

## REVIEW ARTICLE

# Status of research on *Drosophila ananassae* at global level

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### Abstract

*Drosophila*, a dipteran insect, has been found to be the best biological model for different kinds of studies. *D. melanogaster* was first described by Meigen in 1830, is most extensively studied species of the genus *Drosophila* and a number of investigations employing this species have been documented in areas such as genetics, behaviour, evolution, development, molecular biology, ecology, population biology, etc. Besides *D. melanogaster*, a number of other species of the genus *Drosophila* have also been used for different kinds of investigations. Among these, *D. ananassae*, a cosmopolitan and domestic species endowed with several unusual genetic features, is noteworthy. Described for the first time from Indonesia (Doleschall 1858), this species is commonly distributed in India. Extensive research work on *D. ananassae* has been done by numerous researchers pertaining to cytology, genetics, mutagenesis, gene mapping, crossing-over in both sexes, population and evolutionary genetics, behaviour genetics, ecological genetics, sexual isolation, fluctuating asymmetry, trade-offs etc. Genome of *D. ananassae* has also been sequenced. The status of research on *D. ananassae* at global level is briefly described in this review. Bibliography on this species from different countries worldwide reveals that maximum contribution is from India.

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### Introduction

*Drosophila*, the tiny fruit fly, is a well-known model organism. It is well accepted that studies on *Drosophila* are economical and very effective for studies ranging from behaviour to molecules and evolution, cell biology to development, human disorders and even social activities, that too, at molecular levels. It was once considered as ‘cinderella of genetics’ as many studies on genetics were investigated on *Drosophila*. For the common people, *Drosophila* means *D. melanogaster* but there are 1500 species reported in this genus (Singh 2013). Among all species of *Drosophila*, *D. funebris* was first described in 1787 by J. C. Fabricius and was moved into the genus *Drosophila* by C. F. Fallen in 1823 (see Markow and O’Grady 2007). Similarly, *D. melanogaster* was described by Meigen (1830). By the early 1900s, *D. melanogaster* became an established model organism, but researchers realized the importance of several other species of *Drosophila* also. In the late 1930s, Th. Dobzhansky began to use *D. pseudoobscura* and its sibling species *D. persimilis* and *D. miranda* for the classic evolutionary and population genetic studies (Anderson *et al.* 1991).

*D. subobscura* is in many ways the old world counterpart of *D. pseudoobscura* (Powell 1997), and it has also been extensively studied for inversion polymorphism by European researchers such as Prevosti, Sperlich (see Krimbas and Loukas 1980), Rose, Santos, Matos (Simoes *et al.* 2008; Fragata *et al.* 2014) and others. Among all these species, *D. ananassae* has also attracted the attention of a number of *Drosophila* researchers particularly from Japan, USA, France, Germany and India. *D. ananassae* is a cosmopolitan and domestic species, and occupies a unique status among the *Drosophila* species due to certain peculiarities in its cytological and genetic behaviour: spontaneous male recombination, high mutability, segregation distortion, parthenogenesis, absence of genetic coadaptation, varied chromosomal polymorphism and occurrence of spontaneous genetic mosaic. Singh and Mohanty (1992) detected a spontaneous bilateral genetic mosaic in *D. ananassae* having three mutant characters. It was a male fly and mitotic recombination in the zygote considered as the cause of origin for this mosaic (figure 1).

*D. ananassae* belongs to the *ananassae* species complex of the *ananassae* subgroup of the *melanogaster* species group. Its uniqueness has also been proved by a number of studies as it occupies a separate branch in *Drosophila* phylogeny (FlyBase; *Drosophila* 12 Genomes Consortium 2007; Singh 2000, 2010). Also, the polytene chromosomes of

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**Figure 1.** A bilateral spontaneous genetic mosaic in *D. ananassae* (Singh and Mohanty 1992).

*D. ananassae* have six arms because of a pericentric inversion of the Mueller A element which converts the normal acrocentric X-chromosome into a submetacentric chromosome (Schaeffer *et al.* 2008). The *ananassae* species group has 22 described species and three species complexes: *ananassae*, *biplectinata* and *ercepeae* (Matsuda *et al.* 2009; Signor *et al.* 2013) found throughout Southeast Asia, while a few are found in Africa, Indian subcontinent, north-eastern Australia and south Pacific regions (Singh and Sisodia 2013). The *D. ananassae* species complex has 10 species: *D. ananassae*, *D. atripex*, *D. cornixa*, *D. ironensis*, *D. monieri*, *D. nesoetes*, *D. ochrogaster*, *D. pallidosa*, *D. parapallidosa* and *D. phaeopleura* (Matsuda *et al.* 2009; Sawamura *et al.* 2010; Singh and Sisodia 2013). Phylogenetic analysis reveals two major lineages within the *ananassae* subgroup. The first lineage has *ananassae* and *biplectinata* species complexes and the other has *ercepeae* complex and *D. varians* species (Matsuda *et al.* 2009). Among all these species, *D. ananassae* has been most extensively utilized for various kinds of studies at global level, and many laboratories are using this species for comparative studies. The present review summarizes briefly the status of research on *D. ananassae* at global level.

*D. ananassae* was initially described by Doleschall in 1858 from Ambon Island in Indonesia. Although it is cosmopolitan in distribution, it is largely circumtropical and of frequent occurrence in domestic habitats. It is recorded from all six biogeographic zones but is absent in some areas (Singh 2010). Further, it occurs commonly in India. This species came into notice when T. H. Morgan, C. B. Bridges and A. H. Sturtevant compiled data in a volume entitled 'The Genetics of *Drosophila*' in 1925 as *D. caribbea* Sturtevant in the chapter 'other species of *Drosophila*'. Hideo Kikkawa in 1935 verified *D. caribbea* as *D. ananassae* based on the description of Doleschall (1858). Having many similarities at the levels of physiological requirements and rearing conditions with *D. melanogaster*, Moriwaki and Kikkawa (Japan)

started using *D. ananassae* as model organism for the cytogenetic and genetic studies (Tobari 1993). By 1938, they characterized more than 100 mutations of *D. ananassae*; although, the first mutation of this species was curved wing, an autosomal recessive gene on II chromosome, reported by Sturtevant in 1921 (Kikkawa 1938). They have independently discovered several other unique features of this species, namely spontaneous crossingover in males, high mutability and bobbed mutation (affects the size of all bristles, abdominal scalerite, lengthening of larval pupal period, lowered fecundity, fertility and viability) associated with Y and 4th chromosomes. Since then this species has been employed extensively by Japanese researchers for various kinds of studies. In 1930s, research on *D. ananassae* pertaining to its genetics and cytology was initiated by researchers in Japan. Kikkawa (1938) selected *D. ananassae* as material for genetic studies because of its excellent viability, high mutability and certain peculiarities in cytological and genetical behaviours. With the progress of research, it became clear that it is unique among the species in the genus *Drosophila* thus far investigated. Moriwaki (1993) writes 'in conclusion, I am happy to report that *D. ananassae* has secured a position as a unique and valuable organism for genetic research especially characterized by male recombination and high mutability both involving chromosomal and extra chromosomal determinants and by the *Om-tom* system.' A number of researchers from Japan used *D. ananassae* for their research work (see Moriwaki and Tobari 1975; Tobari 1993).

Speciation has also been studied in *D. ananassae* particularly with its sibling, *D. pallidosa* which is endemic to the islands of south Pacific Ocean. Although these species have sympatric distribution, postmating reproductive isolation such as hybrid inviability / hybrid sterility does not occur between them. However, these species show morphological differences in body colouration and the number of rows in the sex comb (Yamada *et al.* 2002). Behavioural isolation has been considered as crucial in maintaining the integrity of gene pool of these two sibling species in spite of their sympatric distribution and absence of postmating sexual isolation (Yamada *et al.* 2008). Thus, the phylogenetic separation between *D. ananassae* and *D. pallidosa* must have been a recent event in the process of speciation of the *melanogaster* species group. Female pheromones induce male courtship behaviour in both these species. Males of both the species strongly court heterospecific females. Females discriminate courting males by acoustic cues whether males are conspecific or heterospecific. The acoustic signals produced by the male wing vibration are critical in achieving sexual isolation between these two species. Courtship songs play an important role in female mate discrimination against courting males. Based on this, it is concluded that the mechanism that prevents gene flow between the two species is mate discrimination and the loci which play crucial role in the development of reproductive isolation have been mapped to distinct position near the *Delta* locus in middle of the left arm of the second chromosome (Doi *et al.* 2001). Evolutionary

relationship has been studied in *D. ananassae* species cluster based on introns of multiple nuclear loci and it has been found that *D. ananassae* and *D. pallidosa* do not share the haplotypes for a specific locus which is close to the proximal breakpoint of In(2L)D. This finding is the basis of suggestion that taxon-specific inversions prevent gene flow, as predicted by the chromosomal speciation hypothesis (Sawamura *et al.* 2010).

In Japan, Kikkawa (1938) conducted detailed studies on genetics and cytology of *D. ananassae*. He suggested that it is one of the tropical species with wide geographical distribution. He also studied metaphase chromosomes and reported a number of mutations, male recombination and chromosome inversions in this species. Genetic determination of Y-4 linkage of bobbed mutation in *D. ananassae* was made by Kikkawa (1938). Moriwaki and his coworkers also utilized this species for their extensive and important studies on *D. ananassae* with respect to detection of mutations, male crossingover, population genetics of inversion polymorphism, balanced polymorphism, cytogenetics, polytene chromosome map preparation and puffing activity (Moriwaki 1940; Moriwaki and Ito 1969; Moriwaki and Tobar 1975; Tobar 1993). The presence of chiasmata at a frequency capable of accounting for the observed recombination values in males of *D. ananassae* has also been demonstrated (Matsuda *et al.* 1983). Sexual behaviour of *D. ananassae* has been studied in detail by Oguma (1993). Several mutator systems have been found in *D. ananassae*, but the 'optic morphology' (*Om*) hypermutability system is quite unique (Tanda *et al.* 1993). Almost all mutations of this system affect eye morphology and because of this reason they are called as *Om* mutations. These mutations are caused by *tom*, a retrotransposon (Tanda *et al.* 1993). A number of Japanese workers have tested the speciation hypothesis using *D. ananassae* and its sibling species *D. pallidosa* (Doi *et al.* 2001; Yamada *et al.* 2002, 2008; Sawamura *et al.* 2010).

*D. ananassae* has also been used by researchers in USA. A number of investigations have been documented with particular reference to cytogenetic studies, inversion polymorphism, mutations, male recombination, taxonomy, speciation, molecular biology and genome sequencing. Kauffmann (1936a, b; 1937a, b) reported a number of interesting and important observations on chromosomes of *D. ananassae* and inversions in hybrids between Alabama and Japanese populations. Seecof (1957) constructed maps of polytene chromosomes and detected a number of chromosomal aberrations from Marshall Islands. Levins (1966) suggested mathematical biogeography as a new field and also considered *D. ananassae* in his studies for model building. Futch (1966) studied speciation in the south Pacific populations of *D. ananassae* and found variation in the degree of inversion polymorphism in light and dark forms of *D. ananassae* with sexual isolation between them. Later, light and dark forms were found to be taxonomically different but sibling species (Bock and Wheeler 1972) showing sexual isolation. Although they are sympatric in distribution and

lacking postmating reproductive isolation, still produce fully fertile hybrids (Futch 1973). Futch (1972) also reported parthenogenesis in *D. ananassae*. Allozyme polymorphism has also been studied in natural populations of *D. ananassae* (Gillespie and Kojima 1968; Johnson 1971). Hinton and Downs (1975) studied mitotic, meiotic and polytene chromosomes of *D. ananassae* and reported a large number of chromosomal aberrations. Further, based on Y-4 association of nucleolus organizer it has been suggested that a translocation of nucleolus organizing region from X to 4 has occurred during speciation of *D. ananassae* (Hinton and Downs 1975). Hinton (1970, 1974, 1979, 1981, 1983, 1984) conducted a series of studies on *D. ananassae* with particular reference to mutagenesis, male recombination, transposable elements and extra-chromosomal inheritance. Markow and Smith (1979) conducted a selection experiment on the phototactic behaviour of this species and suggested a different mechanism than the other group members. Schug and his associates (2007, 2008) used *D. ananassae* for their research work in the area of population and evolutionary genetics. For comparative studies, *D. ananassae* is a favourite model organism in the *melanogaster* group. Recently, researchers from USA have used *D. ananassae* for their molecular work and genome sequencing (Clark *et al.* 2007; Markow and O'Grady 2007; Bosco *et al.* 2007; Signor *et al.* 2013; Choi and Aquadro 2014). It has also been used for the construction of polytene chromosome maps along with other species of *Drosophila* (Schaeffer *et al.* 2008). Allozyme polymorphism has been studied in *D. ananassae* populations by researchers from France (Da Lage *et al.* 1989, 1992, 2000). Population genetics of DNA polymorphism in *D. ananassae* has been studied by Stephan and his associates from Germany (Stephan 1989; Stephan and Langley 1989; Stephan and Mitchell 1992; Stephan *et al.* 1998; Das *et al.* 2004). The expression pattern of Frost, a cold sensitive gene, was compared among eight *Drosophila* species by Bing *et al.* (2012) of Canada at 10 different life stages and was found upregulated in *D. ananassae* also as in other species of the *melanogaster* group. Peculiar gene arrangements were reported in Brazilian natural populations of *D. ananassae* (Freire-Maia 1961). VanKure and Vibrationovski (2014) of Brazil studied sex-biased genes in three species including *D. ananassae*. Beatriz Goni (Uruguay) has collected flies from Brazil, Indonesia and Japan, and demonstrated that crossing-over does occur in natural populations of *D. ananassae* males (Goni *et al.* 2012).

Research on *Drosophila* genetics in India was initiated by Prof. S. P. Ray-Chaudhuri at Calcutta University in 1940s after obtaining the Ph.D. degree under the supervision of Nobel laureate Prof. H. J. Muller, while Prof. Muller was at the Institute of Animal Genetics, University of Edinburgh in 1937–1940. When Prof. Ray-Chaudhuri moved to Zoology Department, Banaras Hindu University in 1960, he continued his research on *Drosophila* with particular reference to population genetics, crossingover, mutagenesis, cytogenetics and taxonomy. The work on genetics of *D. ananassae*

which he initiated at Calcutta University is still being pursued at Zoology Department, Banaras Hindu University by his student. First paper on *D. ananassae* was published by Ray-Chaudhuri and his coworkers (1959) from Calcutta University. Male crossingover was also studied by Mukherjee and his students (Mukherjee 1961; Mukherjee and Das 1971). Few studies on population genetics of *D. ananassae* have been reported by Krishnamurthy and his students (Rajeshwari and Krishnamurthy 1969) as well as Parkash and his students (Parkash *et al.* 1993; Parkash and Shamina 1994). Joshi and Gore (1999) reported latitudinal variations in eclosion rhythm in different geographic strains of *D. ananassae*. Sheeba and her group (Prabhakaran *et al.* 2013; Prabhakaran and Sheeba 2014) demonstrated that *D. ananassae* has a prominent morning activity peak only (unimodal) instead of bimodal activity as found in other closely related *Drosophila* species, thus justifying its uniqueness at biological rhythm level also. Joshi and his group (Bharathi *et al.* 2003, 2004) used *D. ananassae* occasionally for comparative studies of life history traits at various levels. A well-established group of drosophilists at Mysore is also using *D. ananassae* occasionally for studies at various levels (Jayaramu *et al.* 2006; Pratibha and Krishna 2010; Pratibha *et al.* 2011). Shivanna and his team are investigating *D. ananassae* for quantitative and qualitative analyses of accessory gland secretory proteins to compare and correlate among other drosophilids for associated fitness traits (Hiremani and Sivanna 2010; Kudupali and Shivanna 2013). The most extensive research work on *D. ananassae* has been carried out by researchers at the Zoology Department, Banaras Hindu University. Ray-Chaudhuri and Jha (1966, 1967) prepared polytene chromosome maps described chromosomal aberrations and frequencies of inversions in a few natural populations of *D. ananassae*. Ray-Chaudhuri and Kale (1966) reported crossingover in males in certain strains of *D. ananassae*. For the first time, Kale (1969) demonstrated that male recombination in *D. ananassae* is meiotic in origin. Singh (1970) detected cosmopolitan inversions for the first time from natural populations of *D. ananassae*, from Andaman and Nicobar Islands. Singh and Ray-Chaudhuri (1972) presented evidence for balanced polymorphism in laboratory populations of *D. ananassae*. In total, there have been 78 paracentric inversions, 21 pericentric inversions and 48 translocations are reported in *D. ananassae* (Singh 2014). Thus, it exhibits a high degree of chromosomal polymorphisms in its natural populations (Singh 1988a, b; Singh and Singh 2007a). The natural populations of *D. ananassae* have undergone a considerable degree of genetic divergence at the level of chromosomal polymorphism as a consequence of their adaptation to varying environments and natural selection operates to maintain the three cosmopolitan inversions (Singh 1983, 1984, 1989, 1996, 1998a, b; Singh and Singh 2007b, 2008b). The cosmopolitan distributions of three paracentric inversions (AL in 2L, DE in 3L and ET in 3R) have been considered as monophyletic in origin as they are coextensive with the

species. Other inversions have localized distribution (Singh 1970). These cosmopolitan inversions often persist in laboratory stocks also, thus suggest their association with heterotic buffering (Singh 1982). Although inversion polymorphism is subject to selection, inversion frequency may change in laboratory populations due to random genetic drift (Singh 1987, 1988b; Singh and Singh 2008a). Similarly, interracial hybridization experiments suggest that there is absence of genetic coadaptation in geographic populations of *D. ananassae* (Singh 1972, 1985). Therefore, heterosis associated with inversions in *D. ananassae* appears to be simple luxuriance rather than populational heterosis (coadaptation). Using cosmopolitan inversions as marker, extensive population structure study suggests strong genetic differentiation and minimal gene flow showing strong sub-structuring in Indian natural populations of *D. ananassae* (Singh and Singh 2010). Association studies of cosmopolitan inversions in *D. ananassae* strongly correlated with mating propensity in both sexes (Singh and Chatterjee 1986, 1988), in behavioural isolation between two karyotypically different homozygous strains (Nanda and Singh 2011a), in frequency-dependent sexual selection (Som and Singh 2004), in maintaining body size under various selection pressures (Yadav and Singh 2003, 2006) and in recombination (Singh 1973; Singh and Singh 1987, 1988; Singh and Mohanty 1990, 1991). Random genetic drift is the cause of linkage disequilibrium between inversions in isofemale lines of *D. ananassae*. The tight linkage between the two inversions as evidenced by recombination studies support the notion that linkage disequilibrium is caused by random genetic drift (Singh and Singh 1990).

In addition to the significant findings pertaining to inversion polymorphism, a number of other phenomena which are of considerable evolutionary significance have been elucidated in *D. ananassae*. For example, incipient sexual isolation among different populations (Nanda and Singh 2011c), trade-offs in various life history traits (Sisodia and Singh 2002, 2006; Yadav and Singh 2007), adaptive plasticity (Sisodia and Singh 2002), sexual selection (Sisodia and Singh 2004), fluctuating asymmetry (Vishalakshi and Singh 2006, 2008a, b, 2009), founder effect, sexual isolation (Nanda and Singh 2011a, b), pupation site preference (Singh and Pandey 1993a, b), oviposition site preference (Srivastava and Singh 1996), sternopleural bristle phenotypes (Singh and Mathew 1995, 1997), mating propensity and fertility (Singh and Chatterjee 1985, 1987, 1988, 1989), female remating, sperm displacement and polygenic control (Singh and Singh 1999, 2001a, b), rare male mating advantage (Som and Singh 2004) and allozyme polymorphism (Kumar and Singh 2014).

The resistance to different kinds of stress such as heat and cold shocks, starvation and desiccation in populations of *D. ananassae* has been investigated (Sisodia and Singh 2010a, b, 2012). Sisodia and Singh (2010a) investigated cold tolerance in 45 Indian populations of *D. ananassae* from different latitudes. Significant latitudinal differentiation it was

observed for chill-coma recovery in *D. ananassae* populations and it was found that chill-coma recovery was associated with local climatic factors of original populations. These results provide evidence that populations of *D. ananassae* from higher latitudes show more cold resistance than those from low latitudes. These findings suggest that cold adaptation and resistance within *D. ananassae* may vary with latitude which has resulted due to direct/indirect action of natural selection. In *D. ananassae*: (i) there is a positive correlation between starvation resistance and lipid contents; (ii) there is a negative correlation between desiccation and lipid contents and between desiccation and heat resistance; and (iii) flies from lower latitudes (south) show higher starvation resistance, heat resistance and lipid contents, but the pattern is reversed for desiccation resistance. *D. ananassae* flies from different latitudes vary in their susceptibility to starvation because of difference in their propensity to store body lipid. Thus, there is evidence for climatic selection in *D. ananassae* in the Indian subcontinent influenced by latitudinal variation in temperature and humidity (Sisodia and Singh 2010b). Further, the larval nutrition also affects the stress resistance in *D. ananassae*. It has been reported that flies consuming protein-rich diet have higher desiccation and heat-shock resistance whereas flies developed on carbohydrate-rich diet have higher resistance for starvation and cold. Thus *D. ananassae* adapts to different stress tolerance according to the quality of available diet. This is correlated with phenotypic adjustment at anatomical and physiological levels (Sisodia and Singh 2012). Thus, these findings in *D. ananassae* provide evidence that the quality and quantity of nutrients consumed by organisms have strong impact on stress resistance (Singh 2015).

### Conclusion

Thus extensive work has been done on *D. ananassae*, a genetically unique species, at global level. However, when compared with other species the work done on *D. ananassae* comes next to *D. melanogaster*. Further, if research done on *D. ananassae* in different countries is compared, maximum contribution comes from India. It is a good model species for genetical, behavioural, evolutionary and ecological studies, and a number of researchers have started using *D. ananassae* for comparative studies. We suggest that by employing *D. ananassae* as a model species, more emphasis may be given in the field of evolutionary and speciation genetics by researchers and the findings may be utilized to unravel the mechanism of speciation and adaptation.

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