Study of inversion heterozygosity in three Indian populations of *Drosophila ananassae.*

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Abstract

Changing environmental conditions play a major role in bringing mutational modifications in the genetic makeup of the species. On encountering any stress in the surroundings, the organisms may undergo several behavioral changes to adapt to the new environment. Inversion is one such chromosomal aberration wherein the sequence on one or both of the chromosomes gets inverted. Inversion heterozygosity is linked to formation of loops on account of which recombination frequency in the inverted region decreases and the underlying genes escape themselves to a certain amount. Literature suggests that inversions play a significant role in adaptation, speciation, and affects life-history traits of an organism. Inversion polymorphisms are found to be species-specific, therefore often used for phylogenetic analysis and species identification. Inversions are also found to be population specific, thus help to determine the eco-geographic distribution of a species population. *Drosophila* is an ideal model system for studying inversion polymorphism as it possesses polytene chromosomes in its larval stage, which is extensively studied for banding patterns and differential gene expression. The present work was carried out to study inversion heterozygosity in *Drosophila ananassae* belonging to Northern (Ahmedabad, Ghaziabad) and Southern (Kochi) India. The study was further extended to examine the reproductive traits, *e.g.*, ovariole number and fecundity in the Kochi population, and a correlation was established between inversion heterozygosity and reproductive success. The results of the current study revealed that the Kochi population had the maximum inversion heterozygosity. Keywords: Inversion-heterozygotes, *D. ananassae*, India, Reproductive –fitness

Introduction

Inversions, a kind of chromosomal rearrangement, are classified into different categories on the basis of (i) position, *i.e.*, paracentric or pericentric depending on the inclusion of centromere (Griffith *et al.*, 2000); (ii) demography, *i.e.*, common cosmopolitan (frequency > 5%), rare cosmopolitan (< 5%), recurrent endemics (occur in a few individuals), and unique endemics (found only once) (Mettler *et al.*, 1977); (iii) chromosomal arrangement, *i.e.*, homozygous (Inv/Inv) or heterozygous (ST/Inv). The significant role of inversion polymorphism in different evolutionary processes and disease pathogenesis in human beings has been well documented in earlier studies (Feus *et al.*, 2005; Kehrer *et al.*, 2005; Broman *et al.*, 2003). *Drosophila* has been an attractive genetic model since the time of Morgan and contributed immensely to understanding both the classical and molecular aspect of complex biological systems (Beckingham *et al.*, 2007). Various mutations are observed in its natural populations. Successful establishing and maintenance of mutant lines in the laboratory conditions makes it more instrumental in conducting inheritance experiments. The presence of polytene chromosomes in the third instar larval stage of this organism allows its use in cytogenetic studies. The polytene chromosome helps the organism to undergo development and growth at a fast rate and hence is present at the larval stage wherein an organism requires maximum growth. The banding pattern present in the polytene chromosome shows the expression profile of genes and also helps in determining inversion polymorphism. The importance of inversions in *Drosophila*, their population-specific and species-specific occurrence and adaptive nature has been reported in earlier studies (Singh, 2008; Rezende *et al.*, 2010; Singh *et al.*, 2012; Kenig *et al.*, 2015). Inversions are known to help in the process of adaptation (Dolgova *et al.*, 2010), speciation (Noor *et al.*, 2001) and play an important role in species identification (Feder *et al.*, 2014) and also in understanding evolution of sex chromosomes (Ming and Moore, 2007).

Drosophila ananassae is a common cosmopolitan species having a world-wide distribution and that has become a model for various genetic and evolutionary studies due to its unique characteristics, *e.g.* spontaneous male recombination, segregation distortion, mutation (Mukherjee and Das, 1971), occurrence of genetic mosaics for autosomal genes (Singh and Mohanty, 1992), high mutability (Tobari, 1993), and extra-chromosomal inheritance (Sturtevant, 1942). *D.ananassae* is known to harbor a large number of paracentric and pericentric inversions in its four chromosomes (Singh, 1989). The present work was conducted to find the frequency of inversion polymorphism in Indian *D. ananassae* collected from three different regions of Northern (Ghaziabad, Ahmedabad) and Southern (Kochi) India. The study was further extended in one of the populations, *i.e*., Kochi, to find whether there is any correlation between inversion heterozygosity and the variations in life-history traits, *e.g*., fecundity and ovariole number.

Material and Methods

Drosophila *strains used*

D. ananassae flies were collected from Ahmedabad, Kochi, and Ghaziabad between July-October 2015. The flies were collected from fruit orchards by net sweeping method from shaded areas. Each single naturally caught female fly was kept in separate vials to establish iso-female lines. The progeny from each iso-female line were routinely sub-cultured to fresh media vials and a subsequent generation record was maintained for each line. Fly stocks were kept in incubator at 25°C and were transferred to fresh food vials on a weekly basis. Eight iso-female lines from each of these regions were used in the present study.

Setting up of experiment

20 male and female flies were kept in vials and allowed to mate for 4-5 days after which the parental flies were discarded or transferred. To ensure healthy growth of the larvae, yeast paste was added after the 5^{th} day, and the vials were observed on a daily basis for emergence of third instar larvae.

Cytological analysis

Healthy third instar larvae (~20 from each iso-female lines) were taken and the salivary gland was isolated for preparation of polytene chromosome slides by the squashing method. Polytene chromosomes were observed under a microscope. The chromosomal arms with inversion polymorphism were identified by comparing them with the chromosomal maps from FlyBase. Inversions identified on each chromosomal arm were categorised into different types as mentioned above.

Fecundity and ovariole number

Both fecundity and ovariole number were calculated in 4 iso-female lines of the Kochi population. 20 virgin males and females each from 4 days old age were kept in a mating chamber for 48 hours, and the eggs laid during this period were counted to find the fecundity rate. For ovariole number, ~ 8-10 days aged females were dissected for counting of their ovarioles present in both the ovaries.

Results and Discussion

1. Inversion Heterozygosity with respect to different chromosomal arms:

In all studied populations, the 2L chromosome arm was found to be highly polymorphic for inversions as carrying the maximum number of different inversions. Alpha (Figure 1.a.i), a common cosmopolitan inversion, was found to be present at a frequency of 12% in Kochi and Ghaziabad and 4% in Ahmedabad populations (Figure 3). Also, four more inversions were found on the 2L arm out of which one of them (Figure 1.a.ii) was common to both Kochi and Ahmedabad, whereas the third one (Figure 1.a.iii) was common to Kochi and Ghaziabad. The rest of the two were specific for their regional population (Figure 1.a.iv, v). Alpha covers around 60% of the total chromosomal arm whereas the other four inversions cover around 20% of the area. All of the inversions were found to be sub-terminal. The right arm of the second chromosome harbored 2 different inversions, one of them being cosmopolitan, *i.e*., Zeta as shown in Figure 1.b.i (Singh and Mohanty, 1990), was found in all three regional populations, whereas the second one was specific for Kochi

(Figure 1.b.ii). Zeta spans around 50% of the chromosomal arm and the new inversion takes around 20% of the region. The 3L arm of the chromosome has one cosmopolitan inversion, Delta (Figure 2.a.i), which spans around 50% of the arm and was found to be present in Kochi, Ghaziabad, and Ahmedabad populations at a frequency of 41%, 25%, and 17%, respectively (Figure 3). Also a new sub-terminal inversion, which occupies 15% of the region on the 3L arm, was found only in the Kochi population (Figure 2.a.ii). Eta is a cosmopolitan inversion found on 3R, which is located very close to the centromere and occurred at 23%, 49%, and 12% in Kochi, Ghaziabad, and Ahmedabad, respectively (Figure 2.b.i; Figure 3). It spans around 20% of the total chromosomal area and was reported in all three populations. A second inversion, 3R1 (Figure 2.b.ii), found on this arm spans around 20% of the area and was specific for the Ahmedabad region.

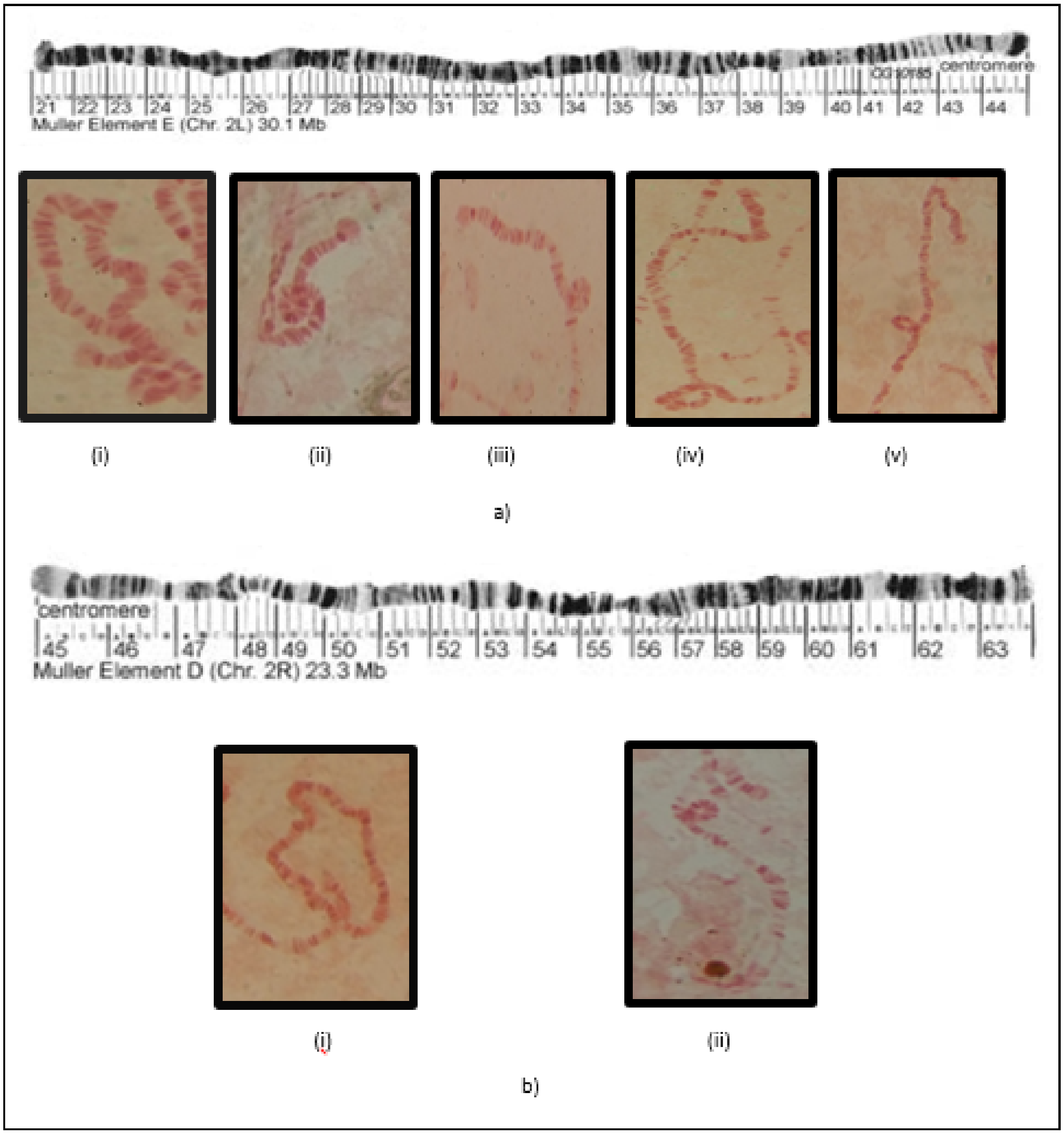


Figure 1. Cytological map and inversions found on the second arm of the chromosome.

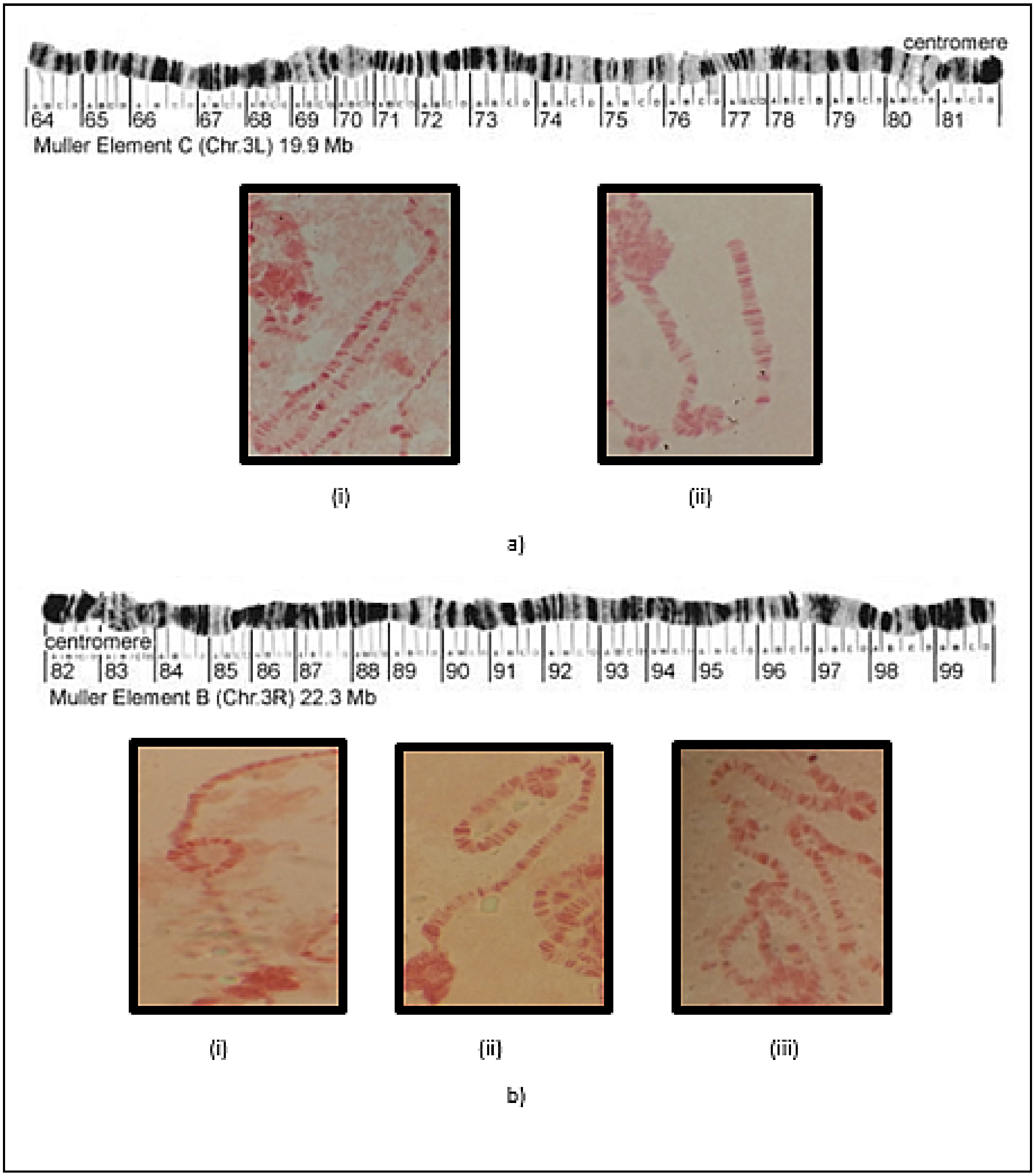


Figure 2. Cytological map and inversions found on the third arm of the chromosome.

2. Clinal variation in inversion heterozygosity

Overall, all three populations reported the presence of cosmopolitan inversions; however, some of the inversions were found to be only region specific. Also by calculating the mean number of inversion heterozygosity, it was found that the Kochi population has higher mean heterozygosity (0.95), followed by Ghaziabad (0.83), and Ahmedabad (0.55) showing the minimum mean heterozygosity. Such a variation in the frequency of inversion heterozygotes prompted us to dig the literature and find if this frequency distribution shows any clinal pattern. One of the works on the Nagaland population of *D. ananassae* by Bovito (2015) reported the frequency distribution of three cosmopolitan inversions (2L, 3L, 3R). Thus, we have analysed the data obtained for these three inversions in the present study and compared it to the data from literature (Figure 3). All these regions show a similar pattern with respect to frequency distribution by showing higher percentage of inversion heterozygotes on the third arm of chromosome (Delta-3L, Eta-3R), which can be contrasted from the one obtained in the Nagaland population, which harbors a higher number of Alpha heterozygotes (Bovito, 2015). Such a variation clearly shows that inversion is an adaptive trait, and it also shows clinal pattern of distribution which was also well established through earlier studies (Anderson *et al*., 2005; Umina *et al*., 2005).

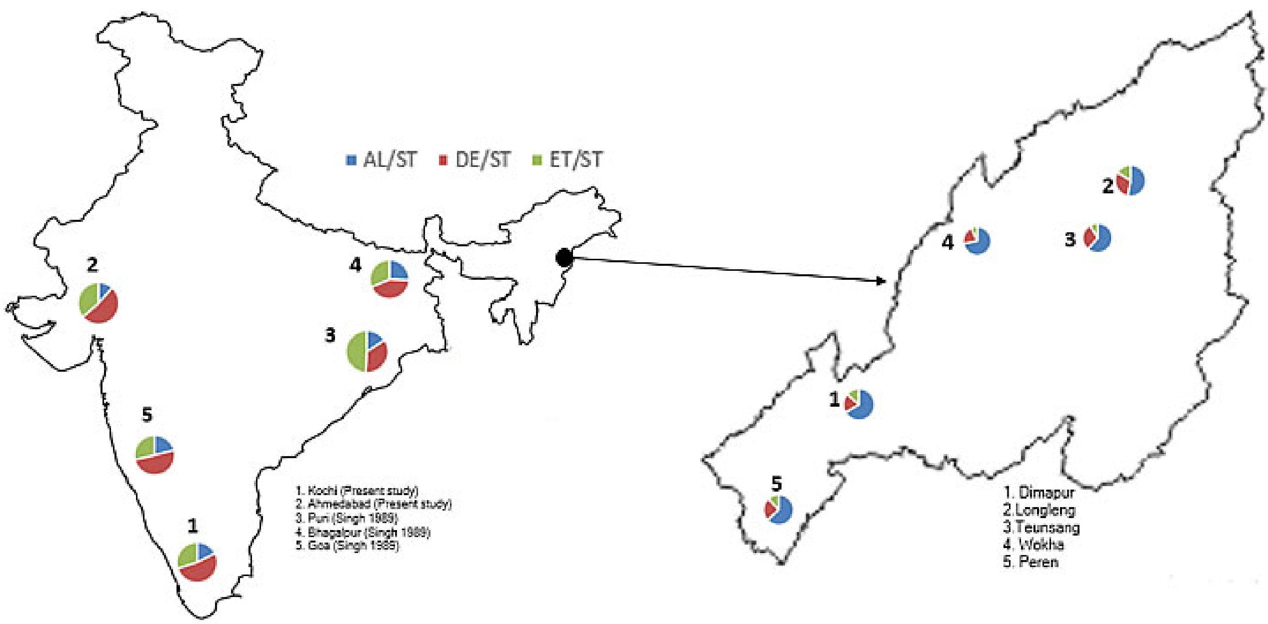


Figure 3. Frequency distribution of cosmopolitan inversions in different regions of India and comparison with Nagaland population frequency distribution (The data for Puri, Bhagalpur, and Nagaland have been taken from literature.).

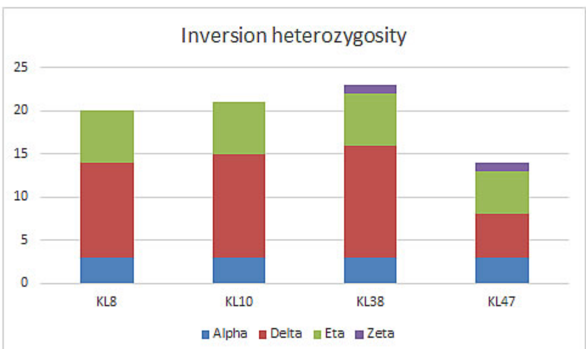
3. Life-history trait analysis in Kochi population

The extended study in the Kochi population revealed that a correlation exists between inversion heterozygosity and rate of fecundity. However, a slight variation was observed with respect to the number of ovarioles when compared with the inversion heterozygosity. Amongst the four iso-female lines of Kochi, KL 38 shows a higher number of inversions and maximum number of ovarioles (average 22 per ovary) and high fecundity values (70 per 20 females in 48 hours) as shown in Figure 4. In contrast, KL 47 has a lower number of inversion heterozygotes, least number of ovarioles, and also less fecundity. Such an occurrence can help us to establish a hypothesis that inversion heterozygosity is linked to reproductive success of the females.

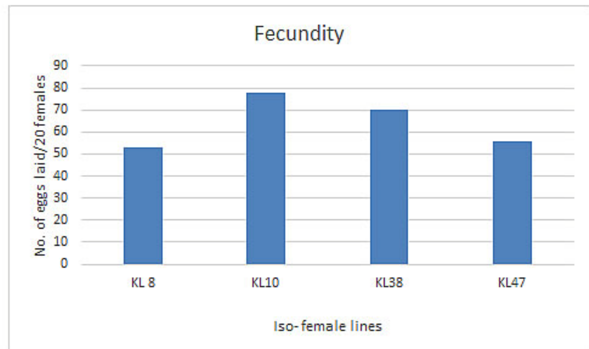
Conclusion

Inversion heterozygosity shows a variation on the basis of population and is an adaptive trait. The results clearly show that the Kochi population shows more inversions as compared to Ahmedabad or Ghaziabad populations. The third chromosome inversions are found to be fixed for all three populations. From the overall comparison between inversion frequency in these different regions of India including

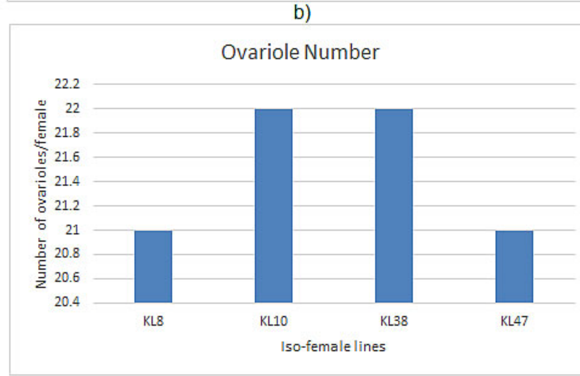
Nagaland, it can be further concluded that inversions are regional-specific and are known to show latitudinal clines. The study involving life-history trait variation along with the inversion heterozygosity in Kochi population evidences that heterozygous inversion can influence the reproductive success of an organism. To come up with a strong inference, the study needs to be extended with an increased sample size. Earlier studies have reported that not only the inversion frequency but the total chromosomal region included in the inversion heterozygote loops also affects the recombination in other chromosomes. Breakpoint analysis of inversions and further digging into the essential genes present in the inversion area may help us to determine the significant role of these inversions on the functional genes and to understand more about the process of mutation and adaptation.



a)



b)



c)

Figure 4. Shows variation in a) inversion heterozygosity, b) fecundity, and c) ovariole number in four iso-female lines of Kochi.

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Magnesium supplementation reduces seizure and paralysis in *technical knockout* and *easily shocked* bang-sensitive mutants.

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Introduction

The idea that diet and nutrition play a role in health disorders is not a new one. Nutritional factors have been shown to be involved in the regulation of electrical activity in the brain (Gaby, 2007). In particular, maintenance of proper electrolyte concentrations are important, since their disruption commonly results in seizure and convulsions (Castilla-Guerra *et al.*, 2006). Epilepsy is a common seizure disorder that is treated by anticonvulsants; however, these drugs have limited efficacy in many patients. Because drugs have been somewhat ineffective, other treatments have been sought including diets. For example, the ketogenic diet, which is high in fat and low in carbohydrates and protein, has been utilized in controlling epilepsy in children (Lutas and Yellen, 2013).

Magnesium (Mg) has been used as an anticonvulsant for the condition of pre-eclampsia (Castilla-Guerra *et al.*, 2006). However, the electrolyte has not been used as a treatment for general epilepsy. There are many properties of Mg that may make it a potentially effective anticonvulsant. Mg is the fourth most common mineral found in the human body and is required for over 300 enzyme systems and as a cofactor for mitochondrial energy production. Magnesium also inhibits NMDA receptors, increases prostaglandin synthesis, stabilizes neuronal membranes, and acts as a calcium channel blocker (Castilla-Guerra *et al.*, 2006). Mg deficiency also has been shown to cause seizures or increase susceptibility to seizures. Sufferers of grand mal seizures have been found to maintain lower serum concentrations of magnesium than controls (Gaby, 2007). In a study of hippocampal seizures in rats, injection of $MgSO_4$ proved to increase the rats' seizure thresholds (Hallak *et al.*, 1992). In addition, Mg content in food has been in decline over the past sixty years and up to 75% of Americans do not take in the recommended dietary allowance of the mineral (Yuen and Sander, 2012).

Our study examines if magnesium supplementation could alter seizure behaviors in bang-sensitive mutants that are models of mitochondrial disease and epilepsy. Bang-sensitive (BS) paralytics exhibit seizures and paralysis in response to mechanical stimulation (Reynolds *et al.*, 2004). They also show a reduced threshold for seizures compared to wild-type in electrophysiology experiments (Kuebler and Tanouye, 2000). Bang-sensitive mutants include *bangsenseless* (*bss*), *bang-sensitive* (*bas*), *easily shocked* (*eas*), and *technical knockout* (*tko*), among others that express the bang-sensitive phenotype, but with differing biological underpinnings. We found that *eas* and *tko* mutants raised on diets supplemented with Mg would experience decreased seizures and shorter recovery times when exposed to stress conditions.

Methods and Procedures

Flies

Laboratory stock cultures of *tko* and *eas* flies as well as the CS wild-type background were used for the experiments. Flies were cultured at 25°C under 12 hr dark/light conditions on standard molasses/yeast/cornmeal (MYC) food with and without 30 mM $MgSO_4$.