flies revealed significant effect of D ($F_{2,522}$=13.46, p<0.0001), G ($F_{2,522}$=33.84, p<0.0001), S ($F_{1,522}$=112.05, p<0.0001) and their interaction (D × G; $F_{4,522}$=26.19, p<0.0001; Table 1). Multiple comparisons of dry wing length by Tukey's test revealed results similar to the normal wing length wherein PR flies at gen 20 had significantly smaller wings than the control (Fig. 3B). Thus, even though PR yield flies with shorter wings at the end of 20 generations (similar to dry body size), it is not true across generations.

Discussion:-

Normal and dry body weight:-

Our results are similar to the results of Vijendravarma et al., [28] that show the effect of diet (parental) on different traits (including body weight) of fruit flies, and it also suggests that these observed differences might be due to the maternal effects and the long-term DR imposition. Further, we did not expect variations in the AL body weight across generations, and convincingly their body weight and relative water content were unaffected, thereby providing convincing results for the control flies. In the PR50 and 70% flies, there might be due to the effect of parental diet on the normal body weight of the flies as suggested elsewhere [28, 29], because the PR flies showed lower body weight at the end of 20 generations. Moreover, the lower body weight of PR flies after 20 generations can be thought to be in line with the study of Kristensen et al., [19], which reported the protein-rich diet for 17 generations yielded bigger and thereby fatter flies as compared to the control. Interestingly, the dry weight of PR males is more stable than the females and is in line with the study reported elsewhere [6]. Thus, long term PR implementation suggests the existence of a plastic response to diet as compared to the genetic effect in case of dry weight and sex [19].

Body size and Pupal size:-

The body size of the PR flies is smaller at generation 20 as compared to their previous generations and control. These results are contrary to the study of Chippindale et al., [31], which reported that bigger adult body size is associated with increased fitness of the flies. Since the fitness of the organism is assessed based on its reproductive capacity and ability to withstand stress, our results might have a positive effect in spite of smaller body size. Surprisingly, the flies with large body size exhibit lower larval viability even though they appear to contribute to the adult fitness [32]. Since the females that mated with smaller males appeared more fecund and also copulated longer [33], the duration of copulation and offspring number are dependent on the female body size and inversely related to the male body size [33, 34]. Moreover, even though the large females differ in size as compared to the smaller females, does not guarantee significant difference in their ovariole number [34], even though yeast restriction reduces ovariole number [25] and high protein diet is known to increase the same with a possible trade-off in egg to adult viability in *D. ananassae* [15]. Since, the males of *D. melanogaster* prefer smaller females for first mating and then undergo adaptive discrimination [35] or plasticity for mate selection by males [36], we can conclude that body size may be one of the many traits that are assessed to choose a potential mate but not a primary one. Hence, the smaller body size of the PR flies might not be a threat for its mate choice, reproductive successor larval viability in our study, even though the fecundity of our flies remains to be tested.

The pupal size at PR50 flies recorded the highest size as compared to the control and PR70 flies, while gen 1 flies of PR50 yielded highest pupal size compared to gen 20. Since high protein diet did not confer any change in the pupal size of the flies, but high carbohydrate diet resulted in smaller pupa [18], it is surprising to see pupal size difference upon protein restriction. This is in line with the results of Deas et al., [37], that suggests more susceptibility of pupal mass change in poor diet than that of rich nutrient diet, in addition to exhibiting effects of parental and grandparental diet [37]. Overall, our results also show that diet and generation have a differential role of different traits as suggested elsewhere [37].

Normal and dry wing length:-

The concentrations of nutrients (yeast and sugar) in the fly diet play an important role in the wing length of females than in males [17]. In our study, since the concentration of sugar was kept constant, the observed variations in wing length show that the yeast alone can modulate this trait. The PR flies showed smaller wings after 20 generations while there existed variable results based on sex. This is contrary to the report of Güler et al., [17], who stated that female's wing length varies with yeast manipulations while males vary with sugar level variations. There are various other factors capable of modulating wing length like temperature and latitudinal clines [3, 38], wherein care was taken to avoid such temperature perturbations. It is also seen that wing length (similar to that of body size) can be influenced by altitude in *Drosophila* species [39, 40]. There exists a difference in the PR and generation effect on the trend of body size and wing length variations and probably, is in contrast to the study of Sokoloff [30], which stated that wing length can serve as a parameter for estimating fly's body size.

Materials and methods:

Fly culture and maintenance:-

The control and DR imposed flies are maintained on 21-day discrete generation cycles with egg collection done exactly on the 21st day of the previous generation cycle. The control flies are fed with AL (*Ad Libitum*) food, while the PR stocks are fed with 50% and 70% yeast as compared to the control (PR50% and PR70%; henceforth). The egg collection for control and PR stocks are done in their respective AL and PR diets (AL diet but with 50% and 70% yeast for PR50 and PR70 stocks respectively). The flies upon eclosion are transferred to plexi-glass cages (25 cm × 20 cm × 15 cm) and are supplemented with their corresponding food. For the following experiments, approximately 30-40 eggs per vial were collected for control and PR and maintained at the temperature of ~25 ºC (±0.5 ºC), the humidity of ~70 %, the light intensity of ~250 lux in 12:12 hr Light/Dark cycles. The diet manipulations were done only in the yeast concentration present in the control food, wherein we used instant dry yeast from Gloripan.

Normal body weight and dry weight:-

We measured the normal and dry body weight of freshly eclosed flies collected in every 2 hr intervals. The eggs were collected from the DR stocks over a 2 h window and kept under LD12:12 h. Post eclosion, the virgin male and female flies were separated by anesthetizing with CO_2. For weighing the normal body weight, flies were weighed post anesthetization using ether (to maintain the flies in the anesthetized state for longer duration), after which the flies were discarded. For the dry body weight assay, the virgin males and females were killed by freezing and were dried for 36 h at 70°C as per the protocol followed elsewhere (Yadav and Sharma, 2014). The normal and dry body weight assay was assessed by weighing a group of 10 males or 10 females per vial, and 5 such vials of randomly chosen flies from the control and the DR stocks were weighed. The body weight of flies was measured using a weighing balance from UniBloc (Shimadzu) AUX220. The relative water content of the flies was calculated by dividing the water content (normal body weight-dry weight) by the normal body weight of the flies as reported elsewhere (Robinson et al., 2000).

Body size/length and wing length:-

The protocol for egg collection until the separation of virgin male and female flies for this assay is similar to that followed for body weight assay. The flies' body size and wing length were measured under a microscope, wherein 30 virgin males and females from the control and DR stocks were assayed. The body size and the wing length of the anesthetized males and females were measured using a microscope from Olympus with a normal ruler (least count 0.5 mm).

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Competing interests:-

The authors declare that they have no conflict of interest.

Author contributions:-

S. K. & P. Y. conceived and designed the experiments; S. K. performed the experiments, analyzed the data and wrote initial versions of the manuscript. S. K. & P. Y. corrected, read and approved the final version of the manuscript.

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References:-

1) F. W. Robertson, The ecological genetics of growth in *Drosophila*. 1. Body size and development time on different diets. *Genetic Res.* **1**, 288–304 (1960).
2) F. W. Robertson, The ecological genetics of growth in *Drosophila* 6. The genetic correlation between the duration of the larval period and body size in relation to larval diet. *genetics Res.* **4**, 74 –92 (1963).
3) G. H. de Moed, G. De Jong, W. Scharloo, Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. *Genet Res.* **70**, 35 –43 (1997).
4) P. Klepsatel, E. Procházka, M. Gáliková, Crowding of *Drosophila* larvae affects lifespan and other life-history traits via reduced availability of dietary yeast. *Exp. Gerontol.* **110**, 298 –308 (2018).

5) L. Nunney, W. Cheung, The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: Evidence for adaptive plasticity. *Evolution.* **51**, 1529 –1535 (1997).
6) D. Karan, J. P. Morina, B. Moreteaua, J. R. David, Body size and developmental temperature in *Drosophila melanogaster*: analysis of body weight reaction norm. *J. Therm. Biol.* **23**, 301 –309 (1998).
7) R. S. Miller, J. L. Thomas, The Effects of Larval Crowding and Body Size on the Longevity of Adult *Drosophila Melanogaster*. *Ecology*. **39**, 118 (1958).
8) S. J. W. Robinson, B. Zwaan, L. Partridge, Starvation resistance and adult body composition in a latitudinal cline of *Drosophila melanogaster*. *Evolution*. **54**, 1819–1824 (2000).
9) P. Yadav, V. K. Sharma, Correlated changes in life history traits in response to selection for faster pre-adult development in the fruit fly *Drosophila melanogaster*. *J. Exp. Biol.* ***217***, 580–589 (2014).
10) K. G. Ormerod, O. K.LePine, P. S. Abbineni, J. M. Bridgeman, J. R. Coorssen, A. J. Mercier, G. J. Tattersall, *Drosophila* development, physiology, behavior, and lifespan are influenced by altered dietary composition. *Fly (Austin).* **11**, 153–170 (2017).
11) T. M. Bass, R. C. Grandison, R. Wong, P. Martinez, L. Partridge, M. D. Piper, Optimization of dietary restriction protocols in *Drosophila*. *J. Gerontol. A Biol. Sci. Med. Sci.* **62**, 1071–1081 (2007).
12) R. C. Grandison, R. Wong, T. M. Bass, L. Partridge, M. D. Piper, Effect of a standardised dietary restriction protocol on multiple laboratory strains of *Drosophila melanogaster*. *PLoS One*. **4**, e4067 (2009).
13) S. D. Katewa, F. Demontis, M. Kolipinski, A. Hubbard, M. S. Gill, N. Perrimon, S. Melov, P. Kapahi, Intramyocellular fatty-acid metabolism plays a critical role in mediating responses to dietary restriction in *Drosophila melanogaster*. *Cell Metab.* **16**, 97–103 (2012).
14) T. Chapman, L. Partridge, Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. *Proc. Biol. Sci.* **263**, 755 – 759 (1996).
15) S. Sisodia, B. N. Singh, Experimental evidence for nutrition regulated stress resistance in *Drosophila ananassae*. *PLoS One*. **7**, e46131 (2012).

16) S. Krittika, A. Lenka, P. Yadav, Evidence of dietary protein restriction regulating pupation height, development time and lifespan in *Drosophila melanogaster*. *Biol. Open*. **8**, bio042952 (2019).
17) P. Güler, N. Ayhan, C. Kosukcu, B. S. Önder, The effects of larval diet restriction on developmental time, pre adult survival, and wing length in *Drosophila melanogaster*. *Turk. J. Zool.* **39**, 395 –403 (2015).
18) T. Reis, Effects of Synthetic Diets Enriched in Specific Nutrients on *Drosophila* Development, Body Fat, and Lifespan. *PLoS One*. **11**, e0146758 (2016).
19) T. N. Kristensen, J. Overgaard, V. Loeschcke, D. Mayntz, Dietary protein content affects evolution for body size, body fat and viability in *Drosophila melanogaster*. *Biol. Lett*. **7**, 269–272 (2011).
20) S. N. Morris, C. Coogan, K. Chamseddin, S. O.Fernandez-Kim, S. Kolli, J. N. Keller, J. H. Bauer, Development of diet-induced insulin resistance in adult *Drosophila melanogaster*. *Biochim Biophys Acta.* **1822**, 1230–1237 (2012).
21) J. Hodin, L. M. Riddiford, Different mechanisms underlie phenotypic plasticity and interspecific variation for a reproductive character in Drosophilids (Insecta: Diptera). *Evolution.* **54**, 1638–1653 (2000).
22) R. M. Udugade, A. A. Deshmukh, K. A. Gajare, Dietary Restriction Initiated from Larval Stage Decreases Maximum Life Span in *Drosophila melanogaster*. *IORS-JPBS*. **6**, 37–42 (2016).
23) C. M. May, A. Doroszuk, B. J. Zwaan, The effect of developmental nutrition on life span and fecundity depends on the adult reproductive environment in *Drosophila melanogaster*. *Ecol. Evol.* **5**, 1156–1168 (2015).
24) M. Stefana, P. C. Driscoll, F. Obata, A. R. Pengelly, C. L. Newell, J. I. MacRae, A. P. Gould, Developmental diet regulates *Drosophila* lifespan via lipid autotoxins. *Nat.Commun*. **8**, 1384 (2017).
25) M. P. Tu, M. Tatar, Juvenile diet restriction and the aging and reproduction of adult *Drosophila melanogaster*. *Aging Cell*. **2**, 327–333 (2003).
26) C. K. Mirth, H. Y. Tang, S. C. Makohon-Moore, S. Salhadar, R. H. Gokhale, R. D. Warner, T. Koyama, L. M. Riddiford, A. W. Shingleton, JH controls body size by altering growth rate. *PNAS USA.* **111**, 7018–7023 (2014).

27) L. M. Matzkin, S. Johnson, C. Paight, T. A. Markow, Preadult parental diet affects offspring development and metabolism in *Drosophila melanogaster*. *PLoS One.* **8**, e59530 (2013).

28) R. K. Vijendravarma, S. Narasimha, T. J. Kawecki, Effects of parental larval diet on egg size and offspring traits in *Drosophila*. *Biol. Lett*. **6**, 238–241(2010).

29) T. M. Valtonen, K. Kangassalo, M. Pölkki, M. J. Rantala, Transgenerational effects of parental larval diet on offspring development time, adult body size and pathogen resistance in *Drosophila melanogaster*. *PLoS One*. **7**, e31611 (2012).

30) A. Sokoloff, Morphological variation in natural and experimental populations of *Drosophila pseudoobscura* and *Drosophila persimilis*. *Evolution.* **20**, 49–71 (1966).

31) A. K. Chippindale, T. J. F. Chu, M. R. Rose, Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution.* **50**, 753–766 (1996).

32) L. Partridge, K. Fowler, Responses and correlated responses to artificial selection on thorax length in *Drosophila melanogaster*. *Evolution*. **47**, 213–226 (1993).

33) S. Pitnick, Male size influences mate fecundity and remating interval in *Drosophila melanogaster*. *Anim. Behav*. **41**, 735–745 (1991).

34) A. Lefranc, J. Bundgaard, The influence of male and female body size on copulation duration and fecundity in *Drosophila melanogaster*. *Hereditas.* **132**, 243–247 (2000).

35) P. G. Byrne, W. R. Rice, Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. *Proc. Biol. Sci.* **273**, 917–922 (2006).

36) D. A. Edward, T. Chapman, Variation in male mate choice in *Drosophila melanogaster*. *PLoS One*. **8**, e56299 (2013).

37) J. B. Deas, L. Blondel, C. G. Extavour, Ancestral and offspring nutrition interact to affect life-history traits in *Drosophila melanogaster*. *Proc. Biol. Sci.* **286**, 20182778 (2019).

38) J. R. David, R. Allemand, J. Van Herrewege, Y. Cohet, “Ecophysiology: abiotic factors” in The Genetics and Biology of *Drosophila*, M. Ashburner, H. L. Carson, J. N. Thompson Jr, Eds. (Academic Press, London, 1983), pp. 105–170.

39) H. D. Stalker, H. L. Carson, An altitudinal transect of *Drosophila robusia* Sturtevant. *Evolution*. **2**, 295–305 (1948).

40) A. O. Tantawy, Studies on natural populations of *Drosophila*. III. Morphological and genetic differences of wing length in *Drosophila melanogaster* and *D. simulans* in relation to season. *Evolution.* **18**, 560–570 (1965).

Figure Legends:-

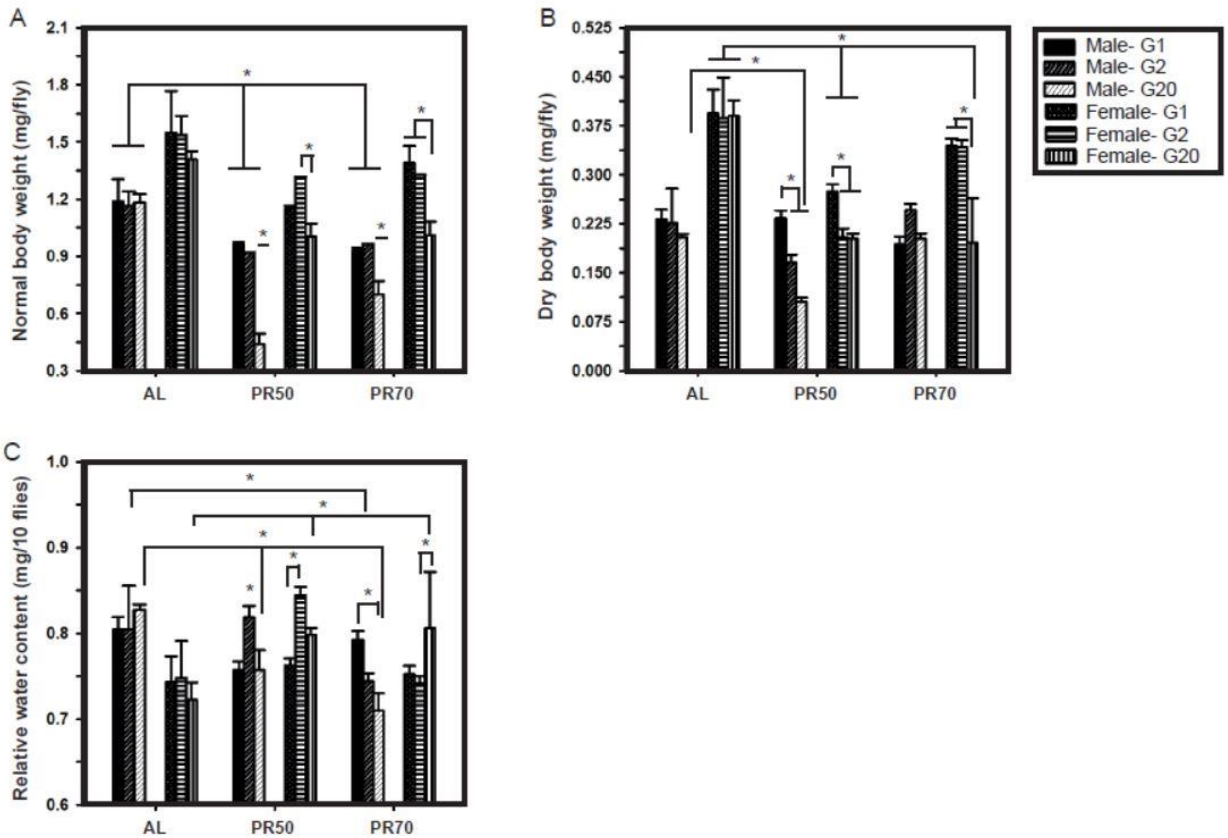

Figure 1: Low weighed males and females under the PR diet for 20 generations. The normal (A) and dry (B) body weight of the flies (varying across generations), shows PR flies weighing lower than that of AL flies at the end of generation 20. The effect of diet pertaining to the relative water content (C) is prominent, wherein after 20 generations of PR diets, male and female flies possessed lower and higher water content as compared to AL respectively. The graph represents diet in the *x*-axis and body weight (A, B), relative water content (C) in the *y*-axis. The bars and error bars are represented as the mean ± standard deviation (SD). The asterisks on the bars indicate significance levels wherein *p*-value is <0.05. G1, G2 and G20 represent generation 1, 2, and 20 respectively.

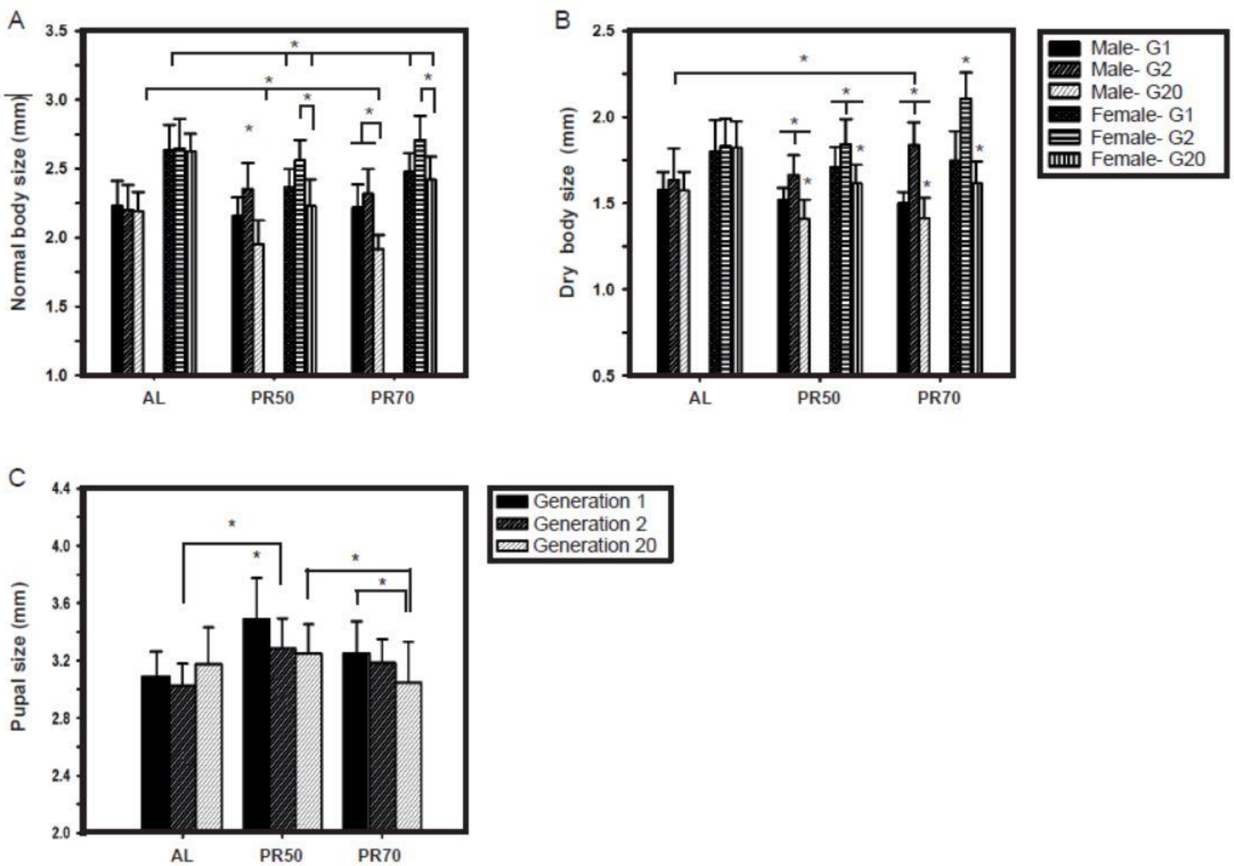

Figure 2: Smaller flies and unaltered pupal size due to the PR diet for 20 generations. The effect of diet and generation on the normal (A) and dry (B) body size of the flies are variable, wherein the normal body size of PR flies is lower than their control at the end of the 20 generations. The pupal size (C) of PR50 flies were the highest at gen 1 as compared to PR70 and control, but after 20 generations were similar to AL. The graph represents diet in the *x*-axis and body size (A, B), pupal size (C) in the *y*-axis. All other details are the same as in Figure 1.

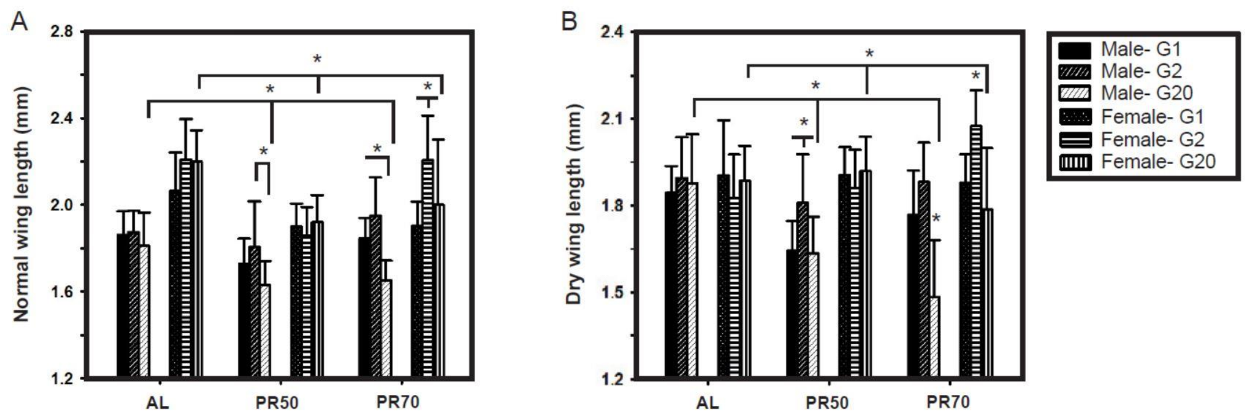

Figure 3: Reduced wing length of flies due to long-term PR diet imposition. The normal (A) and dry wing length (B) of the PR flies are smaller than the AL flies post 20 generations, but the pattern of variations across generation is different from that witnessed for body size. The graph represents diet in the *x*-axis and wing length on the *y*-axis. All other details are the same as in Figure 1.

Table 1: ANOVA details of the normal and dry body weight, size, pupal size and wing length of long-term PR imposed flies.

Assay	**Effect**	**d.f.**	**MS effect**	**d.f. error**	**MS error**	***F***	***P*<**
Normal body weight	Diet (D)	2	114.29	72	0.59	193.04	0.0001
	Gen (G)	2	58.99	72	0.59	99.63	0.0001
	Sex (S)	1	290.52	72	0.59	490.7	0.0001
	Diet × Gen (D × G)	4	8.65	72	0.59	14.61	0.0001
	Diet × Sex (D × S)	2	0.88	72	0.59	1.48	0.2342
	Gen × Sex (G × S)	2	0.34	72	0.59	0.57	0.5654
	Diet × Gen × Sex (D × G × S)	4	5.65	72	0.59	9.54	0.0001
Dry body weight	Diet (D)	2	8.7003	72	0.0815	106.75	0.0001
	Gen (G)	2	3.079	72	0.0815	37.78	0.0001

	Sex (S)	1	23.61	72	0.0815	289.74	0.0001
	Diet × Gen (D × G)	4	1.015	72	0.0815	12.46	0.0001
	Diet × Sex (D × S)	2	2.607	72	0.0815	31.99	0.0001
	Gen × Sex (G × S)	2	0.13	72	0.0815	1.61	0.2064
	Diet × Gen × Sex (D × G × S)	4	0.88	72	0.0815	10.8	0.0001
Relative Water content	Diet (D)	2	0.0077	72	0.00068	11.4	0.0001
	Gen (G)	2	0.002	72	0.00068	3.03	0.0547
	Sex (S)	1	0.0026	72	0.00068	3.87	0.0531
	Diet × Gen (D × G)	4	0.0070	72	0.00068	10.38	0.0001
	Diet × Sex (D × S)	2	0.0225	72	0.00068	33.3	0.0001
	Gen × Sex (G × S)	2	0.0035	72	0.00068	5.12	0.0083
	Diet × Gen × Sex (D × G × S)	4	0.0057	72	0.00068	8.46	0.0001
Normal body size	Diet (D)	2	1.036	522	0.027	38.5	0.0001
	Gen (G)	2	2.641	522	0.027	98.2	0.0001
	Sex (S)	1	16.45	522	0.027	611.6	0.0001
	Diet × Gen (D × G)	4	0.586	522	0.027	21.8	0.0001
	Diet × Sex (D × S)	2	0.471	522	0.027	17.5	0.0001
	Gen × Sex (G × S)	2	0.143	522	0.027	5.3	0.0052
	Diet × Gen × Sex (D × G × S)	4	0.056	522	0.027	2.1	0.083
Dry body size	Diet (D)	2	0.374	522	0.018	21.23	0.0001
	Gen (G)	2	2.870	522	0.018	163.13	0.0001
	Sex (S)	1	6.394	522	0.018	363.37	0.0001
	Diet × Gen (D × G)	4	0.724	522	0.018	41.12	0.0001
	Diet × Sex (D × S)	2	0.025	522	0.018	1.42	0.2438
	Gen × Sex (G × S)	2	0.0004	522	0.018	0.03	0.9729
	Diet × Gen × Sex (D × G × S)	4	0.015	522	0.018	0.86	0.4907

Pupal size	Diet (D)	2	1.452	261	0.049	29.34	0.0001
	Gen (G)	2	0.394	261	0.049	7.96	0.0004
	Diet × Gen (D × G)	4	0.292	261	0.049	5.91	0.0001
Normal wing length	Diet (D)	2	1.757	522	0.024	72.28	0.0001
	Gen (G)	2	0.682	522	0.024	28.07	0.0001
	Sex (S)	1	7.338	522	0.024	301.92	0.0001
	Diet × Gen (D × G)	4	0.258	522	0.024	10.6	0.0001
	Diet × Sex (D × S)	2	0.223	522	0.024	9.19	0.0001
	Gen × Sex (G × S)	2	0.453	522	0.024	18.62	0.0001
	Diet × Gen × Sex (D × G × S)	4	0.112	522	0.024	4.6	0.0012
Dry wing length	Diet (D)	2	0.287	522	0.021	13.46	0.0001
	Gen (G)	2	0.723	522	0.021	33.84	0.0001
	Sex (S)	1	2.393	522	0.021	112.05	0.0001
	Diet × Gen (D × G)	4	0.559	522	0.021	26.19	0.0001
	Diet × Sex (D × S)	2	0.608	522	0.021	28.49	0.0001
	Gen × Sex (G × S)	2	0.224	522	0.021	10.51	0.0001
	Diet × Gen × Sex (D × G × S)	4	0.114	522	0.021	5.33	0.0003