To know the extent of genetic variations and estimates of heritability for mating propensity, fecundity, longevity and triglycerides level in inbred lines of *D. ananassae*:

- The experiments pertaining to above objective were carried out in twenty four full sib mated inbred lines of *D. ananassae*. These inbred lines were originally raised from twenty four isofemale lines. The fitness traits tested were: mating propensity, fecundity, longevity and triglycerides level. Among lines difference in the mean values of different traits reflect the genetic variation persisting in the traits.

- Results indicated that there is significant genetic variation in mating propensity, fecundity and longevity (life history traits) among the twenty four inbred lines of *D. ananassae*. However, triglycerides level (physiological trait), either in females or males did not exhibit significant genetic variation. The values of coefficient of variations are highly variable for all the traits studied. A high coefficient of variation implies that the trait is not experiencing efficient selection to reduce genetic variation in the nature.

- Estimated heritabilities are moderate for all the traits and ranged from 0.094 to 0.221. It is maximum for mating propensity (0.221) followed by triglycerides level in males (0.21) and females (0.194), fecundity (0.164) and life span (0.094) which is consistent with the most estimates of life history traits in natural populations (Lynch and Walsh 1998; Reed *et al.* 2010) suggesting that fitness traits have low heritability.

- Pair-wise correlations were analyzed between different traits of twenty four inbred lines of *D. ananassae*. A significant positive correlation between mating success and fecundity was found. A positive correlation was also found between mating success and triglycerides level but the correlation was not significant. A non-significant negative correlation between triglycerides level and longevity in female was noticed. Triglycerides level and fecundity did not show any
correlation. Correlation analysis between longevity of female and fecundity illustrated a positive correlation though; it was not significant. Longevity of female and mating success did not show any correlation.

**To see the phenotypic reaction norms and genotype-by-drug interactions for mating propensity, fecundity, longevity and triglycerides level in response to Withania somnifera root extract in inbred lines of D. ananassae:**

- Aim of this objective was to demonstrate how individuals of the same genotype show varying response in different environmental conditions, that is, the phenomenon of phenotypic plasticity. When an individual of a particular genotype produces a phenotype that varies as a continuous function of the environmental conditions, the relationship is called a reaction norm (Woltereck 1909). Genotype by drug interactions (G x D; a form of G x E) will occur when there is variation among genotypes in the rank order or relative magnitude of effects in different environments (Falconer and Mackay 1996). The isofemale inbred lines is convenient tool for the analysis of phenotypic plasticity in relation to environmental gradient (David et al. 2005). Thus, again inbred lines of D. ananassae were undertaken as a study material. The environmental gradient provided here was the different concentration of WS root extract.

- Reaction norms for mating propensity, fecundity, longevity and triglycerides level in females and males were plotted by putting environmental gradients on X-axis and trait values on Y-axis. The plots for reaction norms were found to be non horizontal for all the traits, demonstrating the presence of phenotypic plasticity in individuals of D. ananassae.

- In the present study, inbred lines represented different genotypes while treatments with different concentrations of drug represented environmental gradients. Thereby, in the present study G x D is represented as Line X Treatment. Results of two-way ANOVA showed that there were
significant Line X Treatment interactions for mating propensity, fecundity and longevity. However, Line X Treatment interactions were not significant for triglycerides level either in females or in males. Other than, Line X Treatment interactions two-way analysis also indicated significant differences in mating propensity, fecundity and longevity which were due to variation in lines (genotypes). However, line wise variation did not alter triglycerides level significantly in females and males.

- Owing to medicinal importance of *W. somnifera*, effect of WS root extract on fitness traits of *D. ananassae* was observed. It was evident from the two-way analysis that treatment with WS root extract causes significant differences in fecundity, longevity and triglycerides level in females and males except mating propensity. Further, result of one-way ANOVA also shows that WS root extract has significant effect on fecundity, longevity and triglycerides level in both the sexes of *D. ananassae* but it does not affect mating propensity. It was found that fecundity reduces significantly in treatment group at 0.01% concentrations compared to the control while at higher concentration i.e., at 0.05% and 0.1%, significant difference was not found. Similarly, triglycerides level in females and males were observed to be significantly reduced compared to the control; at 0.01% WS root extract treatment. In females triglycerides level fall significantly at 0.1% also with respect to control but in males no significant alteration was found at higher concentration (0.05% and 0.1%). However, longevity gets reduced at all the concentrations of WS root extract. It was noticed that effects of WS root extract occurred in dose-dependent manner. Surprisingly, in case of fecundity and triglycerides assays, effects were more pronounced at low dose which could be explained by low dose effect hypothesis (Kamrin 2007; Vandenberg *et al.* 2012).
Pair-wise correlation analyses were made for different phenotypic traits across all the lines and WS root extract treatments. Results of correlation analyses showed that there is significant positive correlation between mating propensity and fecundity, between fecundity and longevity, between fecundity and triglycerides level, between longevity and triglycerides level in females and between triglycerides in females & triglycerides in males.

To see the effect of temperature on mating propensity, survivorship, triglycerides level and hsp70 expression in different karyotypes associated with alpha inversion in *D. ananassae*:

- In this experiment karyotypically characterised stocks i.e. ST/ST (standard homokaryotype), AL/AL (inverted homokaryotype), ST/AL & AL/ST (heterokaryotypes) of *D. ananassae* were employed.
- Mating propensity of different karyotypes was examined in the flies of control (no exposure to high temperature) and three experimental groups: (1) both sexes exposed to high temperature (2) only males exposed to high temperature (3) only females exposed to high temperature. To compare the relative effect of karyotype and temperature, two-way nested ANOVA was employed and the results indicate that mating success differs significantly among karyotypes as well as within karyotypes due to experimental condition provided. Significant difference for mating success within a karyotype is the consequence of alteration in temperature. The estimate of variance component indicates that the difference in mating success is largely due to karyotypic variations (57% for Gangtok population and 51% for Varanasi population) while temperature causes approximately 30% variation in a given karyotype. Results of one-way ANOVA for each karyotype have shown that there is significant difference in mating success in different experimental conditions for homokaryotypes (ST/ST or AL/AL) while heterokaryotypes (AL/ST or ST/AL) do not show significant alteration in mating success except for the AL/ST karyotype.
of Gangtok population, suggesting heterokaryotypes exhibit greater thermal tolerance than homokaryotypes. Post-hoc analysis (Bonferroni t test) displayed that, in homokaryotypes, males are more influenced by elevated temperature in comparison to females.

- The results of survivorship assay depicted that homokaryotypes (ST/ST or AL/AL) show higher mortality rate than heterokaryotypes (AL/ST or ST/AL) in both the sexes of both the strains. However, significant differences were found only in some cases of pair wise comparisons for survivorships.

- Results of triglyceride estimation demonstrated that there is high triglycerides level in heterokaryotypes than homokaryotypes in both the sexes of both the strains except GT-ST/AL male. However, when they were exposed to high temperature, a decline in triglycerides level was observed, irrespective of homo- or hetero-karyotypes.

- Hsp70 expression was determined in male flies of different karyotypes exposed to 33°C or 35°C. Whole body lysates of control flies raised at 24°C showed no detectable Hsp70 expression in the absence of heat stress. However, Hsp70 expression was found to be elevated in the whole body lysates of heterokaryotypes of both the strains than homokaryotypes when they were given thermal stress.

**To identify the variants of the structural gene loci coding for Esterase enzyme and investigation of role of Esterase-4 locus in reproductive fitness of D. ananassae:**

- Allozyme polymorphism studies in *D. ananassae* have shown that Esterase enzyme is expressed by five distinct loci, *Est-1, Est-2, Est-3, Est-4* and *Est-5* in *D. ananassae*. Out of these five loci; four loci (*Est-2, Est-3, Est-4* and *Est-5*) were polymorphic and each of these polymorphic loci were expressed in two allelic forms. Alleles of *Est-2, Est-3* and *Est-5* were identified as having fast and slow alleles based on their electrophoretic mobility during native PAGE while, *Est-4*
locus was identified as having active and null alleles based on enzymatic activity. Heterozygotes (cross product of \textit{Est-4 active} (+/+)) and \textit{Est-4 null} (-/-)) expressed enzyme activity, indicating \textit{Est-4 active} allele behaving as a dominant allele and these two alleles followed the Mendelian pattern of segregation.

- It was assumed that \textit{Est-4} locus may have direct or indirect role in mating propensity, mating preference, fecundity, fertility and productivity, longevity and lipid metabolism. So, these parameters were assayed in the flies which were either homozygous for \textit{Est-4 active} or \textit{Est-4 null} alleles. In mating propensity assay, an average mating success was found to be 8.4 and 7 for \textit{Est-4 active} and \textit{Est-4 null} genotypes respectively. However, the difference was found to be statistically non-significant.

- Theoretically, two main mechanisms can produce deviations from random mating, sexual selection and sexual isolation due to discrepancy in mating propensity and mate choice. Therefore, heterogeneity \(G\) test for sexual selection effects (GS), sexual isolation effects (GI) and for the combined effects (GT= GI + GS) were assessed. GT, GI and GS values were not statistically significant, providing evidence for random mating between the genotypes of \textit{Est-4}. However, value of GS was very close to the value at \(P = 0.05\), suggesting that a moderate sexual selection might be operating. In this analysis, the PTI coefficients correspond to the combined sexual selection and sexual isolation effects for each mating pair combination, whereas, \(I_{PSI}\) coefficient is the estimate of global sexual isolation. PTI coefficients were not statistically significant for all the possible mating pair combinations except for one, i.e., \textit{Est-4 active} male + \textit{Est-4 null} female combination. An estimate of \(I_{PSI}\) coefficient was also not significant.

- There was a significant difference for the fecundity in females of the two genotypes. The egg laying capacity of \textit{Est-4 active} females were more than \textit{Est-4 null} females.
• Present study revealed that there is strong dependency between genotypes and the rate of fertility. The flies of \textit{Est-4 active} are more fertile than \textit{Est-4 null} flies. The median value of progeny produced by per female for \textit{Est-4 active} and \textit{Est-4 null} was 24 and 20 respectively but the difference was not statistically significant.

• Triglycerides level in haemolymph of \textit{Est-4 active} larvae was found to be significantly more than in \textit{Est-4 null} larvae. Triglycerides content was higher in adult flies of \textit{Est-4 active} than \textit{Est-4 null} in both the sexes as well. However, the difference was not significant.

• The longevity assay showed reduction in the life span of \textit{Est-4 null} compared to the \textit{Est-4 active} flies in both the sexes. Furthermore, reduction in the life span of \textit{Est-4 null} females compared to the \textit{Est-4 active} females was significant indicating role of \textit{Est-4} locus in the regulation of life span.