Population genetics of Drosophila ananassae

PRANVEER SINGH AND BASHISTH N. SINGH*
Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi-221005, India

(Received 10 February 2008 and in revised form 20 May and 3 August 2008)

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 genetic 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...
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 *bipecinata* complex (6 species). In the *ananassae* subgroup, morphological, molecular, karyotypic and behavioural data strongly support a division into three complexes *ananassae*, *bipecinata* and *erecteae* (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 *suzuki* 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, 1985a, 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 46 translocations in its natural populations (Singh & Singh, 2007b). 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, 1993a, b; Srivastava & Singh, 1996; Joshi, 1999; Doi et al., 2001; Yamada et al., 2002a, 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; Vishalaksh & Singh, 2006a). 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. (2002a,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., 2002a,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, 2006b).

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, 2007a). 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, 2007a). 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, 2007a).

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, 1982a, 1983b, 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, 1982a; Tobari & Moriwaki, 1993). However, the degree of heterosis may vary depending on the allelic contents of the chromosome variants (Singh, 1983b). 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, 1974a, 1981, 1985b) 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, 1974a, 1981, 1985b). 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, 1985b). 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, 1983a, 1984). Two linked inversions, namely, delta (3L) and eta (3R) of the third chromosome are associated randomly in natural populations (Singh, 1974a, 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, 1983a, 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, 1982b, 1983a; 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 successions 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 amyrase 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, Acp4, 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 netral 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 (1D), genes experiencing normal amounts of polymorphism, Om (1D) 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*<sub>IS</sub> and *F*<sub>IT</sub>, 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*<sub>ST</sub> 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*<sub>ST</sub> are influenced by the size of subpopulations, which is the major determinant of the magnitude of random changes in allele frequency.

Pair-wise *F*<sub>ST</sub> 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*<sub>ST</sub> 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*<sub>ST</sub> 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 (Loughheed 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 confirmed 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*.

Financial support from CSIR, New Delhi in the form of Senior Research Fellowship to Praveen Singh is highly acknowledged. The research work of B. N. Singh cited in this review has been supported by DST, UGC and CAS in Zoology, Banaras Hindu University. We are thankful to the two anonymous reviewers for their helpful comments on the original draft of the manuscript and to Miss Punita Nanda, Genetics Laboratory, Department of Zoology, Banaras Hindu University for her valuable inputs.

References

Awasaki, T., Juni, N. & Yoshida, K. M. (1996). An eye imaginal disc-specific transcriptional enhancer in the long terminal repeat of the ron reterortransponso is responsible for eye morphology mutations in *Drosophila ananassae*. Molecular and General Genetics 251, 161–166.

Baines, J. F., Das, A., Mousset, S. & Stephan, W. (2004). The role of natural selection in genetic differentiation of worldwide populations of *Drosophila ananassae*. Genetics 168, 1977–1988.

Balicki, E., Dimitri, P., Desset, S., Leblanc, P., Codipietro, D. & Vaury, C. (1997). Genomic distribution of the retrovirus like element ZAM in *Drosophila*. Genetica 100, 131–140.

Bock, I. R. & Wheeler, M. R. (1972). The *Drosophila melanogaster* species group. University of Texas Publication 7213, 1–102.

Cariou, M. L. & Da Lage, J. L. (1993). Isozyme polymorphisms. In *Drosophila ananassae, Genetical and Biological Aspects* (ed. Y. N. Tobari), pp. 160–171. Tokyo: Japanese Scientific Society Press.

Chen, Y., Marsh, B. J. & Stephan, W. (2000). Joint effects of natural selection and recombination on gene flow between *Drosophila* populations. Genetics 155, 1185–1194.

Da Cunha, A. B. (1960). Chromosomal variation and adaptation in insects. Annual Review of Entomology 5, 85–110.

Da Lage, J. L. & Cariou, M. L. (1993). Organization and structure of the amylase gene family. In *Drosophila ananassae, Genetical and Biological Aspects* (ed. Y. N. Tobari), pp. 171–181. Tokyo: Japanese Scientific Society Press.

Da Lage, J. L., Cariou, M. L. & David, J. R. (1989). Geographical polymorphism of amylase in *Drosophila ananassae* and its relatives. Heredity 63, 67–72.

Da Lage, J. L., Lemeunier, F., Cariou, M. L. & David, J. R. (1992). Multiple amylase genes in *Drosophila ananassae* and related species. Genetic Research 59, 85–92.

Da Lage, J. L., Maczkowiak, F. & Cariou, M. L. (2000). Molecular characterization and evolution of the amylase multigene family of *Drosophila ananassae*. Journal of Molecular Biology and Evolution 51, 391–403.

Da Lage, J. L., Kergoat, G. J., Maczkowiak, F., Silvain, J. F., Cariou, M. L. & Lachaise, D. (2007). A phylogeny of Drosophilidae using Amyrel gene: questioning the *Drosophila* species group boundaries. Journal of Zoological Systematics and Evolutionary Research 45, 47–63.

Das, A. (2005). Population genomics and bioinformatic studies reveal evolutionary history of *Drosophila ananassae*. Current Science 89, 1316–1321.

Das, A., Mohanty, S. & Stephan, W. (2004). Inferring the population structure and demography of *Drosophila ananassae* from multilocus data. Genetics 168, 1975–1985.

Doane, W. W. (1969). *Drosophila* amylase and problems in cellular differentiation. In *Problems in Biology. DNA in Development* (ed. E. W. Hanly), pp. 73–109. Salt Lake City, UT: University of Utah Press.

Dobzhansky, T. (1970). *Genetics of Evolutionary Process*. New York: Columbia University Press.

Dobzhansky, T. & Dreyfus, A. (1943). Chromosomal aberrations in Brazilian *Drosophila ananassae*. Proceedings of the National Academy of Sciences of the USA 26, 301–305.

Doi, M., Mastuda, M., Tomaru, M., Matsubayashi, H. & Oguma, Y. (1997). Behavioral response of males to major sex pheromone component, (Z)-5,25-hentriacontadiene, of *Drosophila ananassae* females. Journal of Chemical Ecology 23, 2067–2078.

Doi, M., Mastuda, M., Tomaru, M., Matsubayashi, H. & Oguma, Y. (2001). A locus for female discrimination behaviour causing sexual isolation in *Drosophila*. Proceedings of the National Academy of Sciences of the USA 98, 6714–6719.

Doleschall, C. L. (1858). Derde bijdrage tot de kennis der dipteren fauna van Nederlandsch Indie. Tijdschrift voor Nederlandsch-Indie. 17, 73–128.

Drosophila 12 Genomes Consortium (2007). Evolution of genes and genomes on the *Drosophila* phylogeny. Nature 450, 203–218.

Futch, D. G. (1972). A preliminary note on parthenogenesis in *Drosophila ananassae*. Drosophila Information Service 48, 78.

Futch, D. G. (1966). A study of speciation in South Pacific populations of *Drosophila ananassae*. University of Texas Publication 6615, 79–120.

Futch, D. G. (1975). On the ethological differentiation of *Drosophila ananassae* and *Drosophila pallidosa* in Samoa. Evolution 29, 299–312.

Gillespie, J. H. & Kojima, K. I. (1968). The degree of polymorphism in enzymes involved in energy production compared to that in non-specific enzymes in two
Population genetics of Drosophila ananassae

Drosophila ananassae populations. *Proceedings of the National Academy of Sciences of the USA* **61**, 582–601.

Good, J. M., Hayden, C. A. & Wheeler, T. J. (2006). Adaptive protein evolution and regulatory divergence in *Drosophila*. *Molecular Biology and Evolution* **23**, 1101–1103.

Hartl, D. L. & Clark, A. G. (1997). *Principles of Population Genetics*. Sunderland, MA: Sinauer Associates.

Hedrick, P. W. (2005). *Genetics of Populations*. Jones and Bartlett Publishers.

Hinton, C. W. (1970). Identification of two loci controlling crossing over in males of *Drosophila ananassae*. *Genetics* **66**, 663–676.

Hinton, C. W. (1984). Morphogenetically specific mutability in *Drosophila ananassae*. *Genetics* **106**, 631–653.

Hinton, C. W. & Downs, J. E. (1975). The mitotic, polytene and meiotic chromosomes of *Drosophila ananassae*. *Journal of Heredity* **66**, 353–361.

Jha, A. P., Mishra, D. N. & Pandey, B. N. (1978). *Drosophila* alcohol dehydrogenase isozymes: biochemical genetic differentiation between two geographic strains of *Drosophila ananassae*. *Indian Journal of Experimental Biology* **16**, 1261–1263.

Johnson, F. M. (1971). Isozyme polymorphism in *Drosophila ananassae*. Genetic diversity among island populations in the South Pacific. *Genetics* **68**, 77–95.

Joshi, D. S. (1999). Latitudinal variation in locomotor activity rhythm in adult *Drosophila ananassae*. *Canadian Journal of Zoology* **77**, 865–870.

Kale, P. G. (1969). The meiotic origin of spontaneous crossing over in *Drosophila ananassae* males. *Genetics* **62**, 123–133.

Kaneshiro, K. & Wheeler, M. R. (1970). Preliminary report on the species of the ananassae subgroup. *Drosophila Information Service* **45**, 143.

Kaufmann, B. P. (1937). Morphology of the chromosomes of *Drosophila ananassae*. *Cytologia (Tokyo), Fujii Jubilee Volume*, 1043–1055.

Kikkawa, H. (1938). Studies on the genetics and cytology of *Drosophila ananassae*. *Genetics* **20**, 458–516.

Kim, Y. & Stephan, W. (2000). Joint effects of genetic hitchhiking and background selection on neutral variation. *Genetics* **155**, 1415–1427.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Kojima, K. & Tobiari, Y. N. (1969). Selective modes associated with karyotypes in *Drosophila ananassae*. II. Heterosis and frequency dependent selection. *Genetics* **63**, 639–651.

Krimbas, C. B. & Powell, J. R. (ed.) (1992). *Drosophila Inversion Polymorphism*. Boca Raton, FL: CRC Press.

Lemeunier, F., Dautrillaux, B. & Ashburner, M. (1978). The relationship within the melanogaster subgroup species of genus *Drosophila (Sophophora)* III. The mitotic chromatid rings and the quinacrine fluorescent patterns of polytene chromosomes. *Chromosoma* **69**, 349–361.

Lemeunier, F., David, J., Tsacas, L. & Ashburner, M. (1986). The melanogaster species group. In *The Genetics and Biology of Drosophila* (ed. M. Ashburner, H. L. Carson & J. N. Thompson Jr), pp. 147–256. London: Academic Press.

Lemeunier, F., Aulard, S., Arienti, M., Jallon, J. M., Cariou, M. L. & Tsacas, L. (1997). The eecepea complex: new cases of insular speciation within the *Drosophila ananassae* species subgroup (melanogaster group) and description of two new species (Diptera: Drosophilidae). *Annals of Entomological Society of America* **90**, 28–42.

Lougheed, S. C., Gibbs, H. L., Prior, K. A. & Weatherhood, P. J. (1999). Hierarchical patterns of genetic population structure in black rat snakes (*Elaphe obsolete obsolete*) as revealed by microsatellite DNA analysis. *Evolution* **53**, 1995–2001.

Markow, T. A. & Smith, L. D. (1979). Genetics of phototactic behavior in *Drosophila ananassae*, a member of *Drosophila melanogaster* species group. *Behavior Genetics* **9**, 61–68.

Matsubayashi, H., Tobiari, Y. N. & Hori S. H. (1991). Genetic analysis of Om (2D) in males of *Drosophila ananassae*. *Japanese Journal of Genetics* **66**, 387–397.

Matsuda, M., Imai, H. T. & Tobiari Y. N. (1983). Cytogenetic analysis of recombination in males of *Drosophila ananassae*. *Chromosoma* **88**, 286–292.

Morikawa, D. (1937). A high ratio of crossing-over in *Drosophila ananassae*. *Zeitschrift für Induktive Abstammungs- und Vererbungslehre* **74**, 17–23.

Morikawa, D. (1940). Enhanced crossing-over in the second chromosome of *Drosophila ananassae*. *Japanese Journal of Genetics* **16**, 37–48.

Morikawa, D. & Tobiari, Y. N. (1973). Spontaneous male crossing-over of frequent occurrence in *Drosophila ananassae* from Southeast Asian populations. *Japanese Journal of Genetics* **48**, 167–173.

Morikawa, D. & Tobiari, Y. N. (1975). *Drosophila ananassae*. In *Hand book of Genetics* (ed. R. C. King), pp. 513–535. New York: Plenum Press.

Morikawa, D., Ohnishi, M. & Nakajima, Y. (1956). Analysis of heterosis in populations of *Drosophila ananassae*. *Proceedings of International Genetics Symposium, Cytophlogia Supplementary Volume*, 370–390.

Morikawa, D., Tobiari, Y. N. & Oguma, Y. (1970). Spontaneous crossing-over in the males of *Drosophila ananassae*. *Japanese Journal of Genetics* **45**, 411–420.

Nardon, C., Decelere, G., Levenbruck, C., Weiss, M., Vieira, C. & Biemont, C. (2005). Is genome size influenced by colonization of new environments in dipteran species? *Molecular Ecology* **14**, 869–878.

Navarro, A., Barbadilla, A. & Ruiz, A. (2000). Effect of inversion polymorphism on the neutral nucleotide variability of linked chromosome regions in *Drosophila*. *Genetics* **155**, 685–698.

Nei, M. (1972). Genetic distance between populations. *American Naturalist* **106**, 283–292.

Nei, M. (1973). Analysis of gene diversity in subdivided population. *Proceedings of the National Academy of the Sciences of the USA* **70**, 3321–3323.

Nemoto, T., Doi, M., Oshio, K., Matsubayashi, H., Oguma, Y., Suzuki, T. & Kuwahara, Y. (1994). (Z,Z)-5,27-tritricotriadiene: major sex pheromone of *Drosophila pallidosa* (Diptera: Drosophilidae). *Journal of Chemical Ecology* **20**, 3029–3037.

Parkash, R. & Jyoutsma, R. (1988). Allozyme variation in three *Drosophila* species of *ananaassae* species subgroup. *Current Science* **57**, 1071–1074.

Patterson, J. T. & Stone, W. S. (1952). *Evolution in the Genus Drosophila*. New York: McMillan.

Ray-Chaudhuri, S. P. & Jha, A. P. (1966). Studies on salivary gland chromosomes of Indian *Drosophila ananassae*. *Proceedings of the International Cell Biology Meetings Bombay, India*, pp. 352–383.

Ray-Chaudhuri, S. P. & Jha, A. P. (1967). Genetics of natural populations of Indian *Drosophila ananassae*. *Nucleus* **10**, 81–89.
Roy, V., Monte-Dieu, L., Chaminadac, N., Siljak-Yakovlev, S., Aulard, S., Lemeunier, F. & Montchamp-Moreau, C. (2005). Evolution of the chromosomal RNA genes in two Drosophila species subgroups: ananassae and melanogaster. Heredity 94, 388–395.

Sawamura, K., Tomimura, Y., Sato, H., Yamada, H., Matsuda, M. & Oguma, Y. (2006). Establishing interspecific mosaic genome lines between Drosophila ananassae and D. pallidosa by means of parthenogenesis. Genetical Research 88, 1–11.

Schug, M. D., Regulski, E. E., Pearce, A. & Smith, S. G. (2004). Isolation and characterization of dinucleotide repeat microsatellites in D. ananassae. Genetical Research 83, 19–29.

Schug, M. D., Smith, S. G., Tozier-Pearce, A. & McEvey, S. F. (2007). The genetic structure of Drosophila ananassae populations from Asia, Australia and Samoa. Genetics 175, 1429–1440.

Schug, M. D., Baines, J. F., Amanda, K., Mohanty, S., Das, A., Grath, S., Smith, S. G., Zhargam, S., McEvey, S. F. & Stephan, W. (2008). Evolution of mating isolation between populations of Drosophila ananassae. Molecular Ecology 17, 2706–2721.

Sharma, M., Sharma, S. & Prakash, R. (1993). ADH polymorphism and ethanol tolerance in three species of ananassae species subgroup. Evolutionary Biology 7, 51–62.

Singh, A. K. & Singh, B. N. (1988). An extreme linkage between inversions in Drosophila ananassae. Current Science 57, 400–402.

Singh, A. K. & Singh, B. N. (1989). Further data on interchromosomal associations in Drosophila ananassae. Naturalia 14, 19–29.

Singh, B. N. (1972). The lack of evidence for coadaptation in geographic populations of Drosophila ananassae. Genetica 44, 602–607.

Singh, B. N. (1974a). On the combinations of different gene arrangements in the third chromosome of Drosophila ananassae. Caryology 27, 285–292.

Singh, B. N. (1974b). Persistence of heterosis in crosses between geographic races of Drosophila ananassae. Indian Journal of Experimental Biology 12, 376–377.

Singh, B. N. (1981). Intercalary hybridization in Drosophila ananassae. Genetica 57, 139–142.

Singh, B. N. (1982a). Persistence of chromosomal polymorphism in various strains of Drosophila ananassae. Genetica 59, 151–156.

Singh, B. N. (1982b). The lack of evidence for interchromosomal interactions in Drosophila ananassae. Naturalia 7, 29–34.

Singh, B. N. (1983a). On intra- and interchromosomal associations in Drosophila ananassae. Genetica 60, 231–235.

Singh, B. N. (1983b). Variation in gene arrangement frequencies and the degree of heterosis in laboratory strains of Drosophila ananassae. Brazilian Journal of Genetics 6, 407–414.

Singh, B. N. (1983c). Cosmopolitan inversions in Drosophila ananassae. Caryology 36, 333–343.

Singh, B. N. (1984). Epistatic interaction between linked gene arrangements in Drosophila ananassae. Brazilian Journal of Genetics 7, 175–181.

Singh, B. N. (1985a). Drosophila ananassae – a genetically unique species. Nucleus 28, 169–176.

Singh, B. N. (1985b). Heterosis without selectional coadaptation in Drosophila ananassae. Theoretical and Applied Genetics 69, 437–441.

Singh, B. N. (1987). On the degree of genetic divergence in Drosophila ananassae populations transferred to laboratory conditions. Zeitschrift fur Zoologische Systematik und Evolutionsforschung 25, 180–187.

Singh, B. N. (1996). Population and behaviour genetics of Drosophila ananassae. Genetica 97, 321–329.

Singh, B. N. (2000). Drosophila ananassae: a species characterized by several unusual genetic features. Current Science 78, 391–398.

Singh, B. N. (2001). Patterns of inversion polymorphism in three species of the Drosophila melanogaster species group. Indian Journal of Experimental Biology 39, 611–622.

Singh, B. N. & Chatterjee, S. (1985). Symmetrical and asymmetrical sexual isolation in laboratory strains of Drosophila ananassae. Canadian Journal of Genetics and Cytology 27, 405–409.

Singh, B. N. & Chatterjee, S. (1986). Mating ability of homo- and heterokaryotypes of Drosophila ananassae from natural populations. Heredity 57, 75–78.

Singh, B. N. & Chatterjee, S. (1987). Variation in mating propensity and fertility in isofemale strains of Drosophila ananassae. Genetica 73, 237–242.

Singh, B. N. & Chatterjee, S. (1988). Parallelism between male mating propensity and chromosome arrangement frequency in natural populations of Drosophila ananassae. Heredity 60, 269–272.

Singh, B. N. & Chatterjee, S. (1989). Rare-male mating advantage in Drosophila ananassae. Genetica Selection Evolution 21, 447–455.

Singh, B. N. & Mathew, S. (1997). Greater fertility of Drosophila ananassae flies possessing high number of sternopleural bristles. Current Science 72, 112–114.

Singh, B. N. & Pandey, M. B. (1993a). Selection for high and low pupation height in Drosophila ananassae. Behaviour Genetics 23, 239–243.

Singh, B. N. & Pandey, M. B. (1993b). Evidence for additive polygenic control in pupation height in Drosophila ananassae. Hereditas 119, 111–116.

Singh, B. N. & Ray-Chaudhuri, S. P. (1972). Balanced chromosomal polymorphism in experimental populations of Drosophila ananassae. Indian Journal of Experimental Biology 10, 301–303.

Singh, B. N. & Singh, A. K. (1988). Heterozygous inversions and spontaneous male crossing-over in Drosophila ananassae. Genone 30, 45–450.

Singh, B. N. & Singh, A. K. (1990). Linkage disequilibrium in laboratory strains of Drosophila ananassae is due to drift. Hereditas 112, 201–208.

Singh, B. N. & Singh, A. K. (1991). Non-random associations between independent inversions in laboratory stocks of Drosophila ananassae. Naturalia 16, 11–18.

Singh, P. & Singh, B. N. (2007a). Population genetics of Drosophila ananassae: genetic differentiation among Indian natural populations at the level of inversion polymorphism. Genetical Research 89, 191–199.

Singh, P. & Singh, B. N. (2007b). Chromosomal aberrations in Drosophila ananassae. Drosophila Information Service 90, 49–54.

Singh, P. & Singh, B. N. (2008). Population genetics of Drosophila ananassae: variation in the degree of genetic divergence in populations transferred to laboratory conditions. Zoological Studies (in press).

Singh, S. R. & Singh, B. N. (2001). Female remating in Drosophila ananassae: bi-directional selection for remating speed. Behavior Genetics 31, 361–370.

Singh, S. R. & Singh, B. N. (2003). Behavioral genetics of Drosophila ananassae. Genetica and Molecular Research 2, 394–409.

Sisodia, S. & Singh, B. N. (2002). Effect of temperature on longevity and productivity in Drosophila ananassae. Applied Genetics 54.213.162.168, on 27 Mar 2022 at 23:44:26, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.5016672308009737
Populations genetics of Drosophila ananassae

ananassae: evidence for adaptive plasticity and trade-off between longevity and productivity. *Genetica* 114, 95–102.

Sisodia, S. & Singh B. N. (2004). Size dependent sexual selection in *Drosophila ananassae*. *Genetica* 121, 207–221.

Som, A & Singh, B. N. (2004). Rare male mating advantage for inversion karyotype in *Drosophila ananassae*. *Behavior Genetics* 34, 335–342.

Sperlich, D. & Pfriem, P. (1986). Chromosomal polymorphism in natural and experimental populations. In *The Genetics and Biology of Drosophila* (ed. M. Ashburner, H. L. Carson & J. N. Thompson Jr), pp. 257–309. New York: Academic Press.

Spieth, H. T. (1966). Mating behavior of *Drosophila ananassae* and *ananassae* like flies from the Pacific. *University of Texas Publication* 3, 133–146.

Srivastava, T. & Singh, B. N. (1996). Bidirectional selection for choice of oviposition site in *Drosophila ananassae*. *Korean Journal of Genetics* 18, 295–300.

Stephan, W. (1989). Molecular genetic variation in the centromeric region of the X chromosome in three *Drosophila ananassae* populations. II. The Om (1D) locus. *Molecular Biology and Evolution* 6, 624–635.

Stephan, W. & Langley, C. H. (1989). Molecular genetic variation in the centromeric region of the X chromosome in three *Drosophila ananassae* populations. I. Contrast between the vermillion and forked loci. *Genetics* 121, 89–99.

Stephan, W. & Mitchell, S. J. (1992). Reduced levels of DNA polymorphism and fixed between population differences in the centromeric region of *Drosophila ananassae*. *Genetics* 132, 1039–1045.

Stephan, W., Xing, L., Kirby, D. A. & Braverman, J. M. (1998). A test of background selection hypothesis based on nucleotide data from *Drosophila ananassae*. *Proceedings of the National Academy of the Sciences of the USA* 95, 5649–5654.

Stone, W. S., Wheeler, M. R., Wilson, F. D., Gerstenberg, V. L. & Yang, H. (1966). Genetic studies of natural populations of *Drosophila*. II. Pacific islands populations. *University of Texas Publication* 6615, 1–36.

Tobari, Y. N. (ed.) (1993). *Drosophila ananassae, Genetical and Biological Aspects*. Tokyo: Japanese Scientific Society Press.

Tobari, Y. N. & Kojima, K. (1967). Selective modes associated with inversion karyotypes in *Drosophila ananassae*. I. Frequency dependent selection. *Genetics* 57, 179–188.

Tobari, Y. N. & Kojima, K. (1968). The selective modes associated with two chromosome polymorphisms in *Drosophila ananassae*. *Proceedings 12th International Congress on Genetics, Tokyo* Volume 1, p. 227.

Tobari, Y. N. & Moriwaki, D. (1993). Life cycle. In *Drosophila ananassae, Genetical and Biological Aspects* (ed. Y. N. Tobari), pp. 1–6. Tokyo: Japanese Scientific Society Press.

Tomimura, Y., Matsuda, M., Tobari, Y. N., Cariou, M. L., Da Lage, J. L., Stephan, W. & Langley, C. H. (1993). Population genetics. In *Drosophila ananassae, Genetical and Biological Aspects* (ed. Y. N. Tobari), pp. 139–198. Tokyo: Japanese Scientific Society Press.

Vishalakshi, C. & Singh, B. N. (2006a). Sexual isolation between two sibling species of *Drosophila: D. ananassae* and *D. pallidosa*. *Current Science* 90, 1003–1006.

Vishalakshi, C. & Singh, B. N. (2006b). Fluctuating asymmetry in certain morphological traits in laboratory populations of *Drosophila ananassae*. *Genome* 49, 777–785.

Vogl, C., Das, A., Beaumont, M., Mohanty, S. & Stephan, W. (2003). Population subdivision and molecular sequence variation: theory and analysis of *Drosophila ananassae* data. *Genetics* 165, 1385–1395.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics* 15, 323–353.

Yadav, J. P. & Singh, B. N. (2006). Evolutionary genetics of *Drosophila ananassae*: I. Effect of selection on body size and inversion frequencies. *Journal of Zoological Systematics and Evolutionary Research* 44, 323–329.

Yadav, J. P. & Singh, B. N. (2007). Evolutionary genetics of *Drosophila ananassae*: Evidence for trade-offs among several fitness traits. *Biological Journal of Linnean Society* 90, 669–685.

Yamada, H., Sakai, T., Tomaru, M., Doi, M., Matsuda, M. & Oguma, Y. (2002). Search for species specific mating signal in courtship songs of sympatric sibling species, *Drosophila ananassae* and *Drosophila pallidosa*. *Genes and Genetic Systems* 77, 97–106.

Yamada, H., Matsuda, M. & Oguma, Y. (2002). Genetics of sexual isolation based on courtship song between two sympatric species, *Drosophila ananassae* and *Drosophila pallidosa*. *Genetics* 116, 225–237.