Population genetics of *Drosophila ananassae*

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Summary

Drosophila ananassae Doleschall is a cosmopolitan and domestic species. It occupies a unique status among *Drosophila* species due to certain peculiarities in its genetic behaviour and is of common occurrence in India. Quantitative genetics of sexual and non-sexual traits provided evidence for genetic control of these traits. *D. ananassae* exhibits high level of chromosomal polymorphism in its natural populations. Indian natural populations of *D. ananassae* show geographic differentiation of inversion polymorphism due to their adaptation to varying environments and natural selection operates to maintain three cosmopolitan inversions. Populations do not show divergence on temporal scale, an evidence for rigid polymorphism. *D. ananassae* populations show substantial degree of sub-structuring and exist as semi-isolated populations. Gene flow is low despite co-transportation with human goods. There is persistence of cosmopolitan inversions when populations are transferred to laboratory conditions, which suggests that heterotic buffering is associated with these inversions in *D. ananassae*. Populations collected from similar environmental conditions that initially show high degree of genetic similarity have diverged to different degrees in laboratory environment. This randomness could be due to genetic drift. Interracial hybridization does not lead to breakdown of heterosis associated with cosmopolitan inversions, which shows that there is lack of genetic co-adaptation in *D. ananassae*. Linkage disequilibrium between independent inversions in laboratory populations has often been observed, which is likely to be due to suppression of crossing-over and random genetic drift. No evidence for chromosomal interactions has been found in natural and laboratory populations of *D. ananassae*. This strengthens the previous suggestion that there is lack of genetic co-adaptation in *D. ananassae*.

1. Introduction

Population genetics is the study of the mechanisms by which genetic changes are affected in a population. Since, evolution has been defined as any change in the genetic composition of a population, population genetics is of considerable importance to the understanding of the elemental forces of evolution. Population genetics concerns both, investigations on the origin of genetic diversity (mutations and chromosomal variability) and investigations on the spread of genetic diversity (selection, drift and migration). Population genetical studies have involved mainly concealed genetic variability caused due to deleterious genes, chromosomal variability, allozyme and DNA polymorphisms. In the 1920s and 1930s, Sir Ronald Fisher, Sewall Wright and J. B. S. Haldane contributed to the birth of population genetics while, G. H. Hardy (Great Britain) and W. Weinberg (Germany) in 1908 led to its mathematical foundation via the Hardy–Weinberg principle or the binomial square law. This law defines the condition of genetic equilibrium under the absence of evolutionary forces such as, selection, drift, mutation and migration.

Since the last review by Singh (1996), which was meant to give an overview of the field until 1996, there has been a pronounced concentration of activity around the Globe, particularly in the field of population genetics of *Drosophila ananassae*. The present review documents the work done on population

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genetical aspects of various evolutionary phenomena in *D. ananassae* carried out to date.

2. *D. ananassae*

D. ananassae was first described by Doleschall (1858) from Ambon (=Ambonia), Indonesia, and is largely distributed in tropical and subtropical regions with occurrence in all the six zoogeographic regions of the world (Patterson & Stone, 1952). Previous studies have provided strong evidence that the geographic origin of *D. ananassae* is in Southeast Asia, an area called Sunda shelf, and peripheral populations in Asia and South Pacific represent migration since the time the sea level rose 20 000 years ago since glaciation and human migration to Oceania (Das *et al.*, 2004; Schug *et al.*, 2007). It is one of the most common species, especially in and around the places of human habitations and appears to qualify as a polytypic species (Tobari, 1993). Kaneshiro & Wheeler (1970) reported that the *ananassae* species subgroup is divisible into the *ananassae* complex (5 species) and the *bipectinata* complex (6 species). In the *ananassae* subgroup, morphological, molecular, karyotypic and behavioural data strongly support a division into three complexes *ananassae*, *bipectinata* and *ercepeae* (Bock & Wheeler, 1972; Lemeunier *et al.*, 1978, 1986, 1997; Roy *et al.*, 2005). Da Lage *et al.* (2007) proposed to raise the species subgroups *ananassae* and *montium* to the rank of species group, and to restrict the *melanogaster* species group to the *melanogaster* subgroup plus the 'Oriental' subgroups, among which the *suzukii* subgroup is polyphyletic.

The mitotic chromosome complement of *D. ananassae* consists of two pairs of large, a pair of small V-shaped metacentric autosomes and a pair of medium-sized V-shaped metacentric sex chromosomes in females. In males, one of the two X chromosomes is replaced by J-shaped Y chromosome (Kaufmann, 1937; Kikkawa, 1938; Futch, 1966; Hinton & Downs, 1975). *D. ananassae* is an organism of choice in evolutionary genetics, population genetics, behaviour genetics, recombination (Moriwaki & Tobari, 1975; Tobari 1993; Singh, 1996) and ecology. It is amongst 12 other *Drosophila* genomes that have been sequenced and assembled (*Drosophila* 12 Genomes Consortium, 2007).

(i) *A genetically unique species*

D. ananassae occupies a unique status among *Drosophila* species due to certain peculiarities in its genetic behaviour (Singh, 1985*a*, 2000). It exists in highly structured populations in Asia and South Pacific (Johnson, 1971; Stephan, 1989; Stephan & Langely, 1989; Tomimura *et al.*, 1993; Stephan *et al.*, 1998; Vogl *et al.*, 2003; Das *et al.*, 2004; Schug *et al.*, 2007,

2008) and its biogeographical history is well characterized. Other peculiar characteristics are the existence of spontaneous crossing-over in males, which is meiotic in origin (Kikkawa, 1937; Moriwaki, 1937, 1940; Moriwaki *et al.*, 1970; Moriwaki & Tobari, 1973; Matsuda *et al.*, 1983; Kale, 1969; Hinton, 1970; Singh & Singh, 1988); presence of chromosome rearrangements, such as, pericentric inversions, translocations, transpositions, deficiencies and extrabands, reflecting high mutability in *D. ananassae* (Kikkawa, 1938). *D. ananassae* harbours a large number of chromosome rearrangements, 78 paracentric inversions, 21 pericentric inversions and 48 translocations in its natural populations (Singh & Singh, 2007*b*). Most of these paracentric inversions have restricted distribution, while three cosmopolitan inversions (Futch, 1966), namely, alpha (AL) in 2L, delta (DE) in 3L and eta (ET) in 3R show worldwide distribution (Singh, 1996). The same inversions were given different names by other investigators. In the present paper, nomenclature by Ray-Chaudhuri & Jha (1966) as, alpha (AL), delta (DE) and eta (ET) will be followed. The *optic morphology* (*Om*), hyper-mutability system (Hinton, 1984; Matsubayashi *et al.*, 1991; Awasaki *et al.*, 1996); ZAM, a retrovirus-like element has also been reported in *D. ananassae* (Baldrich *et al.*, 1997); spontaneous bilateral genetic mosaic, which was characterized by three mutant characters (*cu*, *e*, *se*) on the left side and all normal characters on the right side was detected in *D. ananassae*; parthenogenesis has been reported in the light and dark forms of *D. ananassae* by Futch (1972); segregation distortion; Y-4 linkage of nucleolus organizer (contrary to X–Y nucleolus organizer in *Drosophila*) has also been reported (Hinton & Downs, 1975).

3. Behaviour and quantitative genetics

Non-sexual behaviour like, phototactic activity, eclosion rhythm, oviposition site preference and pupation site preference (fitness and survival determining behaviours) are supposed to be under polygenic control and are influenced by additive genetic variation (Markow & Smith, 1979; Singh & Pandey, 1993*a*, *b*; Srivastava & Singh, 1996; Joshi, 1999; Doi *et al.*, 2001; Yamada *et al.*, 2002*a*, *b*; see Singh, 1996; Singh & Singh, 2003 for references).

Sexual isolation, maintained by strong mating preferences has been reported in the light and dark forms of *D. ananassae* in laboratory stocks (Spieth, 1966; Futch, 1966, 1973; Doi *et al.*, 2001; Sawamura *et al.*, 2006; Vishalakshi & Singh, 2006*a*). These forms were later found to be sibling species (*D. ananassae* and *Drosophila pallidosa*) of *ananassae* complex. Das *et al.* (2004) had found only 12 fixed nucleotide differences over 10 loci between these two sibling species. Per-site divergence averaged over all loci and populations was

found to be constant and very low with no remarkable variation among samples. This shows that the separation of these two species has been a recent event in the speciation history of the *melanogaster* group (Bock & Wheeler, 1972). *D. ananassae* and *D. pallidosa* are therefore, good case of species pair, suitable for the study of sexual isolation. *D. ananassae* is a cosmopolitan in tropical and subtropical regions, and *D. pallidosa* is endemic to New Caledonia, Samoa, Tonga and Fiji Islands, where these two species are sympatric in these areas (Futch, 1966; Stone *et al.*, 1966; Tobari, 1993). *D. pallidosa* has specific inversions on XL, 2L, 2R and 3R, not found in sympatric strains of *D. ananassae* (Futch, 1966; Tobari, 1993), suggesting that these species could be genetically isolated in nature. Although, female cuticular hydrocarbons function as sex pheromones, inducing male courtship behaviour in *D. ananassae* and *D. pallidosa* (Nemoto *et al.*, 1994; Doi *et al.*, 1997). Females of both species must discriminate courting males by acoustic cues whether males are conspecific or heterospecific, as males possess species specificity in songs, and genetic factors are involved in song generation. Strong sexual isolation exists between the species, but interspecific hybrids of both sexes are viable and fertile (Spieth, 1966; Futch, 1973).

Doi *et al.* (2001) mapped genes contributing to the female discrimination behaviour and showed significant effects of second and third chromosomes leading to sexual isolation. Yamada *et al.* (2002 *a*, *b*) reported that a very narrow region on the second chromosome was involved in controlling the female's discriminatory behaviour among courting males in *D. ananassae*. These investigators also recorded and analysed male courtship songs in several strains of *D. ananassae* and *D. pallidosa*, and observed species specificity in the courtship song parameters (Yamada *et al.*, 2002 *a*, *b*). It was suggested that these parameters play a role in mate recognition that enforces sexual isolation.

In *D. ananassae*, mate discrimination varies considerably throughout the species range, being higher among the populations outside the ancestral Indonesian range and highest in South Pacific. Results suggest that colonization and genetic differentiation affect the evolutionary origin of mate discrimination (Schug *et al.*, 2008). The patterns of marked geographical population structure that are a feature of *D. ananassae* (Tobari, 1993; Vogl *et al.*, 2003; Das *et al.*, 2004; Schug *et al.*, 2007) populations appeared to be accompanied by a structure in pattern of mate discrimination as well (Schug *et al.*, 2008). A phylogeographic approach clarifies the ancestral relation between the populations from the South Pacific that show particularly strong mate discrimination and that they may be in early stage of speciation (Schug *et al.*, 2008). In *D. ananassae*, the degree of sexual isolation is stronger in isofemale lines than in natural populations and may involve genetic bottlenecks (Singh & Chatterjee, 1985). Laboratory strains of *D. ananassae* have developed behavioural reproductive isolation as a result of genetic divergence (Singh & Singh, 2003). There is evidence for rare-male mating advantage in *D. ananassae* (Singh & Chatterjee, 1989; Som & Singh, 2004).

The genetics of various quantitative traits have been widely used in assessing the effect of artificial and natural selection to shed light on the genetic constitution of natural populations. There is a positive correlation between mating propensity, sternopleural bristle numbers and fertility in *D. ananassae* (Singh & Chatterjee, 1987; Singh & Mathew, 1997). Size-assortative mating, which provides evidence for size-dependent sexual selection has also been reported in *D. ananassae* (Sisodia & Singh, 2004). Evidence for adaptive plasticity and trade-off between longevity and productivity is also reported in *D. ananassae* (Sisodia & Singh, 2002). Correlated responses to bi-directional selection on thorax length, examined on several life-history traits and chromosome inversion polymorphisms, have revealed apparent trade-offs in *D. ananassae* (Yadav & Singh, 2006, 2007). Chromosomes occurring in high frequency were associated with higher mating activity, and heterosis was found to be associated with alpha inversion and male mating activity (heterokaryotypic males were superior in mating activity than homokaryotypes). Thus, inversion polymorphism may have a partial behavioural basis and males are more subjected to intrasexual selection than females (Singh & Chatterjee, 1986, 1988). Remating behaviour in *D. ananassae* has shown it to be prevalent in male and there are inter-strain variations in male remating time. In addition, sperm displacement and bi-directional selection for female remating speed indicate that post-mating behaviour may also be under genetic control in *D. ananassae* (Singh & Singh, 2001).

Fluctuating asymmetry (FA) study was also performed in laboratory populations of *D. ananassae* to study departure from perfect symmetry of bilaterally symmetrical metrical traits. Results show that FA exists in controlled laboratory environment; it occurs in both sexual and non-sexual traits; males have higher FA level for sexual traits; and sexual traits are better indicators of developmental stress than non-sexual traits (Vishalakshi & Singh, 2006 *b*).

4. Genetic polymorphisms

(i) *Inversion polymorphism in natural populations*

Since the establishment of the modern synthesis, inversions have been a privileged system to study such diverse subjects as phylogenies, geographical clines, temporal cycles and meiotic drive, and, of course, to

look for evidence of natural selection (Krimbas & Powell, 1992). Study of chromosomal polymorphism in populations show the interplay of evolutionary factors in the maintenance and improvement of their adaptation to the environment. The development of polymorphism through natural selection is one of the ways through which a population may improve its capacity to utilize the environment and survive through temporal changes of it. In natural populations of *Drosophila*, chromosomal polymorphism due to inversions is common and is an adaptive trait (Da Cunha, 1960; Dobzhansky, 1970; Sperlich & Pfriem, 1986).

Studies on chromosomal polymorphism in Indian populations of *D. ananassae* were initiated by Ray-Chaudhuri & Jha (1966, 1967); since then, a number of investigations on chromosomal polymorphism in Indian populations of *D. ananassae* have been carried out. Quantitative data on the frequencies of three cosmopolitan inversions in Indian natural populations of *D. ananassae* show that there are significant variations in the frequencies of these inversions (also showing north–south trends) and the level of inversion heterozygosity among the populations, and that the natural populations are geographically differentiated at the level of inversion polymorphism (see review by Singh, 1996; Singh & Singh, 2007 *a*). Populations from the similar eco-geographic regions show similar trends in inversion frequencies and level of inversion heterozygosity. There is no strong positive relation between genetic differentiation and geographic distance although many pair-wise comparisons show that populations separated by small geographic distances show higher genetic identity (see review by Singh, 1996; Singh & Singh, 2007 *a*). However, in the study by Das *et al*. (2004), genetic differentiation was found to correlate significantly with geographic distance.

These three cosmopolitan inversions differ in their distribution and prevalence and do not show variation with geographical and other parameters as revealed by correlation and regression analysis. No temporal divergence was found between spatially similar but temporally different populations (sampled at different times), i.e. none of the populations showed long-term directional changes. This reinforces the concept of rigid polymorphism in natural populations of *D. ananassae*, as such a system does not show temporal variation or variation with geographical parameters (Singh & Singh, 2007 *a*).

Nei's (1973) gene diversity estimates were calculated using quantitative data on the frequencies of three cosmopolitan inversions in 45 Indian natural populations of *D. ananassae* to deduce the distribution of genetic differentiation when populations were grouped according to the time of collection (years and months), regions (coastal and mainland regions) and seasons. Major proportion of this diversity is distributed among populations of different groups rather than within-populations of the same group. The association of genetic variation with environmental and geographical heterogeneity could be due to natural selection operating on chromosomal variability in *D. ananassae* (Singh & Singh, unpublished).

Singh (2001) reviewed the work done on inversion polymorphism in Indian natural populations of three species, viz. *Drosophila melanogaster*, *Drosophila bipectinata* and *D. ananassae*, which clearly demonstrates that these three species vary in their patterns of inversion polymorphism and have evolved different mechanisms for adjustment to their environments, although they belong to the same species group.

Thus, there is geographic variation of chromosomal polymorphism in *D. ananassae* populations due to their adaptation to varying environment and natural selection operates to maintain inversions. Since, the three cosmopolitan inversions in *D. ananassae* are widely distributed and occur in high frequencies, they may be regarded as very old in the evolutionary history of the fly and adaptively important for the species.

(ii) *Inversion polymorphism in laboratory populations*

Chromosomal polymorphism due to three cosmopolitan inversions often persists in laboratory populations of *D. ananassae* established from females collected from nature (Singh, 1982 *a*, 1983 *b*, *c*, 1987). These laboratory populations when compared with the corresponding natural populations show both increasing and decreasing trends in inversion frequencies and level of inversion heterozygosity though most of the populations have maintained more or less similar trends (Singh & Singh, 2008). This demonstrates that heterotic buffering is associated with these inversions and chromosomal polymorphism is balanced due to adaptive superiority of inversion heterozygotes (Moriwaki *et al*., 1956; Singh & Ray-Chaudhuri, 1972; Singh, 1982 *a*; Tobari & Moriwaki, 1993). However, the degree of heterosis may vary depending on the allelic contents of the chromosome variants (Singh, 1983 *b*). Genetic identity (I) and genetic distance (D) values calculated following the formula of Nei (1972) to determine the degree of genetic divergence between natural and laboratory populations indicate that there is variation in the degree of genetic divergence in *D. ananassae* populations transferred and maintained for several generations under laboratory conditions. Populations collected from similar environmental conditions that initially show high degree of similarity have diverged to different degrees. This randomness could be due to genetic drift, though inversions in this species are subject to selection (Singh & Singh, 2008).

(iii) *Genetic co-adaptation*

The results obtained in *D. ananassae* with respect to the phenomenon of genetic co-adaptation (Singh, 1972, 1974*b*, 1981, 1985*b*) conflicts with what has been found in other species of *Drosophila*. In *D. ananassae*, the inversion heterozygotes produced by chromosomes derived from distant localities exhibit heterosis. Evidence for persistence of heterosis associated with cosmopolitan inversions in interracial hybridization experiments has been presented, involving chromosomally polymorphic and monomorphic strains of *D. ananassae* (Singh, 1972, 1974*b*, 1981, 1985*b*). Based on these findings, it has been suggested that heterosis associated with cosmopolitan inversions in *D. ananassae* appears to be simple luxuriance rather than population heterosis (co-adaptation), and thus luxuriance can function in the adjustment of organisms to their environment (Singh, 1985*b*). This provides evidence against selectional co-adaptation hypothesis.

(iv) *Lack of evidence for intra- and interchromosomal interactions*

Inversion polymorphism found in different species of *Drosophila* offers a good material for testing epistatic interactions. The phenomenon of non-random associations between linked inversions is documented in *D. ananassae* (Singh, 1983*a*, 1984). Two linked inversions, namely, delta (3L) and eta (3R) of the third chromosome are associated randomly in natural populations (Singh, 1974*a*, 1984; Singh & Singh, unpublished). However, the same two inversions show non-random association in laboratory stocks, which could be due to suppression of crossing-over and random genetic drift (Singh, 1983*a*, 1984; Singh & Singh, 1988, 1990, 1991, unpublished). For unlinked inversions, no evidence of interchromosomal interactions has been found in *D. ananassae* in both natural and laboratory populations (Singh, 1982*b*, 1983*a*; Singh & Singh, 1989, unpublished).

Tobari & Kojima (1967, 1968) and Kojima & Tobari (1969) studied the selective modes of inversion polymorphism of a single pair of arrangements of either II or III chromosomes singly and of two pairs of the arrangements of both the chromosomes jointly. Their results indicate that interaction between arrangements of 2L and 3L can be responsible for the differences in the succession of frequencies approaching equilibrium between populations containing different genetic conditions, however balanced polymorphisms were established in all populations. The fitness of the karyotypes gradually changes, depending upon the frequencies of the karyotypes, which are successively changing in the population. Thus, the fitness of the karyotypes is a function of their frequencies in the population (Tomimura *et al.*, 1993).

(v) *Allozyme polymorphism*

Enzyme polymorphism has been used mainly to detect selection acting on specific loci, to understand genetic structure of populations, and to analyse the patterns of geographic differentiation. Results of amylase electrophoresis in *D. ananassae* (Doane, 1969) revealed some polymorphism and striking geographic pattern throughout the world (Da Lage *et al.*, 1989). African populations were much more polymorphic than those from far East and showed multibanded phenotypes, suggesting multiplication of *Amy* structural gene, with at least 4 copies per haploid genome in certain populations. Nine other species of *D. ananassae* subgroup exhibited weak amylase activity (Da Lage *et al.*, 1989). Unlike the case in *D. melanogaster*, the isozymes in this species show considerable temporal variation in expression (Da Lage & Cariou, 1993). Though, *D. ananassae* is in the *melanogaster* group, it has evolved a very different set of regulatory patterns for amylase than *D. melanogaster*, though both are, ancestrally, tropical fruit breeders. Number of copies and allozymic variations are higher in *D. ananassae* (Da Lage *et al.*, 1992; Cariou & Da Lage, 1993). An analysis of *D. ananassae* subgroup including *D. ananassae* itself has shown that a maximum of 30 % of the loci are polymorphic and that even the most polymorphic (*Estc, Acph, Ca, Pgm*) loci show similar variability in all species (see Tobari, 1993 for references).

Similar studies on *Adh* isozymes between different geographic strains show biochemical genetic differentiation (Jha *et al.*, 1978; Parkash & Jyoutsna, 1988; Sharma *et al.*, 1993).

Considering enzyme polymorphism, the main conclusion emerging is that populations of *D. ananassae* have a moderate level of genetic variability in spite of their worldwide distribution (Tobari, 1993). Compared to allozymes, the picture of geographic differentiation appears to be different for chromosomes, which are more variable and more differentiated even over short distances. This could be due to the fact that allozymes in general are more neutral than chromosome arrangements (Tobari, 1993).

In numerous studies of allozyme variation in *D. ananassae* (Gillespie & Kojima, 1968; Johnson, 1971), authors have often attempted to detect linkage disequilibrium between loci, reasoning that if selection affects these polymorphisms, one might expect such disequilibrium at least in some loci. The conclusion from all these studies is that virtually no linkage disequilibrium exists among allozyme loci.

(vi) *DNA polymorphism*

Using data of DNA sequence variation, theories of population genetics and evolution can be tested more rigorously than with previously available methods (Tobari, 1993). Das *et al.* (2004) had inferred the population structure and demography of *D. ananassae* using multilocus DNA sequence (10 netural loci) and 16 populations covering entire species range (Asia, Australia and America). Using putatively neutral nuclear DNA sequence polymorphisms from 10 independent loci, central populations were discerned from the peripheral populations. The levels of nucleotide diversity, the number and frequency of haplotypes, and the amount of linkage disequilibrium vary among the populations. In comparison with the previous studies of two neutral loci [*Om* (1D) and forked] with samples from Asia and South America (Stephan, 1989; Stephan & Langley, 1989; Stephan *et al.*, 1998), their (Das *et al.*, 2004) analysis finds lower estimates of nucleotide diversity. In *D. ananassae*, reduced recombination is associated with low levels of DNA polymorphism. In other studies (Stephan, 1989; Stephan & Langley, 1989; Stephan & Mitchell, 1992) of DNA polymorphism in *D. ananassae* at four loci of the X-chromosome: vermilion (*v*), furrowed (*fw*), forked (*f*) and *Om* (ID), genes experiencing normal amounts of polymorphism, *Om* (ID) and forked (*f*), were found to be 10 times more variable than genes located in regions of very low recombination, furrowed (*fw*) and vermilion (*v*). These effects are due to restricted migration between populations and differences in recombination rates of the chromosome regions in which the various loci lie. Recombination is the main factor determining nucleotide variability in different regions of the genome. Chromosomal inversions are known to reduce and redistribute recombination, and thus their specific effect on nucleotide variation may be of major importance as an explanatory factor for levels of DNA variation (Navarro *et al.*, 2000). Reduction in average heterozygosity in the *v* and *fw* regions can be explained based on the models of directional selection and genetic hitchhiking. Recurrent fixation of few alleles will wipe out standing variation in a population by this process and thus reduce the level of heterozygosity, if the recombination rate is low. The hitchhiking effect is less strong in regions with intermediate or high recombination rates, such as *f* and *Om* (1D). Effect of population subdivision on variation among populations with different distances from the species centre in Southeast Asia, i.e. Myanmar, India and Brazil, was also examined. The between-population differences in average heterozygosity may be explained via neutral theory of molecular evolution (Kimura, 1983), which predicts that average nucleotide heterozygosity is proportional to effective population

size, so that average heterozygosity follows the order Myanmar > India > Brazil. Since, *D. ananassae* spreads from its zoogeographical centre in Southeast Asia (Myanmar) to India and then to Central America and South America via restricted migration, hence reduced population size and average heterozygosity (Stephan, 1989; Stephan & Langley, 1989; Stephan & Mitchell, 1992). Natural selection may have a strong influence on the broad expanses of genome in populations from Northern versus Southern Asia (Stephan *et al.*, 1998; Chen *et al.*, 2000; Kim & Stephan, 2000; Baines *et al.*, 2004) and because of the obvious genetic drift that may accompany South Pacific Islands and potentially some of the ancestral populations from Southeast Asia that surround the ancestral geographic range in Indonesia. However, the young age of population makes it extremely unlikely that natural selection have a role in DNA sequence variation (Das *et al.*, 2004), but evidence of adaptive evolution was inferred from the pattern of DNA sequence variation in northern versus southern populations of Asia (Stephan *et al.*, 1998; Chen *et al.*, 2000; Kim & Stephan 2000; Baines *et al.*, 2004). These studies (Stephan *et al.*, 1998; Chen *et al.*, 2000; Baines *et al.*, 2004) suggest that extensive physical and genetic maps based on molecular markers and detailed studies of population structure may provide insights into the degree to which natural selection affects DNA sequence polymorphism across broad regions of chromosome. In other studies (Vogl *et al.*, 2003; Das *et al.*, 2004; Schug *et al.*, 2004, 2007), we found that the level of molecular variation is quite variable among the populations. Populations in ancestral range in Indonesia and peripheral range in Asia and Australia show lower genetic differentiation than populations from the Pacific Island (Schug *et al.*, 2008). Molecular variation varies considerably among the ancestral, peripheral and South Pacific populations consistent with the previous studies of intron polymorphism (Vogl *et al.*, 2003; Das *et al.*, 2004; Schug *et al.*, 2007) and microsatellites (Schug *et al.*, 2007). In contrast to polymorphism, divergence between *D. ananassae* populations and its sibling species *D. pallidosa* is constant across loci.

Genome size differences are usually attributed to the amplification and deletion of various repeated DNA sequences, including transposable elements (TEs), when species encounter a new environment. Nardon *et al.* (2005) conducted a study to find out whether genome size is influenced by colonization of new environments in Dipteran species, including *D. ananassae*. Results show that *D. ananassae* does not display obviously smaller average genomes in their probable region of origin, and variability in genome size of Indian populations of *D. ananassae* have been found. This could be due to different colonization routes followed by this species and different

environmental conditions encountered by the populations.

Using genomic data from five closely related species of *Drosophila* (*D. melanogaster*, *Drosophila simulans*, *Drosophila yakuba*, *Drosophila erecta* and *D. ananassae*), a maximum likelihood framework was applied to calculate rates of protein evolution and to test the evidence of positive selection. In all comparisons, weak positive correlation between expression divergence and protein evolution was found (Good *et al*., 2006).

5. Population sub-structuring

Natural population displays geographic population sub-structure, which is due to the differences in allele and genotype frequencies from one geographic region to other. Population subdivision is centrally important for evolution and affects estimation of all evolutionary parameters from natural and domestic populations (Hartl & Clark, 1997). In subdivided populations, random genetic drift results in genetic divergence among subpopulations. Migration (movement of individuals among subpopulations) acts as a sort of genetic glue that holds subpopulations together and sets a limit to how much genetic divergence can occur (Hedrick, 2005).

D. ananassae exhibits more population structure than both *D. melanogaster* and *D. simulans* (Vogl *et al*., 2003; Das, 2005). This species is characterized by high incidence of interpopulation migration (Dobzhansky & Dreyfus, 1943). Although, populations are separated by major geographical barriers such as mountains and oceans, recurrent transportation by human activity may lead to genetic exchange (Schug *et al*., 2008). Due to extensive population structure, *D. ananassae* can be used for analysing the effect of population subdivision on genetic variation. Past molecular analyses of the effect of population subdivision on genetic variation are limited to few loci and populations (Stephan, 1989; Stephan & Langley, 1989; Stephan & Mitchell, 1992; Stephan *et al*., 1998; Das *et al*., 2004; Schug *et al*., 2007, 2008).

Singh & Singh (unpublished) employed inversions as chromosomal markers for the first time for population structure analysis (genetic variability estimates, *F*-statistics and gene flow). Population structure analysis was done using traditional *F*-statistics following Wright (1951). Values of F_{IS} and F_{IT}, the most inclusive measure of inbreeding, are found close to zero in most of the cases. Thus, set of populations as a whole, shows no sign of inbreeding. Values of F_{ST} show that range-wise population subdivision, possibly due to drift accounts for approximately 4·6–64·2 % of the total genetic variation. Presumably, values of F_{ST} are influenced by the size of subpopulations, which is the major determinant

of the magnitude of random changes in allele frequency.

Pair-wise F_{ST} values show that Indian populations of *D. ananassae* exhibit strong genetic differentiation, display population sub-structuring and exist as semi-isolated populations. In other studies, estimates of F_{ST} for mtDNA (Schug *et al*., 2008) found is lower than that for X-linked loci (Das *et al*., 2004) although it does not indicate inconstancies, but it could be due to profound effect of purifying selection at mtDNA. Gene flow between populations was estimated as the number of migrants exchanged between populations per generation (Nm). Nm values were derived from one approach using F_{ST} values, following the island model of Wright (1951) with a small level of migration. Our gene flow estimates were low and only slightly above the range shown by rat snakes (Lougheed *et al*., 1999). This suggests that populations of *D. ananassae* are highly differentiated, display population sub-structuring and exist as semi-isolated populations. This is despite the fact that *D. ananassae* is co-transported with human goods frequently. Genetic distance (*D*) approach was also utilized in determining the pattern of geographic variation and 'isolation by distance' among Indian natural populations of *D. ananassae*. Lowermost *D* values correspond to geographically closest populations, whereas, 'isolation by distance' effect was not conformed statistically as genetic distance and geographic distances are insignificantly correlated. Similar studies (Vogl *et al*., 2003; Schug *et al*., 2007) done earlier at molecular level in *D. ananassae* have arrived at the same conclusion. However, in other studies, after taking genetic differentiation and geographical distance into account for ancestral populations, a significant pattern of 'isolation by distance' is found at mtDNA (Schug *et al*., 2008) and X-linked loci (Das *et al*., 2004).

Similar to observations from previous studies with different molecular markers (Johnson, 1971; Stephan, 1989; Stephan & Langley, 1989; Stephan & Mitchell, 1992; Stephan *et al*., 1998; Das *et al*., 2004; Schug *et al*., 2007, 2008), it could be said that, populations of *D. ananassae* show strong sub-structuring due to genetic differentiation of their natural populations, migration and demographic processes such as past events of population expansion and/or bottlenecks. Given limited gene flow, populations are expected to diverge genetically due to drift. Low levels of gene flow coupled with high degrees of genetic differentiation might have occurred historically and is being maintained currently. Demographic properties, historical and contemporary events and other factors are more important in shaping the patterns of population sub-structuring, genetic differentiation and gene flow than mere terrestrial habitat characteristics (un)favorable for migration.

6. Conclusions

The results of investigations on chromosomal polymorphism in *D. ananassae* demonstrate that this species presents a high degree of chromosomal variability in its natural populations. There is geographic differentiation of inversion polymorphism, which must have developed in response to the ecological conditions existing in different geographical localities. Since, the three cosmopolitan inversions in *D. ananassae* are widely distributed and occur in high frequencies, they may be regarded as very old in the evolutionary history of the fly and adaptively important for the species. *D. ananassae* populations show substantial sub-structuring and exist as semi-isolated populations. Gene flow is low despite co-transportation of flies with human goods. There is persistence of cosmopolitan inversions when populations are transferred to laboratory conditions, which suggests that heterotic buffering is associated with these inversions in *D. ananassae*. Populations collected from similar environmental conditions that initially show high degree of genetic similarity have diverged to different degrees in the laboratory environment. This randomness could be due to genetic drift. No evidence for chromosomal interactions has been found in natural and laboratory populations of *D. ananassae*. This strengthens the previous suggestion that there is lack of genetic co-adaptation in *D. ananassae*.

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