BIODIVERSITY AND GENETICS OF CERTAIN *DROSOPHILA* SPECIES OF NAGALAND STATE, INDIA

Thesis

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Certificate

This is to certify that the thesis entitled **"Biodiversity and genetics of certain *Drosophila* species of Nagaland state, India"** is a record of original research work done by **Mr. Bovito Achumi** under my supervision. He is a registered research scholar (*Regd. No-476/2012*) of the Department and has fulfilled all the requirements of Ph.D regulations of Nagaland University for the submission of thesis. The work is original and neither the thesis nor any part of it has been submitted elsewhere for the award of any degree or distinctions. The thesis is therefore, forwarded for adjudication and consideration for the award of degree of Doctor of Philosophy in Zoology under Nagaland University.

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ABSTRACT

The fruit fly *Drosophila* is one of the most intensively studied organisms in biology that serves as a model system for investigations of many developmental, cellular processes, disease(s), adaptation, diversity and evolution; whose underlying fundamental principles are comparable to higher eukaryotes, including man (Reviewed in Devineni *et al.* 2013). *Drosophila,* with its cosmopolitan nature and complexities in species composition, is an excellent model for studying the eco-distributional patterns of various species.

The family Drosophilidiae comprises of more than 3,500 described species including the genus *Drosophila* (Bachli 1998). In this family *Drosophila* is the most abundant genus and comprises of 1500 species in the world (Bachili 1999-2008). The review of literature shows that more than 200 *Drosophila* species have been reported from India (Hegde *et al.* 2000).

Nagaland is one of the sub-Himalayan hilly states of north-east India which is blessed with tremendous floral and faunal diversity. However very little work has been done to understand *Drosophila* diversity. Collections from wild localities of all the 11 district headquarters of Nagaland revealed a total of 16 *Drosophila* species belonging to four subgenera (*Sophophora*, *Drosophila*, *Dorsilopha* and *Scaptodrosophila*). Observations illustrate the fact that *Drosophila* fauna of Nagaland state shows similarity not only with South Asia but also with that of East Asia which can be explained from geographical location of this north eastern state.

In order to understand distributional pattern of a species or related group of species in space and time, altitudinal and seasonal variation in *Drosophila* species of mount Japfu in Nagaland were studied. A total of 4,680 *Drosophila* flies belonging to 19 species of 4 subgenera were collected at altitudes of 1500, 1800, 2100, 2400 and 2700 m a.s.l. The subgenus *Sophophora* was predominant with 10 species, followed by subgenus *Drosophila*, with 4 species. Subgenus *Dorsilopha* and subgenus *Scaptodrsophila* were represented by 1 species each. The remaining 3 species are under identification. Cluster analysis and constancy methods were used to analyze species occurrence. Altitudinal changes in the population densities and relative abundances of different species across seasons were also studied. The diversity of *Drosophila* community was assessed by applying Simpson's diversity index. At 1800 m the

Simpson's index was low (0.09301), suggesting high *Drosophila* diversity at this altitude. The density of *Drosophila* changed significantly during different seasons (F= 26.72; df=2: p<0.0001). The results suggest that distributional pattern of a species or related group of species is uneven in space and time (Achumi *et al.* 2013).

In the present study basing on morphological markers and internal characters a new species, *Drosophila hegdii* was identified (Achumi *et al.* 2011). New species status and its molecular phylogeny were understood with the help of "DNA Barcoding." Neighbour-Joining trees were constructed using MEGA5 (Neighbor-Joining (NJ) method with bootstrap test (1000 replicates) using the Kimura 2-parameter model, with gaps treated by pair wise deletion). Molecular analysis indicates that *D. hegdii* and *D. jambulina* belonging to the same cluster with strong boot strap support of 86. The ancestor of *D .vulcana* and *D. hegdii* clade was estimated to have appeared about 0.02296 Mya, the divergence between *D. jambulina* and *D. hegdii* was estimated to be 0.02223 Mya. Molecular analysis confirms the observation made through morphological markers that *Drosophila hegdii* is a new species.

Chromosomal rearrangements are sources of genetic variation. Dobzhansky (1950) suggested that the chromosomal polymorphism is a device to cope with the diversity of environments. Swanson (1974) demonstrated that paracentric inversions due to their high adaptive value in heterozygous condition have been positively selected in animals. Present study aimed at understanding probable significance of multiple cosmopolitan inversions in *D. ananassae* in adapting to various climatic and geographical factors of Nagaland. The polymorphic grade is remarkably high in Dimapur population with 81% heterokaryotypes having 0.81 mean inversion heterozygotes. Mon population exhibits least polymorphic with 37% heterokaryotypes. Remaining nine populations- Longleng, Phek, Tuensang, Mokokchung, Wokha, Zunheboto, Kohima, Peren and Kiphire are intermediate, ranging from 49%-76% heterokaryotes. The populations that exhibit the three common inversions frequencies are computed for the X^2 homogeneity test. Results show that the differences in the frequencies of multiple inversions exhibited by different populations are found to be significant ($p < 0.05$), indicating that the populations under study are distinct from one another with regard to the degree of variability. In order to understand the influence of multiple eco-geographical factors such as altitude, humidity, rainfall and temperature on inversion frequencies; patterns of variation in inversion frequencies were examined by means of correlation analysis using SPSS 16.0, considering inversion frequencies (2LA, 3LA and

3RA) as dependent variables on climatic variables (humidity, rainfall and temperature) and geographical variables (altitude). Results reveal lack of significant correlation between presence of multiple inversions and certain climatic and geographical variables (humidity and longitude). However, significant correlation exists with reference to certain climatic indicators such as rainfall (negative correlation) and temperature (positive correlation). Basing on these observations, polymorphism of 2LA, 3LA and 3RA inversions in *Drosophila ananassae* populations of Nagaland state and their adaptive significance is discussed in the present work. Results point out the adaptive significance between genomic rearrangements such as frequency of 2LA inversion and certain climatic factors such as temperature and rainfall; which explains the gene environmental interaction as a survival strategy.

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PREFACE

Preface

The fruit fly *Drosophila* is one of the most intensively studied organisms in biology that serves as a model system for investigations of many developmental, cellular processes, disease(s), adaptation, diversity and evolution; whose underlying fundamental principles are comparable to higher eukaryotes, including man (Reviewed in Devineni *et al.* 2013). Genus *Drosophila*, with its cosmopolitan nature and complexities in species compositions is an excellent model for studying the eco-distributional pattern of various species (Carson 1965). Systematic study concerning variations in the species compositions and the patterns of distribution of various members of the genus *Drosophila* in different geographical regions of the earth will enable understand the principles underlying adaptive radiation and certain mechanisms involved in speciation (Muniyappa 1981).

Significant progress has been made in the field of taxonomy and systematics of the family Drosophilidae (Diptera) in India. However, vast area of great ecological interest still either awaits exploration or is poorly explored. Particularly, very little is known regarding *Drosophila* fauna of North-Eastern region of the Indian subcontinent. This region with its diverse climatic conditions, variable altitudes, deep valleys, luxuriant flora, running streams and moist surroundings makes one of the richest repositories of biodiversity in the world. It provides an ideal location for the colonization of several *Drosophila* species (Singh and Gupta 1977, Dwivedi and Gupta 1979, Gupta and Singh 1979, Dwivedi *et al.* 1979, 1980, Singh 1987; Yenisetti *et al.* 2002; Achumi *et al.* 2011, 2013).

Nagaland is one of the sub-Himalayan hilly states blessed with tremendous floral and faunal diversity, but very little work has been done to understand *Drosophila* diversity. Singh (1987) conducted a pioneering preliminary survey on Drosophilids of Dimapur, Medziphema and Kohima of Nagaland. A preliminary report on Drosophilids of Mokokchung town was published by Yenisetti *et al.* (2002). But for these maiden attempts no systematic comprehensive study has been done on Drosophilids of Nagaland. As most parts of Nagaland are unexplored virgin areas, it is possible that new *Drosophila* species can be identified from this region. In the present work chapter I focuses on the occurrence and distribution of *Drosophila* species of Nagaland.

The ecological and biological diversity of an ecosystem determines the presence or absence of a species in an ecological niche. Apart from physical and biotic factors, the topography and season also affect animal distribution. As elevation is one of the important aspects of topography, it is important to look at animal distribution from that perspective. Efforts have been made to collect *Drosophila* at different altitudes, but these data were not considered with an ecological perspective (Reddy and Krishnamurthy 1977). According to Reddy and Krishnamurthy (1977), physical and biotic factors are the sole determinants of animal distribution. This idea logically denotes that elevation and season have no influence on animal distribution. In the present study, goal was to determine if elevation affects distribution.

According to Gause's competitive exclusion theory, two related species competing for the same resources cannot co-exist together in the same ecological niche (Gause 1934). However, laboratory experiments questioned the validity of this principle (Ayala 1969). The presence of taxonomically or phylogenetically related species in an ecological niche indicates their coexistence, and absence of such related species infers competitive exclusion (Guruprasad *et al.* 2010). Present study sought to understand whether taxonomically or phylogenetically related *Drosophila* species coexist in nature (Achumi *et al.* 2013). Current study was undertaken to understand the altitudinal and seasonal variation of *Drosophila* species on Mount Japfu (15 km from Kohima town, the capital of Nagaland state), which has a peak altitude of about 3015.6 meter (Achumi *et al.* 2013). Observations of this study constitute Chapter II.

A new species, *Drosophila hegdii* was discovered from Lumami, Nagaland state in this study (Achumi *et al*. 2011). Chapter III describes this new species and explores its molecular phylogeny with the help of "DNA barcoding."

In every organism or a population there is a continued interaction between the genotype and the environment to attain a better homeostatic stability. This stability is achieved by several ways and one is by initiating change in the karyotype (Slavica *et al.* 2006). Chromosomal rearrangements are sources of genetic variation. Chromosomal rearrangements have been implicated in adaptation and speciation in a wide variety of taxa

(Coghlan *et al*. 2005). This can be understood by looking into the chromosomal polymorphism mainly due to inversions in their natural populations. Dobzhansky and Pavan (1950) suggested that the chromosomal polymorphism is a device to cope with the diversity of environments. Swanson (1974) demonstrated that paracentric inversions due to their high adaptive value in heterozygous condition have been positively selected in animals. Present study aims at understanding the significance of multiple cosmopolitan inversions in *D. ananassae* in adapting to various climatic and geographical factors. Chapter IV focuses on inversion polymorphism and its adaptive significance in Nagaland populations of *Drosophila ananassae.*

In nut shell current study provides an insight into the species diversities and pattern of distribution of the members of the *Drosophila* in Nagaland; explains whether taxonomically or phylogenetically related *Drosophila* species coexist in nature; deciphers the molecular phylogeny of a new species- *Drosophila hegdii* from Nagaland and further reveals the adaptive significance of genomic rearrangements in *Drosophila ananassae* populations of Nagaland, a sub-Himalayan hilly state of north-east India.

CHAPTER I

OCCURANCE AND DISTRIBUTION OF *DROSOPHILA* SPECIES IN NAGALAND, A SUB-HIMALAYAN HILLY STATE OF NORTH-EAST INDIA

INTRODUCTION:

The fruit fly *Drosophila* is one of the most intensively studied organisms in biology that serves as a model system for investigations of many developmental, cellular processes, disease, adaptation, diversity and evolution; whose underlying fundamental principles are comparable to higher eukaryotes, including man (Reviewed in Devineni *et al*. 2013). Culturing *Drosophila* is easy and inexpensive and they could be kept in large numbers. It has a short life cycle, hence could be used for any study which needs observation over generations. Libraries of several *Drosophila* species and mutant stocks of many species are available at different laboratories in the world. Presence of polytene chromosomes added advantage for taxonomic and genetic studies. *Drosophila* genome has also been sequenced. Because of these advantages, *Drosophila* is valuable in understanding the basic principles of genetics, molecular biology, adaptation and evolution. Fruit fly has also been extensively used to appreciate many intricacies concerned with the relationships between the ecological factors and population fluctuations (Da Cunha *et al*. 1951; Parshad and Paika 1964; Parshad and Duggal 1966; Heed 1968; Gupta and Ray Chaudhuri 1970a,b; Rajeshwari 1971; Reddy and Krishnamurthy 1968, 1973; Siddaveere Gowda *et al.* 1977; Hegde and Krishnamurthy 1979; Prakash and Reddy 1980; Bizzo and Sene 1982; Brncic *et al.* 1985; Singh and Chatterjee 1987; Putman 1995; Begon *et al.* 1996; Hegde *et al.* 2000; Nagabhushan 2002; Yenisetti *et al*. 2002; Mateus *et al.* 2006; Toress and Ravazzi 2006; Guru Prasad 2008 and Achumi *et al*. 2013).

The family Drosophilidiae comprises of more than 3,500 described species including the genus *Drosophila* (Bachli 1998). It is estimated that there are more than 2240 biologically valid species of *Drosophila* (Wheeler 1986). Indian subcontinent with its vast array of vegetation and climates harbors variety of *Drosophila* species. Though studies on Indian Drosophilids was started by Bezzi (cf. Sturtevant 1921) much of our knowledge on eco-distribution of *Drosophila* in India was acquired only after 1964 (Parshad and Paika1964; Gupta and Ray Chaudhuri 1970a,b; Rajeshwari 1971; Godbole and Vaidya 1972; Ranganath and Krishnamurthy 1972a,b; Nirmala and Krishnamurthy 1973; Gupta 1974; Nirmala and Reddy 1975; Dwivedi *et al*. 1979; Dwivedi and Gupta 1980; Prakash and

Reddy 1980; Gai and Krishnamurthy 1983; Hegde *et al.* 2000; Vasudev *et al.* 2001; Yenisetti *et al*. 2002; Guru prasad 2008 and Achumi *et al*. 2013).

The review of literature shows that more than 200 *Drosophila* species were reported from India (Hegde *et al*. 2000). Some of them are endemic to certain regions of the country and a few are cosmopolitan. Parshad and Paika (1964) reported 7 species along with 3 new species and a subspecies. Parshad and Duggal (1965, 1966) collected *Drosophila* from various parts of Kashmir and they were able to record 20 species. Reddy and Krishnamurthy (1968, 1970, and 1974) in addition to describing 2 new spices (*D. rajasekari* and *D. mysorensis*) reported 21 species in and around Mysore, Karnataka, Nilgiri and Palni Hills of Tamilnadu. Gupta and Ray Chaudhuri (1970a,b) made an extensive survey in few wild and domestic localities of north India, as well as few parts of Andaman-Nicobar Islands and reported 29 species, of which 8 were new to science. Jha *et al*. (1971) recorded 7 species from Darjeeling. Godbole and Vaidya (1972) reported 10 species in and around Poona. Nirmala and Krishnamurthy (1973) described 2 new species, namely, *D. neonasuta* and *D. chamundiensis* from Mysore. Reddy and Krishnamurthy (1973) described 2 new species *D. anamelani* and *D. coonoresis* from Karnataka. Nirmala and Reddy (1975) described 2 new species, *D. mundagensis* from Mundage and *D. krishnamurthhii* from Soundatti, Karnataka. Prakash and Reddy (1979b) reported *D. sahyadrii* from Western Ghats of Karnataka. Prakash and Reddy (1977, 1978b, and 1980) described few new species *D. girienesis*, *D. agumbensis*, *D. gundensis* and *D. nagaraholensis* from Karnataka. Muniyappa (1981) reported a new species *D. sampagiensis* from Karnataka. Muniyappa and Reddy (1981) discovered *D. gangotrii* and *D. madikerii* from Coorg district of Karnataka. Muniyappa and Reddy (1982) described *D. brahmagiriensis* and *D. cauverii* from Coorg district of Karnataka. Gai and Krishnamurthy (1983) described *D. septacoila* from South Canara district of Karnataka. Hegde *et al.* (1989) described *D. longivittata* from Salem, Tamilnadu. Hegde *et al.* (2000) described *D. palniensis* from Tamilnadu.

Significant progress has been made in the field of taxonomy and systematics of the family Drosophilidae (Diptera) in India. However, a vast area of great ecological interest still

either awaits exploration or is poorly explored. Particularly, very little is known regarding *Drosophila* fauna of North-Eastern region of the Indian subcontinent. This region with its diverse climatic conditions, variable altitudes, deep valleys, luxuriant flora, running streams and moist surroundings, one of the richest repositories of biodiversity in the world. It provides an ideal location for the colonization of several *Drosophila* species (Singh and Gupta 1977, Dwivedi and Gupta 1979, Gupta and Singh 1979, Dwivedi *et al.* 1979, Singh 1987; Yenisetti *et al.* 2002; Achumi *et al*. 2011, 2013).

Few *Drosophila* species were reported from North-Eastern region of the country. Dwivedi and Gupta (1979) reported two new species of subgenus *Drosophila* (*D. guptai* and *D. ramamensis*) from Darjeeling, West Bengal. Gupta and Singh (1979) reported 7 species and two new species of *Drosophila* (*D. novaspinofera* and *D. penispina*) from Shillong, Meghalaya. Gupta and Singh (1980) reported two new and two unrecorded species of *Drosophila* from Kurseong, Darjeeling, West Bengal. Gupta and Singh (1981) reported two new species (*D. paralongifera* and *D. neomakinoi*) from Rimbick, West Bengal. Kumar and Gupta (1983) reported 18 species from Meghalaya and Arunachal Pradesh. Singh (1987) reported 11 species of *Drosophila* from Dimapur, Medziphema and Kohima of Nagaland. Yenisetti *et al.* (2002) reported 8 species of *Drosophila* from Mokokchung, Nagaland. Achumi *et al.* (2011) reported one new species of *Drosophila* (*Drosophila hegdii*) from Lumami Nagaland. Achumi *et al.* (2013) also reported 19 species from mount Japfu of Nagaland.

Nagaland state is one of the 'Eight Sisters' of North-East India. It is bordered by state of Assam in the west, by state of Arunachal Pradesh and part of Assam towards the north, on the east by the country of Burma and by the state of Manipur on towards the south. Nagaland is situated at the foot hills of Himalayas. Naga Hills are covered with the tropical evergreen and sub tropical forest that are endowed with rich flora and fauna. Geographically, Nagaland state lies between 26º 60' N and 27º40' N latitude and 93º20' E and 95º15' E longitude (area of about 16,579 sq. Kilometers). Nagaland is popular for the fact that its climate remains salubrious throughout the year. Annual average rainfall varies from 175 cm to 250 cm. Temperature varies from 4º C to 31ºC.

Very little work is done on *Drosophila* diversity of Nagaland. Singh (1987) conducted a pioneering preliminary survey on Drosophilids of Dimapur, Medziphema and Kohima of Nagaland. Yenisetti *et al*. (2002) published a preliminary report on Drosophilids of Mokokchung town. But for these maiden attempts no systematic comprehensive study was done on *Drosophila* of Nagaland. As most parts of Nagaland are unexplored virgin areas, it is possible that new *Drosophila* species can be identified from this region. In order to understand occurance and distribution of *Drosophila* species, collections were made from wild localities of all the eleven districts of Nagaland state.

MATERIALS AND METHODS:

Collections were performed in eleven district head quarters of Nagaland state during post-monsoon months of 2012.

Drosophila collections were made following two methods:

1) Bottle trapping method: For bottle trapping method, milk bottles of 200 ml capacity containing a smashed ripe banana sprayed with yeast were tied to the twigs underneath small bushes at the height of three to five feet above the ground. Ten traps were kept in an area of 1 Km radius. After 2 days the mouth of each bottle was plugged with cotton and removed from the bushes. The flies which were attracted by the bait were collected in the bottles (soon after sun rise or just before sun set) and were transferred to fresh bottles containing wheat cream agar medium (medium was prepared by adding 100 g of sugar (jaggery) to 500 ml of water and boiled by gentle stirring till jaggery dissolved. Then, 500 ml of water, 100 g of wheat powder (soji), and 8 g of agar-agar were added to the boiling sugar water mixture. When the medium turns sticky 7.5 ml propionic acid (anti fungal agent) was added while continuous stirring the medium. This medium then becomes a thick fluid. It was then distributed to sterilized jam bottles (200 ml milk bottles) or vials of 1'x3' size. The mouth of the bottles/vials was kept closed with cotton. Next day moisture was removed from bottles/vials and two drops of yeast solution was added to the medium. This medium was used after 24 hours).

2) Net sweeping method: For net sweeping method, a hand made *Drosophila* net containing a fine cloth cone tied to the rim of the net was used. Sweeping was made on fermenting fruits (crushed banana were spread in shaded areas of the bushes in the wild and flies were collected after 2 days) that were spread under four shady regions in an area of 1Km radius. After each sweep (three sweepes were performed) flies were collected at the bottom of the cone of the net and were transferred to the bottles containing freshly prepared wheat cream agar medium.

The flies were then brought to the laboratory, isolated and sex was identified. The males were directly used for identification of species basing on morphological characters such as

presence or absence of the sex comb; if present, the number of sex comb rows and teeth in each row and studying the characterestics of the genital plate. Individual females were kept in separate food vials and allowed to produce isofemale lines. The males of the F1 progeny of these gravid females were used for species identification.

Categorization of the collected *Drosophila* flies were made to respective taxonomic groups by employing the parameters as suggested by Bock (1971), Patterson and Stone (1952), Sturtevant (1921) and Throckmorton (1962). The most important parameters employed to identify the species are the morphological features like colour and size of imagoes, number and nature of aristal branches, nature and arrangement of genital arch, nature and number of acrostichal hairs, length of the wings and its indices, the internal characters of the adults, the shape and number of egg filaments, pupal characters, pupal spiracles, and behavior were also taken into consideration for species identification.

Flora at collection sites of Dimapur:

Black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); timburni, *Diospynum* spp (Ericales: Ebenaceae); deer-eye beans, *Mucna perita* (Adans) (Fabales: Fabaceae); tapioca-root, *Maninot utilissema* (Crantz) (Malpighiales: Euphorbiaceae); carrion flower, *Smilax* spp (Liliales: Smilacaceae); khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); yellow himalayan raspberry, *Rubus* spp (Rosaceae); wormwood, *Artemisisia vulgaris* (L.) (Asterales: Asteraceae); begger-ticks, *Bidens* spp (Asterales: Asteraceae); blueberry ash, *Elaeocarpus* spp (Oxalidales: Elaeocarpaceae); blady grass, *Imperata cylindrica* (Drauv) (Poales: Poaceae); etc.

Flora at collection sites of Kiphire:

Blueberry ash, *Elaeocarpus* spp (Oxalidales: Elaeocarpaceae); deer-eye beans, *Mucna perita* (Adans) (Fabales: Fabaceae); bologi, *Crossocephalum s*pp (Asterales: Asteraceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); blady grass, *Imperata cylindrica* (Drauv) (Poales: Poaceae); cowich, *Mucuna pruriens* (L.) (Fabales: Fabaceae); kamraj, *Helminthostachys zeylanica* (L.) (Ophioglossales: Ophioglossaceae); carrion flower, *Smilax* spp (Liliales: Smilacaceae); shaking bake, *Pteris* spp (Polypodiales:

Pteridaceae); Brahmi booti, *Centella asiatica* (L.) (Apiales: Apiaceae); khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); etc.

Flora at collection sites of Kohima:

Banana, *Musa* spp (Zingiberales: Musacae); yellow himalayan raspberry, *Rubus* spp, (Rosaceae); jackfruit, *Artocarpus hetrophyllus* (Lam), (Rosales: Moraceae); *Makania* spp; carrion flowers, *Simlax* spp (Liliales: Smilacaceae); pinyin, *Stemona* spp (Pandanales: Stemonaceae); currant tomato, *Solanum* spp (Solanales: Solanaceae); maibau, *Alnus nepalensis* (Don) (Fagales: Betulaceae); marda, *Termenalia elliptice* (Wright and Arn) (Myr-tales: combretaceae); khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); etc.

Flora at collecton sites of Longleng:

Bamboo, *Bambusa* spp, (Poales: Poaceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); timburni, *Diospynum* spp (Ericales: Ebenaceae); deer-eye beans, *Mucna perita* (Adans) (Fabales: Fabaceae); tapioca-root, *Maninot utilissema* (Crantz) (Malpighiales: Euphorbiaceae); carrion flowers, *Simlax* spp (Liliales: Smilacaceae); *Rubus* spp; khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); wormwood, *Artemisisia vulgaris* (L.) (Asterales: Asteraceae); thoroughworts, *Eupatorium* spp; begger-ticks, *Bidens* spp (Asterales: Asteraceae); etc.

Flora at collection sites of Mokokchung:

Blueberry ash, *Elaeocarpus* spp; (Oxalidales: Elaeocarpaceae); deer-eye beans, *Mucna perita* (Adans) (Fabales: Fabaceae); bologi, *Crossocephalum s*pp (Asterales: Asteraceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); blady grass, *Imperata cylindrica* (Drauv) (Poales: Poaceae); kamraj, *Helminthostachys zeylanica* (L.) (Ophioglossales: Ophioglossaceae); carrion flowers, *Simlax* spp (Liliales: Smilacaceae); banana, *Musa* spp (Zingiberales: Musacae); etc.

Flora at collection sites of Mon:

Sow thistles, *Sonchus* spp; banana, *Musa* spp (Zingiberales: Musacae); *Polygonum* spp; wormwood, *Artemisisia vulgaris* (L.) (Asterales: Asteraceae); beggar-ticks, *Biden* spp (Asterales: Asteraceae); bamboo, *Bambusa* spp, (Poales: Poaceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); timburni, *Diospynum* spp, (Ericales: Ebenaceae); cowich, *Mucuna pruriens* (L.) (Fabales: Fabaceae); tapioca-root, *Maninot utilissema* (Crantz) (Malpighiales: Euphorbiaceae); carrion flowers, *Simlax* spp (Liliales: Smilacaceae); yellow himalayan raspberry, *Rubus* spp, (Rosaceae); khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); etc.

Flora at collection sites of Peren:

Blueberry ash, *Elaeocarpus* spp (Oxalidales: Elaeocarpaceae); deer-eye beans, *Mucna perita* (Adans) (Fabales: Fabaceae); bologi, *Crossocephalum s*pp (Asterales: Asteraceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); blady grass, *Imperata cylindrica* (Drauv) (Poales: Poaceae); kamraj, *Helminthostachys zeylanica* (L.) (Ophioglossales: Ophioglossaceae); carrion flowers, *Simlax* spp (Liliales: Smilacaceae); shaking bake, *Pteris* spp (Polypodiales: Pteridaceae); Brahmi booti, *Centella asiatica* (L.) (Apiales: Apiaceae); etc.

Flora at collection sites of Phek:

Butterfly bush, *Buddleja* spp (Lamiales: Scrophulariaceae); brahmi booti, *Centella asiatica* (L.) (Apiales: Apiaceae); sirib large, *Entada pursathea* (Roux) (Fabales: Fabaceae); knotwood, *Polygonum* spp (Caryophyllales: Polygonaceae); maibau, *Alnus nepalensis* (Don) (Fagales: Betulaceae); khang, *Acacia pinnata* (Miller) (Fabales: Fabaceae); bologi, *Crossocephalum s*pp (Asterales: Asteraceae); himalayan nettle, *Girardinia heterophylla* (Vahl) (Rosales: Urticaceae); banana, *Musa* spp (Zingiberales: Musacae); currant tomato, *Solanum* spp (Solanales: Solanaceae); blady grass, *Imperata cylindrica* (Drauv) (Poales: Poaceae); etc.

Flora at collection sites of Tuensang:

Banana, *Musa* spp (Zingiberales: Musacae); himalayan raspberry, *Rubus* spp (Rosaceae); jackfruit, *Artocarpus hetrophyllus* (Lam), (Rosales: Moraceae); carrion flower, *Simlax* spp (Liliales: Smilacaceae); pinyin, *Stemona* spp (Pandanales: Stemonaceae); currant tomato, *Solanum* spp (Solanales: Solanaceae); marda, *Termenalia elliptice* (Wright and Arn) (Myrtales: combretaceae); cowich, *Mucuna pruriens* (L.) (Fabales: Fabaceae); begger-ticks, *Bidens* spp (Asterales: Asteraceae); wormwood, *Artemisisia vulgaris* (L.) (Asterales: Asteraceae); etc.

Flora at collection sites of Wokha:

Sirib large, *Entada pursathea* (Roux) (Fabales: Fabaceae); knotwood, *Polygonum* spp (Caryophyllales: Polygonaceae); maibau, *Alnus nepalensis* (Don) (Fagales: Betulaceae); banana, *Musa* spp; (Zingiberales: Musacae); timburni, *Dryopteris* spp (Ericales: Ebenaceae); cowich, *Mucuna pruriens* (L.) (Fabales: Fabaceae); himalayan nettle, *Girardinia heterophylla* (Vahl) (Rosales: Urticaceae); begger-ticks, *Bidens* spp (Asterales: Asteraceae); etc.

Flora at collection sites of Zunheboto:

Wormwood, *Artemisisia vulgaris* (L.) (Asterales: Asteraceae); begger-ticks, *Bidens* spp (Asterales: Asteraceae); butterfly bush *Buddleja* spp (Lamiales: Scrophulariaceae); brahmi booti, *Centella asiatica* (L.) (Apiales: Apiaceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); cowich, *Mucuna pruriens* (L.) (Fabales: Fabaceae); butterfly bush *Buddleja* spp (Lamiales: Scrophulariaceae); carrion flowers, *Simlax* spp (Liliales: Smilacaceae); pinyin, *Stemona* spp (Pandanales: Stemonaceae); currant tomato, *Solanum* spp (Solanales: Solanaceae); etc.

OBSERVATIONS:

The list of *Drosophila* species collected in 11 districts of Nagaland and their taxonomic position is shown in table 1. Total of 16 species were collected belonging to four subgenera viz. *Sophophora, Drosophila, Dorsilopha* and *Scaptodrosophila*. Pooled data collected from 11 districts has yielded a total of 2326 individuals. Out of these 1463 individuals (62.89%) belonged to 10 species of sub genus *Sophophora.* 569 individuals (24.46%) belonged to 4 species of the subgenus *Drosophila.* 121 individuals (5.20%) belonged to 1 species of subgenus *Dorsilopha.* The remaining 173 individuals (7.43%) belonged to 1 species of subgenus *Scaptodrosophila*.

***Drosophila* fauna at Dimapur:**

The list of *Drosophila* species collected in Dimapur (headquarters of Dimapur district of Nagaland) and their taxonomic position is shown in table 1. Analysis of *Drosophila* collection of 279 flies from this locality revealed the occurrence of 14 species representing four subgenera viz. *Sophophora, Drosophila, Dorsilopha* and *Scaptodrosophila* of the genus *Drosophila*. Out of these 187 individuals (67.02%) belonged to 8 species of sub genus *Sophophora,* namely *D. bipectinata, D. eugracilis, D. jambulina, D. Kikkawai, D. melanogaster, D. malerkotliana, D. parvula and D. takahashii.* 59 individuals (21.14%) belonged to 4 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta, D. parvula* and *D. paraimmigrans*). 22 individuals (7.88%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*). The remaining 11 individuals (3.94%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna of Kiphire:**

The list of *Drosophila* species collected in Kiphire (headquarters of Kiphire district of Nagaland) and their taxonomic position was given in table 1. Total of 11 species were collected comprising of four subgenera viz. *Sophophora, Drosophila, Dorsilopha* and *Scaptodrosophila*. Pooled data collected from Kiphire district has yielded a total of 160 individuals. Out of these 109 individuals (68.12%) belonged to 6 species of sub genus *Sophophora* (*D. bipectinata, D. kikkawai, D. malerkotliana, D. melanogaster, D. parvula* and *D. rajasekari*). 36 individuals (22.5%) belonged to 3 species of the subgenus

Drosophila (D. immigrans, D. paraimmigrans and *D. repleta*). 3 individuals (1.87%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*). The remaining 12 individuals (7.5%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna at Kohima:**

The list of *Drosophila* species collected at Kohima (headquarters of Kohima district of Nagaland) and their taxonomic position was given in table 1. Sample analyzed revealed a total of 15 species representing four subgenera viz. *Sophophora, Drosophila, Dorsilopha* and *Scaptodrosophila*. Pooled data collected from Kohima has yielded a total of 333 individuals. Out of these 209 individuals (62.76%) belonged to 9 species of sub genus *Sophophora* (*D. bipectinata, D. eugracilis, D. jambulina, D. kikkawai, D. malerkotliana, D. melanogaster, D. parvula, D. rajasekari* and *D. takahashii*). 95 individuals (28.52%) belonged to 4 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta, D. paraimmigrans* and *D. repleta*). 7 individuals (2.10%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*). The remaining 22 individuals (6.60%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*). *D. takahashii* and *D. bipectinata* were found to dominate in this locality.

***Drosophila* fauna of Longleng:**

The list of *Drosophila* species collected in Longleng (headquarters of Longleng district of Nagaland) and their taxonomic position was given in table 1. Total of 8 species were collected belonging to three subgenera viz. *Sophophora Drosophila* and *Scaptodrosophila*. Pooled data collected from Longleng district has yielded a total of 172 individuals. Out of these 100 individuals (58.13%) belonged to 4 species of sub genus *Sophophora* (*D. eugracilis, D. malerkotliana, D. melanogaster* and *D. rajasekari*). 56 individuals (32.55%) belonged to 3 species of the subgenus *Drosophila* (*D. immigrans, D. paraimmigrans,* and *D. repleta*). 16 individuals (9.30%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna of Mokokchung:**

The list of *Drosophila* species collected in Mokokchung (headquarters of Mokokchung district of Nagaland) and their taxonomic position was shown in table 1. Total of 11 species were collected belonging to four subgenera viz. *Sophophora*, *Drosophila*, *Dorsilopha* and *Scaptodrosophila*. Pooled data collected from Mokokchung district has yielded a total of 187 individuals. Out of these 135 individuals (72.19%) belonged to 7 species of sub genus *Sophophora* (*D. bipectinata D. eugracilis, D. jambulina, D. melanogaster, D. malerkotliana, D. parvula* and *D. takahashii*). 23 individuals (12.29%) belonged to 2 species of the subgenus *Drosophila* (*D. immigrans,* and *D. nasuta*). 13 individuals belong to 1 species of subgenus *Dorsilopha* (*D. buskii*). 10 individuals (5.34%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna at Mon:**

The list of *Drosophila* species collected at Mon (headquarters of Mon district of Nagaland) and their taxonomic position was given in table 1. A survey of *Drosophila* fauna here yielded a total of 227 flies comprising 13 species representing four subgenera viz. *Sophophora*, *Drosophila, Dorsilopha* and *Scaptodrosophila*. Out of these, 142 individuals (62.55%) belonged to 7 species of sub genus *Sophophora* (*D. eugracilis, D. jambulina, D. kikkawai, D. malerkotliana, D. melanogaster, D. parvula,* and *D. takahashii*). 51 individuals (22.46%) belonged to 4 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta, D. paraimmigrans* and *D. parvula*). 11 individuals (4.84%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*). The remaining 23 individuals (10.13%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna of Peren:**

The list of *Drosophila* species collected in Peren (headquarters of Peren district of Nagaland) and their taxonomic position was given in table 1. Analysis of the sample revealed the presence of 10 species comprising of four subgenera viz. *Sophophora*, *Drosophila, Dorsilopha* and *Scaptodrosophila*. Pooled data collected from Peren district has yielded a total of 223 individuals. Out of these 104 individuals (46.63%) belonged to 5 species of sub genus *Sophophora* (*D. bipectinata, D. eugracilis, D. jambulina, D. malerkotliana* and *D. takahashii*). 75 individuals (33.63%) belonged to 3 species of the

subgenus *Drosophila* (*D. paraimmigrans, D. nasuta* and *D. repleta*). 24 individuals (10.76%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*). The remaining 20 individuals (8.96%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna of Phek:**

The list of *Drosophila* species collected in Phek (headquarters of Phek district of Nagaland) and their taxonomic position was given in table 1. Total of 10 species were collected comprising of three subgenera viz. *Sophophora*, *Drosophila* and *Dorsilopha*. Pooled data collected from Phek has yielded a total of 183 individuals. Out of these 112 individuals (61.20%) belonged to 6 species of sub genus *Sophophora* (*D. bipectinata, D. eugracilis, D. jambulina, D. kikkawai, D. melanogaster* and *D. takahashii*). 49 individuals (26.77%) belonged to 3 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta* and *D. paraimmigrans*). 22 individuals (12.02%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*).

***Drosophila* fauna of Tuensang:**

The list of *Drosophila* species collected in Tuensang (headquarters of Tuensang district of Nagaland) and their taxonomic position is shown in table 1. Total of 11 species were collected belonging to three subgenera viz. *Sophophora, Drosophila,* and *Scaptodrosophila*. Pooled data collected from Tuensang district has yielded a total of 187 individuals. Out of these 121 individuals (32.35%) belonged to 7 species of sub genus *Sophophora* (*D. bipectinata, D. eugracilis, D. jambulina, D. kikkawai, D. malerkotliana, D. rajasekari* and *D. takahashii*). 44 individuals (11.76%) belonged to 3 species of the subgenus *Drosophila* (*D. immigrans, D. paraimmigrans* and *D. repleta*). 22 individuals (5.88%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna of Wokha:**

The list of *Drosophila* species collected in Wokha (headquarters of Wokha district of Nagaland) and their taxonomic position was given in table 1. Total of 11 species were collected. Comprising of four subgenera viz. *Sophophora, Drosophila, Dorsilopha* and *Scaptodrosophila*. Pooled data collected from Wokha district has yielded a total of 179

individuals. Out of these 118 individuals (65.92%) belonged to 7 species of sub genus *Sophophora* (*D.bipectinata, D. eugracilis, D. jambulina, D. malrekoltiana, D. melanogaster, D. parvula* and *D. takahashii*). 25 individuals (13.96%) belonged to 2 species of the subgenus *Drosophila* (*D. immigrans*, and *D. paraimmigrans*). 13 individuals (7.26%) belonged to 1 species of subgenus *Dorsilopha* (*D.buskii*). 23 individuals (12.84%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*).

***Drosophila* fauna of Zunheboto:**

The list of *Drosophila* species collected in Zunheboto (headquarters of Zunheboto district of Nagaland) and their taxonomic position was given in table 1. Total of 11 species were collected belonging to three subgenera viz. *Sophophora*, *Drosophila* and *Scaptodrosophila*. Pooled data collected from Zunheboto district has yielded a total of 196 individuals. Out of these 126 individuals (64.28%) belonged to 7 species of sub genus *Sophophora* (*D. bipectinata, D. hegdii, D. jambulina, D. kikkawai, D. melanogaster, D. malerkotliana,* and *D. takahashii*). 56 individuals (28.57%) belonged to 3 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta* and *D. repleta*). 14 individuals (7.14%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*). A new species (*Drosophila hegdii)* identified from this collection (Description of this new species and its molecular phylogeny is discussed in Chapter III).

Table 1. Occurrence and distribution of *Drosophila* species in 11 districts of Nagaland

Species	Dimapur	Kiphire	Kohima	Longleng	Mokokchung	Mon	Peren	Phek	Tuensang	Wokha	Zunheboto	Total
Genus*: Drosophila*	Total											
Subgenus*: Sophophora*												
1 .D. bipectinata	25	26	40	--	14	--	27	19	30	11	28	220
2. D. eugracilis	34	--	24	35	19	18	20	21	11	13	--	195
3. D. jambulina	23	--	18	--	27	25	10	12	7	21	19	162
4. D. kikkawai	28	19	17	--	--	30	--	31	23	--	27	175
5. D. malerkotliana	22	18	30	42	17	16	29	--	26	33	--	233
6. D. parvula	15	12	25	--	29	29	18	--	--	10	12	150
7. D. melanogaster	12	15	9	6	11	10	--	20	--	7	12	102
8. D. rajasekari	--	--	3	17	--	--	--	--	3	--	--	23
9. D. takahashii	28	19	43	--	18	14	--	9	21	23	24	199
10. D. hegdii	--		--			--		--			4	4
Total	187	109	209	100	135	142	104	112	121	118	126	1463
Subgenus *Drosophila*												
1. D. immigrans	28	18	38	28	14	15	--	18	14	14	19	206
2. D. nasuta	12	10	22	--	9	15	24	8	--	--	22	122
3. D. paraimmigrans	7	--	21	17	--	11	38	23	24	11	--	152
4. D. repleta	12	8	14	11	--	10	13	--	6	--	15	89
Total	59	36	95	56	23	51	75	49	44	25	56	569
Subgenus *Dorsilopha*												
1. D. buskii	22	3	7	--	19	11	24	22	--	13	--	121
Total	22	3	7	--	19	11	24	22	--	13	--	121
Subgenus *Scaptodrosophila*												
1. D. nigra	11	12	22	16	10	23	20	--	22	23	14	173
Total	11	12	22	16	10	23	20	--	22	23	14	173
Grand total	279	160	333	172	187	227	223	183	187	179	196	2326

Figure 1. Geographical location of Nagaland state, India in Asia

(adapted from Googlemaps)

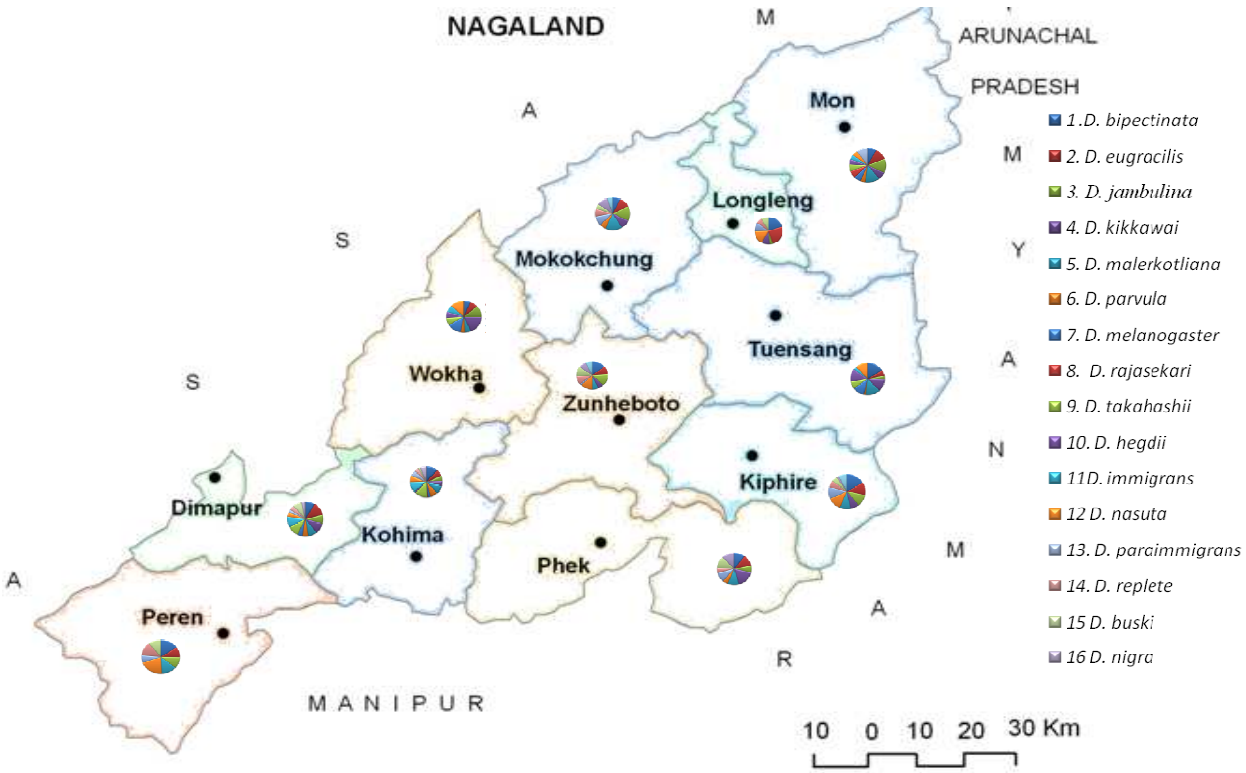


Figure 2. Map showing the localities of collections of *Drosophila* species in 11 districts of Nagaland (adapted from Google maps)

DISCUSSION:

The study of evolution in any group of animals or plants implies knowledge of the number and distribution of the species involved and the population structure and habits of the species in relation to their environment (Heed 1957). Genus *Drosophila* with its cosmopolitan nature and complexities in species compositions provides an excellent material to understand the eco distributional pattern of various species. Systematic study concerning the variations in species composition and the distributional pattern of the members of this genus in different geographical regions of the earth will enable us to understand the principles underlying adaptive radiation and certain mechanisms involved in speciation (Dobzhansky 1937). The occurrence and the distributional pattern not only be correlated with the type of vegetation and climatic conditions of the area under consideration but also with the colonizing abilities of the species concerned (Prakash 1979).

Present study reveals that not only the numbers of *Drosophila* species vary among different places; but also the number of individuals belonging to same species differs among different places under study.

The abundance of *Drosophila* species collected in 11 district headquarters of Nagaland and their taxonomic position was shown in Table 1. Total of 16 species (including one new species *Drosophila hegdii*) were collected. Pooled data collected from 11 districts has yielded a total of 2326 individuals, belonging to 4 subgenera namely, *Sophophora*, *Drosophila*, *Dorsilopha* and *Scaptodrosophila*. The subgenus S*ophophora* was represented by 10 species, namely *D. bipectinata, D. eugracilis D. jambulina, D. kikkawai, D. malerkotliana, D. melanogaster, D. parvula, D. rajasekari, D. takahashii* and *D. hegdii. Drosophila* was represented by 4 species namely *D. immigrans, D. nasuta, D. paraimmigrans* and *D. repleta. Dorsilopha* was represented by *D. buskii* and *Scaptodrosophila* was represented by *D. nigra*. Thus the study indicates that the *Drosophila* fauna of Nagaland is diverse.

In Dimapur a total of 279 individuals were collected, out of these 187 individuals (67.02%) belonged to 8 species of sub genus *Sophophora*, namely *D. bipectinata*, *D. eugracilis, D. jambulina, D. kikkawai, D. malerkotliana, D. melanogaster, D. parvula* and *D. takahashii*. 59 individuals (21.14%) belonged to 4 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta D. parvula and D. paraimmigrans*). 22 individuals (7.88%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*). The remaining 11 individuals (3.94%) belonged to 1 species of subgenus *Scaptodrosophila* (*D.nigra*). Singh (1987) surveyed Dimapur locality and reported 10 species namely- *D. buski, D. nasuta, D. immigrans D. lacertosa, D. kikkawai, D. bipectinata, D. ananassae, D. melanogaster, D. malerkotliana* and *D. jambulina*. Of the above mentioned species *D. kikkawai, D.buskii, D. immigrans, D. nasuta, D. bipectinata, D. malerkotliana* and *D. jambulina* were observed in the present study. However *D. lactertosa* and *D. nepalensis* were absent in the present collection.

Collection analyzed from Kohima revealed a total of 15 species. Pooled data collected from Kohima district has yielded a total of 333 individuals. Out of these 209 individuals (62.76%) belonged to 9 species of sub genus *Sophophora* (*D. bipectinata, D. eugracilis, D. jambulina, D. kikkawai, D. malerkotliana, D. melanogaster, D. parvula, D. rajasekari* and *D. takahashii*). 95 individuals (28.52%) belonged to 4 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta, D. paraimmigrans* and *D. repleta*). 7 individuals (2.10%) belonged to 1 species of subgenus *Dorsilopha* (*D. buskii*). The remaining 22 individuals (6.60%) belonged to 1 species of subgenus *Scaptodrosophila* (*D. nigra*). Singh (1987) surveyed Kohima locality and found 8 species namely, *D. buskii, D. nasuta, D. immigrans D. lacertosa, D. kikkawai D. ananassae, D. melanogaster D. nepalensis.* Of the above mentioned species *D. kikkawai, D. buskii, D. immigrans* and *D. nasuta* were observed in the present study too. However, *D. lactertosa* and *D. nepalensis* were absent.

Catch analyzed from Mokokchung has yielded a total of 187 individuals. Out of these 135 individuals (72.19%) belonged to 7 species of sub genus *Sophophora* (*D. bipectinata, D. eugracilis, D. jambulina, D. melanogaster, D. malerkotliana, D. parvula,* and *D. takahashii*). 23 individuals (12.29%) belonged to 2 species of the subgenus *Drosophila* (*D. immigrans, D. nasuta*). 13 individuals belong to 1 species of subgenus *Dorsilopha* (*D. buskii*). 10 individuals (5.34%) belonged to 1 species of subgenus *Scaptodrosophila* (*D.*

nigra). *D. parvula* and *D. jambulina* were found in abundance in this locality. Yenisetti *et al.* (2002) surveyed Mokokchung locality and identified 6 species namely, *D. immigrans, D. kikkawai, D. nasuta, D. nepalensis, D. Suzuki, and D. takahashii* and two picture winged *Drosophila* species (unidentified). Of the above mentioned species *D. takahashii, D. immigrans* and *D. nasuta* were observed in the present study. However, *D. nepalensis* and *D. Suzuki* and picture winged *Drosophila* species were absent.

Present study on *Drosophila* fauna of Nagaland reveals the presence of *D. malerkotliana* in abundance. *D. malerkotliana* is considered to be sub-cosmopolitan and widespread in South-East Asia, Borneo, West Malaysia Australia and several island groups in the Oriental region (O'Grady and Markow 2006). It is important to note the fact that this species was reported in all previous collections made by multiple workers from north eastern parts of the country (Dwivedi and Gupta 1979; Gupta and Singh 1979; Gupta and Singh 1980; Gupta and Singh 1981; Singh 1987; Yenisetti *et al*. 2002 and Achumi *et al.* 2013). This observation suggests that Nagaland's *Drosophila* diversity denotes confluence of South Asia and East Asia. Dominance of *D. malerkotliana* over others can be due to its ecological versatility to exploit diverse habitats.

Present collection reveals that *D. bipectinata* is the second dominant species in Nagaland. Literature review denotes that *D. bipectinata* is widespread in Kula Lampur west Malaysia. This species also widely distributed in Borneo, Philipins, Thailand, West Malaysia, Nepal, Japan, Taiwan and India (O'Grady and Markow 2006).

In present collection *D. immigrans* found to be the third abundant species. *D. immigrans* is a cosmopolitan species and is found from Taiwan to Southeast Asia, Indonesia, India (O'Grady and Markow 2006). From their studies on *Drosophila* diversity on Western Ghats, Ranganath and Krishnamurthy (1972b) observed that *D. immigrans* is abundant at higher altitudes. In light of this observation, presence of *D. immigrans* in sub-Himalayan hilly regions suggests its adaptability to higher altitudes.

Present collection reports *D. takahashii, D. parvula, D. nasuta, D. repleta, D. eugracilis, D. jambulina, D. kikkawai, D. rajasekari, D. nigra* and *D. paraimmigrans* in Nagaland populations. *D. takahashii* is most widespread being found from India to Japan and into Micronesia; *D. parvula* is found from South East Asia, west Malaysia and Thailand; *D. nasuta* is thought to be from eastern Africa although this species has become widespread from South East Asia into Micronesia; *D. repleta* species is cosmopolitan in distribution; *D. eugracilis* is widespread in Indian subcontinent, found throughout South East Asia and into Australia; *D. jambulina* is widespread from India to southern China to South East Asia; *D. kikkawai* is circumtropical in distribution; *D. rajasekari* is found in India, Combodia and Thailand; *D. nigra* is found in Ausralia, new Guina, Borneo, Phillippines, Malaysia, Thailand and India; and *D. paraimmigrans* is found in India, Taiwan to Southeast Asia (O'Grady and Markow 2006).

Carson (1965) based on the pattern of distribution of various members of *Drosophila*, has recognized three distinct groups, namely 1. Virtually cosmopolitan species, 2. Species having a tendency for wide spreading but not cosmopolitan and 3. Species having restricted distribution (endemic species). He included five species namely, *D. melanogaster, D. simulans, D. ananassae, D. buskii* and *D. repleta* as truly cosmopolitan. Incidentally, *D. melanogaster, D. simulans, and D. ananassae* are domestic, while *D. buskii* and *D. repleta* are semi-domestic. In the present study *D. melanogaster, D. repleta* and *D. buskii* were present. Though *Drosophila simulans* is cosmopolitan in distribution (O'Grady and Markow 2006), in the present study in none of the eleven locations of Nagaland this species was found. Contrarily David *et al.* (2007) observed that *D. simulans* was absent from most of West Africa and was very rare in the Cote d' Ivoire and was absent in most parts of East Asia (David *et al.* 2007). Collections in Nagaland state were made from wild localities. This can be the rationale behind the absence of *D. ananassae* and *D. simulans.*

The striking feature of the collection was that in all the eleven locations of Nagaland state *Drosophila* species belonging to two sub genera namely *Sophophora and Drosophila* dominated in their relative proportions of different species and their densities. This finding is in agreement with the observation of Bock and Wheeler (1972) who stated that if

Drosophila collection made in any part of the South East Asian or New Guinean area, only two species groups comprise all or practically of all the catch, that is the *melanogaster* species group of the subgenus *Sophophora* and *immigrans* species group of the subgenus *Drosophila*. They further supplemented their argument by hypothesizing that the *melanogaster* and *immigrans* species group might have originated in South East Asia and then colonized in other regions. As Nagaland is a sub Himalayan hilly state, which is a part of South East Asia, the prevalence of *Sophophora* and *immigrans* species groups in the present collection reaffirms the observation made by Bock and Wheeler (1972).

Present study reveals the fact that *Drosophila* fauna of Nagaland state shows similarity not only with South Asia but also with that of East Asia which can be explained from geographical location of this north eastern state.

CHAPTER II

ALTITUDINAL AND SEASONAL VARIATION IN *DROSOPHILA* SPECIES ON MOUNT JAPFU OF NAGALAND

INTRODUCTION:

The fruit fly *Drosophila* (Diptera: Drosophilidae) has richly contributed to our understanding of pattern of inheritance, variation, speciation and evolution. Genus *Drosophila,* with its cosmopolitan nature and complexities in species compositions is an excellent model for studying the eco-distributional pattern of various species (Carson 1965). Systematic study concerning the variations in the species compositions and the patterns of distribution of various members of the genus *Drosophila* in different geographical regions of the earth will enable understand the principles underlying adaptive radiation and certain mechanisms involved in speciation (Muniyappa 1981).

Significant progress has been made in the fields of taxonomy and systematics of the family Drosophilidae in India. This family is composed of more than 3,500 species throughout the world (Bachli 1998). About two hundred species belonging to twenty genera have been reported from different parts of India. However, very little is known regarding the *Drosophila* fauna of the northeastern region of the Indian subcontinent. This region is one of the richest repositories of biodiversity in the world, with its diverse climatic conditions, variable altitudes, deep valleys, luxuriant flora, running streams and moist surroundings. So, it provides an ideal location for the colonization of several *Drosophila* species (Singh and Gupta, 1977; Dwivedi and Gupta, 1979; Gupta and Singh, 1979; Singh and Gupta, 1980; Singh, 1987). This region includes eight hill states, namely Assam, Arunachal Paradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura.

The study of Indian Drosophilids was initiated by Bezzi (cf. Sturtevant 1921). Later several Indian workers studied the ecology, distribution, taxonomy, cytology and genetics of *Drosophila* (Parshad and Paika 1964; Parshad and Duggal 1965; Gupta and Ray Chaudhuri 1970a,b; Rajeshwari 1971; Nirmala and Krishnamurthy 1972, 1973, 1975; Siddaveere Gowda and Krishnamurthy 1971; Ranganath and Krishnamurthy 1972a,b; Godbole and Vaidya 1972; Parshad and Alicchio 1973; Reddy and Krishnamurthy, 1968, 1970, 1973; Prakash and Reddy 1977, 1978a,b, 1979a,b,c, 1980; Hedge 1979; Muniyappa and Reddy 1980a,b, 1981, 1982; Muniyappa *et al.* 1981; Gai and Krishnamurthy 1982;

Dasmohapatra *et al.* 1982; Gupta and Panigrahy 1990; Singh and Chatterjee 1992; Singh and Sisodia 1995; Naseerulla 1993; Banerjee and Singh 1996; Krishna 1997; Jayshankar 1998; Yenisetti *et al.* 2002). However, little work is done on Drosophilids of North-East India (Singh and Gupta 1977; Dwivedi and Gupta 1979; Gupta and Singh 1979; Singh and Gupta 1980; Singh 1987).

Sub tropical, semi evergreen forests of Naga Hills are biologically diverse habitats on earth. It is possible that new *Drosophila* species can be identified from this region. *Drosophila* is a pollinator for economically important plants, such as *Ceropegia* (Chaturvedi 2006). It is also possible that novel *Drosophila* pollinators for other economically important plants can be identified in these subtropical rain forests.

The ecological and biological diversity of an ecosystem determines the presence or absence of a species in an ecological niche. Apart from physical and biotic factors, the topography and season also affects animal distribution. As elevation is one of the important aspects of topography, it is important to look at animal distribution from that perspective. Efforts have been made to collect *Drosophila* from different altitudes, but these data were not considered with an ecological perspective (Reddy and Krishnamurthy 1977). According to Reddy and Krishnamurthy (1977), physical and biotic factors are the sole determinants of animal distribution. This idea logically denotes that elevation and season have no influence on animal distribution. In the present study, goal was to determine if elevation affects distribution.

According to Gause's competitive exclusion theory, two related species competing for the same resources cannot co-exist together in the same ecological niche (Gause 1934). However, laboratory experiments questioned the validity of this principle (Ayala 1969). The presence of taxonomically or phylogenetically related species in an ecological niche indicates their coexistence, and absence of such related species infers competitive exclusion (Guruprasad *et al*. 2010). Present study sought to understand whether taxonomically or phylogenetically related *Drosophila* species coexist in nature. Present study also has been undertaken to understand the altitudinal and seasonal variation of

Drosophila species on Mount Japfu, which is situated 15 Km from Kohima, the capital of the sub-Himalayan hilly state Nagaland, India.

MATERIALS AND METHODS:

The altitudinal and seasonal fluctuation in *Drosophila* fauna was studied in five different wild localities of mount Japfu, which has a peak altitude of about 3015.60 m. Its slopes are covered with thick vegetation. The selected collection spots were located at 25º11'N latitude and 94º55'E longitude. Monthly collections of flies were made at the altitudes of 1500, 1800, 2100, 2400 and 2700 m a.s.l from April 2010 to March 2011. Both bottle trapping and net sweeping methods were used. For bottle trapping, method milk bottles of 200 ml capacity containing a smashed ripe banana sprayed with yeast were tied to the twigs underneath small bushes at the height of 1-1.5 m above the ground. Fifteen traps were kept in each site. After 2 days, the mouth of each bottle was plugged with cotton and removed from the bushes. The flies that were attracted by the bait and collected in the bottles were transferred to fresh bottles containing wheat cream agar medium (*Drosophila* culture medium was prepared as explained in Chapter I). Net sweeping was done on rotting fruits (crushed banana were spread beneath shaded areas of bushes one day before collection). After each sweep, collected flies were transferred to bottles containing freshly prepared wheat cream agar medium.

The flies were then brought to the laboratory, isolated, identified and sexed. Categorization of the collected *Drosophila* flies was made to respective taxonomic groups by employing the parameters as suggested by Sturtevant (1921), Patterson and Stone (1952), Throckmorton (1962), and Bock (1971). The most important parameters employed to identify the species are the morphological features like colour and size of imagoes, number and nature of aristal branches, nature and arrangement of genital arch, nature and number of acrostichal hairs, length of the wings and its indices, presence or absence of the sex comb, if present the number of sex comb rows and teeth in each row. The internal characters of the adults, the shape and number of egg filaments, pupal characters, pupal spiracles and behavior were also taken into consideration for identification. To study seasonal variation, the entire year was divided into three seasons: pre-monsoon, extending from January through March; monsoon, from April through September; and post-monsoon, from October through December.

Flora of collection sites

Flora at 1500 m a.s.l: Maibau, *Alnus nepalensis* (Don) (Fagales: Betulaceae); begger-ticks, *Bidens* spp, (Asterales: Asteraceae); *Makania* spp; sow thistles, *Sonchus* spp; butterfly bush *Buddleja* spp (Lamiales: Scrophulariaceae); brahmi booti, *Centella asiatica* (L.) (Apiales: Apiaceae); sirib large, *Entada pursathea* (Roux) (Fabales: Fabaceae); banana, *Musa* spp (Zingiberales: Musacae); carrion flowers, *Simlax* spp (Liliales: Smilacaceae); pinyin, *Stemona* spp (Pandanales: Stemonaceae); currant tomato, *Solanum spp* (Solanales: Solanaceae); marda, *Termenalia elliptice* (Wright and Arn) (Myr-tales: combretaceae); etc.

Flora at 1800 m a.s.l: Jackfruit, *Artocarpus hetrophyllus* (Lam), (Rosales: Moraceae); yellow himalayan raspberry, *Rubus* spp, (Rosaceae); wormwood, *Artemisisia vulgaris* (L.) (Asterales: Asteraceae); begger-ticks, *Bidens* spp; bamboo, *Bambusa* spp, (Poales: Poaceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); timburni, *Diospynum* spp, (Ericales: Ebenaceae); deer-eye beans, *Mucna perita* (Adans) (Fabales: Fabaceae); tapioca-root, *Maninot utilissema* (Crantz) (Malpighiales: Euphorbiaceae); *Smilax* spp; khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); knotwood, *Polygonum* spp (Caryophyllales: Polygonaceae); etc.

Flora at 2100 m a.s.l: *A. nepalensis* (Don); khang, *Acacia pinnata* (Miller) (Fabales: Fabaceae); thickhead, *Crossecephalum spp* (Asterales: Asteraceae); himalayan nettle, *Girardinia heterophylla* (Vahl) (Rosales: Urticaceae); *Rubus* spp; blady grass, *Imperata cylindrica* (Drauv) (Poales: Poaceae); *Musa* spp; etc.

Flora at 2400 m a.s.l: bologi, *Crossocephalum s*pp (Asterales: Asteraceae); *A. vulgaris*; white weed, *Ageratum conzyoids* (L.); thoroughworts, *Eupatorium* spp; *Biden* spp; blueberry ash, *Elaeocarpus* spp (Oxalidales: Elaeocarpaceae); shaking brake, *Pteris* spp (Polypodiales: Pteridaceae); *I. cylindrica*; *C.asiatica; P. insularies*; knotwood, *Polygonum* spp; cowich, *Mucuna pruriens* (L.) (Fabales: Fabaceae); etc.

Flora at 2700 m a.s.l: *Crossocephalum* spp; *Curculigo* spp; *I. cylindrica*; kamraj, *Helminthostachys zeylanica* (L.) (Ophioglossales: Ophioglossaceae); *Polygonum* spp; *Smilax* spp; timburni, *Dryopteris* spp (Ericales: Ebenaceae); rhododendron, *Rhododendron* spp (Ericaceae); etc.

Data analysis:

The relationship between the abundance, richness and diversity of all groups of flies collected throughout the year was calculated by Simpson's diversity (Simpson 1949). Simpson diversity index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species was calculated using the formula.

Formula to calculate Simpson index is as follows:

$$D = \frac{\sum n\,(n-1)}{N\,(N-1)}$$

Where, n= the total number of organisms of a particular species

N= the total number of organisms of all population

In order to verify the qualitative distribution of different species, the occurrence constancy method (Dijoz 1983) was used. The constancy value (C) was obtained by dividing the number of collections in which one species occurred by the total number of collections, and then multiplying that result by 100. Based on constancy value, the species collected were grouped as constants, when C ≥ 50, accessory species when C ≥ 25 and < 50, accidental species when C< 25. Species that occurred in only one area were considered exclusive.

To understand the difference in seasonal variation of *Drosophila* flies at mount Japfu, one way analysis of variance (ANOVA) was performed using GraphPad Prism5 software (www.graphpad.com).

Custer analysis was performed using WinSTAT software (www.winstat.com) to design, analyze and compare different *Drosophila* populations, as described by Giri *et al.* (2007). In the cluster study, Euclidean distance was chosen to measure the similarity between different species, and Ward's strategy (Giri *et al.* 2007) was performed to unite two clusters.

OBSERVATIONS:

The list of *Drosophila* species collected at different altitudes of mount Japfu from April, 2010 through March 2011 and their taxonomic position was given in table 1. A total of 19 species were collected, including 16 species of *Drosophila* belonging to four subgenera (*Sophophora, Drosophila, Dorsilopha* and *Scaptodrosophila*). The remaining 3 species were not identified. Pooled data on monthly collections of *Drosophila* yielded a total of 4680 individuals. Out of these, 2889 individuals (61.73%) belonged to 10 species of sub genus *Sophophora,* 1578 (33.71%) individuals belonged to 4 species of the subgenus *Drosophila.* 100 (2.13%), 3 were unidentified, 62 (1.32%) individuals belonged to 1 species of subgenus *Scaptodrosophila,* and 51 (1.07%) belonged to1species of subgenus *Dorsilopha.*

The value of Simpson's index, indicating the abundance, richness and diversity of *Drosophila* flies at different altitudes was given in table 3. At the lowest altitude (1500 m a.s.l) Simpson's index was 0.10903, and at highest altitude (2700 m a.s.l) it was 0.141355. The altitudinal variation of the *Drosophila* population was depicted in Figure 1. The number of *Drosophila* flies decreased with increasing altitude. The altitudinal variations of the most abundant *Drosophila* species were shown in Figure 2.

The seasonal variation in the population density of *Drosophila* is depicted in Figure 3. The density was low in pre-monsoon period, increased in monsoon period, and then decreased in post-monsoon period. The analysis of variance calculated for pre-monsoon, monsoon and post- monsoon seasons showed significant differences between them ($F=26.72$; $df=2$, $p<0.0001$).

The constancy value (C) of all species at all altitudes along with absolute numbers (A) and relative abundance (r) are presented in Table 2. Constant species ($C \geq 50$) represented 36.84% of the total collected species (7 out of 19), while 8 species were considered as accessory (42.10%), and 4 species were considered accidental (21.05%). Constant species were *D. bipectinata* (Duda) (Diptera:Drosophilidia), *D. eugracilis* (Bock and Wheeler), *D.*

kikkawai (Burla), *D. malerkotliana* (Parshad and Paika), *D. takahashii* (Sturtevant), *D. immigrans* and *D. paraimmigrans;* accessory species were *D. jambulina* (Parshad and Paika), *D. parvula* (Bock and Wheeler), *D. rajasekari* (Reddy and Krishnamurthy), *D. trileuta* (Bock and Wheeler), *D. nasuta* (Lamb), *D. repleta* (Wollaston), *D. buskii* (Coquilett), and unidentified species (1); accidental species were *D. agumbensis* (Prakash and Reddy), *D. nigra* (Grimshaw), unidentified (2) and unidentified (3).

In the cluster analysis (Figure 10) the accidental species stands first in the cluster, followed by the accessory species, and the bottom is occupied by constant species. *D. agumbenesis, D. jambulina, D. rajasekari, D. trileuta* belong to *melanogaster* species group of the subgenus *Sophophora. D. nigra* belongs to subgenus *Scaptodrosophila. D. agumbenesis* and *D. jambulina* belong to *montium* subgroup and *D. bipectinata* belongs to the *ananassae* subgroup. *D. repleta, D. buskii* of the same cluster belongs to subgenus *Drosophila.* In the second cluster *D. eugracilis, D. kikkawai* and *D. parvula* belong to the *melanogaster* species group of the subgenus *Sophophora* and *D. paraimmigrans, D. immigrans* of the same cluster belong to subgenus *Drosophila. D. takahashii* belongs to *melanogaster* species group of subgenus *Sophophora.*

***Drosophila* fauna at 1500 m a.s.l:**

The monthly census data of *Drosophila* collected during April, 2010-March, 2011; at 1500 m a.s.l altitude of mount Japfu was presented in Table 4, along with the meteorological data of this locality. The pooled data for the year 2010-2011 yielded a total of 1130 individuals. Out of these 663 (58.15%) individuals belonged to 10 species of subgenus *Sophophora*; 414 individuals (36.31%) belonged to 4 species of subgenus *Drosophila*; 35 individuals (3.07%) belonged to a species of subgenus *Dorsilopha;* 18 (1.57%) belonged to 2 unidentified species. At this altitude a maximum of 16 species were collected during 2010-11. At 1500 m a.s.l the most common and abundant species was *D. immigrans* with 190 individuals (16.66%). The second common and abundant species was *D. takahashii*, consisting of 183 individuals (16.05%). The third common and abundant species was *D. malearkotliana* consisting of 143 individuals (12.54%). Of the remaining species *D. eugracilis* was represented by 140 flies (12.28%); *D. paraimmigrans* by 135 flies

(11.84%); *D. repleta* by 89 flies (7.80%); *D. bipectinata* by 55 flies; *D. buskii* by 35 flies. October collection yielded the highest number of flies (146), while collection in January was least with only 41 individuals. The density of the *Drosophila* species started increasing from April, there was a steep increase from June onwards reaching maximum density in the month of October. At 1500 m altitude *D. nasuta, D. nigra* and unidentified species 1 were found to be absent.

The composition and the frequencies of different species were highly variable from month to month in different seasons (Figure 5). January-March constitute pre-monsoon season, April-September constitute monsoon season and October-December constitute post-monsoon season. 174 individuals of the *Drosophila* species were collected in pre-monsoon period, 607 in monsoon and 349 in post-monsoon periods comprising 9, 13, and 13 species respectively. Among the common and abundant species *D. immigrans* occurred throughout the year except in January and September; *D. takahashii* occurred throughout the year except in February and April. *D. malerkotliana* was found to be absent in the months of January, February, April, May, October, and November. The density *of D. immigrans* was lowest (07 individuals) in December and increased steadily until July (30 individuals) and then declined. Thus the density of this species was highest during monsoon season. *D. takahashii* also showed a peak in monsoon period. A maximum of 34 flies could be collected in the month of May and a minimum of 07 flies could be collected in the month of January and March. *D. malerkotliana* also showed a peak in monsoon period. A maximum of 37 flies could be collected in the month of July and the minimum of 12 flies could be collected during the month of December.

***Drosophila* fauna at 1800 m a.s.l:**

The monthly collection of *Drosophila* at 1800 m a.s.l altitude of Japfu peak during 2010-11 and the number of individuals of different species collected was given in table 5, along with the meteorological data of this locality. At 1800 m a.s.l altitude a total of 1130 individuals were collected. Out of this 631 individuals (55.84%) belonged to 9 species of subgenus *Sophophora;* 425 individuals (37.61%) belonged to four species of subgenus *Drosophila;* 47 individuals (4.15%) belonged to unidentified species (1) and 15 (1.32%) belonged to one species of subgenus *Scaptodrosophila.* The remaining 12 (1.06%)

belonged to a species of subgenus *Dorsilopha*. At this altitude a maximum of 16 species were collected.

The most common and abundant species was *D. immigrans* consisting 177 individuals (15.66%). The second common and abundant species was *D. bipectinata* consisting 145 individuals (12.83%). The third common and abundant species was *D. kikkawai* consisting 136 individuals (12.03%). 672 individuals (59.46%) represented 12 other species namely *D. eugracilis, D. jambulina, D. malerkotliana, D. parvula, D. rajasekari, D. takahashii, D. trileuta, D. nasuta, D. repleta, D. buskii, D. nigra* and unidentified species (1). A maximum of 139 flies could be collected in the month of August while minimum of only 42 individuals were found in January. The population size increased gradually from the month of May and then suddenly increased reaching maximum density in the month of August with 139 individuals. This peak of activity gradually declined and finally the density of the *Drosophila* reached minimum during January (Figure 6). Among common and abundant species none of the species occurred throughout the year. *D. immigrans* was found to be absent in the month of November and December. *D. bipectinata* was found to be absent in the month of April, May, June and October. *D. kikkawai* was found to be absent in the months of January, February, April and November. The composition and the frequencies of different species were highly variable from month to month in different seasons. 188 individuals of the *Drosophila* species were collected in pre-monsoon, 692 in monsoon and 250 in post-monsoon period comprising 9,12,11 species respectively.

D. immigrans showed a peak in the post-monsoon period, confined to October (30 flies). The density of this species was low in the month of April. *D. bipectinata* showed maximum of 25 individuals in monsoon period confined to September. The population density was low in the month of March (03 individuals), pre-monsoon period. *D. kikkawai* presented a small peak in monsoon period. The population growth was maximum in the month of June. It was interesting to note that the population was found to be minimum with only 5 individuals in the month of July.

***Drosophila* fauna at 2100 m a.s.l:**

The monthly collection of *Drosophila* at 2100 m a.s.l altitude of Japfu peak during 2010-11 yielded a total of 877 individuals. The number of individuals of different species was given in table 6, with meteorological data of the locality. Out of the total 877 individuals collected, 681 individuals (77.65%) belonged to 9 species of subgenus *Sophophora*; 160 individuals (18.24%) belonged to 3 species of subgenus *Drosophila;* 20 individuals (2.28%) belong to unidentified species (1) and (2); 12 individuals (1.36%) belonged to a species of subgenus *Scaptodrosophila* and 4(0.45%) belonged to a species of subgenus *Dorsilopha*. At this altitude a maximum of 16 species were collected. The most common and abundant species was *D. malerkotliana* with 148 individuals (16.87%). The second common and abundant species was *D. bipectinata* with 143 individuals (16.30%). The third common and abundant species was *D. takahashii* with 107 individuals (12.20%). 479 flies (38.09%) represented 14 species namely *D. paraimmigrans, D. parvula, D. jambulina, D. kikkawai, D. eugracilis, D. rajasekari, D. trileuta, D. immigrans, D. nasuta, D. buskii, D. nigra,* unidentified species (1) and unidentified species (2). A maximum of 119 flies could be collected during the month of June, while minimum of only 11 flies could be collected during the month of April 2010 at this altitude. Among the common and abundant species none of the species occurred throughout the year. *D. malerkotliana* showed a peak in the month of June, *D. bipectinata* is found to occur maximum in the month of October and *D.takahashii* was found to occured maximum during the months of May and June.

191 individuals of the *Drosophila* species were collected during pre-monsoon, 438 in monsoon and 248 in post-monsoon periods comprising 12, 11 and 9 species respectively (Figure 7). Among the common and abundant species *D. malerkotliana* showed a peak in the monsoon period confined to June with 29 individuals and started declining and reached minimum in the month of January with only 03 individuals. *D. bipectinata* showed maximum number during the month of October with 27 individuals and minimum during the month of February and June. *D. takahashii* showed a peak in the monsoon period confined to May and June with 26 individuals each and started declining and reached minimum in the month of April.

***Drosophila* fauna at 2400 m a.s.l:**

The monthly collection of *Drosophila* at 2400 m a.s.l altitude of Japfu peak during the year 2010-2011, yielded a total of 847 individuals. The number of individuals of different species collected was given in table 7, along with the meteorological data of this locality. Out of the 847 individuals collected, 527 (62.84%) belonged to 10 species of subgenus *Sophophora*; 295 (35.17%) belonged to 3 species of subgenus *Drosophila*; 16 (1.88%) belonged to a species of subgenus *Scaptodrosophila* and 9 (1.06%) belonged to unidentified species (1). Maximum of 15 species were collected. At this altitude the most common and abundant species was *D. takahashii* with 158 individuals (18.65%). The second common and abundant species was *D. immigrans* with 150 individuals (17.70%). The third common and abundant species was *D. paraimmigrans* with 121 individuals (14.28%). 448 flies (49.84%) represented *D. malerkotliana, D. eugracilis, D. kikkawai, D. bipectinata, D. agumbenesis, D. jambulina, and D. parvula. D. rajasekari, D. trileuta, D. nasuta, D. nigra* and unidentified species (1). At this altitude *D. repleta, D. buskii* and unidentified species (2), (3) were found to be absent. A maximum of 103 flies could be collected during the month of November while minimum of only 35 flies during April. The composition and the frequencies of different species were highly variable from month to month in different seasons. 186 flies of the *Drosophila* species were collected in pre-monsoon; 413 in monsoon and 284 in post-monsoon periods comprising of 9, 11, and 9 species respectively (Figure 8). Among the 3 common and abundant species *D. takahashii* and *D. immigrans* were observed in all the seasons of the year, while *D. paraimmigrans* was found to be absent during the months of January, February and April. *D. takahashii* was found to be maximum in number during monsoon period with 20 individuals in the month of September and started declining and reached minimum in pre-monsoon period, confined to March with only 3 individuals. *D. immigrans* showed a peak in the month of June with 30 individuals and minimum with 3 individuals in the month of March. *D. paraimmigrans* showed a peak in monsoon period with 26 individuals in the month of June.

***Drosophila* fauna at 2700 m a.s.l:**

The monthly collection of *Drosophila* at 2700 m a.s.l altitude at Japfu peak during the year 2010-2011, yielded 696 individuals. The number of individuals of different species was given in table 8 along with the meteorological data of this locality. Out of this 387

(50.60%) belonged to 9 species of subgenus *Sophophora*; 284 (35.63%) belonged to 3 species of subgenus *Drosophila*; 19 (2.72%) belonged to a species of subgenus *Scaptodrosophila*; 6 (0.86%) belonged to unidentified species (1). Maximum of 14 species were collected. At this altitude the most common and abundant species were *D. takahashii* and *D. paraimmigrans* with 136 individuals each (19.54%). The second common and abundant species was *D. immigrans* with 131 individuals (18.82%). The third common and abundant Species was *D. malerkotliana* with 96 individuals (13.79%). Remaining 197 individuals (28.3%) were represented by 11 species. At this altitude *D. trileuta, D. buskii*, unidentified species (2) and (3) were found to be absent. Among the 3 common and abundant species none of the species occurred throughout the year. *D. takahashii* was found to be absent during the months of January, February and July. *D. immigrans* was found to be absent during the months of January, February, March. *D. malerkotliana* was found to be absent during February.

During April, June, July and November *D. trileuta, D. repleta*, unidentified species (2) and (3) were found to be absent at this altitude. The composition and the frequencies of different species were highly variable from month to month in different seasons. 89 flies of the *Drosophila* species were collected in pre-monsoon, 462 in monsoon and 145 in post-monsoon periods comprising of 8, 15 and 5 species respectively (Figure 9).

Among the common and abundant species *D. takahashii* and *D. paraimmigrans* showed a total of 136 individuals each. *D. takahashii* showed a peak in the monsoon period confined to June and showed minimum in the early monsoon period. Population density started increasing from May and reached maximum in June and September and then started declining in the month of April. *D. paraimmigrans* showed peak in the month of July with 27 individuals. The population growth was initiated during the month of July and declined during the month of January. *D. malerkotliana* showed peak in monsoon period; the population growth was initiated during the month of September and declined by December. *D. immigrans* showed maximum population size in the month of July with 34 individuals (monsoon period) and started declining and reached minimum in the month of May.

Table 1. Showing the list of species of *Drosophila* and their numbers collected at different altitudes of mount Japfu during 2010-2011. (m-meters)

Species	1500 m	1800 m	2100 m	2400 m	2700 m	Total
Genus:*Drosophila*						
Subgenus: *Sophophora*						
1.D. agumbensis	17	0	0	1	1	19
2.D. bipectinata	55	145	143	28	52	423
3.D. eugracilis	140	80	86	57	15	378
4.D . jambulina	7	15	4	8	25	59
5.D. kikkawai	16	136	64	48	43	307
6..D. malerkotliana	143	57	148	117	96	561
7 D. parvula	36	76	74	67	17	270
8. D. rajasekari	20	1	18	8	2	49
9.D. trileuta	46	19	37	35	0	137
10.D. takahashii	183	102	107	158	136	686
Total						2889
Subgenus *Drosophila*						
1.D. immigrans	190	177	63	150	131	711
2.D. nasuta	0	58	27	24	17	126
3.D. paraimmigrans	135	134	70	121	136	596
4.D. repleta	89	56	0	0	0	145
Total						1578
Subgenus *Dorsilopha*						
D. buskii	35	12	4	0	0	51
Total						51
Subgenus *Scaptodrosophila*						
1.D. nigra	0	15	12	16	19	62
Total						62
Unidentified (1)	17	47	19	9	6	98
Unidentified (2)	0	0	1	0	0	1
Unidentified (3)	1	0	0	0	0	1
Total	1130	1130	877	847	696	4680

Table 2. Absolute (A) and relative abundance (r) and constancy values(c) for each species collected at different altitudes of mount Japfu from April 2010 to March 2011

Species	1500 m			1800 m			2100 m			2400 m			2700 m		
	A	r	c	A	r	c	A	r	c	A	r	c	A	r	c
Subgenus															
Sophophora															
D.agumbenesis	17	0.02	8.33	0	..	..	0	..	..	1	0	8.33	1	0	8.33
D.bipectinata	55	0.05	25	145	0.12	66.7	143	0.16	75	28	0.03	25	52	0.07	58.33
D.eugracilis	140	0.12	75	80	0.07	41.7	86	0.1	58.3	57	0.09	33.3	15	0.02	16.67
D.jambulina	7	0.01	25	15	0.01	16.7	4	0	8.33	8	0.01	8.33	25	0.04	25
D.kikkawai	16	0.01	16.7	136	0.12	66.7	64	0.07	50	48	0.06	41.7	43	0.06	25
D.malerkotliana	143	0.12	50	57	0.05	33.3	148	0.17	75	117	0.14	75	96	0.13	58.33
D.parvula	36	0.03	16.7	76	0.07	41.7	74	0.08	41.7	67	0.08	41.7	17	0.02	25
D.rajasekari	20	0.02	33.3	1	0	8.33	18	0.02	25	8	0.01	8.33	2	0	8.33
D.trileuta	46	0.04	16.7	19	0.01	16.7	37	0.04	33.3	35	0.04	16.7	0	..	..
D.takahashii	183	0.17	83.3	102	0.09	66.7	107	0.12	66.7	158	0.19	100	136	0.2	75
Total	663			631			681			527			387		
Subgenus															
Drosophila															
D.immigrans	190	0.17	83.3	177	0.16	83.3	63	0.07	50	150	0.18	100	131	0.18	75
D.nasuta	0	0	..	58	0.05	33.3	27	0.03	16.7	24	0.02	16.7	17	0.02	8.33
D.paraimmigrans	135	0.11	66.7	134	0.11	66.7	70	0.07	50	121	0.14	75	136	0.2	83.33
D.repleta	89	0.08	41.7	56	0.05	33.3	0	..	..	0	..	..	0	..	..
Total	414			425			160			295			284		
Subgenus															
Dorsilopha															
D.buskii	35	0.03	33.3	12	0.01	8.33	4	0	8.33	0	..	..	0	..	..
Total	35			12			4			0			0		
Subgenus *Scaptodrosophila*															
D.nigra	0	..	..	15	0.01	8.33	12	0.01	8.33	16	0.01	16.7	19	0.08	16.67
Total	0			15			12			16			19		
Unidentified (1)	17	0.02	16.7	47	0.04	33.3	19	0.02	25	9	0.01	16.7	6	0.01	8.33
Unidentified (2)	0	..	..	0	..	..	1	0	8.33	0	..	..	0	..	..
Unidentified (3)	1	0.01	8.33	0	..	..	0	..	..	0	..	..	0	..	..
Total	18			47			20			9			6		
Grand total	1130	..	..	1130	..	..	877	..		847	..	..	696	..	..

Table 3. Simpson Index (D) according to the altitude of the mount Japfu

Altitude	Simpson Index (D)
1500m	0.10903
1800m	**0.09301**
2100m	0.10362
2400m	0.11733
2700m	0.141355

Table 4. Monthly collection record of different *Drosophila* species collected at an altitude of 1500 m a.s.l of mount Japfu along with the meteorological data from April 2010 to March 2011

Months	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total
Temperature in ℃	23.9	24.8	24.9	25	25.4	24.9	22.7	19.9	16.8	16.3	18.1	20.7	
Humidity	64.3	72.2	80.8	82.1	84.2	82.1	81.1	69.3	67.9	59.4	61.3	61.5	
Rainfall in mm	107.8	117.5	218.6	291	358.4	347.4	154.6	5.2	52.2	14	14.5	54.9	
Genus:*Drosophila*													
Subgenus *Sophophora*													
1.D. agumbensis	0	0	0	0	0	0	0	0	0	0	17	0	17
2.D. bipectinata	18	0	0	0	0	0	17	0	0	20	0	0	55
3.D. eugracilis	16	0	0	7	21	18	23	19	14	0	11	11	140
4.D. jambulina	0	0	0	0	0	0	0	0	0	2	4	1	7
5.D. kikkawai	2	14	0	0	0	0	0	0	0	0	0	0	16
6.D. rajasekari	0	0	0	3	4	7	6	0	0	0	0	0	20
7.D. malerkotliana	0	0	27	37	19	26	0	0	12	0	0	22	143
8.D. parvula	0	9	0	0	0	0	0	27	0	0	0	0	36
9.D. trileuta	0	0	0	0	0	0	19	0	27	0	0	0	46
10.D. takahashii	0	34	30	8	9	33	22	20	13	7	0	7	183
Subgenus *Drosophila*													
1.D. immigrans	19	15	27	30	22	0	23	23	7	0	15	9	190
2.D. paraimmigrans	18	8	12	28	25	16	0	16	0	12	0	0	135
3.D. repleta	0	5	0	0	0	0	24	0	25	0	22	13	89
Subgenus *Dorsilopha*													
1.D. buskii	7	0	0	0	14	10	4	0	0	0	0	0	35
Unidentified (1)	9	0	0	0	0	0	8	0	0	0	0	0	17
Unidentified (3)	0	0	0	0	0	0	0	0	0	0	0	1	1
Total	89	85	96	113	114	110	146	105	98	41	69	64	1130

Table 5. Monthly collection record of different *Drosophila* species collected at an altitude of 1800 m a.s.l of mount Japfu along with the meteorological data from April 2010 to March 2011

Months	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total
Temperature in ℃	23.9	24.8	24.9	25	25.4	24.9	22.7	19.9	16.8	16.3	18.1	20.7	
Humidity	64.3	72.2	80.8	82.1	84.2	82.1	81.1	69.3	67.9	59.4	61.3	61.5	
Rainfall in mm	107.8	117.5	218.6	291	358.4	347.4	154.6	5.2	52.2	14	14.5	54.9	
Genus:*Drosophila*													
Subgenus *Sophophora*													
1. D. bipectinata	0	0	0	17	21	25	0	24	24	17	14	3	145
2..D. eugracilis	0	10	28	12	15	0	0	15	0	0	0	0	80
3.D. jambulina	0	0	0	0	14	0	0	0	0	1	0	0	15
4 D. kikkawai	0	18	28	5	12	17	23	0	16	0	0	17	136
5. D. rajasekari	0	0	0	0	0	0	0	1	0	0	0	0	1
6. D. malerkotliana	18	0	0	0	0	0	24	0	10	0	5	0	57
7..D. parvula	15	0	0	15	0	0	25	0	0	6	0	15	76
8 D. trileuta	12	0	0	0	0	0	0	0	0	7	0	0	19
9. D. takahashii	9	32	0	10	14	0	0	0	8	4	12	13	102
Subgenus *Drosophila*													
1.D. immigrans	6	17	26	26	26	20	30	0	0	7	9	10	177
2.D .paraimmigrans	0	20	20	15	20	15	0	21	0	0	15	8	134
3.D. repleta	17	0	0	0	0	20	14	0	0	0	0	5	56
4.D. nasuta	0	0	0	18	0	20	0	0	0	0	6	14	58
Subgenus *Dorsilopha*													
1.D.buskii	0	0	0	0	0	12	0	0	0	0	0	0	12
Subgenus *Scaptodrosophila*													
1.D. nigra	0	0	0	0	0	0	15	0	0	0	0	0	15
Unidentified (1)	19	0	0	7	17	4	0	0	0	0	0	0	47
Total	96	97	102	125	139	133	131	61	58	42	61	85	1130

Table 6. Monthly collection record of different *Drosophila* species collected at an altitude of 2100 m a.s.l of mount Japfu along with the meteorological data from April 2010 to March 2011

Months	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total
Temperature in °C	23.9	24.8	24.9	25	25.4	24.9	22.7	19.9	16.8	16.3	18.1	20.7	
Humidity	64.3	72.2	80.8	82.1	84.2	82.1	81.1	69.3	67.9	59.4	61.3	61.5	
Rainfall in mm	107.8	117.5	218.6	291	358.4	347.4	154.6	5.2	52.2	14	14.5	54.9	
Genus:*Drosophila*													
Subgenus: *Sophophora*													
1.D. bipectinata	0	0	8	10	0	18	27	25	11	11	8	25	143
2.D. eugracilis	0	7	0	0	0	12	8	21	5	11	22	0	86
3.D .jambulina	0	0	0	0	0	0	0	0	0	0	4	0	4
4.D. kikkawai	3	5	18	0	6	0	21	0	0	0	0	11	64
5. D. malerkotliana	0	0	29	22	25	18	0	11	17	3	16	7	148
6. D. parvula	0	10	30	8	14	12	0	0	0	0	0	0	74
7.D. rajasekari	0	0	8	4	6	0	0	0	0	0	0	0	18
8. D. takahashii	4	26	26	14	0	12	8	0	12	5	0	0	107
9.D. trileuta	0	0	0	0	0	12	8	0	12	5	0	0	37
Subgenus *Drosophila*													
1.D. immigrans	4	0	0	14	10	0	8	17	0	0	10	0	63
2.D. paraimmigrans	0	24	0	0	0	0	16	7	10	0	4	9	70
3.D. nasuta	0	0	0	0	0	0	0	0	0	13	0	14	27
Subgenus *Dorsilopha*													
1.D.buskii	0	0	0	0	0	0	4	0	0	0	0	0	4
Subgenus *Scaptodrosophila*													
1.D.nigra	0	0	0	0	0	0	0	0	0	0	0	12	12
Unidentified (1)	0	4	0	7	0	8	0	0	0	0	0	0	19
Unidentified (2)	0	0	0	0	0	0	0	0	0	0	1	0	1
Total	11	76	119	79	61	92	100	81	67	48	65	78	877

Table 7. Monthly collection record of different *Drosophila* species collected at an altitude of 2400 m a.s.l of mount Japfu along with the meteorological data from April 2010 to March 2011

Months	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total
Temperature in °C	23.9	24.8	24.9	25	25.4	24.9	22.7	19.9	16.8	16.3	18.1	20.7	
Humidity	64.3	72.2	80.8	82.1	84.2	82.1	81.1	69.3	67.9	59.4	61.3	61.5	
Rainfall in mm	107.8	117.5	218.6	291	358.4	347.4	154.6	5.2	52.2	14	14.5	54.9	
Genus:*Drosophila*													
Subgenus *Sophophora*													
1.D. agumbensis	1	0	0	0	0	0	0	0	0	0	0	0	1
2.D. bipectinata	0	0	13	0	8	0	7	0	0	0	0	0	28
3.D. eugracilis	0	0	0	0	0	0	17	0	0	14	12	14	57
4.D. jambulina	8	0	0	0	0	0	0	0	0	0	0	0	8
5.D. kikkawai	0	12	0	7	0	0	0	13	0	9	0	7	48
6.D. rajasekari	8	0	0	0	0	0	0	0	0	0	0	0	8
7.D. malerkotliana	0	27	0	16	9	0	16	16	9	5	9	10	117
8.D. parvula	0	15	17	0	0	0	0	15	13	7	0	0	67
9.D. trileuta	0	0	0	0	0	0	0	19	16	0	0	0	35
10.D. takahashii	11	17	16	10	10	20	15	19	17	13	7	3	158
Subgenus *Drosophila*													
1.D. immigrans	4	6	30	16	14	28	11	7	8	13	10	3	150
2.D. paraimmigrans	0	14	26	10	14	17	8	14	8	0	0	10	121
3.D. nasuta	0	0	0	0	0	0	0	0	0	0	13	11	24
Subgenus *Scaptodrosophila*													
1.D.nigra	0	0	0	0	0	0	0	0	0	0	11	5	16
Unidentified (1)	3	6	0	0	0	0	0	0	0	0	0	0	9
Total	35	97	102	59	55	65	74	103	71	61	62	63	847

Table 8. Monthly collection record of different *Drosophila* species collected at an altitude of 2700 m a.s.l of mount Japfu along with the meteorological data from April 2010 to March 2011

Months	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total
Temperature in °C	23.9	24.8	24.9	25	25.4	24.9	22.7	19.9	16.8	16.3	18.1	20.7	
Humidity	64.3	72.2	80.8	82.1	84.2	82.1	81.1	69.3	67.9	59.4	61.3	61.5	
Rainfall in mm	107.8	117.5	218.6	291	358.4	347.4	154.6	5.2	52.2	14	14.5	54.9	
Genus:*Drosophila*													
Subgenus *Sophophora*													
1.D. agumbensis	1	0	0	0	0	0	0	0	0	0	0	0	1
2.D. bipectinata	9	0	0	0	0	13	14	5	3	0	3	5	52
3.D. eugracilis	0	0	0	7	0	0	0	0	0	8	0	0	15
4.D. jambulina	15	0	0	0	0	0	0	0	0	0	6	4	25
5.D. kikkawai	0	10	19	0	0	14	0	0	0	0	0	0	43
6.D. rajasekari	2	0	0	0	0	0	0	0	0	0	0	0	2
7.D. malerkotliana	0	21	0	0	12	22	14	0	4	10	0	13	96
8.D. parvula	0	0	0	7	7	0	0	0	0	3	0	0	17
9..D. takahashii	7	15	26	0	9	26	12	17	13	0	0	11	136
Subgenus *Drosophila*													
1.D. immigrans	12	6	14	34	16	16	7	9	17	0	0	0	131
2.D. paraimmigrans	9	7	7	27	22	14	21	9	0	7	13	0	136
3.D. nasuta	0	0	0	0	17	0	0	0	0	0	0	0	17
Subgenus *Scaptodrosophila*													
1.D.nigra	0	0	0	0	13	0	0	0	0	0	6	0	19
Unidentified (1)	0	0	0	6	0	0	0	0	0	0	0	0	6
Total	55	59	66	81	96	105	68	40	37	28	28	33	696

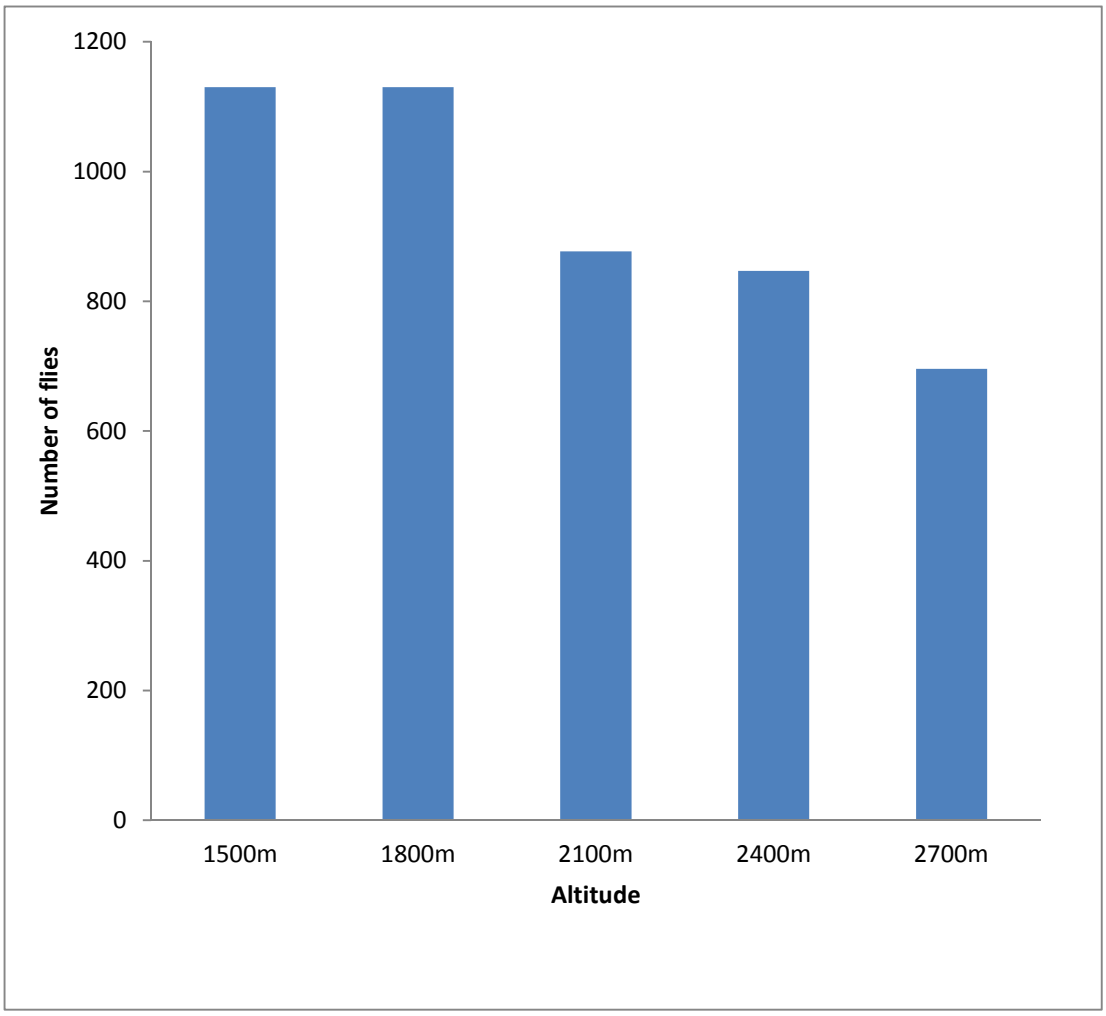


Figure 1. Altitudinal variation of *Drosophila* population at different altitudes of mount Japfu

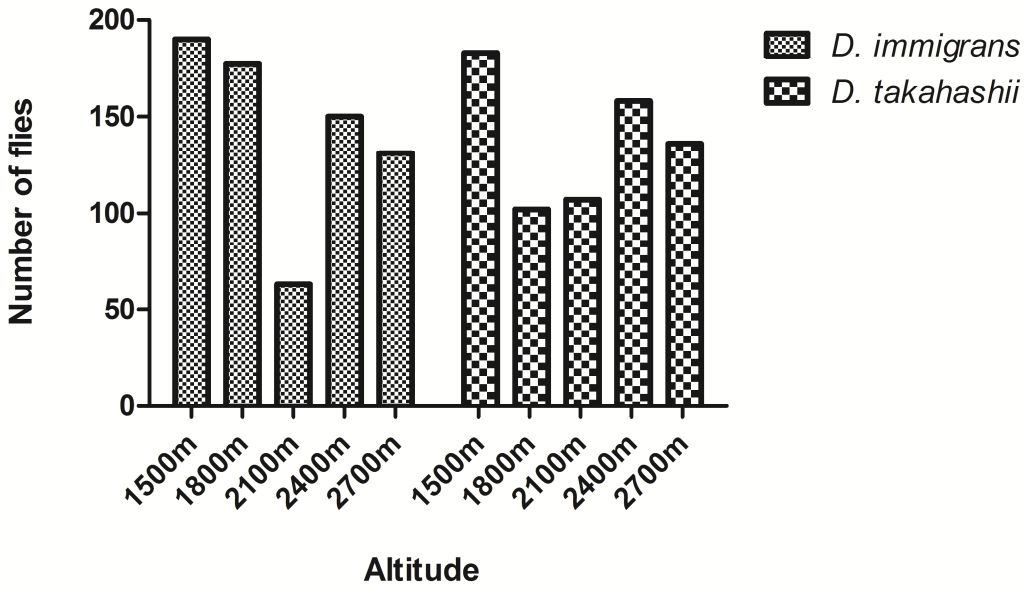


Figure 2. Altitudinal variation in the population of most abundant *Drosophila* species of mount Japfu

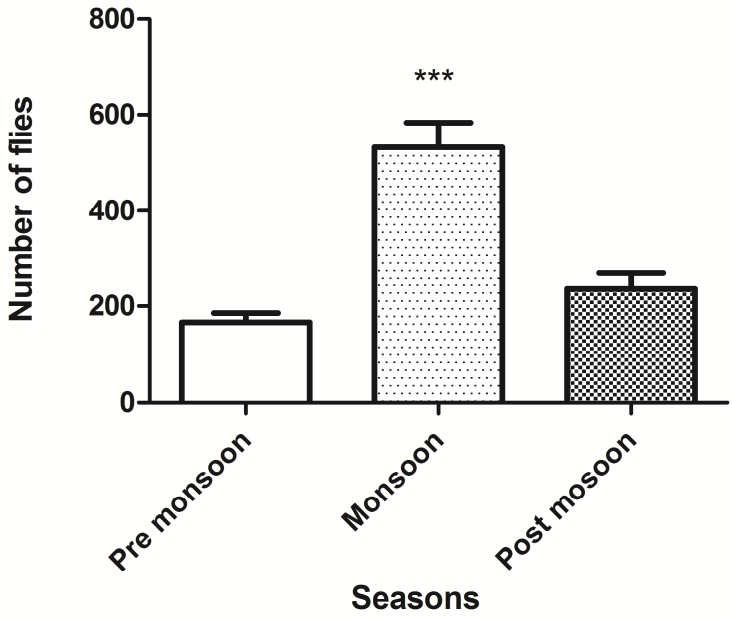


Figure 3. Seasonal variation in *Drosophila* flies collected from Japfu mountain (F=26.72; df=2; P<0.0001)

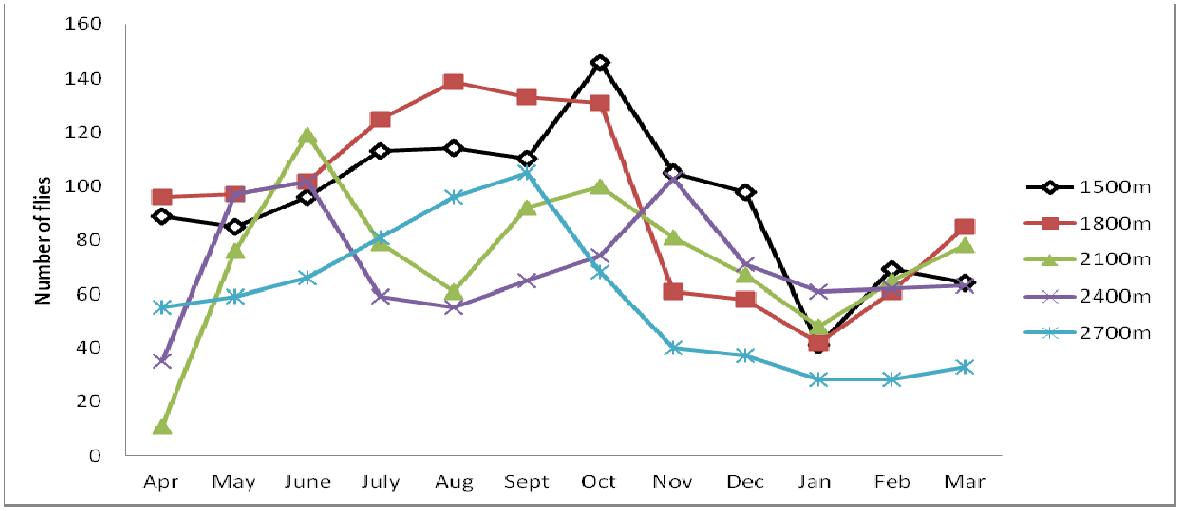


Figure 4. Seasonal fluctuation in the total population size of different species of *Drosophila* at different altitudes of mount Japfu

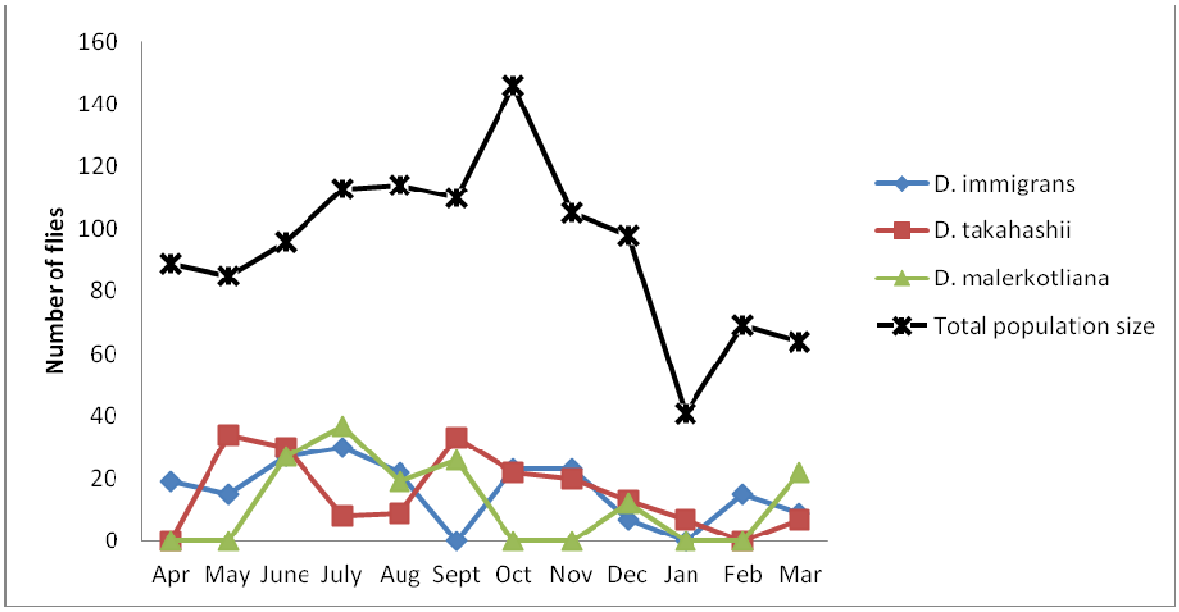


Figure 5. Seasonal fluctuation in the total population size and those of the three common and abundant species of *Drosophila* at 1500 m a.s.l altitude of mount Japfu

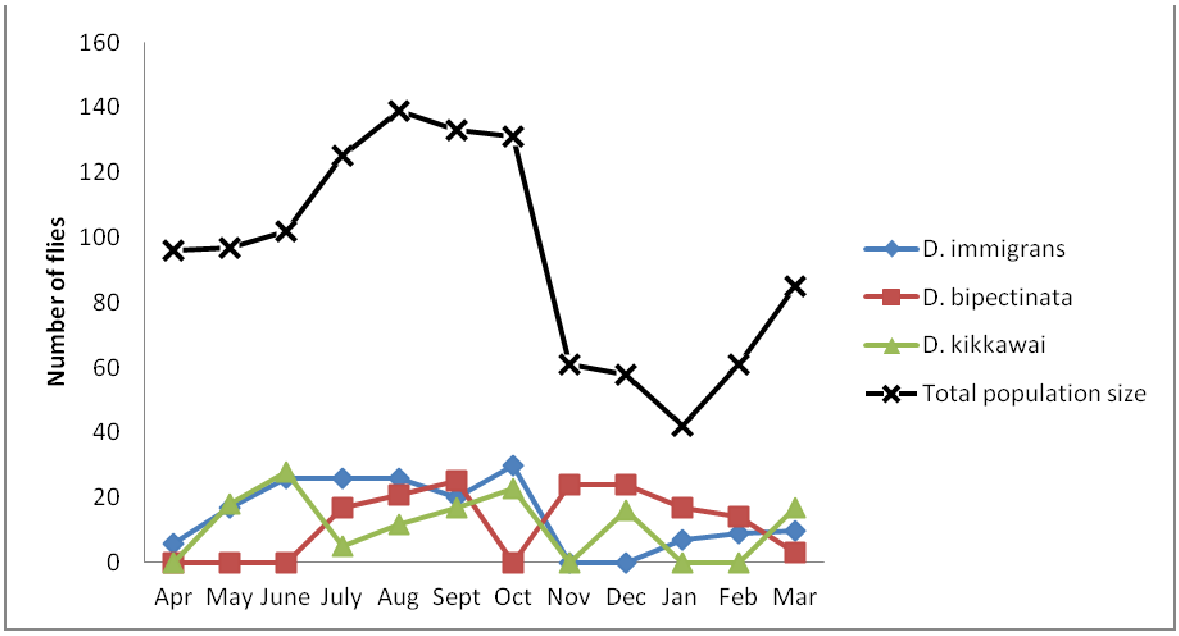


Figure 6. Seasonal fluctuation in the total population size and those of the three common and abundant species of *Drosophila* at 1800 m a.s.l altitude of mount Japfu

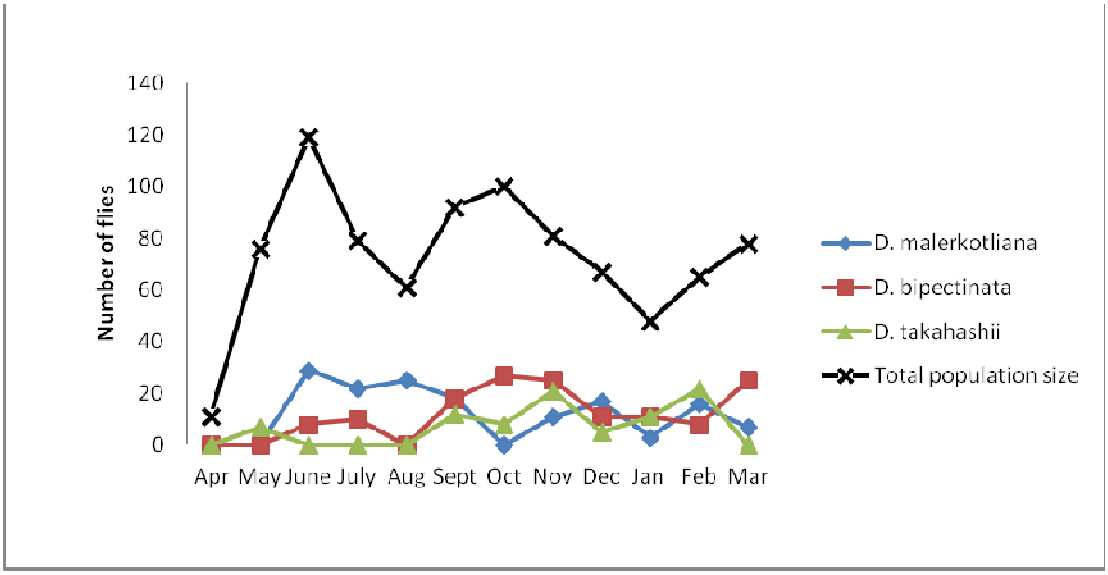


Figure 7. Seasonal fluctuation in the total population size and those of the three common and abundant species of *Drsophila* at 2100 m a.s.l altitude of mount Japfu

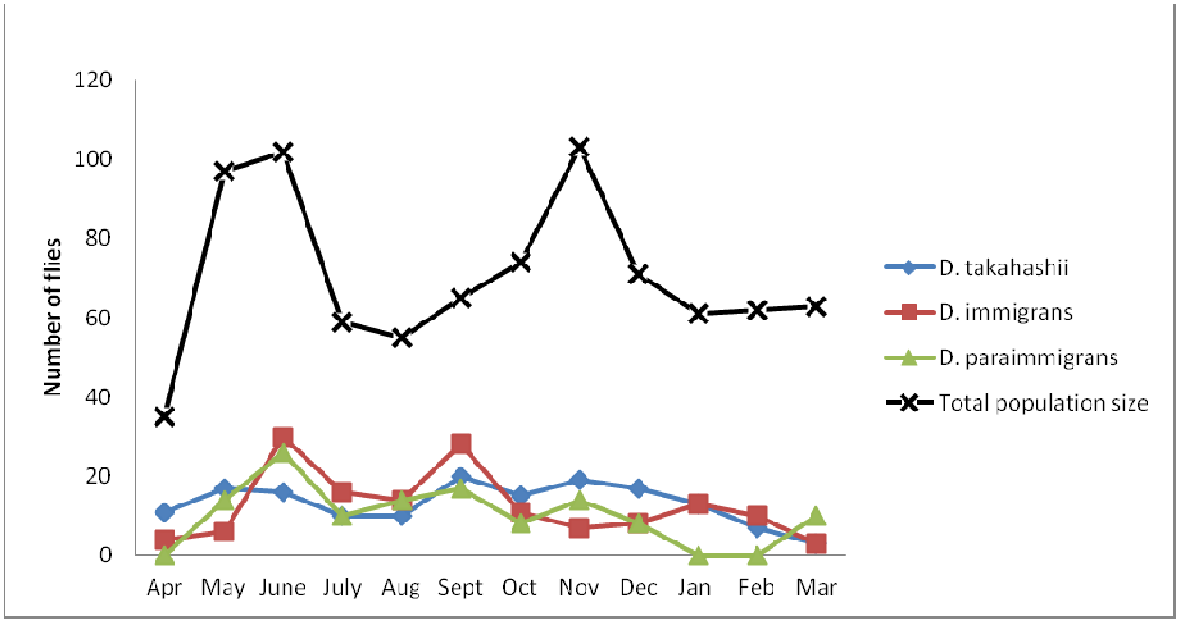


Figure 8. Seaonal fluctuation in the total population size and those of the three common and abundant species of *Drosophila* at 2400 m a.s.l altitude of mount Japfu

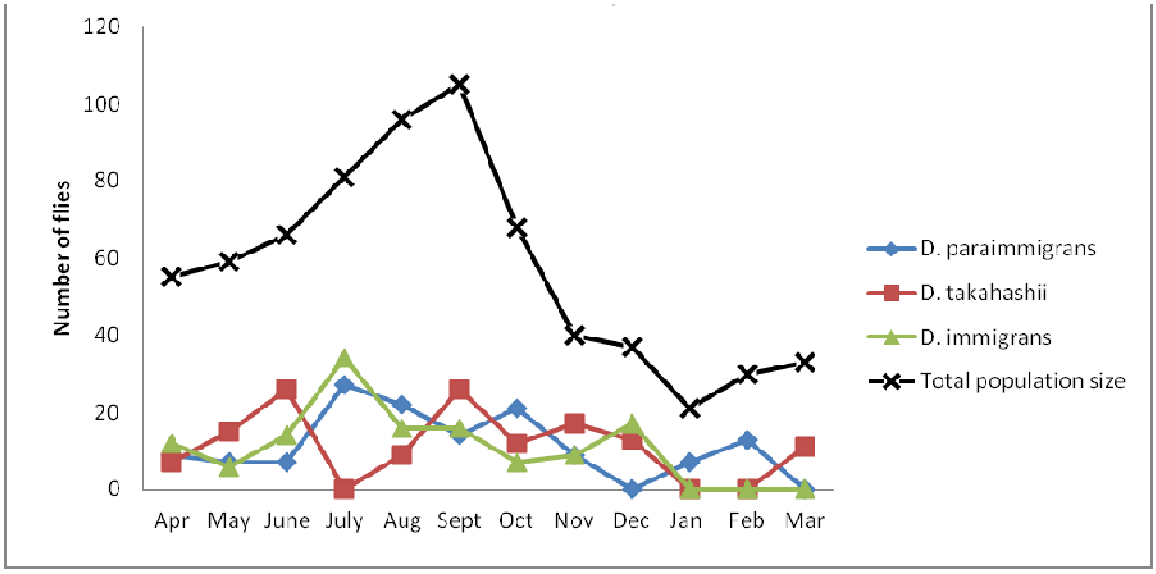


Figure 9. Seasonal fluctuation in the total population size and those of the three common and abundant species *of Drosopila* at 2700 m a.s.l altitude of mount Japfu

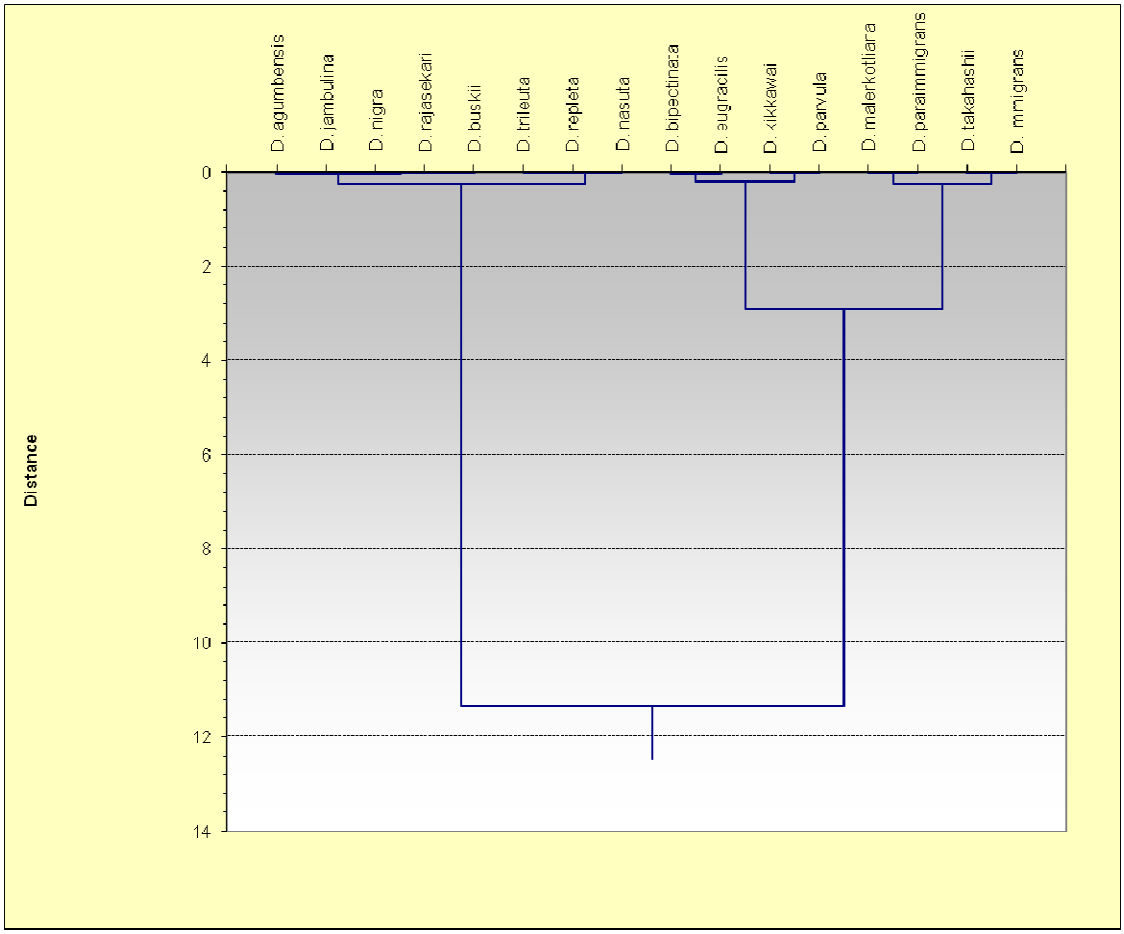


Figure 10. The cluster analysis of *Drosophila* species found in Japfu Mountain: (Dendrogram using Ward method)

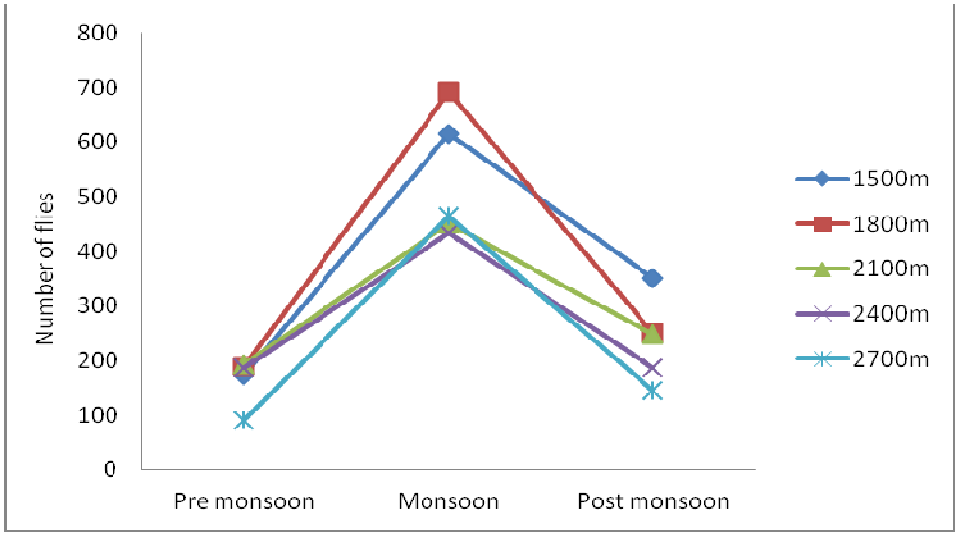


Figure 11. Variation of *Drosophila* species in different seasons at Japfu Mountain

DISCUSSION:

Species of an animal or plant found all over the world is frequently adjudged as biologically successful (Carson 1965). *Drosophila* is one such species constellation, which is distributed throughout the world. It is estimated that there are more than 2240 biologically valid species of *Drosophila* (Wheeler 1986). In the present study 19 species are recorded from mount Japfu. Pooled data on monthly collection of *Drosophila* made from 1500 m a.s.l, 1800 m a.s.l, 2100 m a.s.l, 2400 m a.s.l and 2700 m a.s.l altitudes of mount Japfu has yielded a total of 4680 individuals. Out of these 2889 individuals (61.73%) belong to 10 species of sub genus *Sophophora*. Out of the remaining 1791 flies, 1578 (33.71%) individuals belong to 4 species of the subgenus *Drosophila*. Of the remaining 213 flies, 62 (1.32%) individuals belong to a species of subgenus *Scaptodrosophila*; 100 (2.13%) belong to 3 unidentified species and the remaining 51 (1.07%) belong to a species of subgenus *Dorsilopha.*

Carson (1965) based on the pattern of distribution of various members of *Drosophila*, has recognized three distinct groups, namely- 1) virtually cosmopolitan species 2) species having a tendency for wide spreading but not cosmopolitan and 3) species having restricted distribution (endemic species). He has included five species namely *D. melanogaster, D. simulans, D. ananassae, D. buskii* and *D. repleta* as truly cosmopolitan. Incidentally *D. melanogaster, D. simulans and D. ananassae* are domestic while *D. buskii* and *D. repleta* are semi-domestic. In the present study, of the 19 species collected only *D. buskii* and *D. repleta* are cosmopolitan species, while others are either widespread or endemic. The absence of *D. melanogaster*, *D. simulans* and *D. ananassae* in the present wild area collections can be attributed to the fact that they are fully domestic species.

According to couple of preliminary reports on Drosophilids of Nagaland (Singh 1987; Yenisetti *et al.* 2002) of the total 16 subgenera of *Drosophila* known, only three subgenera namely, *Sophophora*, *Drosophila* and *Dorsilopha* were found in Nagaland. However in the present study a species of subgenus *Scaptodrosophila* is also found.

Careful analysis of the pattern of distribution of various species collected at 5 wild localities has revealed a total of 19 species of *Drosophila* (Table 1). Out of the total 16 subgenera of *Drosophila* known, members of 4 subgenera namely, *Sophophora, Drosophila, Dorsilopha* and *Scaptodrosophila* are found in mount Japfu. Subgenus *Sophophora* is represented by 10 species, namely *D. agumbenesis, D. bipectinata, D. eugracilis, D. jambulina, D. kikkawai, D. malerkotliana, D. parvula, D. rajasekari, D. takahashii and D. trileuta.* Subgenus *Drosophila* is represented by four species of which *D. immigrans* and *D. paraimmigrans* belong to *immigrans* subgroup. *D. nasuta* belongs to *nasuta* subgroup and *D. repleta* to *repleta* group. Subgenus *Dorsilopha* is represented by *D. buskii* and subgenus *Scaptodrosophila* is represented by *D. nigra.* Remaining three are unidentified species. Thus the study indicates that the *Drosophila* fauna of mount Japfu is highly diverse.

The density of *Drosophila* on mount Japfu decreased with increasing altitude. The density was high at 1500 and 1800 m a.s.l., but was low at 2700 m a.s.l. (Figure 1). The results indicate that *Drosophila* community was affected by elevation. Wakahama (1961, 1962) has reported similar altitudinal variation in the distribution of *Drosophila* on Mt. Dakesan in Japan. He noticed that the total density of all species decreases with increasing altitude. Reddy and Krishnamurthy (1977) also noticed such altitudinal variation in *Drosophila* populations in Jogimatii hills of Karnataka. Guruprasad *et al.* (2010) also observed seasonal and altitudinal variation in *Drosophila* populations of Chamundi hill of Mysore, Karnataka, India. The reasons behind the observed phenomenon can be attributed to changes that occur as one ascends an altitudinal transect, potentially involving changes in temperature, precipitation, partial pressure of atmospheric gases, atmospheric turbulence and wind speed, and radiation input, including short-wave ultra violet radiation at different wavelengths (Barry 1992). According to Hodkinson (2005), the above-mentioned changes are often strongly interactive and together create environmental envelope within which insect species survive and reproduce. Hodkinson (2005) further emphasizes that the above mentioned parameters combine to produce a general decrease in the overall structural complexity of the insects' habitat with increasing altitude.

According to Hegde *et al.* (2000), the growth and size of the population depends on several environmental factors in addition to genetic structure. In the present study, consideration of the common and abundant species shows that numerical variation exists in regard to these species at all five altitudes. *D. immigrans, D. takahashii, D .malerkotliana* is found to be common and abundant species at 1500 m a.s.l. At 1800 m a.s.l *D. immigrans, D. bipectinata, D. kikkawai,* at 2100 m a.s.l, *D. malerkotliana, D. bipectinata,* and *D. takahashii,* at 2400 m a.s.l *D. takahashii, D. immigrans, and D. paraimmigrans* and at 2700 m a.s.l *D. takahashii, D. paraimmigrans, D. immigrans* are found to be common and abundant species. *D. immigrans* rank first at 1500 m a.s.l and 1800 m a.s.l and second at 2400 m a.s.l and 2700 m a.s.l whereas at 2100 m a.s.l *D. malerkotliana* rank first. At 2400 m *D. takahashii* rank first and at 2700 m a.s.l *D. paraimmigrans* occupies the first position. The occurrence of dominance of one species over the others in any given area can be correlated with the dominant species' ecological versatility to exploit the conditions available in those habitats. The present study corroborates with the work of Muniyappa and Reddy (1981), Hegde *et al.* (2001), and Vasudev *et al*. (2001). There may be many other unknown microclimatic conditions that could also affect the density of *Drosophila.* Present results are in concurrence with the work of Cooper and Dobzhansky (1956), Reddy and Krishnamurthy (1977), Hegde *et al.* (2001) all of which have shown the influence of microclimatic conditions on the diversity of *Drosophila*. The present findings are also in agreement with the work of Cooper and Dobzhansky (1956) on species of *Drosophila* inhabiting the Sierra Nevada Mountains of Yosemite region of California, where some of the species occurred at all elevations at which collections were made (259-3353 m a.s.l.). The results of the present study are also in agreement with the work of Guruprasad *et al*. (2010), who showed that the number and density of *Drosophila* species decreased with increasing altitude at Chamundi Hill in Mysore, Karnataka. In the present study, the presence of more species at lower altitudes can be attributed to the existence of thick vegetation, which provided good sources of food, and a more congenial environment at lower altitudes than at the higher altitudes.

Significant variations in the densities of *Drosophila* were noticed during different seasons of the year on mount Japfu. The density was highest during monsoon seasons at all altitudes. During this season at 1500 m a.s.l, 607 flies; at 1800 m a.s.l, 692 flies, at 2100 m a.s.l, 438 flies; at 2400 m a.s.l, 413 flies and at 2700 m a.s.l, 462 flies are collected. The

density was lowest during the pre monsoon season. With 174, 188, 186, 89 flies respectively at 1500, 1800, 2100, 2400 and 2700 m a.s.l., of mount Japfu. Possible reasons for the high density during monsoon season could be the availability of adequate food in the form of rotting fruits and congenial climate for multiplication of the flies. The fact that the fruiting season of many plants in the area coincides with monsoon offers support for this conclusion. The monsoon season is characterized by heavy rains, reduction in temperature, and increases in humidity. As the monsoon recedes rainfall and humidity decrease, leading to a dry climate. The population density also starts declining in post-monsoon season (349, 250,248,248 and 145 flies respectively at 1500 m a.s.l, 1800 m a.s.l, 2100 m a.s.l, 2400 m a.s.l and 2700 m a.s.l altitudes), reaching its minimum during pre-monsoon season. Thus, the fluctuations in population size of *Drosophila* are closely related with wet and dry seasons. However, in temperate regions, the population density declines to an extremely low level during cold winter months indicating the influence of temperature on the regulation on population size, as is the case in several *Drosophila* species inhabiting temperate regions (Dobzhansky 1943; Patterson and Wagner 1943; Dobzhansky and Pavan 1950; Williams and Miller 1952; Wakahama 1961).

The common and abundant species encountered at all altitudes are not the same. At 1500 m a.s.l., *D. immigrans, D. takahashii* and *D .malerkotliana* are found to be common and abundant species. At 1800 m a.s.l., *D. immigrans, D. bipectinata* and D. *kikkawai*; at 2100 m a.s.l., *D. malerkotliana, D. bipectinata* and *D. takahashii;* at 2400 m a.s.l., *D. takahashii, D. immigrans, D. paraimmigrans;* at 2700 m a.s.l., *D. takahashii, D. paraimmigrans, D. immigrans* are found to be common and abundant species.

At 1500 m a.s.l., the total population size of *D. immigrans* exhibits two peaks (population size), one during monsoon and one during post-monsoon period; *D. takahashii* also exhibits a peak during monsoon and post-monsoon. However there is a decline in population size of *D. takahashii* during June and July. This decline can be attributed to microclimatic conditions. *D. malerkotliana* also show two peaks one during monsoon period and one during post-monsoon period.

At 1800 m a.s.l., *D. immigrans* reach the maximum population size during post-monsoon period. There is a steep decrease in the later part of the post-monsoon and pre-monsoon period. *D. bipectinata* which has small population size in the later part of pre-monsoon period gradually increases to reach the maximum during the monsoon period. *D. kikkawai* reaches a maximum population size in the monsoon period and in post-monsoon period. It is observed that *D. kikkawai* is absent in January, February, April and November collections.

At 2100 m a.s.l., altitude, *D. malerkotliana* shows a peak of population size during monsoon period and a minimum flies can be collected during pre-monsoon period. *D. bipectinata* shows maximum population size during post-monsoon and a minimum flies can be collected in the early part of the pre-monsoon and early part of monsoon. *D. takahashii* reaches maximum population size during monsoon period and start declining and reach minimum during pre-monsoon period and post-monsoon period.

At 2400 m a.s.l., *D. takahashii* reaches maximum population size during monsoon period and a minimum number of flies can be collected during pre-monsoon period, *D. immigrans* shows two peaks one during early part of the monsoon and one during later part of the monsoon season. *D. Paraimmigrans* also shows a peak during monsoon period.

At 2700 m a.s.l., among the common and abundant species, *D. takahashii* shows two peaks, one during monsoon period and another during post monsoon period. It shows minimum number of flies during pre-monsoon period. However there is a decline in the population size during the month of July, which may be due to invisible microclimatic conditions. *D. paraimmigrans* also shows two peaks one during monsoon and other during post-monsoon period and population size decline during pre-monsoon period. *D. immigrans* shows one peak during monsoon period and a minimum number of flies can be collected during pre-monsoon and post-monsoon period. Thus the maximum population size is build up during monsoon (April-September).

This situation can be correlated to the flowering and fruiting promoted many kinds of fungus that provide fertile breeding and feeding sites for different species of *Drosophila* and hence found to be maximum in population density during monsoon seasons. The pre monsoon (January- March) on the other hand, characterized by dry weather causing a decline in the population density; similarly during post-monsoon (October- December) the climatic condition is not congenial and hence there is also a decline in the population densities of various species. Heed (1968) in the course of his studies on *Drosophildae* of Hawaiian Islands has pointed out that the main controlling factors that influence the pattern of distribution of *Drodophilids* are wind intensity, humidity, temperature, rainfall and acceptable ovipositional sites.

The seasonal fluctuations in regard to population densities of various species of *Drosophila* under study have also revealed that some species build up large populations during certain seasons of the year and others are completely out of sight during certain months of the year (parts of pre-monsoon and post- monsoon periods). This does not necessarily mean that they are completely absent in these months. Nowaza (1956) has identified four phases of population growth in *Drosophila* namely, (1) hibernating phase (2) first active phase (3) summer resting phase and (4) second active phase. There are number of factors that may influence the species richness of a community. They may be classified as 1) geographical (e.g. latitude and longitude) 2) environmental (an environment with a greater variety of species) and 3) biological (the relationships of predation, competition and population density etc.). These factors may have important consequences on the number of species in a given ecosystem. The changes in the natural environment caused by the alteration of seasons would result in the change in relative frequency of different species from season to season (figure 11). In tropical areas, especially in Brazil, changes in the environment are caused by the alteration between the dry and rainy seasons (Dobzhansky and Pavan 1950). It should be emphasized that the months with higher species richness occur during the rainy season. These differences suggest that at different altitudes the capacity to support *Drosophila* species varies. Thus the existence of seasonal variation in *Drosophila* species is quite evident by the presence of greater numbers of species in monsoon compared to pre and post monsoon periods.

Patterson and Wagner (1943) have shown that the population size of various *Drosophila* species in Mexico fluctuate and decline enormously during winter. Wakahama (1956, 1957) demonstrated a considerable variation in population densities of certain *Drosophila* species inhabiting the botanical gardens of University of Sappara, Japan. All these studies have highlighted that the environment plays a major role in regulating these fluctuations. Reddy and Krishnamurthy (1977) have also shown temporal changes in population size of various *Drosophila* species in four wild and on domestic locality in and around Mysore city.

According to the constant, accessory and accident species, as well as the cluster analysis, present study indicates several species that coexisted had similar ecological preferences.

In Simpson's index (D), 0 represent infinite diversity, and 1 represents no diversity, i.e. the greater the value of D, the lower is the diversity. Applying this index to understand the measures of biodiversity of flies at different altitudes of mount Japfu, shows that at the second lowest altitude studied (1800 m a.s.l) had the lowest D-value, indicating more biodiversity compared to other altitudes. Hodkinson (2005) suggested that the altitudinal distribution of an insect species is controlled by its environmental tolerances, with maximum population size being achieved at some optimum elevation and population density declining with altitude above and below the optimum. Present results suggest that the optimum elevation on mount Japfu for *Drosophila* biodiversity is at 1800 m a.s.l. From the eco-distributional analysis of *Drosophila* species in mount Japfu, it is clear that the distributional pattern of a species or related group of species is uneven in space and time. The *Drosophila* community of mount Japfu is highly diverse and depended on several environmental factors in addition to the genetic structure of the species present in it.

CHAPTER III

DROSOPHILA HEGDII (DIPTERA: DROSOPHILIDAE), A NEW SPECIES FROM NAGALAND: ITS MOLECULAR PHYLOGENY

INTRODUCTION:

North-Eastern region of Indian subcontinent (this region includes eight hill states, namely Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura) with its diverse climatic conditions, variable altitudes, deep valleys, luxuriant flora, running streams and moist surroundings is one of the richest repositories of biodiversity in the world. Hence it provides an ideal location for the colonization of several *Drosophila* species (Singh and Gupta 1977; Dwivedi and Gupta 1979; Gupta and Singh 1979; Singh and Gupta 1980; Singh 1987; Yenisetti *et al.* 2002 and Achumi *et al.* 2011, 2013).

Nagaland is one of the sub-Himalayan hilly states of biodiversically rich north eastern part of the country. However very little work was done to understand *Drosophila* diversity in this part of the country. A preliminary survey on Drosophilids of Dimapur, Medziphema, and Kohima of Nagaland state was conducted (Singh 1987). Yenisetti *et al.* (2002) published a report on Drosophilids of Mokokchung town. In spite of existence of potential possibility, till today no new *Drosophila* species were reported from this sub-Himalayan hilly state bordering Indo-Burma region.

Survey of *Drosophila* was undertaken in Lumami (Lumami is a hamlet in Zunheboto district of Nagaland and is head quarters of the Nagaland University) which is situated at 94˚.28'E Longitude and 26˚.33' N Latitude, having an altitude of 940 m above sea level. In Lumami temperature ranges from 8˚C to 30˚C and average annual rainfall is about 200 cm. The torrential monsoon rain is an integral feature of the weather in this place. Heavy rainfall during the monsoon favours the growth of thick forest, fruit bearing trees, providing favorable natural habitats for the colonization by the members of the genus *Drosophila.* In the present study 16 species were collected, belonging to 4 subgenera (*Sophophora*, *Drosophila*, *Dorsilopha* and *Scaptodrosophila*). Three species were unidentified. Of the three unidentified, one was recognized as a new species basing on morphological markers such as head, thorax, wings, legs, abdomen of male and female; internal characters such as periphallic organ, phallic organ, egg guide, egg and pupa

(Achumi *et al*. 2011). It is named after Prof. S.N. Hegde (retired) of Mysore University, Karnataka, India (Prof. Hegde made significant contribution to cytotaxonomy and genetics of Indian Drosophilids) as *Drosophila hegdii*.

"DNA barcoding" is identified as a promising tool not only for rapid identification of known species, that is "species identification," but also for discovery and delimitation of species, that is, "species discovery" or "DNA taxonomy" (Hebert *et al*. 2003a,b; Jinbo *et al*. 2011). Simon (1991) observed that when focusing on very closely related species, one should select rapidly evolving regions, for example, mitochondrial genes as markers. Mitochondrial cytochrome c oxidase subunit I *(COI)* gene has been widely used as DNA barcoding for "species identification": its 648 base pair fragment is the standard marker in the Barcode of Life Project (Hebert *et al.* 2003a, b). In the present study by employing mitochondrial cytochrome c oxidase subunit I marker, effort was made to establish the independent species status of *Dropsophila hegdii.* Confirmation of new species status and understanding its molecular phylogeny was done with the help of "DNA barcoding." Molecular analysis confirms the observations made through morphological markers that *Drosophila hegdii* is a new species.

MATERIALS AND METHODS:

Drosophila collections were made by following two methods: 1) Bottle trapping method and 2) Net sweeping method (detailed procedure of these methods was explained in chapter I).

The flies were then brought to the laboratory, isolated and sex was identified. The males were directly used for identification of species basing on morphological charterers such as presence or absence of the sex comb; if present the number of sex comb rows and teeth in each row and characterestics of genital plate. Individual females were kept in separate food vials and isofemale lines were generated. The males of the F1 progeny of these gravid females were used for species identification.

Categorization of the collected *Drosophila* flies were made to respective taxonomic groups by employing the parameters as suggested by Bock (1971), Patterson and Stone (1952), Sturtevant (1921) and Throckmorton (1962). The most important parameters employed to identify the species are the morphological features like colour and size of imagoes, number and nature of aristal branches, nature and arrangement of genital arch, nature and number of acrostichal hairs, length of the wings and its indices, the internal characters of the adults, the shape and number of egg filaments, pupal characters, pupal spiracles and behavior were also taken into consideration for species identification.

Genomic DNA extraction and estimation:

Genomic DNA was extracted from single adult male fly. The fly was freezed on ice and homogenized using crusher in extraction buffer (5% sucrose; 80mM NaCl; 100mM Tris, pH 8.5; 0.5% SDS; 50mM EDTA). The contents were mixed well and kept on ice, incubated at 65°C for 30 minutes and immediately chilled on ice. 8M potassium acetate was added and placed on ice after mixing well. Pellet was precipitated by centrifugation for 10 minutes at 10000 rpm at 4°C. The supernatant was transferred to a clean tube and DNA was precipitated with chilled ethanol. The tube was then centrifuged for 10 minutes at 10000rpm at 4°C to pellet DNA. The pellet was washed with 70% ethanol and dried. The pellet was re-suspended in double distilled water and kept for overnight at 55°C-60°C.

The DNA obtained was stored at 4°C until use. The quality of genomic DNA was checked with the help of A260/A280 ratio.

Calculation: 1.0 OD at 260 mm corresponds to 50 µg/ml of double-stranded DNA
dsDNA concentration= 50 µg x OD_{260} x dilution factor.

The primer sequences:

Set I

F: 5'-GCT CAA CAAATCATAAAGATATTGGC-3'

R: 5'-TAAACT TTA GCG TGA CCA AAA AAT CA-3'

Set II

F: 5'-ATTGAA CCA ATC ATA AGG ATA TTG C-3'

R: 5'-TAAACT TGT GGA TGT CAAAAAATCG-3'

(Set I and Set II were mixed in 1:1 ratio)

The DNA extracted was amplified with PCR using above mentioned oligos. The target regions were amplified on a thermal Cycler (Bio-Rad). The PCR amplification was carried out with the following conditions:

94° C for 2 minutes

94° C for 30 seconds
40° C for 40 seconds
72° for 1 minute
} 5 cycles

94° C for 30 seconds
45° C for 40 seconds
72° for 1 minute
} 35 cycles

72°C for 10 minutes

4° C for α

PCR products were extracted from agarose gel and cleaned using DNA Gel Extraction Kit (Qiagen). The amplicons were purified by precipitation with isopropanol and then subjected to sequencing reaction using BigDye Terminator v3.1 Cyclke Sequencing Kit (Applied Biosystems) following the recommended protocol. The sequences were analyzed on the 3100-Genetic Analyzer (Applied Biosystems).

Phylogenetic analysis:

DNA sequences were edited and aligned using ClustalW (Figure 5). ClustalW is a widely used system for aligning any number of homologous nucleotide or protein sequences. For multi-sequence alignments, ClustalW uses progressive alignment methods. In these, the most similar sequences, that is, those with the best alignment score are aligned first. Then progressively more distant groups of sequences are aligned until a global alignment is obtained. This heuristic approach is necessary because finding the global optimal solution is prohibitive in both memory and time requirements. ClustalW performs very well in practice. The algorithm starts by computing a rough distance matrix between each pair of sequences based on pairwise sequence alignment scores. These scores are computed using the pairwise alignment parameters for DNA and protein sequences. Next, the algorithm uses the neighbor-joining method with midpoint rooting to create a guide tree, which is used to generate a global alignment. The guide tree serves as a rough template for clades that tend to share insertion and deletion features. This generally provides a close-to-optimal result, especially when the data set contains sequences with varied degrees of divergence, so the guide tree is less sensitive to noise.

Phylogenetic trees and molecular evolutionary analyses were performed using MEGA 5 (Tamura *et al*. 2011) (Neighbor-Joining Tree (NJ) method with bootstrap test (1000 replicates) using the Kimura 2-parameter model, with gaps treated by pairwise deletion. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.05102 was shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) was shown next to the branches (Felsenstein 1985). The tree was drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were

computed using the p-distance method (Nei and Kumar 2000) and are in the units of the number of base differences per site. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 519 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

OBSERVATIONS:

Description of morphological markers/characteristics of *D. hegdii:*

Head:

Arista with 4 branches above, 3 below, plus the terminal fork. Antenna dark, basal segment of the antenna bears a pair of dark bristles. Vibrissae with two anterior and two posterior bristles. In between the anterior and posterior bristles are 10-12 small bristles. Palp with a large and many small bristles. Anterior orbital proclinate, median orbital half the size of anterior orbital, posterior equal to anterior. Anterior verticles direct inward, posterior convergent. Ocular triangle with a pair of dark bristles, eyes red.

Thorax:

Mid to dark brown, acrosticals in 8 regular rows, dorsocentrals convergent, anterior dorsocentrals are shorter than the posterior- approximately 2/3rd the length of the posterior, anterior scutellar convergent, posterior scutellar convergent and crossed. Both anterior and posterior scutellars are of equal length. Two humerals, upper humerals half the length of the lower, posterior allars longer than anterior. Notopleurals and stenopleurals are of equal length. Notopleural and supra allars are of equal length. There are about 2-3 smaller bristles along the anterior and posterior stenopleurals. Halters translucent.

Wings:

Transparent, wing length of male is 97 mm and female is 100 mm. Wing indices are calculated following the formula of Okada (1956) and presented in table 1.

Legs: [Figure 2(1)]

Sex comb present in male on first and second tarsal segment. First tarsal consists of about 25-27 teeth and second tarsal consists of 16 teeth. Teeth are uniform and slightly curved.

Abdomen of male and female:

First four tergites of male are shiny yellow-brown with broad dark apical band; last two segments are completely black. Tergites of female are shiny dark brown with broad darker apical bands.

Internal characters:

Female reproductive parts consist of ovarioles with 5-6 ventral receptacles that are transparent with 2-3 coils, spermetheca roundish colourless, [Figure 2(4)]. Male testis is short showing 2-3 coils and light yellow in colour, paragonia spherical transparent [Figure 2(3)].

Periphallic organ: [Figure 2(2)]

Ependrium broad, dorsally and laterally. Primary and secondary claspers present, primary claspers with a lateral row of about 5 teeth and a ventral medial cluster of teeth one elongated, toe with 3-4 bristles; secondary claspers oval, partially separated from anal plate with 3 black teeth, two are prominent and one is rudiment and about 1-8 small bristles along the ventral lateral and dorsal borders. Cerci rounded on the outer side and slightly curved on inner side and with about 25 long and short bristles.

Phallic organ: [Figure 2(5)]

Adeagus and anterior gonopophysis not fused. Anterior gonopophysis protrude dorsally. Novasternum with prominent median convexity of variable thickness bearing a pair of spines, ventral fragma broad and concave, basal apodeme is thick and short.

Egg guide: [Figure 2(6)]

Brown in colour with about 9-10 marginal and 1-2 discal teeth at the tip, teeth are dark in colour.

Egg: [Figure 2(7)]

White in colour with two filaments present at the anterior.

Pupa: [Figure 2(8)]

Yellow with 9-10 spiracle filaments. At the posterior end there are 3 pairs of projections- one pair is lateral, second pair is ventral and third pair is dorsal.

Holotype-Male: India, Nagaland, Lumami, 14.xi.11 Coll. Bovito Achumi and Sarat Chandra Yenisetti, Deposited in the *Drosophila* vivarium of Department of Zoology, University of Mysore, Manasagangatori, Mysore- 5700 006, India.

Allotype- Female: Same as above.

Paratype- 5♂♂ and 5♀♀, India, Nagaland, Lumami; Coll. Bovito Achumi and Sarat Chandra yenisetti.

DNA barcoding of *Drosophila hegdii:*

Phylogenetic relationship of the unidentified species was analyzed using mitochondrial cytochrome c oxidase I *(COI)* DNA sequence. The nucleotide sequences of cytochrome c oxidase subunit of *Drosophila hegdii* were submitted to GenBank (NCBI: National Centre for Biotechnology Information, Bethesda, USA). GenBank Accession nos of the new species: JX492316 and JX492317. The new species *COI* sequence data was compared with those of *D. jambulina* (GenBank accession No. AY737610.1); *D. vulcana* (courtesy: Dr. Maxi Polihronakis Richmand, *Drosophila* species stock center, University of California, San Diego, USA) and *D. melanogaster* (GenBank accession No. AF200846).

DNA sequences were edited and analyzed using MEGA 5 (Tamura *et al*. 2011). Phylogenetic trees and molecular evolutionary analyses were performed by the Neighbor-Joining (NJ) method with bootstrap test (1000 replicates) using the Kimura 2-parameter model, with gaps treated by pairwise deletion. The tree was drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed

for each sequence pair. There were a total of 898 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

A phylogenetic tree was constructed by applying the method of Neighbor-Joining Tree (NJ) (Figure 6) for *Drosophila hegdii, Drosophila jambulina*, *Drosophila vulcana* and *Drosophila melanogaster*. According to Tamura *et al.* (2004) *Melanogaster* and *Montium* species groups diverged from one another 41.3 Mya ago. The tree indicates *D. hegdii* and *D. jambulina* belong to the same cluster with strong boot strap support of 86. The ancestor of *D.vulcana* and *D. hegdii* clade was estimated to have appeared about 0.02296 Mya, the divergent between *D. jambulina* and *D. hegdii* was estimated to be 0.02223 Mya.

The number of base differences per site between sequences was shown in Table 2. The evolutionary divergences between *D. hegdii* and *D. jambulina* were 0.11, *D. hegdii* and *D. vulcana* was 0.057 and *D. hegdii* and *D. melanogaster* was 0.88. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (10000 replicates). The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 898 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

Maximum Likelihood Estimate of Substitution Matrix was shown in Table 3. Each entry is the probability of substitution (*r*) from one base (row) to another base (column). Substitution pattern and rates were estimated under the Tamura-Nei (1993) model. The nucleotide frequencies are A = 29.43%, T/U = 39.88%, C = 14.11%, and G = 16.57%.

Estimates of base composition bias difference between sequences were shown in Table 4. The difference in base composition bias per site was shown according to Kumar and Gadagkar (2001). It was observed that even when the substitution patterns are homogeneous among lineages, the compositional distance will correlate with the number of differences between sequences. The analysis involved 4 nucleotide sequences. Codon

positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 519 positions in the final dataset.

Table 1. Wing indices of *D. hegdii* (Mean value of 10 flies)

Sex	Costal index	4V index	4C index	5X index
Male	2.69	1.15	2.55	2.62
Female	2.81	1.1	2.75	2.5

Table 2. Estimates of Evolutionary Divergence between CO I sequence of *D. melanogaster, D. jambulina, D. vulcana* and *D. hegdii*

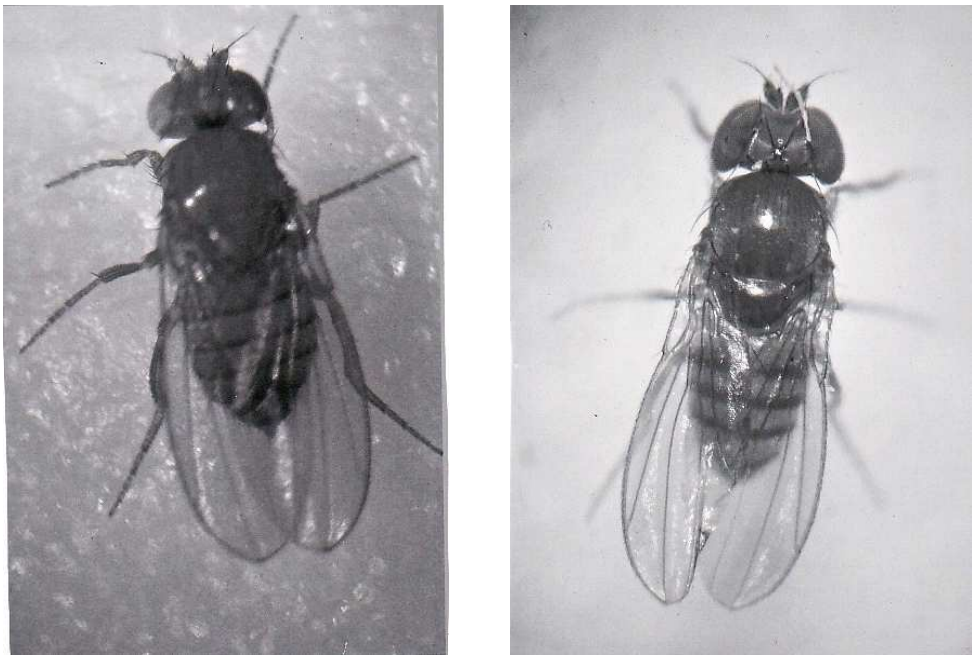
	1	2	3	4
1. Drosophila hegdii		0.004323728	0.010135592	0.011800173
2. Drosophila jambulina	0.011560694		0.009643225	0.011796287
3. Drosophila vulcana	0.057803468	0.050096339		0.013121109
4. Drosophila melanogaster	0.088631985	0.088631985	0.113680154	

Table 3. Maximum Likelihood Estimate of Substitution Matrix with reference to *D. hegdii, D. jambulina, D. vulcana and D. melanogaster*

	A	**T/U**	**C**	**G**
A	-	*10.98*	*3.88*	**6.63**
T/U	*8.10*	-	**6.94**	*4.56*
C	*8.10*	**19.62**	-	*4.56*
G	**11.77**	*10.98*	*3.88*	-

Table 4. Estimates of Base Composition Bias Difference between CO I sequence of *D. melanogaster*, *D. jambulina*, *D. vulcana* and *D. hegdii*

	1	2	3	4
1. Drosophila hegdii				
2. Drosophila jambulina	0.013487476			
3. Drosophila vulcana	0.109826590	0.129094412		
4. Drosophila melanogaster	0.025048170	0.025048170	0.050096339	



a) Female b) Male

Figure 1. Female and male *D. hegdii*

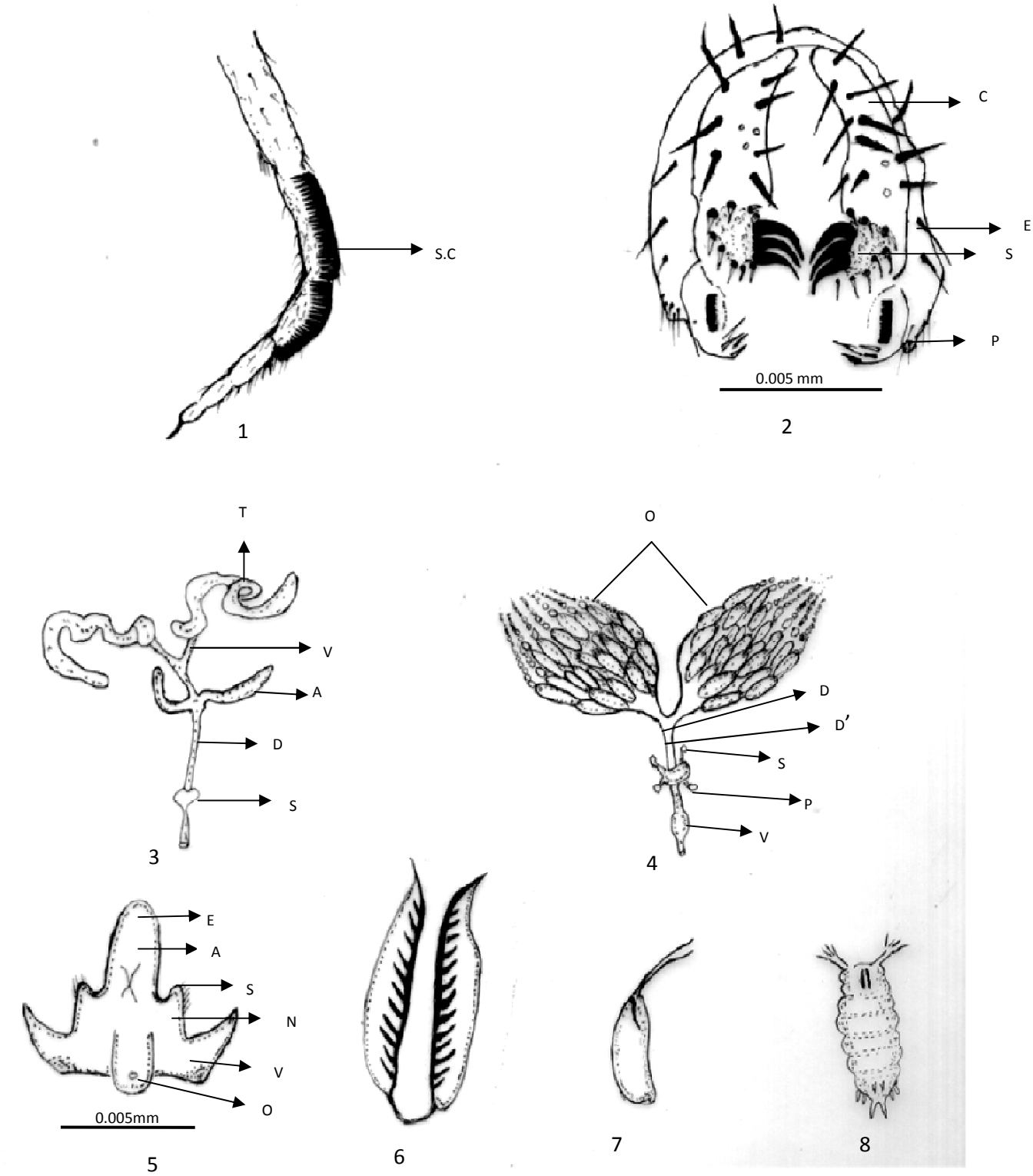


Figure 2. Morphological and anatomical characterestics of *Drosophila hegdii.* (1) S.C sex comb in first & second tarsal segment: (2) Periphallic Organ: E-Epandrium; P-Primary Claspers; S-Secondary Clasper; C-Anal Cercus; (3) Male reproductive system: T-Testis; V-Vasa deferentia; A-Paragonia; D-Anterior ejaculatory duct;S-Sperm pump; (4) Female reproductive system: O-Ovaries; D-Oviduct; D'-Common oviducts; S-Spermathecae; P-Paravaria; V-Vagina; (5) Phallic Organ: E-Edeagus; A-Anterior gonopophysis; S-Spines; N-Novasternum; O-Basal apodeme; V-Ventral fragma; (6) Egg guide; (7) Egg; (8) Pupa

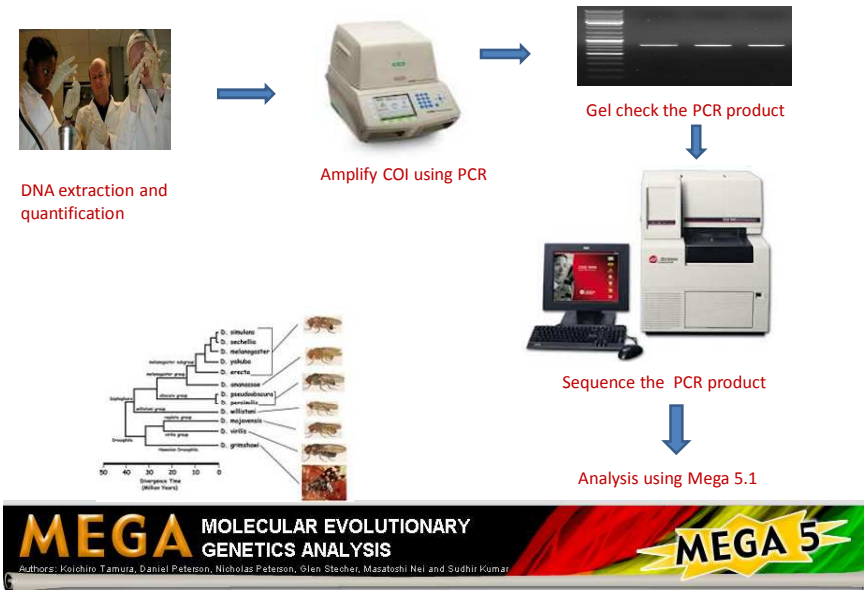


Figure 3. DNA Barcoding and Molecular Phylogeny of *Drosophila hegdii*

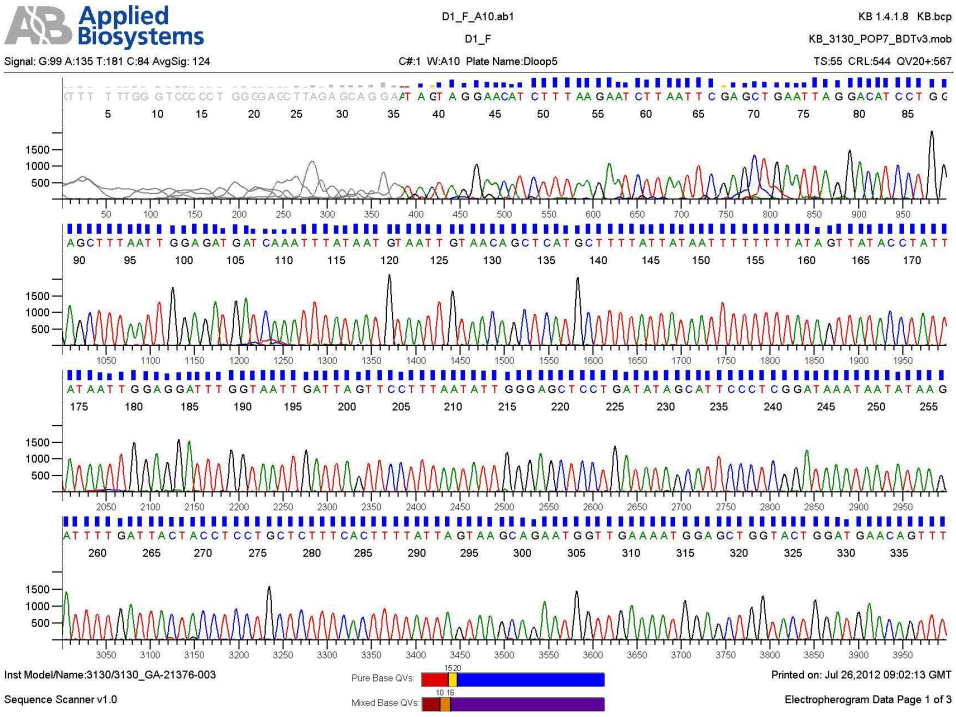


Figure 4. CO I sequence of *Drosophila hegdii*

Figure 5. Sequence alignment (using ClustalW) of *D. hegdii*, *D. jambulina*, *D. vulcana* and *D. melanogaster*

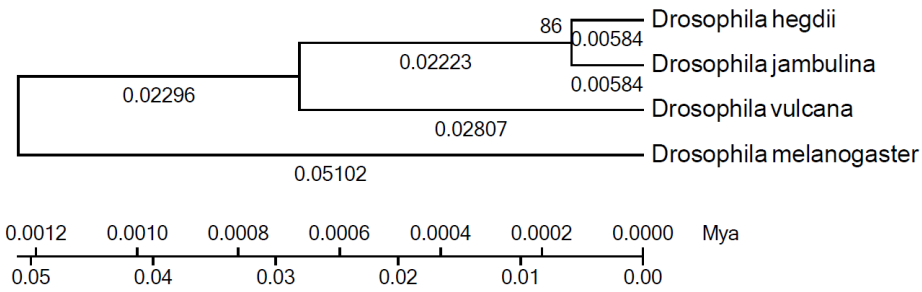


Figure 6. NJ tree inferred from the concatenated sequences of *D. melanogaster*, *D. jambulina*, *D. vulcana* and *D. hegdii*. It is estimated that *Melanogaster* and *Montium* species groups were diverged at 41.3 Mya (Time scale was given in Mya), which is considered as calibration point (Tamura *et al.* 2004). MEGA 5 was used for constructing the NJ tree (1000 replications; model: Kimura 2-parameter; gaps: treated by pairwise deletion)

DISCUSSION:

Taxonomic status/Phylogeny of *Drosophila hegdii* basing on morphological and molecular markers:

The nature of the banding pattern of the abdominal tergites, the presence of 2 egg filaments and the puparia warrant *Drosophila hegdii* inclusion in the subgenus *Sophophora*. The presence of long ventral receptacle, coiled testis, convergent scutellars and two pairs of malphigian tubules qualify its inclusion in the *melanogaster* species group (Patterson and Stone 1952). Further the presence of sex comb extending beyond the tips of the tarsal joint, the presence of primary claspers and secondary claspers with curved black teeth permit its inclusion in the *montium* sub group (Bock and Wheeler 1972).

Basing on the morphological markers, unidenfied species is recognised as a new species- *Drosophila hegdii* (Achumi *et al.* 2011). *Drosophila hegdii* resembles *D. vulcana* and *D. jambulina* in the general colouration of the body, but differed in other morphological characters such as the number of teeth in sex-combs, the nature of arrangement of teeth in the sex comb, the prominent teeth, sex comb extending beyond the tips of the tarsal joints, the prominent teeth in the secondary claspers, number of rows of acrostical hairs, wing indices, periphallic and phallic organ. In addition the new species differed from other known species of *montium* sub group in characters such as the number of teeth in sex comb, and abdominal banding pattern.

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species (Hebert 2003a). It differs from molecular phylogeny in that the main goal is not to determine patterns of relationship but to identify an unknown sample in terms of a pre-existing classification (Kress *et al.* 2005). The most commonly used barcode region, for animals, at least, is a segment of approximately 600 base paris of the mitochondrial gene cytochrome oxidase I *(COI).* DNA sequences are obtained for above mentioned marker and these sequences are compared to a DNA database to determine to which species or other taxonomic unit the specimen belongs. DNA barcoding is, in one form or another, widely used in conservation

genetics and molecular ecology (Duminil *et al.* 2006; Rubinoff 2006; Ward *et al*. 2008) but is also used in a number of other areas including forensic applications (Dawnay *et al.* 2007) and ancient DNA studies (Willerslev *et al*. 2007). It has often been associated with methods for delineating and defining species based on DNA evidence (Floyed *et al*. 2002; Hebert *et al.* 2003a; Remigio and Hebert 2003).

A final taxonomic system for the animal kingdom will probably include at least 10 million species partitioned among more than a million genera. Given such high diversity, there is a growing realization that it is critical to seek technological assistance for its initial description and its subsequent recognition (Godfray 2002; Blaxter 2003). Recent investigations have suggested the feasibility of creating identification systems reliant on the analysis of sequence diversity in small segments of DNA (Tauztz *et al*. 2003). Hebert *et al.* (2003a) proposed a DNA barcoding system for animal life that is based upon sequence diversity in cytochrome c oxidase subunit I *(COI).* They established that diversity in the amino acid sequences coded by the 5' section of this mitochondrial gene was sufficient to reliably place species into higher taxonomic categories (from phyla to orders). They also found that diversity in nucleotide sequences of the same gene region regularly permitted the inequity of closely allied species of lepidopterans, a group with modest rates of molecular evolution and high species diversity. As such, these insects provided a challenging test for the ability of *COI* diversity to resolve species boundaries (Hebert *et al.* 2003b).

In present study the extents of *COI* divergence for *Drosophila hegdii, Drosophila jambulina, Drosophila vulcana* and *Drosophila melanogaster* was examined by using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.05102 was shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) was shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. DNA barcoding based on standard markers (sequence) fragment of *COI* inferred phylogenetic tree based on the concatenated sequences of the four species support

the monophyly of the *melanogaster* complex. *Melanogaster* and *Montium* species groups diverged from one another 41.3 Mya ago (Tamura *et al.* 2004).

D. jambulina D. vulcana and *D. hegdii* belong to *montium* species subgroup of the *melanogaster* species group, and *D. melanogaster* belong to *melanogaster* species subgroup of the *melanogaster* species group. The tree indicates *D. hegdii* and *D. jambulina* belonging to the same cluster with strong boot strap support of 86. The ancestor of *D.vulcana* and *D.hegdii* clade was estimated to have appeared about 0.02296 Mya, the divergent between *D. jambulina* and *D. hegdii* was estimated to be 0.02223 Mya.

The number of base differences per site between sequences is shown in Table 2. The evolutionary divergence between *D. hegdii* and *D. jambulina* was 0.11, *D. hegdii* and *D. vulcana* was 0.057 and *D. hegdii* and *D. melanogaster* was 0.088. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (10000 replicates). The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 898 positions in the final dataset.

Substitution pattern and rates were estimated under the Tamura-Nei (1993) model (Table 3). Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in *italics*. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 100, the nucleotide frequencies are A = 29.43%, T/U = 39.88%, C = 14.11%, and G = 16.57%. The maximum Log likelihood for this computation was -1027.464. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 519 positions in the final dataset.

The base composition different between sequences per site showed *D. jambulina- D. hegdii* was 0.013, *D. vulcana-D.hegdii* was 0.109 and *D. melanogaster- D. hegdii* was 0.025.

Thus speciation order seems to be similar with the morphological differentiation among the three species by a diagnostic morphological characters such as the number of teeth in sex-combs, the nature of arrangement of teeth in the sex comb, the prominent teeth, sex comb extending beyond the tips of the tarsal joints, the prominent teeth in the secondary claspers, number of rows of acrostichal hairs, wing indices, periphallic and phallic organ. In addition the new species differed from other known species of *montium* subgroup in characters such as the number of teeth in sex comb, and abdominal banding pattern. The nature of the banding pattern of the abdominal tergites, the presence of 2 egg filaments and the puparia warrant its inclusion in the subgenus *Sophophora*. The presence of long ventral receptacle, coiled testis, convergent scutellars and two pairs of malphigian tubules qualify its inclusion in the *melanogaster* species group (Patterson and Stone 1952). Further the presence of sex comb extending beyond the tips of the tarsal joint, the presence of primary claspers and secondary claspers with curved black teeth permit its inclusion in the *montium* subgroup (Bock and Wheeler 1972). Molecular analysis confirms the observation made through morphological markers that *Drosophila hegdii* is a new species.

CHAPTER IV

INVERSION POLYMORPHISM AND ITS ADAPTIVE IMPLICATION IN NAGALAND POPULATIONS OF *DROSOPHILA ANANASSAE*

INTRODUCTION:

In every organism or a population of organisms there is a continued interaction between the genotype and the environment to attain a better homeostatic stability. This stability is achieved by several ways and one is by initiating change in the karyotype (Slavica *et al.* 2006).

Chromosomal rearrangements have been implicated in adaptation and speciation in a wide variety of taxa (Coghlan *et al.* 2005). In vertebrates, the role of chromosomal rearrangements in evolution has been studied for many decades, although most of the evidence is based on observations that closely related species differ in chromosome number or morphology (White 1978).

Two main requirements for natural selection to take place are that there must be variation within a species, and that this variation must be stably transmitted from the parents to the progeny (Darwin 1859). Unfortunately for Darwin, the underlying form of the heritable variation remained a mystery during his time. Dobzhansky found large rearrangements of *Drosophila pseudoobscura* chromosomes that varied between strains of the same species. Later he realized that these intraspecific differences in chromosomal rearrangements in *D. pseudoobscura* were the raw material for evolution to occur and populations to become adapted to their environment (Dobzhansky 1937).

The chromosomal changes, especially varied types of structural changes reported from natural populations of various organisms are in the form of paracentric inversions, pricentric inversions, translocations, duplications, deficiencies, and polyploidy. Though duplications and deficiencies do exist in several species of the genus *Drosophila*, they have not contributed significantly to the evolution of the genus (Wallace 1953).

Paracentric and pericentric inversions have been reported in several members of the genus *Drosophila* (Da Cunha 1955; Rajeshwari 1971; Reddy and Krishnamurthy 1972; Reddy 1973; Sperlich and Pfriem 1986; Krimbas and Powell 1992; Singh 2001; Bartolome and Charlesworth 2006; Mena 2009 and Font 2010). The paracentric inversions are by far the most common type found in various natural populations of different species. The pericentric inversions though are instrumental in shifting the position of centromers and thus responsible for the variation in karyotype, are rare (Alexander 1952; Pattersson and Stone 1952). This is because crossing over within them leads to formation of gametes with duplications and deficiencies resulting in lower fertility and reduced genetic recombination. In some they may be preserved and shielded from immediate elimination, if they are associated with paracentric inversions. Many species of *Drosophila* harbor a vast array of paracentric inversions as they are able to avoid the disadvantages due to the lack of crossing over in males and also due to the specific shunting of the dicentric amd acentric chromatids into the polar bodies in meiosis. It has been demonstrated by Swanson (1974) that in animals with long generation time, the special types of translocations called centric fusions and paracentric inversions have played a major role in restructuring the karytotypes, while the paracentric inversions due to their high adaptive values in heterozygous condition have been positively selected in the animals with ephemeral generations. Further paracentric inversions confer superior adaptive value by their integrated co-adapted gene complexes sheltered from recombination (Schaeffer *et al.* 2003; Munte *et al.* 2005).

Chromosomal inversions that result from two breakages and rejoining events in DNA are the agents of gene order change. Examination of the polytene chromosomes of *Drosophila* salivary glands provided the first glimpse into the structural mutations that alter the genome (Painter 1934). The results of the intensive investigations on the natural populations of *Drosophila* have brought to light, that several species are polymorphic for varied gene arrangements. This variability is mainly due to the inverted sections in the chromosomes. It has been shown that polymorphism for the inverted gene arrangements are one of the most efficient ways by which the carrier populations of the species adapt to the environmental vicissitudes (Brncic 1972).

There are two main hypotheses of how inversions increase in frequency in a population due to adaptation: 1) Dobzhansky's co-adaptation model (1970) proposed that loci within the inversion have epistatic effects on fitness. That is to say, combinations of loci are co-adapted and in combination generate higher fitness than expected from the sum of their independent effects. An inversion suppresses recombination with other chromosomal arrangements and therefore contributes to the maintenance of linkage disequilibrium among alleles. 2) Kirkpatrick and Barton (2006) proposed an alternative model that does not require loci within the inversion to have epistatic interactions. Under this model an inversion will be favored in a population if it contains two or more locally adapted alleles with additive effects on fitness. However, it is important to recognize the fact that these two models are not necessarily mutually exclusive adaptive mechanisms. Although the models differ theoretically, the underlying mechanisms are currently indistinguishable in terms of sequence variation.

There is considerable evidence for shifts in inversion frequencies in populations (Hoffmann and Rieseberg 2008). In humans, a rare inversion that affects fitness components has been identified: A 900-kb polymorphic inversion in chromosome 17 generated by non-allelic recombination between homologous sequences (NAHR) between complex blocks segmental duplications of 200-500 kb, is associated to a region of extended linkage disequilibrium that presents two main haplotypes that diverged as much as 3 million years ago. This inversion is found at a 20% frequency in European populations, while it is absent from African populations, a distribution consistent with the action of positive selection. In fact, in the Icelandic population, carrier females of the above mentioned inverted arrangement attributed to an increased fertility, with around 3% more children and have higher recombination rate than non- carriers (Stefansson *et al.* 2005).

Further, paracentric inversions have been detected in mammals, including humans, where connections to health have been made (Inayama *et al.* 1997; Levy *et al*. 2007; Korbel *et al.* 2007; Kidd *et al.* 2008; Antonacci *et al.* 2009; Durbin *et al.* 2010). In Mucopolyscchridosis type II Hunter syndrome (Bondeson *et al*. 1995) where recurrent inversions have been shown to lead to a disease phenotype is the disruption of the

idunorate 2-sulphatase gene. Microdeletion syndrome, a genetically characterized form of mental retardation harbours a 970 kb inversion polymorphism found at high frequency in European populations (Stefansson *et al.* 2005).

It has also been observed that chromosomal inversions are widespread among members of the *Anopheles gambiae sensu lato (s.l.)* complex of sibling species, some of which are the most important vectors of human malaria in sub- Saharan Africa (Coluzzi *et al.* 2002). In this complex, paracentric inversions are found both as fixed genetic markers differentiating the species and as floating polymorphisms within species. It has been hypothesized that inversion polymorphisms may be responsible for much of the adaptive ecological potential in this species complex (Pombi *et al.* 2008). The chromosomal polymorphisms with the largest geographical distribution are those involving inversions on the left and right arm of chromosome 2 (The 2La and 2Rb arrangements, respectively). These have been extensively studied in *A. gambiae sensu stricto (s.s.),* and found to correlate with factors such as aridity (Coluzzi *et al.* 1979; Powell *et al.* 1999). Brook et al (2002) showed that *A. gambiae* complex has two widespread paracentric inversions (2La and 2Rb) that were associated with dieldrin resistance (2La) and DDT resistance (2Rb) (Brooke *et al.* 2002).

In *Drosophila* model- chromosomal polymorphism as a result of inversions is worked out extensively in many species (Da Cunha 1960; Sperlich and Pfriem 1986; Singh 1996; Jayshankar 1998; Yadav and Singh 2003; Singh and Singh 2008, 2010a). It has been shown that degree of chromosomal variability varies in different species and also in different populations of the same species (Da Cunha 1960; Dobzhansky 1970).

Few workers tried to understand chromosomal polymorphism as a result of paracentric inversions and their role in adaptation using *Drosophila* model (Dobzhansky 1970; Sperlich and Pfriem 1986; Hoffmann *et al.* 2004; Soto *et al*. 2010). In *Drosophila* it has been shown that clines for chromosomal rearrangements are a manifestation of local adaptation (Hoffmann and Rieseberg 2008). Clines for chromosomal inversions are often interpreted in terms of climatic selection (Lee *et al.* 2002). However, the selective factors involved are poorly understood. Latitudinal clines for inversion frequencies are repeated

in different hemispheres and continents (Krimbas and Powell 1992). For example, *Drosophila subobscura* originated in Europe, where there are documented clines for inversions (Sole *et al*. 2002). Inversion clines that mirror the European clines have been established along the west coasts of South and North America (Prevosti *et al.* 1988). In *D. melanogaster* regular gene clines in the frequencies of some allozymes and four common cosmopolitan inversions have been reported from several parts of the world (Lemeunier *et al.* 1986).

In natural populations of India the mean number of inversions in *Drosophila nasuta*, showed geographical and altitudinal clines. Further, heterokaryotypes usually represent more than fifty per cent of individuals in these populations. Thus, *D. nasuta* represents an example of "flexible polymorphism'' with heteroselection (Ranganath and Krishnamurthy 1975, 1978). Contrary to the above mentioned observation, in natural populations of *Drosophila bipectinata* (Gupta and Panigrahy 1990) inversions occur at low frequency and there is no evidence for geographic differentiation which lends support for "rigid inversion polymorphism". Reddy and Krishnamurthy (1974) detected altitudinal clines in the natural population of southwestern India in *D. ananassae* with respect to inversion polymorphism. Das and Singh (1991) also detected a similar pattern of inversion clines in different populations of *D. melanogaster* from India.

Temperature is one of the most important variables that determines distribution and abundance of species (Cossins and Bowler 1987). *Drosophila* is a widely used and well suited model system for studying evolutionary responses to extreme temperatures (Maynard-Smith 1956; Hollingsworth and Bowler 1966; David *et al.* 1983; Hoffmann and Parsons, 1991). Change in inversion frequency related to the direct or indirect effects of temperature shifts due to global warming has been reported for *D. subobscura (*Balanya *et al*. 2006).

Drosophila ananassae, a member of the *ananassae* species complex of the *ananassae* subgroup of the *melanogaster* species group (Bock and Wheeler 1972). It shows high degree of chromosomal polymorphism (Singh 1996). In tropical and sub-tropical regions

of the world, *D. ananassae* is one of the most common species, especially in and around places of human habitations and appears to qualify as a polytypic species (Tobari 1993). It occupies a unique status in the whole genus of *Drosophila* due to certain peculiarities in its genetical behavior such as viability, high mutability and segregation distortion (Singh 2000). *D. ananassae* presents a high degree of chromosomal polymorphism and a total of 78 paracentric inversions are known to occur in its natural populations. However, only three paracentric inversions (2LA, 3LA and 3RA) which have been called cosmopolitan inversion by (Futch 1966) have become coextensive with the species.

Drosophila ananassae has been investigated extensively for its inversion polymorphism. It carries three well knit co-extensive inversions namely 2LA on the left arm of the 2nd chromosome, 3LA on the left arm of the 3rd chromosome and 3RA of the right arm of the 3rd chromosome. Various populations of *Drosophila ananassae* have been variedly studied with special reference to their chromosomes and their genetic polymorphism and their adaptive significance (Kaufman 1936; Kikkawa 1938; Dobzhansky and Dreyfus 1943; Shirai and Moriwaki 1952; Ohinishi and Nakajima 1956; Freire-Maia 1961; Carson 1965; Futch 1966; Siddaveers Gowda and Krishnamurthy 1971; Sajjan and Krishnamurthy 1972; Hinton and downs 1975; Sperlich and Pfriem 1986; Singh 1988, 1989, 1998, 2010; Singh and Anand 1995; Powel 1997; Yadav and Singh 2003; Singh and Singh 2007, 2008, 2010a and b).

Studies on chromosomal polymorphism in Indian populations of *D. ananassae* were initiated by Ray-Chaudhuri and Jha (1967). Since then, a number of investigations on chromosomal polymorphism in Indian populations of *D. ananassae* have been carried out (Singh and Singh 1988, 1989, 2007, 2008; Singh 1972, 1974, 1982, 1983, 1988, 1996, 2000, 2001, 2010). Quantitative data on the frequencies of three cosmopolitan inversions in Indian natural populations of *D. ananassae* shows that there are significant variations in the frequencies of these inversions (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 (Singh 1996; Singh and Singh 2007). These three inversions often persist in laboratory populations due to

heterotic buffering associated with these inversions (Singh and Ray- Chaudhuri 1972; Singh 1982) and their frequencies may change due to random genetic drift (Singh 1998).

Population dynamics of inversion polymorphism with particular reference to three cosmopolitan inversions in Indian populations of *D. ananassae* has been extensively studied by Singh (2010) and he suggested that polymorphic inversions in *D. ananassae* have adaptive role by influencing the body size by different mechanisms like dominance and epistatic interaction in different populations. There are few studies concerning the relation between inversion polymorphism and morphometric traits. Singh and Mathew (1996) have presented evidence in *D. ananassae* that high number of sternopleural bristles is highly correlated with standard (ST) (2L), ST (2R) ZE (2R) and ST (3L) chromosome arrangements and low number of bristles is correlated with alpha (AL) (2L), ST (2R) and delta (DE) (3R). Das *et al.* (1994) studied on inversion polymorphism and extra bristles in Indian population of *D. ananassae.* Three commonly occurring inversions were found in the populations with varying frequencies as the number of individuals with extra bristles. Female individuals were more often found to carry extra scutellar bristles than were males. This result reveals that polygenic loci responsible for the determination of extra bristles are widespread in east coastal region of India natural populations of *D. ananassae*. A significant positive correlation between the inversion frequency and the number of individuals with extra bristles was detected in the isofemale lines of all the five populations. Bhubaneswar, Cuttack, Ratnagiri, Balasore and Howrah localities of east coastal region of India. The 2L inversion was found to be closely associated with individuals with the extra bristles phenotype. These findings provide evidence that a significant gene activity affecting bristle number is present in both major autosomes (II and III) of *D. ananassae.*

Geographically, Nagaland state lies between 26º 60’ N and 27º40’ N latitude and 93º20’ E and 95º15’ E longitude. It is located in the north eastern part of India and having an area of about 16,579 sq. Kilometers. Nagaland is popular for the fact that its climate remains salubrious throughout the year. Annual average rainfall varies from 175 cm to 250 cm. Temperature varies from 4ºC to 31ºC. Nagaland is one of the ‘Eight Sister’ states of northeast India situated in the north eastern part of India. It is bordered by the state of

Assam in the west, by the state of Arunachal Pradesh and part of Assam towards the north, on the east by the country of Burma and by the state of Manipur on towards the south. The mountainous slopes of the state of Nagaland is rich in the growth of natural vegetation the state is covered with the evergreen tropical and sub tropical forest that are endowed with rich flora and fauna.

In spite of existence of such an interesting ecogeographical conditions in this sub-Himalayan hilly state of north eastern part of the diverse Indian subcontinent, absolutely no effort was made to understand the interaction between genomic rearrangements and their adaptive role using *Drosophila ananassae* as a model system.

Present study aims at exploring the extent of inversion polymorphism and its possible role in adaptation. Study areas include eleven localities (all 11district headquarters) viz. Dimapur, Kiphire, Kohima, Longleng, Mon Mokokchung, Peren, Phek, Tuensang, Wokha, and Zunheboto.

MATERIALS AND METHODS:

Geographic locations from where *Drosophila ananassae* populations were collected depicted in Fig 1. Flies were collected from domestic localities.

Single gravid females of *D. ananassae* collected from different localities were isolated and placed individually in separate vials containing the culture medium (Wheat cream, agar and Jaggery) seeded with yeast. Minimum 20 females from each collection were kept individually in a fresh food vial and F1 larva was squashed by lacto-aceto-orcein method. Chromosomal analyses of larvae were performed by taking 5 larvae from each vial (20 x 5=100). Larvae from each of these vials constituted the material for the present study on inversion polymorphism.

The larvae were dissected in 0.7% sodium chloride solution (invertebrate physiological saline). Salivary glands were removed and fixed in 1N HCL for 2-3 minutes. Then glands were transferred to 2% lacto-aceto orcein stain and left for 30-40 minutes. The stained glands were then placed on a clean slide with two drops of 45% acetic acid and squashed gently by placing a clean cover glass. By applying pressure on the glands uniform spreading of the chromosomes was achieved. Immediately after squashing, the edges of the cover slip were sealed with paraffin lanolin mixture. The slides were observed for the inversions if any under low (10X) and high (40x) magnification with Olympus microscope MLX- B.

OBSERVATIONS:

Geographic variability in multiple inversion frequencies:

Figure 1. Shows location of all districts of Nagaland and frequencies of three cosmopolitan inversions (2LA, 3LA and 3RA) in each population.

It has been observed that there are three paracentric inversions which are frequently encountered in natural populations. These are 2LA-located on the left arm of the 2nd chromosome (Fig. 3); 3LA- located on the left arm of the 3rd chromosome (Fig. 4) and 3RA- located on the right arm of the 3rd chromosome (Fig.5). Inversions do exist in combinations. In present study 2LA inversion observed in combination with 3LA (Fig.6) and 3LA inversion in combination with 3RA (Fig.7). The populations under study revealed the presence of a total of three cosmopolitan inversions- 2LA, 3LA, 3RA and combination of 2LA and 3LA and 3LA and 3RA.

Table 1 shows the place of collection along with their altitudes and the various chromosomal rearrangements and Figure 8 depicts multiple inversion frequencies as encountered in eleven populations of *D. ananassae* in Nagaland. The three coextensive inversions are highly variable with regard to their occurrence, distribution and frequencies in the populations under study. Multiple paracentric inversions and their frequencies in different natural populations of Nagaland are as following: The population at an altitude of 260 meters (Dimapur) has shown 54% of 2LA, 16% of 3LA and 11% of 3RA. Population at 896 meters (Kiphire) has shown 48% of 2LA, 21% of 3LA and 7% of 3RA. Population at 897 meters (Mon) has shown 21% of 2LA, 13% of 3LA and 3% of 3RA. While population at 1,066 meters (Longleng) reveals 26% of 2LA, 15% of 3LA and 8% 3RA. Population at an altitude of 1,313 meters (Wokha) has shown 41% of 2LA, 12% of 3LA and 4% of 3RA. The population at an altitude of 1,325 meters (Mokokchung) has shown 36% of 2LA, 17% of 3LA and 15% of 3RA. Population at 1,371 meters (Tuensang) has

shown 35% of 2LA, 17% of 3RA and 5% of 3RA. Population at an altitude of 1,444 meters (Kohima) has revealed 32% of 2LA, 28% of 3LA and 12% of 3RA. The population at an altitude of 1,445 meters (Peren) has shown 47% of 2LA, 18% of 3LA and 9% of 3RA. While population at an altitude of 1,524 meters (Phek) has shown 41% of 2LA, 12% of 3LA and 1% of 3RA. Population at an altitude of 1,874 meters (Zunheboto) has shown 27% of 2LA, 22% of 3LA and 13% of 3RA.

Three cosmopolitan inversions are not only present independently; but also in combination (double heterokaryotypes). Six out of eleven populations (Dimapur, Kohima, Longleng, Peren, Tuensang and Wokha) exhibit double heterokaryotypes (2LA and 3LA, 3LA and 3RA). The combination of 2LA and 3LA was found (3.33%) in three (Dimapur, Kohima and Tuensang) out of eleven populations, while 3LA and 3RA combination was observed (2.25%) in four (Dimapur Longleng Peren and Wokha) out of eleven populations. The 2LA and 3LA combination of double heterokaryotype was present in 1% in Dimapur population; 3% in Kohima population; 6% in Tuensang population. The 3LA and 3RA combination of double heterokaryotype was present in 4% of Longleng population; 2% in Wokha population; 2% in Dimapur population and 1% in Peren population. Only in Dimapur Population both the combinations (2LA and 3LA; 3LA and 3RA) were observed.

Table 1 also incorporates the data on percentage of heterokaryotypes, number of inversions in each population, mean number of heterozygotes per individual and percentage of heterozygotes. The polymorphic grade was remarkably high in Dimapur population with 81% heterokaryotypes having 0.81 mean inversion heterozygotes. Mon population was least polymorphic with 37% heterokaryotypes having 0.37 mean heterozygotes per individual. The remaining nine populations from Longleng, Phek, Tuensang, Mokokchung, Wokha, Zunheboto, Kohima, Peren and Kiphire are intermediate, ranging from 49% to 76% hetero- karyotypes with 0.49 to 0.76% mean hetero-karyotypes per individual.

Chi-square test was applied to determine whether there is a statistically significant difference between populations and among (2LA, 3LA and 3RA) inversion frequencies

(Table 2). The populations that exhibit the three common inversions frequencies were computed for the X^2 homogeneity test. Results show that the differences in the frequencies of multiple inversions exhibited by different populations were found to be significant ($p < 0.05$), indicating that the populations under study are distinct from one another with regard to the degree of variability.

In order to understand the influence of multiple eco-geographical factors such as altitude, humidity, rainfall and temperature on inversion frequencies; patterns of variation in inversion frequencies were examined by means of correlation analysis using SPSS 16.0. Inversion frequencies (2LA, 3LA and 3RA) as dependent variables on climatic variables (humidity, rainfall and temperature) and geographical variables (altitude) as independent variables (Table 3-6).

Results reveal lack of significant correlation between presence of multiple inversions and certain climatic (humidity) and geographical (altitude) variables. However, significant correlation exists with reference to certain climatic indicators such as rainfall (negative correlation) and -temperature (positive correlation).

Results point out existence of significant correlation between presence of 2LA and climatic variable such as temperature (positive correlation). However there exists no correlation between presence of 2LA and certain climatic and geographical variables (humidity and altitude).

Results reveal lack of significant correlation between presence of 3LA inversion and observed climatic (humidity, temperature and rainfall) and geographical (altitude) variables. Results further reveal lack of significant correlation between presence of 3RA inversion and climatic (humidity, temperature and rainfall) and geographical (altitude) variables.

Table 1. Altitudinal variations in the frequencies of various inversions arrangements as found individually and in combinations in 11 different populations of *D. ananassae* of Nagaland

Populations	Altitudes in meters	No. of Larva examined	No. of Larva with inversion	No. of Larva without inversion						Total and frequency of heterozygotes			Mean No. of heterozygotes per individual	% of heterozygotes
					2LA	3LA	3RA	2LA+3LA	3LA+3RA	2LA%	3LA%	3RA%		
Dimapur	260	100	81	19	53	13	9	1	2	54	16	11	0.81	81
Kiphire	896	100	76	24	48	21	7	--	--	48	21	7	0.76	76
Mon	897	100	37	63	21	13	3	--	--	21	13	3	0.37	37
Longleng	1,066	100	49	51	26	11	4	--	4	26	15	8	0.49	49
Wokha	1,313	100	57	43	41	10	2	--	2	41	12	4	0.57	57
Mokokchung	1,325	100	68	32	36	17	15	--	--	36	17	15	0.68	68
Tuensang	1,371	100	57	43	29	11	5	6	--	35	17	5	0.57	57
Kohima	1,444	100	72	28	29	25	12	3	--	32	28	12	0.72	72
Peren	1,445	100	74	26	47	17	8	--	1	47	18	9	0.74	74
Phek	1,524	100	54	46	41	12	1	--	--	41	12	1	0.54	54
Zunheboto	1,874	100	62	38	27	22	13	--	--	27	22	13	0.62	62

Table 2. Observed, expected and Chi- square values of 2LA, 3LA and 3RA inversion heterozygotes of *Drosophila ananassae*

Populations		**2LA**	**3LA**	**3RA**
Mon	Obs.	21	13	3
	Exp.	21.97	10.28	4.73
Longleng	Obs.	26	15	8
	Exp.	29.1	13.62	6.27
Tuensang	Obs.	35	17	5
	Exp.	33.85	15.84	7.3
Kiphire	Obs.	48	21	7
	Exp.	45.13	21.12	9.73
Zunheboto	Obs.	27	22	13
	Exp.	36.82	17.23	7.94
kohima	Obs.	32	28	12
	Exp.	42.75	20.01	9.22
Phek	Obs.	41	12	1
	Exp.	32.06	15.01	6.91
Peren	Obs.	47	18	9
	Exp.	43.94	20.57	9.47
Mokokchung	Obs.	36	17	15
	Exp.	21.37	18.9	8.71
Wokha	Obs.	41	12	4
	Exp.	33.85	15.84	7.3
Dimapur	Obs.	54	16	11
	Exp.	48.1	22.51	10.37
Total		20.857	9.379	18.018

X^2 = 48.2 df= 20 $P < 0.05$

Table 3. Correlation analysis of inversion frequencies on climatic variables and Geographical variables

Correlations						
		Altitude	Humidity	Rainfall	Temperature	% of Heterozygotes
Altitude	Pearson Correlation	1	-.667*	0.08	-.719*	-0.193
	Sig. (2-tailed)		0.025	0.814	0.013	0.569
	N	11	11	11	11	11
Humidity	Pearson Correlation	-.667*	1	-0.242	0.403	0.226
	Sig. (2-tailed)	0.025		0.473	0.22	0.504
	N	11	11	11	11	11
Rainfall	Pearson Correlation	0.08	-0.242	1	-0.397	-.607*
	Sig. (2-tailed)	0.814	0.473		0.226	0.048
	N	11	11	11	11	11
Temperature	Pearson Correlation	-.719*	0.403	-0.397	1	.644*
	Sig. (2-tailed)	0.013	0.22	0.226		0.033
	N	11	11	11	11	11
% of Heterozygotes	Pearson Correlation	-0.193	0.226	-.607*	.644*	1
	Sig. (2-tailed)	0.569	0.504	0.048	0.033	
	N	11	11	11	11	11
*. Correlation is significant at the 0.05 level (2-tailed).						

Table 4. Correlation analysis of inversion frequencies of 2LA on climatic variables and geographical variables

Correlations						
		Altitude	Temperature	Humidity	Rainfall	2LA
Altitude	Pearson Correlation	1	-.719*	-.667*	0.08	-0.422
	Sig. (2-tailed)		0.013	0.025	0.814	0.196
	N	11	11	11	11	11
Temperature	Pearson Correlation	-.719*	1	0.403	-0.397	.685*
	Sig. (2-tailed)	0.013		0.22	0.226	0.02
	N	11	11	11	11	11
Humidity	Pearson Correlation	-.667*	0.403	1	-0.242	0.31
	Sig. (2-tailed)	0.025	0.22		0.473	0.354
	N	11	11	11	11	11
Rainfall	Pearson Correlation	0.08	-0.397	-0.242	1	-.585*
	Sig. (2-tailed)	0.814	0.226	0.473		0.05
	N	11	11	11	11	11
2LA	Pearson Correlation	-0.422	.685*	0.31	-0.585	1
	Sig. (2-tailed)	0.196	0.02	0.354	0.059	
	N	11	11	11	11	11
*. Correlation is significant at the 0.05 level (2-tailed).						

Table 5. Correlation analysis of inversion frequencies of 3LA on climatic variables and geographical variables

Correlations						
		Altitude	Humidity	Rainfall	Temperature	3LA
Altitude	Pearson Correlation	1	-.667*	0.08	-.719*	0.251
	Sig. (2-tailed)		0.025	0.814	0.013	0.457
	N	11	11	11	11	11
Humidity	Pearson Correlation	-.667*	1	-0.242	0.403	0.027
	Sig. (2-tailed)	0.025		0.473	0.22	0.937
	N	11	11	11	11	11
Rainfall	Pearson Correlation	0.08	-0.242	1	-0.397	-0.072
	Sig. (2-tailed)	0.814	0.473		0.226	0.833
	N	11	11	11	11	11
3LA	Pearson Correlation	0.251	0.027	-0.072	0.039	1
	Sig. (2-tailed)	0.457	0.937	0.833	0.909	
	N	11	11	11	11	11
Temperature	Pearson Correlation	-.719*	0.403	-0.397	1	0.039
	Sig. (2-tailed)	0.013	0.22	0.226		0.909
	N	11	11	11	11	11
*. Correlation is significant at the 0.05 level (2-tailed).						

Table 6. Correlation analysis of inversion frequencies of 3RA on climatic variables and geographical variables

Correlations							
		Altitude	Humidity	Rainfall	Temperature		3RA
Altitude	Pearson Correlation	1	-.667*	0.08	-.719*		0.066
	Sig. (2-tailed)		0.025	0.814	0.013		0.847
	N	11	11	11	11		11
Humidity	Pearson Correlation	-.667*	1	-0.242	0.403		-0.005
	Sig. (2-tailed)	0.025		0.473	0.22		0.989
	N	11	11	11	11		11
Rainfall	Pearson Correlation	0.08	-0.242	1	-0.397		-0.391
	Sig. (2-tailed)	0.814	0.473		0.226		0.234
	N	11	11	11	11		11
Temperature	Pearson Correlation	-.719*	0.403	-0.397	1		0.36
	Sig. (2-tailed)	0.013	0.22	0.226			0.277
	N	11	11	11	11		11
3RA	Pearson Correlation	0.066	-0.005	-0.391	0.36		1
	Sig. (2-tailed)	0.847	0.989	0.234	0.277		
	N	11	11	11	11		11

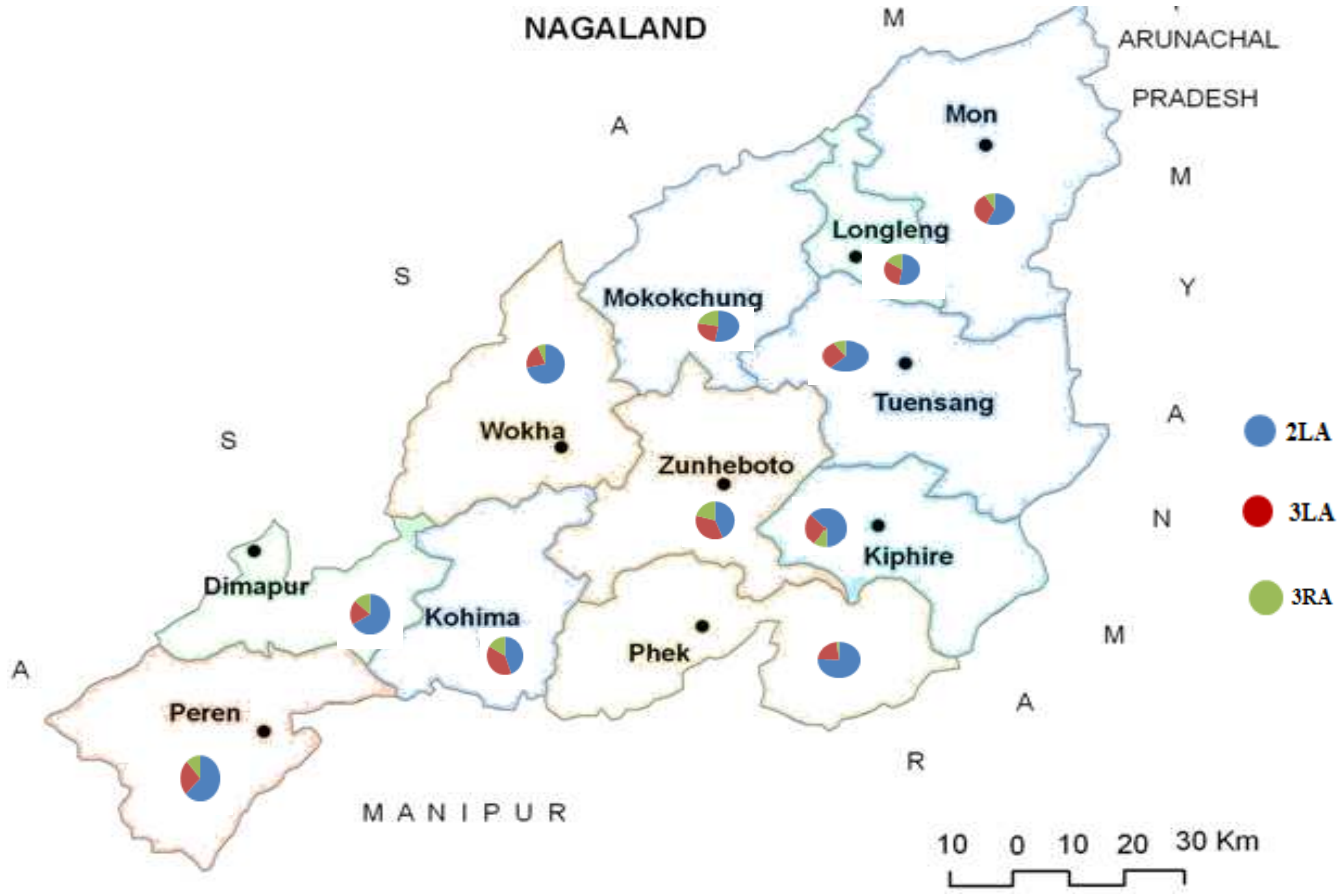


Figure1. Map of Nagaland showing the localities of collections of *Drosophila ananassae*

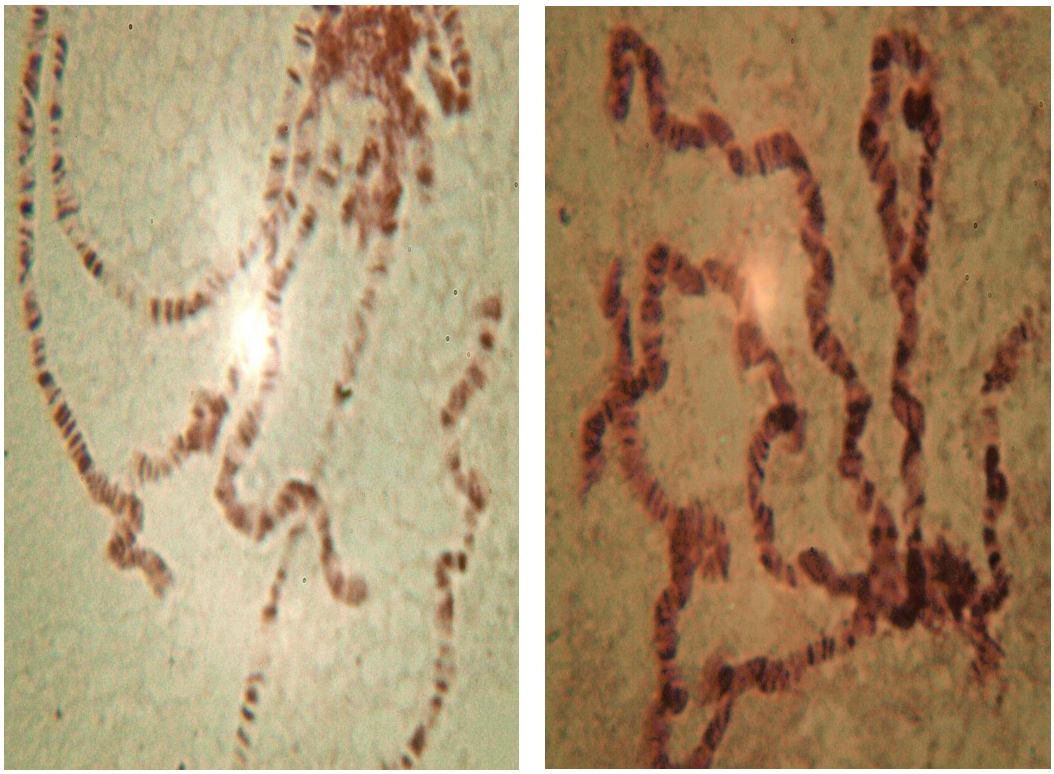


Figure 2. Arms of the chromosome showing without inversion and with inversion in *Drosophila ananassae*

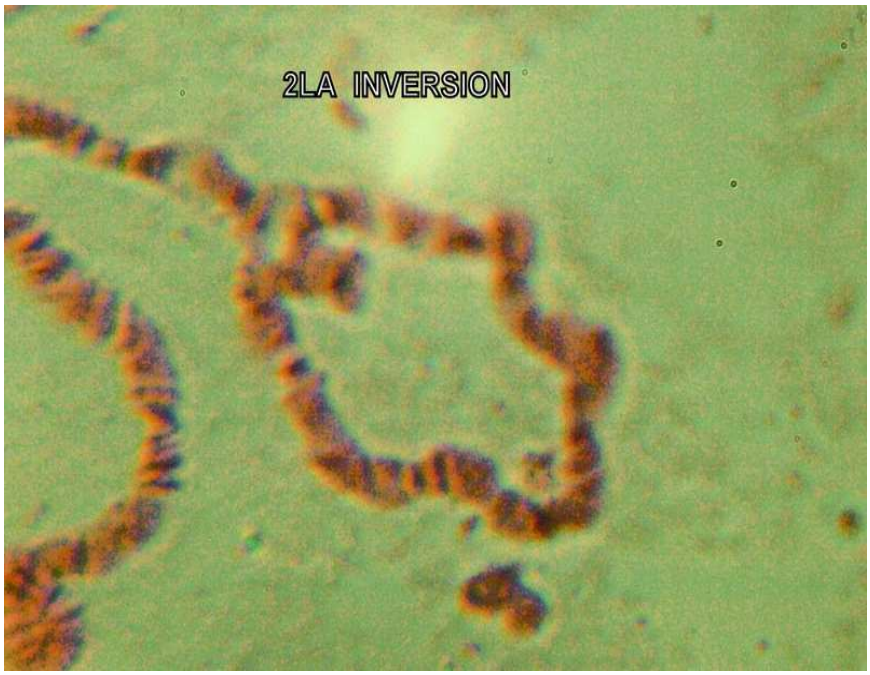


Figure 3. Inversion 2LA

Figure 4. Inversion 3LA

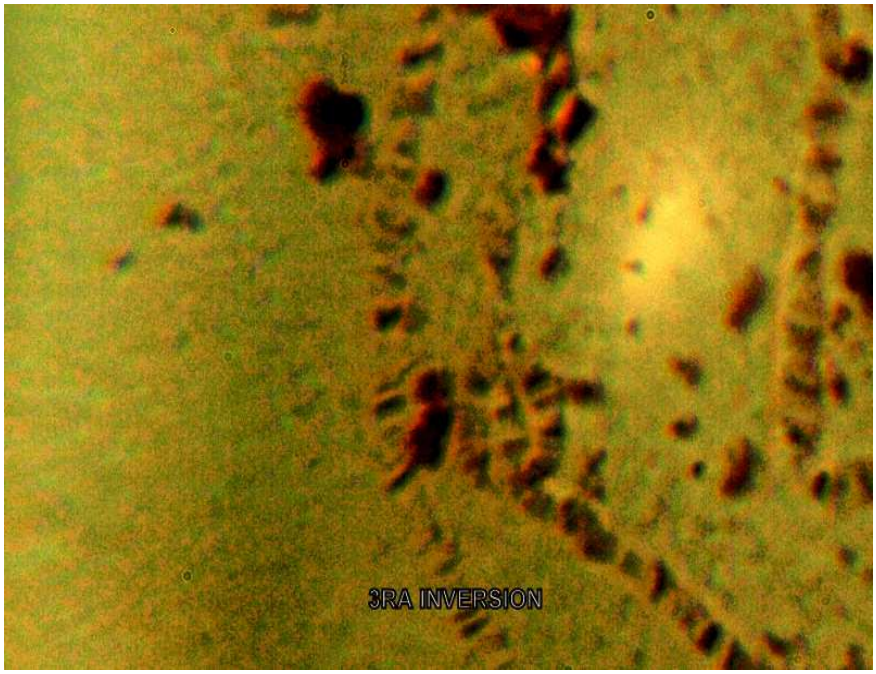


Figure 5. Inversion 3RA

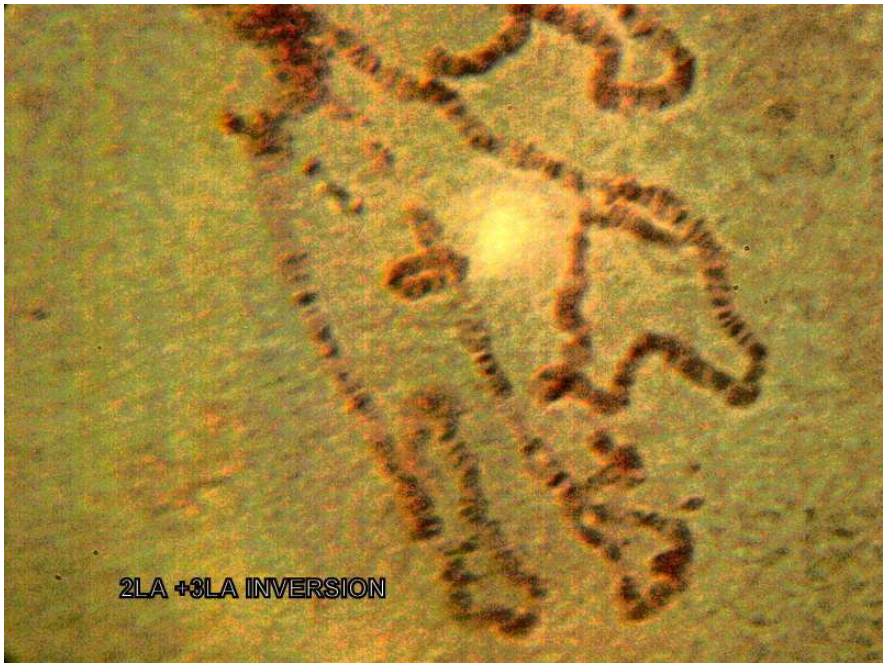


Figure 6. Inversion 2LA+3LA

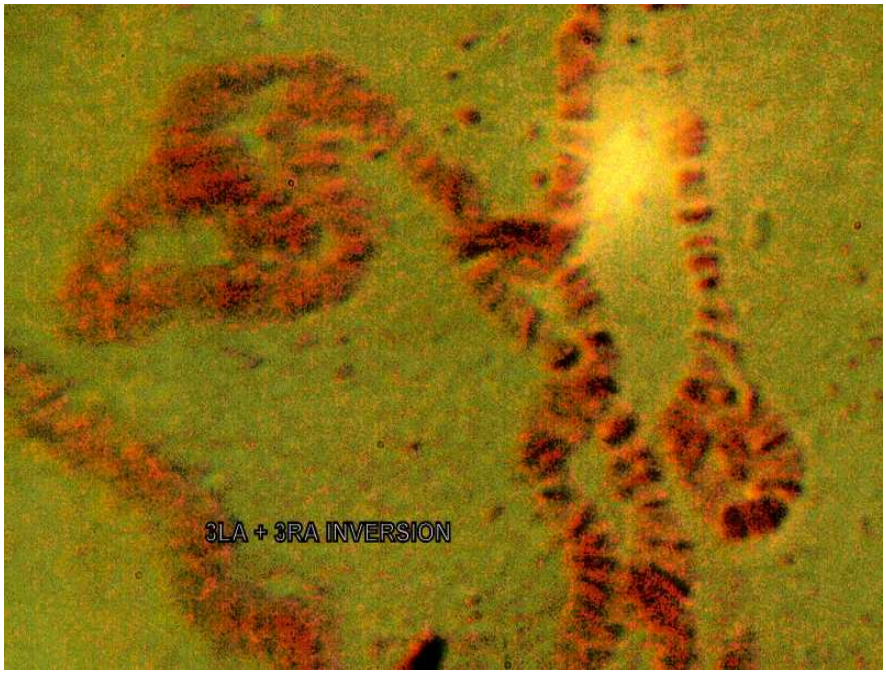


Figure 7. Inversion 3LA+3RA

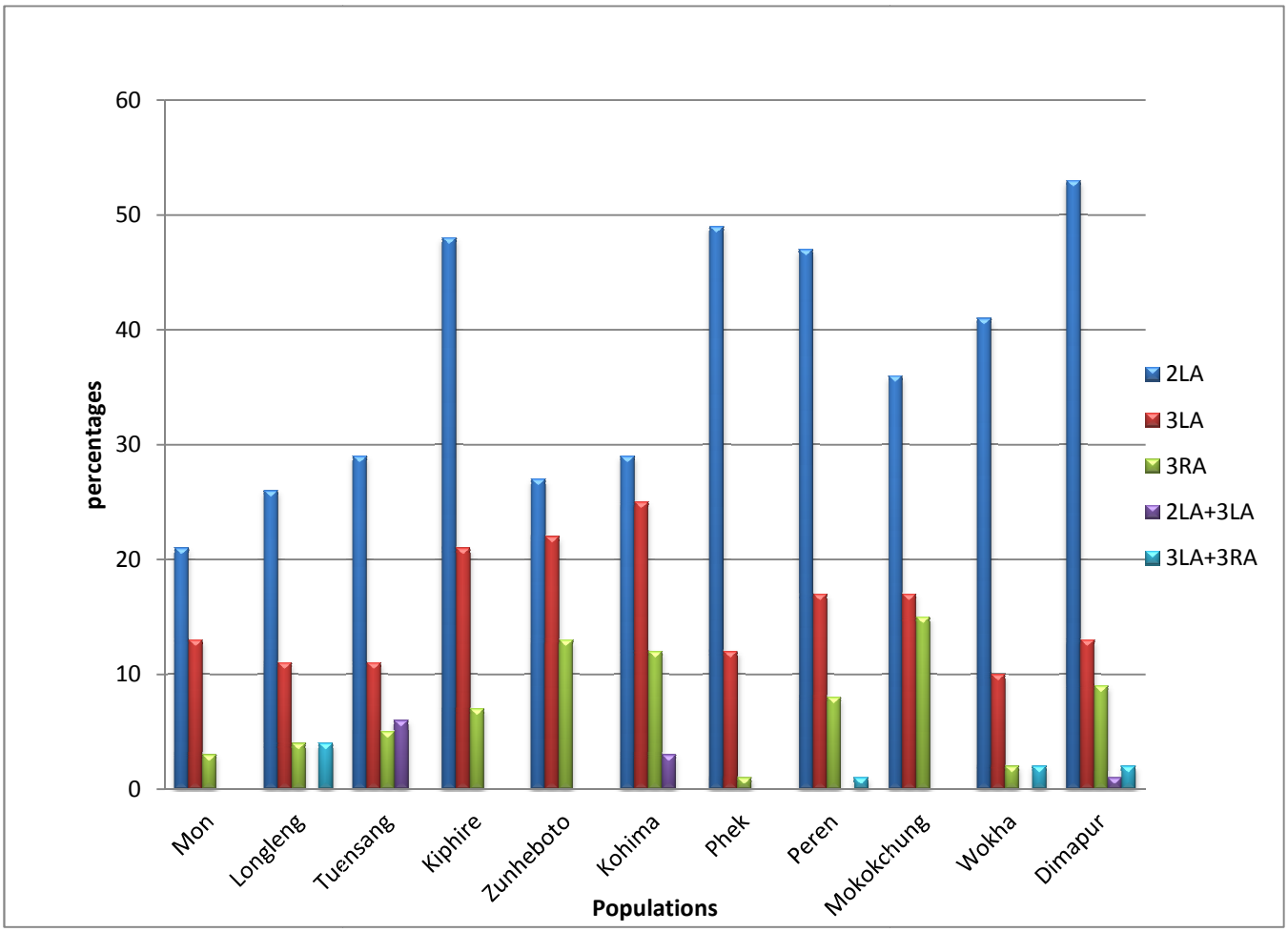


Figure 8. Five different inversions and their frequencies as found in 11 populations of *Drosophila ananassae*

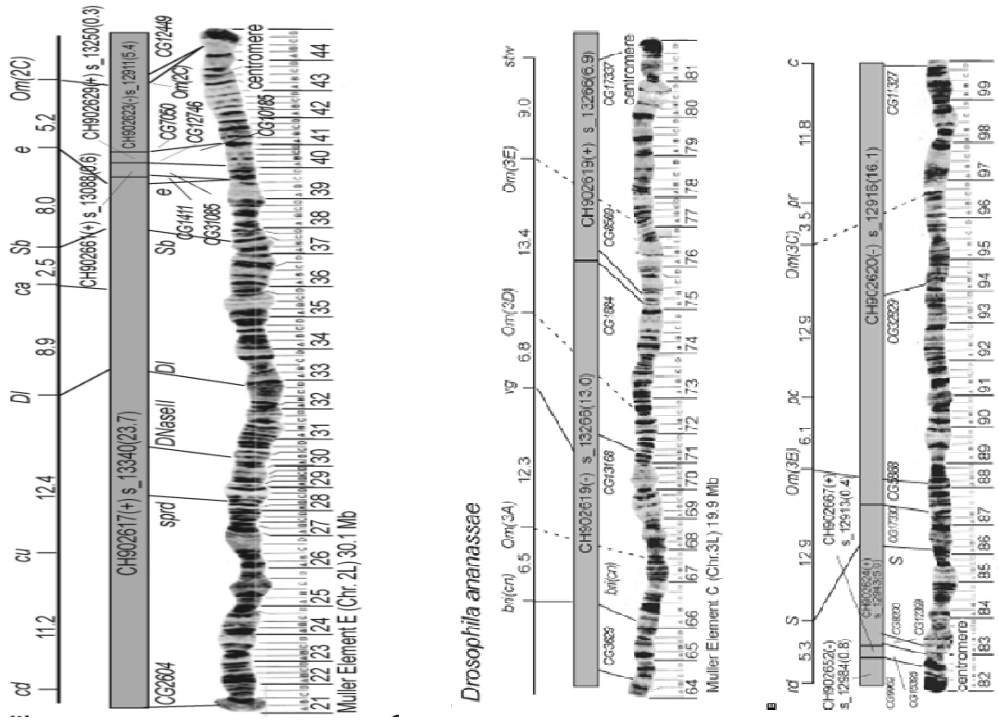


Figure 9. Photomap of the Salivary gland chromosomes of *Drosophila ananassae.* (Adapted from Tobari 1993)

DISCUSSION:

Various animal populations are known to exhibit both concealed genetic variability as well as chromosomal variability (Kaufmann 1936; Kikkawa 1938; Dobzhansky and Dreyfus 1943; Freire-Maia 1961; Ray Chaudhuri and Jha 1965, 1967; Rajeswari and Krishnamurthy 1969; Sajjan and Krishnamurthy 1970; Siddaveere Gowda and Krishnamurthy 1971; Singh *et al*. 1971; Reddy 1973; Ashburner and Lemeuner 1975; Prakash 1979; Singh and Das 1991; Lemeuner and Aulard 1992; Jayshankar 1998; Rieseberg 2001; Hoffmann *et al.* 2004; Vanoore and Kirkapatrick 2007; and Diego *et al*. 2011). While both these are considered as important raw materials of evolution, the potential adaptive ability of a genotype to variable environment, changes in space and time determines the structure, composition as well as the survival (Dobzhansky 1970; Coluzzi *et al.* 1985; Sperlich and Pfriem 1986; Hoffmann *et al.* 2004; Hoffmann and Willi 2008; Soto *et al*. 2010). Genetic polymorphism as such is one of the most efficient ways by which the populations of a species adapt by interacting with evolutionary factors. Such heterogeneous population can utilize many ecological opportunities and can exploit the environment much more successfully than a homogeneous population (Moriwaki *et al.* 1956; Moriwaki and Tobari 1963; Ray chaudhuri 1972; Ranganath and Krishnamurthy 1978; Singh 1982; Singh and Singh 2008). Da Cunha (1955) has stated that a polymorphic population being genetically plastic is also able to respond to temporal changes of the environment than a homogeneous population. This is further supported by Dobzhansky and Wallace (1953) who have stated that heterogeneous individuals have a better homeostasis than the homozygous ones.

Perusal of literature has shown that the paracentric inversions in *Drosophila* have played a major role in adaptation (Prevosti *et al.* 1988; Krimbas and Powell 1992; Levitan 2001; Hoffmann *et al.* 2004; Balanya *et al.* 2006; Hoffmann and Rieseberg 2008). Polymorphic inversions are very common in a great number of *Drosophila* species and many of them were soon found to be under selection, since latitudinal and altitudinal clines, as well as seasonal variations in inversion frequencies have been reported for several species (Krimbas and Powell 1992). For example, *Drosophila subobscura* originated in Europe, where there are documented clines for inversions (Sole *et al*. 2002). In *D. subobscura*

inversion clines that mirror the European clines have been established along the west coasts of South and North America (Prevosti *et al*. 1988). In addition, inversion polymorphism in *D. robusta* exhibit similar altitudinal clines (Levitan 2001). Seasonal variation in inversion polymorphism has been described in *D. pseudoobscura (*Krimbas and Powell 1992) and in *D. subobscura* (Rodriguez-Trelles *et al*. 1996). The reason underlying these variable geographical or temporal distribution of the distinct chromosomal arrangements within a species could be that each arrangements is better adapted to distinct environmental condition and that the natural selection favours a higher frequency of one or the other arrangement depending on the local or temporal conditions where each population is found (Font 2010).

It is noteworthy that the widely distributed species exhibits higher polymorphism than endemic less extensive populations or species (Dobzhansky and Wallace 1953; Carson 1965). *Drosophila ananassae*, a widespread and domestic species, has been found to contain numerous inversions in its natural populations (Singh 1982). Da Cunha and Dobzhansky (1954), Dobzhansky (1950) found a good correspondence between the mean number of heterozygous inversions and an index expressing environmental heterogeneity in natural populations of *Drosophila willistoni*. This led them to suggest that chromosomal polymorphism is a device to cope with the diversity of environments.

Drosophila ananassae, a widespread species, has been found to contain numerous inversions in its natural populations (Singh 1982). Out of several inversions detected, three (2LA, 3LA and 3RA) are cosmopolitan in distribution (Shirai and Moriwaki 1952, Futch 1966 and Singh 1970). The chromosomal analysis of 11 populations of Nagaland has shown that the frequency of inversions and the level of inversion heterozygosity vary among populations and also vary within a population. Present investigation reveals that 9 out of 11 (Dimapur, Kiphire, Wokha, Mokokchung, Tuensang, Kohima, Peren, Phek and Zunheboto) natural populations of this species exhibit presence of inversion heterozygotes above 50%. The percentage of inversion heterozygotes is remarkably high in Dimapur population with 81% while Mon population is least polymorphic with 37% heterozygotes (Table 1). Chromosomal analysis of twelve natural *D. ananassae* populations of India reveals the presence of all the three cosmopolitan inversions (Singh 1989). Significant

differences in the frequencies of various gene arrangements and in overall inversion heterozygotes were observed in *D. ananassae* populations of India. In some of the populations, the chromosomes with inverted gene arrangements occur at a frequency higher than 80 %. For example, in Agra population overall inversion heterozygotes reach 83%, in Birlapur it is 94% and in Madurai too it is 94%. In the present study natural populations of *D. ananassae* exhibit presence of inversion heterozygotes above 50%. These findings reveal that *Drosophila ananassae* maintains a higher number of inversion heterozygotes in natural populations because of obvious inversion heterozygotes superiority.

Data in regard to frequencies of the inversions 2LA, 3LA and 3RA in eleven populations of *Drosophila ananassae* of Nagaland was presented in Table 1. 2LA inversion was most frequent (408 out of total 687 inversions, amounting to 59.39%) followed by 3LA (191out of total 687 inversions, amounting to 27.80%) and least is 3RA (88 out of total 687 inversions, amounting to 12.80%). The frequency of a specific inversion varies in different populations (Figure 8). 2LA frequency was highest in Dimapur population (54%) and lowest in Mon population (21%). 3LA frequency was highest in Kohima population (28%) and lowest in Wokha and Phek population (12%). 3RA frequency was highest in Mokokchung with 15% and lowest in Phek population (1%). Dobzhansky and Dreyfus (1943) showed that the 2LA, 3LA and 3RA are present in 50%, 40% and 30% respectively in Brazilian population of *D. ananassae*. Chromosomal analysis of twelve natural populations of *D. ananassae* in India (Singh 1989) revealed that the frequency of 2LA is 70%, 3LA is 60% and 3RA is 20%. It is also observed that the frequency of a specific chromosome arrangement varies widely in different populations. For example, frequency of 2LA inversion varies from 23 to 96%. The frequency of 3LA varies from 12% to 94% and the frequency of 3RA varies from 3 to 37%. However Sreerama Reddy (1973) analyzed 14 populations of *D. ananassae* from Karnataka state and observed that the frequency of 2LA was 27%, 3LA was 52% and 3RA was 25%. He further observed that the frequency of a specific chromosome arrangement varies widely in different populations. For example, frequency of 2LA inversion varies from 3 to 49%. The frequency of 3LA varies from 11 to 59% and the frequency of 3RA varies from 7 to 45%. Thus the populations under present study present a similar picture from those of the Brazilian and other Indian populations with regard to the frequencies of cosmopolitan

inversions. Hence present study are in conformity with the observations of Dobzhansky (1943) who postulated that the different inversions and their frequencies are never uniform as evidenced by studies on the natural populations of many species of *Drosophila* and thus reflect the flexibility of their genotypes to adjust to their environmental diversity which they confront. This argument may be one of the reasons for the observed variation in overall and individual rearrangements in *Drosophila ananassae* populations belonging to 11 geographical regions of Nagaland state. According to Carson (1965) who coined the term "ubiquitous polymorphism" to include the species that are characterized by the occurrence of aberrant gene sequences in highly variable frequencies and geographically widespread, categorized in this group *D. ananassae* along with *D. busckii, D. hydei, D. immigrans* and *D. melanogaster*. The pattern of chromosomal variability as encountered in *D. ananassae* populations under present study is in conformity with the concept of Carson. In the present study, the three cosmopolitan inversions 2LA, 3LA and 3RA are found in all the populations. Dobzhansky and Dreyfus (1943), Singh (1998, 2010) also reported presence of all three cosmopolitan inversions in all Brazilian and Indian populations respectively. Such a widespread occurrence of these inversions cannot be explained by their independent origin. Townsend (1952) suggested two possibilities to explain the widespread occurrence of inversions- (1) the first possibility is that these inversions arose somewhere at sometime, became widespread being favoured by natural selection (2) The alternative possibility is that the whole species with all its widespread inversions arose in a limited area and then extended its range of distribution. The second suggestion is more plausible and indicates the monophyletic origin of those inversions, with these facts, it is reasonable to expect these common inversions in all the populations.

In the present study 6 populations (Dimapur, Kohima, Longleng, Peren, Tuensang and Wokha) exhibit double heterokaryotypes (2LA and 3LA, 3LA and 3RA). Frequency of 2LA and 3LA combination double karyotype is 1% in Dimapur population; 3% in Kohima population; 6% in Tuensang population. 3LA and 3RA combination was present in 4% of Longleng population; 2% in Wokha population; 2% in Dimapur population and 1% in Peren population. Only in Dimapur Population shows both the combinations (2LA and 3LA; 3LA and 3RA) were observed.

Carson and Stalker (1947) and Mayer (1963) suggested that inversions having limited range of distribution may be of recent origin and did not have enough time to spread to the entire area of the species range. Thus the occurrence of inversions which are in association such as 2LA and 3LA, 3LA and 3RA which is present in low frequencies in the present studies and number of other endemic inversion reported by earlier workers in low frequencies support the above idea. They further argue that, alternatively inversions with limited distribution and low frequency might have lower adaptive value and probably blocked their way to become widespread. It would be interesting to work further and understand into which type of above mentioned alternative ideas the populations that exhibit double hetero-karyotypes in Nagaland would fit in.

The urban population of Dimapur (only urban area in Nagaland state) is distinct from the rest of the populations as far as prevalence of high frequency (81%) of inversion heterozygotes when compared to the rest of the populations and combination of inversions i.e. 2LA and 3LA (1%) and 3LA and 3RA (3%) were observed only in this population. Kenig *et al.* (2010) analyzed inversion polymorphisms of *Drosophila subobscura* in Serbia from the urban area of Belgrade and from the locality, Deliblato, which is not under strong anthropogenic influence, were studied with the aim to characterize and compare their genetic structure by examining chromosomal inversion polymorphism with several other populations from different habitats in the central Balkans. Inversion A_2 (inversion at acrocentric chromosome of *D. subobscura*) was present in the urban Belgrade population in low frequency but it was not registered in the rural Deliblato population. The complex gene arrangements $^{E1+2+9+12}$ inversion $^{E1+2+9+12}$ (multiple inversions at acrocentric chromosome in *D. subobscura*) were found in the Belgrade population but not in that of Deliblato. O_{st} and O^{3+4} inversions (inversions at acrocentric chromosome of *D. subobscura*) have higher frequency in the Belgrade population. The obtained results indicate higher heterozygosity in urban population from Blegrade. Urban areas are considered as environments that are unpredictable and prone to sudden fluctuations of ecological factors. The greatest variety of microhabitats is characteristic for this type of environment. In light of this a higher degree of heterozygosity is expected in populations that inhabit urban environments compared to populations from other habitats (Kenig *et al.* 2010). The observed over all high inversion frequency and inversion combinations in urban Dimapur population can be interpreted as a consequence of selection interacting with

factors related to geographical conditions. The degree of urbanization leads to the increase of ecological niches and consequently to higher chromosomal variability (Singh 1994; Valiati and Valente 1997; Valente *et al.* 1993, 2003). This can be one of the possible explanations for the observed variation in the above explained inversion polymorphism in Dimapur population.

The populations that exhibit the three common inversions frequencies are computed for the X^2 homogeneity test (Table 2). To measure the differences in inversions frequencies (2LA, 3LA and 3RA) among natural populations. Results showed that the differences in the frequencies of multiple inversions exhibited by different populations are found to be significant ($p < 0.05$), indicating that the populations under study are distinct from one another with regard to the degree of variability.

In the present study 2LA inversion having positive correlation with temperature (Table 4). However no correlation is observed between 3LA and 3RA frequency and temperature. Temperature is one of the most important variables that determines distribution and abundance of species (Cossins and Bowler 1987). Changes in inversion frequencies have now been related to direct or indirect effects of temperature shifts in *D. subobscurra* (Rodriguez-Trelles and Rodriguez 1998) *D. melanogaster* (Van Delden and Kamping 1989; Umina *et al.* 2005). Surveys on Spanish populations of *Drosophila subobscura* (Rodriguez-Trelles and Rodriguez 1998) have also indicated that inversion arrangements typical of warm latitudes had increased in frequency at a local level. Van Delden and Kamping (1991) observed in *Drosophila melanogaster* that frequency of *In(2L)t* and also the frequencies of another, short, inversion on the left arm of the second chromosome increased in laboratory populations kept at 29.5°C and 33°C. When populations transferred to lower temperature (20°C and 25°C), the frequencies of *In(2L)t* was finally lost, suggesting existence of positive correlation between *In(2L)t* frequency and ambient temperature. Subsequent tests of egg-to-adult survival at different temperatures proved that at high temperature, survival of individuals possessing *In(2L)t*, either in the homokaryotypic or the heterokaryotypic state, was higher than of the standard (ST) karyotype. Thus it seemed that *In(2L)*t had a selective advantage at high temperature. Kamping and Vandelden (1999) analyzed the maintenance of inversion *In(2L)t* in

Drosophila melanogaster fitness differences among *In(2L)t* and standard (*ST*) homo and heterokaryotypes under high-temperature conditions were determined. Viabilities were measured for high temperature treatment started at different juvenile stages. The capacity to restore fertility after high temperature treatment was measured for adults and juveniles. Genetic adaptation for increased temperature resistance for these traits was determined for strains which were reared at 33°C for 10 generations. Larva-pupa survival rates were high, juvenile mortalities were highest and strongest karyotypic effects were observed during the pupal stage when preceding larval stages were reared at 33°C. Standard karyotypes showed lowest viabilities. Sterility was induced for females and males after high-temperature treatment of adults as well as juveniles. Subsequent transfer to 25° C, however, resulted in restore fertility in some of the individuals, depending on the length of the recovery period. Fertility restoration was significantly higher for heterokaryotype male and females. Heterokaryotype superiority for restored fertility as well as for viability was positively correlated with severity of the treatment. Ten generations of selection at 33°C resulted in significant improvement of juvenile survival and fertility restoration for all karyotypes. These fitness components were positively correlated. It is concluded that the capacity to restore fertility after heat stress is an important fitness component, especially with respect to the *In(2L)t* polymorphism. All these studies suggest that chromosomal rearrangements confer adaptability to changes to temperature in *Drosophila*. The fact that 2LA inversions having positive correlation with temperature imply possible adaptive value of this genome rearrangement in certain *D. ananassae* populations that thrive at higher temperature zones of Nagaland state.

The present study reveals lack of significant correlation between presence of 3LA and 3RA inversions and altitude. Altitude is one of the ecological factors which may influence the genetic structure of natural populations. Singh and Singh (2007) analyzed forty-five natural populations of *D. ananassae* from different ecogeographic regions of India for cosmopolitan chromosomal inversions and concluded that there is no correlation between inversion frequency and altitude. However studies on *D. melanogaster* (Das and Singh 1991) and *D. mediopunctata* (Ananina *et al.* 2004) show altitudinal clines with reference to inversion polymorphism which can be attributed to adhering to multiple strategies by different species of *Drosophila* to the certain geographical parameters such as altitude.

Hence present study is in conformity with the observations made by Singh and Singh (2007).

Present study points out the existence of significant negative correlation between frequency of 2LA and rainfall. However no correlation is observed between 3LA and 3RA frequency and rainfall. It is further observed that no correlation exists between all the three inversion frequencies and humidity. Rodrigues-Trelles *et al.* (1996) studied time series analysis of seasonal changes of the O inversion polymorphism of *Drosophila Subobscura* in a natural population of Spain over 15 years by time series analysis and observed that the O inversion polymorphisms varied on two different timescales: short-term seasonal changes repeated over the years superimposed on long-term directional trends. All the common arrangement (O_{3+4+7}, O_{st}, O_{3+4} and O_{3+4+8}) showed significant cyclic seasonal changes but one of these arrangement (O_{3+4+7}) showed significant long-term trends. Moreover, the degree of seasonality was different for different arrangements. The O_{3+4+7} and O_{st} gene arrangements showed the highest seasonality, which accounted for 47% of their total variances respectively. The seasonal changes in the frequencies of chromosomal arrangements were significantly associated with the seasonal variation of the climate (temperature, rainfall, humidity and isolation). Multiple regression analysis of the frequency of the O arrangements on the climatic variables indicates that O_{3+4+7} gene arrangements showed positive correlation with temperature, negative correlation with humidity and no correlation with rain. O_{st} gene arrangement indicates negative correlation with temperature, positive correlation with humidity and no correlation with rain. O_{3+4+8} gene arrangements indicate negative correlation with temperature no correlation with humidity and rain. O_{3+4} gene arrangements indicate no correlation with temperature and humidity and negative correlation with rain. Results suggest that the climate may play a primary role in the seasonal changes of the O arrangements as multiple regression of the frequency of the most common arrangements on the weather records for the sampling dates revealed a significant association between the seasonal changes of the O arrangements and the seasonal variation of the climate. They made an interesting observation that *HSP70* coding sequences controlling the most active heat shock protein in *D. subobscura* have been located on the O_{3+4} region. Ward *et al.* (1973) analyzed the correlation of climate and host plant morphology with a geographic gradient of an inversion polymorphism in *Drosophila pachea* in Arizona, USA. Results showed correlation of 7A (paracentric inversion) gene arrangement frequencies with rainfall was positive in summer (June, July and August) and fall (October, November and December) while negative in winter

(December, January and February) and spring (March, April and May). The months of the collection were analyzed to determine whether 7A gene arrangements was sensitive to the effects of short term climatic variation the partial correlation for the month of collection revealed a significant association between rainfall and 7A frequency of gene arrangement. These correlations suggest that the 7A arrangement is either wet or dry adapted responding only to the extremes in rainfall Strick-Berger and Wills (1966) detected significant fluctuations of third chromosome gene arrangement in *D. pseudoobscura* with rainfall in Strawberry Canyon, Berkley California. This locality has relatively mild wet winter and cool dry summer. They found one gene arrangement (ST) to be negatively correlated with rainfall, while another gene arrangement (CH) was negatively correlated with temperature. All these observations tell us that in different species of *Drosophila* inversion polymorphism confer adaptive value to climatic factors such as rainfall and humidity. Present study upholds observations of multiple workers and defend that 2LA may confer adaptive advantage to temperature and rainfall.

Present study aims at understanding probable significance of multiple cosmopolitan inversions in *D. ananassae* in adapting to various climatic and geographical factors. Present study upholds observations of multiple workers and defends that 2LA may confer adaptive advantage to temperature and rainfall. In future, it would be interesting to understand alteration in gene expression levels in these inverted regions which will throw light on molecular basis of adaptation through inversion polymorphism in *Drosophila ananassae.*

References

Summary and Conclusion

The fruit fly *Drosophila* is one of the most intensively studied organisms in biology that serves as a model system for investigations of many developmental, cellular processes, disease(s), adaptation, diversity and evolution; whose underlying fundamental principles are comparable to higher eukaryotes, including man (Reviewed in Devineni *et al.* 2013). *Drosophila,* with its cosmopolitan nature and complexities in species composition, is an excellent model for studying the eco-distributional patterns of various species.

The family Drosophilidiae comprises of more than 3,500 described species including the genus *Drosophila* (Bachli 1998). In this family *Drosophila* is the most abundant genus and comprises of 1500 species in the world (Bachli 2008). The review of literature shows that more than 200 *Drosophila* species were reported from India (Hegde *et al.* 2000).

Nagaland is one of the sub-Himalayan hilly states which is blessed with tremendous floral and faunal diversity. However very little work is done to understand *Drosophila* diversity. In the present study *Drosophila* species collected from the wild localities of 11 districts of Nagaland. In the present study a total of 16 species were collected belonging to four subgenera (*Sophophora*, *Drosophila, Dorsilopha* and *Scaptodrosophila*). Present study reveals the fact that *Drosophila* fauna of Nagaland state shows similarity not only with South Asia but also with that of East Asia which can be explained from geographical location of this north eastern state.

Effort was made to understand altitudinal and seasonal variation in *Drosophila* species of mount Japfu in Nagaland. A total of 4,680 *Drosophila* flies belonging to 19 species of 4 subgenera were collected at altitudes of 1500, 1800, 2100, 2400 and 2700 m a.s.l. The subgenus *Sophophora* Sturtevant was predominant, with 10 species, followed by subgenus *Drosophila,* with 4 species. Subgenus *Dorsilopha* and subgenus *Scaptodrsophila* were represented by 1 species each. The remaining 3 species were not identified. Cluster analysis and constancy methods were used to analyze the species occurrence qualitatively.

Altitudinal changes in the population densities and relative abundances of the different species at different seasons were also studied. The diversity of *Drosophila* community was assessed by applying Simpson's diversity index. At 1800 m the Simpson's index was low (0.09301), suggesting high *Drosophila* diversity at this altitude. The density of *Drosophila* changed significantly during different seasons ($F= 26.72$; $df=2$: $p<0.0001$). The results suggested the distributional pattern of a species or related group of species was uneven in space and time.

In the present study basing on morphological markers, internal characters a new species, *Drosophila hegdii* was identified (Achumi *et al.* 2011). New species status and its molecular phylogeny were understood with the help of "DNA Barcoding." Neighbour joining trees were constructed using MEGA5 (Neighbor-Joining (NJ) method with bootstrap test (1000 replicates) using the Kimura 2-parameter model, with gaps treated by pairwise deletion). Molecular analysis indicates that *D. hegdii* and *D. jambulina* belonging to the same cluster with strong boot strap support of 86. The ancestor of *D.vulcana* and *D.hegdii* clade was estimated to have appeared about 0.02296 Mya, the divergence between *D. jambulina* and *D. hegdii* was estimated to be 0.02223 Mya. Molecular analysis confirms the observation made through morphological markers that *Drosophila hegdii* is a new species.

Chromosomal rearrangements are sources of genetic variation. Dobzhansky (1950) suggested that the chromosomal polymorphism is a device to cope with the diversity of environments. Swanson (1974) demonstrated that paracentric inversions due to their high adaptive value in heterozygous condition have been positively selected in animals. Present study aimed at understanding probable significance of multiple cosmopolitan inversions in *D. ananassae* in adapting to various climatic and geographical factors of Nagaland. The polymorphic grade is remarkably high in Dimapur population with 81% heterokaryotypes having 0.81 mean inversion heterozygotes. Mon population exhibits least polymorphic with 37% heterokaryotypes. Remaining nine populations- Longleng, Phek, Tuensang, Mokokchung, Wokha, Zunheboto, Kohima, Peren and Kiphire are intermediate, ranging from 49%-76% heterokaryotes. The populations that exhibit the three common inversions frequencies are computed for the X^2 homogeneity test. Results show that the differences in

the frequencies of multiple inversions exhibited by different populations are found to be significant ($p < 0.05$), indicating that the populations under study are distinct from one another with regard to the degree of variability. In order to understand the influence of multiple eco-geographical factors such as altitude, humidity, rainfall and temperature on inversion frequencies; patterns of variation in inversion frequencies were examined by means of correlation analysis using SPSS 16.0, considering inversion frequencies (2LA, 3LA and 3RA) as dependent variables on climatic variables (humidity, rainfall and temperature) and geographical variables (altitude). Results reveal lack of significant correlation between presence of multiple inversions and certain climatic and geographical variables (humidity and longitude). However significant correlation exists with reference to certain climatic indicators such as rainfall (negative correlation), temperature (positive correlation). Polymorphism of 2LA, 3LA and 3RA inversions in *Drosophila ananassae* populations of Nagaland state and their adaptive significance is discussed in the present work. Results point out the adaptive significance between genomic rearrangements such as frequency of 2LA inversion to certain climatic factors such as temperature and rainfall; which explains the gene environmental interaction as a survival strategy. In future, it would be exciting to understand alteration in gene expression levels in these inverted regions which will throw light on molecular basis of adaptation.

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Altitudinal and seasonal variation in *Drosophila* species on mount Japfu of Nagaland, a sub-Himalayan hilly state of India

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Abstract

Drosophila (L.) (Diptera: Drosophilidae) has richly contributed to the understanding of patterns of inheritance, variation, speciation, and evolution. *Drosophila*, with its cosmopolitan nature and complexities in species compositions, is an excellent model for studying the eco-distributional patterns of various species. This study analyzed the altitudinal and seasonal variation in *Drosophila* species of Mount Japfu in Nagaland, a sub-Himalayan hilly state of northeast India, over the course of one year. A total of 4,680 *Drosophila* flies belonging to 19 species of 4 subgenera were collected at altitudes of 1500, 1800, 2100, 2400, and 2700 m a.s.l. The subgenus *Sophophora* Sturtevant was predominant, with 10 species, followed by subgenus *Drosophila*, with 4 species. Subgenus *Dorsilopha* and subgenus *Scaptodrosophila* were represented by 1 species each. The remaining 3 species were not identified. Cluster analysis and constancy methods were used to analyze the species occurrence qualitatively. Altitudinal changes in the population densities and relative abundances of the different species at different seasons were also studied. The diversity of the *Drosophila* community was assessed by applying Simpson's diversity index. At 1800 m a.s.l., the Simpson's index was low (0.09301), suggesting high *Drosophila* diversity at this altitude. The density of *Drosophila* changed significantly during different seasons ($F = 26.72$; $df = 2$; $p < 0.0001$). The results suggest the distributional pattern of a species or related group of species was uneven in space and time.

Keywords: cluster analysis, occurrence constancy method, Simpson's diversity index
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Introduction

The fruit fly *Drosophila* (L.) (Diptera: Drosophilidae) has richly contributed to the understanding of patterns of inheritance, variation, speciation, and evolution. Genus *Drosophila*, with its cosmopolitan nature and complexities in species compositions, is an excellent model for studying the eco-distributional patterns of various species (Carson 1965). Systematic study concerning the variations in the species compositions and the patterns of distribution of various members of the genus *Drosophila* in different geographical regions of the world will enable understanding of the principles underlying adaptive radiation and certain mechanisms involved in speciation (Muniyappa 1981).

Significant progress has been made in the fields of taxonomy and systematics of the family Drosophilidae in India. This family is composed of more than 3,500 species throughout the world (Bachli 1998). About 200 species belonging to 20 genera have been reported from different parts of India. However, very little is known regarding the *Drosophila* fauna of the northeastern region of the Indian subcontinent. This region is one of the richest repositories of biodiversity in the world, with its diverse climatic conditions, variable altitudes, deep valleys, luxuriant flora, running streams and moist surroundings. So, it provides an ideal location for the colonization of several *Drosophila* species (Singh and Gupta 1977; Dwivedi and Gupta 1979; Gupta and Singh 1979; Singh and Gupta 1980; Singh 1987). This region includes eight hill states, namely Assam, Arunachal Paradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura. A preliminary survey on Drosophilids of Dimapur, Medziphema, and Kohima of Nagaland state was conducted (Singh 1987). A preliminary report was published by Yenisetti et al. (2002) on Drosophilids of Mokokchung town. However, no systematic comprehensive study has been done on Drosophilids of Nagaland, a sub-Himalayan hilly state in the northeast region of India. It is possible that new *Drosophila* species can be identified from this region. *Drosophila* is a pollinator for economically important plants, such as *Ceropegia* (Chaturvedi 2006). It is possible that novel *Drosophila* pollinators for other economically important plants can be identified in these subtropical rain forests.

The ecological and biological diversity of an ecosystem determines the presence or absence of a species in an ecological niche. Apart from physical and biotic factors, the topography and season also affect animal distribution. As elevation is one of the important aspects of topography, it is important to look at animal distribution from that perspective. Efforts have been made to collect *Drosophila* from different altitudes, but these data were not considered with an ecological perspective (Reddy and Krishnamurthy 1977). According to Reddy and Krishnamurthy (1977), physical and biotic factors are the sole determinants of animal distribution. This idea logically denotes that elevation and season have no influence on animal distribution. In the present study, our goal was to determine if elevation affects distribution.

According to Gause's competitive exclusion theory, two related species competing for the same resources cannot co-exist together in the same ecological niche (Gause 1934). However, laboratory experiments questioned the validity of this principle (Ayala 1969). The presence of taxonomically or phylogenetically related species in an ecological niche indicates their coexistence, and absence of such related species infers competitive exclusion

(Guruprasad et al. 2010). Our study sought to understand whether taxonomically or phylogenitically related *Drosophila* species coexist in nature. Our study also has been undertaken to understand the altitudinal and seasonal variation of *Drosophila* species on Mount Japfu, which is situated 15 km from Kohima, the capital of the sub-Himalayan hilly state Nagaland, India.

Materials and Methods

The altitudinal and seasonal fluctuation in *Drosophila* fauna was studied in five different wild localities of Mount Japfu, which has a peak altitude of about 3015.60 m a.s.l. Its slopes are covered with thick vegetation. The selected collection spots were located at 25° 11' N latitude and 94° 55' E longitude. Monthly collections of flies were made at the altitudes of 1500, 1800, 2100, 2400, and 2700 m a.s.l. from April 2010 to March 2011. Both bottle trapping and net sweeping methods were used. For bottle trapping, milk bottles of 200 mL capacity containing a smashed ripe banana sprayed with yeast were tied to the twigs underneath small bushes at the height of 1–1.5 m above the ground. Fifteen traps were kept at each site. After 2 days, the mouth of each bottle was plugged with cotton and removed from the bushes. The flies that were attracted by the bait and collected in the bottles were transferred to fresh bottles containing wheat cream agar medium. The medium was prepared by adding 100 g of sugar (jaggery) to 500 mL of water and boiling it. Then, 500 mL of water, 100 g of wheat powder (soji), and 8 g of agar-agar were added to the boiling sugar water mixture. When the medium turned sticky, 7.5 mL propionic acid (anti-fungal agent) was added while continuously stirring the medium.

Net sweeping was done on rotting fruits (crushed banana spread beneath shaded areas of bushes 1 day before collection). After each sweep, collected flies were transferred to bottles containing freshly prepared wheat cream agar medium.

The flies were then brought to the laboratory, isolated, identified, and sexed. Categorization of the collected *Drosophila* flies was made to respective taxonomic groups by employing the parameters as suggested by Sturtevant (1921), Patterson and Stone (1952), Throckmorton (1962), and Bock (1971). To study seasonal variation, the entire year was divided into three seasons: pre-monsoon, extending from January through March, monsoon, from April through September, and post-monsoon, from October through December.

Flora of the collection sites:
Following is a brief description of the flora available in each of the collection spots.

Flora at 1500 m a.s.l.: maibau, *Alnus nepalensis* (Don) (Fagales: Betulaceae); beggarticks, *Bidens* spp (Asterales: Asteraceae); bittervine, *Makania* spp; sow thistles, *Sonchus* spp; butterfly bush, *Buddleja* spp (Lamiales: Scrophulariaceae); brahmi booti, *Centella asiatica* (L.) (Apiales: Apiaceae); sirib large, *Entada pursathea* (Roux) (Fabales: Fabaceae); banana, *Musa* spp (Zingiberales: Musacae); carrion flowers, *Smilax* spp (Liliales: Smilacaceae); pinyin, *Stemona* spp (Pandanales: Stemonaceae); currant tomato, *Solanum* spp (Solanales: Solanaceae); marda, *Termenalia elliptice* (Wright & Arn) (Myrtales: Combretaceae); etc.

Flora at 1800 m a.s.l.: jackfruit, *Artocarpus hetrophyllus* (Lam) (Rosales: Moraceae); yellow Himalayan raspberry, *Rubus* spp (Rosaceae); wormwood, *Artemisisia vulgaris*

(L.) (Asterales: Asteraceae); beggar-ticks, *Bidens* spp; bamboo, *Bambusa* spp, (Poales: Poaceae); black musale, *Curculigo* spp (Asparagales: Hypoxidaceae); timburni, *Diospynum* spp, (Ericales: Ebenaceae); deer-eye beans, *Mucuna perita* (Adans) (Fabales: Fabaceae); tapioca-root, Maninot utilissema (Crantz) (Malpighiales: Euphorbiaceae); *Smilax* spp; khasi pine, *Pinus insularies* (Gordon) (Pinales: Pinaceae); knotwood, *Polygonum* spp (Caryophyllales: Polygonaceae); etc.

Flora at 2100 m a.s.l.: *A. nepalensis* (Don); khang, *Acacia pinnata* (Miler) (Fabales: Fabaceae); thickhead, *Crossocephalum* spp (Asterales: Asteraceae); Himalayan nettle, *Girardinia heterophylla* (Vahl) (Rosales: Urticaceae); *Rubus* spp; blady grass, *Imperata cylindrica* (Brauv) (Poales: Poaceae); *Musa* spp; etc.

Flora at 2400 m a.s.l.: bologi, *Crossocephalum* spp (Asterales: Asteraceae); *A. vulgaris*; white weed, *Ageratum conyzoids* (L.); thoroughworts, *Eupatorium* spp; *Bidens* spp; blueberry ash, *Elaeocarpus* spp (Oxalidales: Elaeocarpaceae); shaking brake, *Pteris* spp (Polypodiales: Pteridaceae); *I. cylindrica*; *C. asiatica*; *P. insularies*; knotwood, *Polygonum* spp; cowitch, *Mucuna pruriens* (L.) (Fabales: Fabaceae); etc.

Flora at 2700 m a.s.l.: *Crossocephalum* spp; *Curculigo* spp; *I. cylindrica*; kamraj, *Helminthostachys zeylanica* (L.) (Ophioglossales: Ophioglossaceae); *Polygonum* spp; *Smilax* spp; timburni, *Dryopteris* spp (Ericales: Ebenaceae); rhododendron, *Rhododendron* spp (Ericaceae); etc.

Data analysis

The relationship between the abundance, richness, and diversity of all groups of flies collected throughout the year was calculated by Simpson's diversity index (Simpson 1949). Simpson's diversity index (D) measures the probability that 2 individuals randomly selected from a sample will belong to the same species, and was calculated using the following formula:

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

Where n = the total number of organisms of a particular species, and N = the total number of organisms of all populations.

In order to verify the qualitative distribution of different species, the occurrence constancy method (Dijoz 1983) was used. The constancy value (C) was obtained by dividing the number of collections in which one species occurred by the total number of collections, and then multiplying that result by 100. Based on constancy value, the species collected were grouped as constants when $C \geq 50$, accessory species when $C \geq 25$ and < 50, and accidental species when $C < 25$. Species that occurred in only one area were considered exclusive.

To understand the difference in seasonal variation of *Drosophila* flies at Mount Japfu, one-way analysis of variance was performed using GraphPad Prism5 software (www.graphpad.com).

Cluster analysis was performed using WinSTAT software (www.winstat.com) to design, analyze, and compare different *Drosophila* populations, as described by Giri et al. (2007). In the cluster study, Euclidean distance was chosen to measure the similarity between different species, and Ward's strategy (Giri et al. 2007) was performed to unite two clusters.

Table 1. The list of species of *Drosophila* collected, and the number of *Drosophila* collected at different altitudes (m a.s.l.) of Mount Japfu from April 2010 to March 2011.

Genus	Subgenus	Species	Altitude					Total
			1500	1800	2100	2400	2700	
Drosophila	*Sophophora*	*D. agumbensis*	17	0	0	1	1	19
		D. bipectinata	55	145	143	28	52	423
		D. eugracilis	140	80	86	57	15	378
		D . jambulina	7	15	4	8	25	59
		D. kikkawai	16	136	64	48	43	307
		D. malerkotliana	143	57	148	117	96	561
		D. parvula	36	76	74	67	17	270
		D. rajasekari	20	1	18	8	2	49
		D. trileuta	46	19	37	35	0	137
		D. takahashii	183	102	107	158	136	686
		Total						2889
	Drosophila	*D. immigrans*	190	177	63	150	131	711
		D. nasuta	0	58	27	24	17	126
		D. paraimmigrans	135	134	70	121	136	596
		D. repleta	89	56	0	0	0	145
		Total						1578
	Dorsilopha	*D. buskii*	35	12	4	0	0	51
		Total						51
	Scaptodrosophila	*D. nigra*	0	15	12	16	19	62
		Total						62
	Unidentified (1)		17	47	19	9	6	98
	Unidentified (2)		0	0	1	0	0	1
	Unidentified (3)		1	0	0	0	0	1
	Total		1130	1130	877	847	696	4680

Results

The list of *Drosophila* species collected at different altitudes of Mount Japfu from April 2010 through March 2011 and their taxonomic position are given in Table 1. A total of 19 species were collected, including 16 species of *Drosophila* belonging to 4 subgenera (*Sophophora*, *Drosophila*, *Dorsilopha* and *Scaptodrosophila*). The remaining 3 species were not identified. Pooled data on monthly collections of *Drosophila* yielded a total of 4680 individuals. Out of these, 2889 individuals (61.73%) belonged to 10 species of subgenus *Sophophora*, 1578 (33.71%) individuals belonged to 4 species of the subgenus *Drosophila* 100 (2.13%), 3 were unidentified, 62 (1.32%) individuals belonged to 1 species of subgenus *Scaptodrosophila*, and 51 (1.07%) belonged to 1 species of subgenus *Dorsilopha*.

The value of the Simpson's index, indicating the abundance, richness, and diversity of *Drosophila* flies at different altitudes, is given in (Table 3). At the lowest altitude (1500 m a.s.l.), the Simpson's index was 0.10903, and at the highest altitude (2700 m) it was 0.141355.

The altitudinal variation of the *Drosophila* population is depicted in (Figure 1). The number of *Drosophila* flies decreased with increasing altitude. The altitudinal variations of the most abundant *Drosophila* species are shown in (Figure 2).

The seasonal variation in the population density of *Drosophila* is depicted in Figure 3. The density was low in the pre-monsoon period, increased in the monsoon period, and then decreased in the post-monsoon period. The analysis of variance calculated for pre-monsoon, monsoon, and post-monsoon seasons showed significant differences among them ($F = 26.72$; $df = 2$, $p < 0.0001$).

Table 2. Absolute (A) and relative abundance (r) and constancy values (c) for each species collected at different altitudes of Mount Japfu from April 2010 to March 2011.

Subgenus	Species	1500 m a.s.l.			1800 m a.s.l.			2100 m a.s.l.			2400 m a.s.l.			2700 m a.s.l.		
		A	r	c	A	r	c	A	r	c	A	r	c	A	r	c
Sophophora	*D. agumbenesis*	17	0.02	8.33	0		-	0		-	1	0	8.33	1	0.001	8.33
	D. bipectinata	55	0.05	25	145	0.12	66.67	143	0.16	75	28	0.03	25	52	0.07	58.33
	D. eugracilis	140	0.12	75	80	0.07	41.67	86	0.1	58.33	57	0.09	33.33	15	0.02	16.67
	D. jambulina	7	0.01	25	15	0.01	16.67	4	0.004	8.33	8	0.01	8.33	25	0.04	25
	D. kikkawai	16	0.01	16.67	136	0.12	66.67	64	0.07	50	48	0.06	41.67	43	0.06	25
	D. malerkotliana	143	0.12	50	57	0.05	33.33	148	0.17	75	117	0.14	75	96	0.13	58.33
	D. parvula	36	0.03	16.67	76	0.07	41.67	74	0.08	41.67	67	0.08	41.67	17	0.02	25
	D. rajasekari	20	0.02	33.33	1	0	8.33	18	0.02	25	8	0.01	8.33	2	0.002	8.33
	D. trileuta	46	0.04	16.67	19	0.01	16.67	37	0.04	33.33	35	0.04	16.67	0		-
	D. takahashii	183	0.17	83.33	102	0.09	66.67	107	0.12	66.67	158	0.19	100	136	0.2	75
	Total	663		-	631		-	681		-	527		-	387		-
Drosophila	*D. immigrans*	190	0.17	83.33	177	0.16	83.33	63	0.07	50	150	0.18	100	131	0.18	75
	D. nasuta	0		-	58	0.05	33.33	27	0.03	16.67	24	0.02	16.67	17	0.02	8.33
	D. paraimmigrans	135	0.11	66.67	134	0.11	66.67	70	0.07	50	121	0.14	75	136	0.2	83.33
	D. repleta	89	0.08	41.67	56	0.05	33.33	0		-	0		-	0		-
	Total	414		-	425		-	160		-	295		-	284		-
Dorsilopha	*D. buskii*	35	0.03	33.33	12	0.01	8.33	4	0.004	8.33	0		-	0		-
	Total	35		-	12		-	4		-	0		-	0		-
Scaptodrosophila	*D. nigra*	0		-	15	0.01	8.33	12	0.01	8.33	16	0.01	16.67	19	0.08	16.67
	Total	0		-	15		-	12		-	16		-	19		-
Unidentified (1)		17	0.02	16.67	47	0.04	33.33	19	0.02	25	9	0.01	16.67	6	0.008	8.33
Unidentified (2)		0		-	0		-	1	0.001	8.33	0		-	0		-
Unidentified (3)		1	0.01	8.33	0		-	0		-	0		-	0		-
Total		18		-	47		-	20		-	9		-	6		-
Grand total		1130		-	1130		-	877		-	847		-	696		-

Table 3. Simpson's diversity index (D) according to the altitude of Mount Japfu.

Altitude (m a.s.l.)	Simpson Index (D)
1500	0.10903
1800	0.09301
2100	0.10362
2400	0.11733
2700	0.141355

The constancy value (C) of all species at all altitudes along with absolute numbers and relative abundance are presented in Table 2. Constant species ($C \geq 50$) represented 36.84% of the total collected species (7 out of 19), while 8 species were considered accessory (42.10%), and 4 species were considered accidental (21.05%). Constant species were *Drosophila bipectinata* (Duda) (Diptera: Drosophilidae), *D. eugracilis* (Bock and Wheeler), *D. kikkawai* (Burla), *D. malerkotliana* (Parshad and Paika), *D. takahashii* (Sturtevant), *D. immigrans* and *D. paraimmigrans*; accessory species were *D. jambulina* (Parshad and Paika), *D. parvula* (Bock and Wheeler), *D. rajasekari* (Reddy and Krishnamurthy), *D. trileuta* (Bock and Wheeler), *D. nasuta* (Lamb), *D. replete* (Wollaston), *D. buskii* (Coquilett), and unidentified species (1); accidental species were *D. agumbensis* (Prakash and Reddy), *D. nigra* (Grimshaw), unidentified (2) and unidentified (3). In the cluster analysis (Figure 4), the accidental species stand first in the cluster, followed by the accessory species, and the bottom is occupied by constant species. *D. agumbenesis, D. jambulina, D .rajasekari, D. trileuta* belong to *melanogaster* species group of the subgenus *Sophophora. D. nigra* belongs to subgenus *Scaptodrosophila. D. agumbenesis* and *D. jambulina* belong to *montium* subgroup and *D. bipectinata* belongs to the *ananassae* subgroup. *D. repleta, D. buskii* of the same cluster belongs to subgenus *Drosophila.* In the second cluster *D. eugracilis, D. kikkawai* and *D. parvula* belong to the *melanogaster* species group of the subgenus *Sophophora* and *D. paraimmigrans, D. immigrans* of the same cluster belong to subgenus *Drosophila. D. takahashii* belongs to *melanogaster* species group of subgenus *Sophophora.*

Discussion

The density of *Drosophila* on Mount Japfu decreased with increasing altitude. The density was high at 1500 and1800 m a.s.l., but was low at 2700 m a.s.l. (Figure 1). The results

indicate that the *Drosophila* community was affected by elevation. Wakahama (1961, 1962) has reported similar altitudinal variation in the distribution of *Drosophila* on Mt. Dakesan in Japan. He noticed that the total density of all species decreased with increasing altitude. Reddy and Krishnamurthy (1977) also noticed such altitudinal variation in *Drosophila* populations in the Jogimatii hills of Karnataka. Guruprasad et al. (2010) also observed seasonal and altitudinal variation in *Drosophila* populations of Chamundi Hill in Mysore, Karnataka, India. The reasons behind the observed phenomenon can be attributed to changes that occur as one ascends an altitudinal transect, potentially involving changes in temperature, precipitation, partial pressure of atmospheric gases, atmospheric turbulence and wind speed, and radiation input, including short-wave ultra-violet radiation at different wavelengths (Barry 1992). According to Hodkinson (2005), the above-mentioned changes are often strongly interactive and together create an environmental envelope within which insect species survive and reproduce. Hodkinson (2005) further emphasizes that the above-mentioned parameters combine to produce a general decrease in the overall structural complexity of the insects' habitat with increasing altitude.

According to Hegde et al. (2000a), the growth and size of a population depends on several environmental factors in addition to genetic structure. In the present study, consideration of the common and abundant species shows that numerical variation exists in regard to these species at all five altitudes. The occurrence of the dominance of one species over the others in any given area can be correlated with the dominant species' ecological versatility to exploit the conditions available in those habitats. The present study corroborates with the work of Muniyappa and Reddy (1981), Hegde et al. (2001), and Vasudev et al. (2001). There may be many other unknown microclimatic conditions that could also affect the density of *Drosophila*. The results of our study are in concurrence with the work of Cooper and Dobzhansky (1956), Reddy and Krishnamurthy (1977), Hegde et al. (2001), all of which have shown the influence of microclimatic conditions on the diversity of *Drosophila*. The present findings are also in agreement with the work of Cooper and Dobzhansky (1956) on species of *Drosophila* inhabiting the Sierra Nevada Mountains of the Yosemite region of California, where some of the species occurred at all elevations at which collections were made (259–3353 m a.s.l.). The results of our study are also in agreement with the work of Guruprasad et al. (2010), who showed that the number and density of *Drosophila* species decreased with increasing altitude at Chamundi Hill in Mysore, Karnataka. In our study, the presence of more species at lower altitudes can be attributed to the existence of thick vegetation, which provided good sources of food, and a more congenial environment at lower altitudes than at the higher altitudes.

Significant variations in the density of *Drosophila* were noticed during different seasons of the year on Mount Japfu. The density was highest during monsoon season at all altitudes and lowest during the pre-monsoon season. Possible reasons for the high density during monsoon season could be the availability of adequate food in the form of rotting fruits and the congenial climate for multiplication of the flies. The fact that the fruiting season of many plants in the area coincides with the monsoon season offers support for this conclusion. The monsoon season is characterized by heavy rains, reductions in temperature, and increases in humidity. As the monsoon season recedes, rainfall and humidity decrease, leading to a

dry climate. The population density also starts declining in post-monsoon season, reaching its minimum during the pre-monsoon season. Thus, the fluctuations in population size of *Drosophila* could be closely related to the wet and dry seasons. However, in temperate regions, the population density declines to an extremely low level during cold winter months, indicating the influence of temperature on the regulation of population size, as is the case in several *Drosophila* species inhabiting temperate regions (Dobzhansky 1943; Patterson and Wagur 1943; Dobzhansky and Pavan 1950; Williams and Miller 1952; Wakahama 1961).

According to the constant, accessory, and accident species, as well as the cluster analysis, our study indicates several species that coexisted had similar ecological preferences.

In Simpson's diversity index (D), 0 represents infinite diversity, and 1 represents no diversity, i.e., the greater the value of D, the lower the diversity. Applying this index to understand the measures of biodiversity of flies at different altitudes of Mount Japfu shows that the second lowest altitude studied (1800 m a.s.l.) had the lowest D-value, indicating more biodiversity compared to other altitudes. Hodkinson (2005) suggested that the altitudinal distribution of an insect species is controlled by its environmental tolerances, with maximum population size being achieved at some optimum elevation and population density declining with altitude above and below the optimum. The results of our study suggest that the optimum elevation on Mount Japfu for *Drosophila* diversity is at 1800 m a.s.l. From the eco-distributional analysis of *Drosophila* species on Mount Japfu, it is clear that the distributional pattern of a species or related group of species is uneven in space and time. The *Drosophila* community of Mount Japfu was highly diverse and depended on several environmental factors in addition to the genetic structure of the species present in it.

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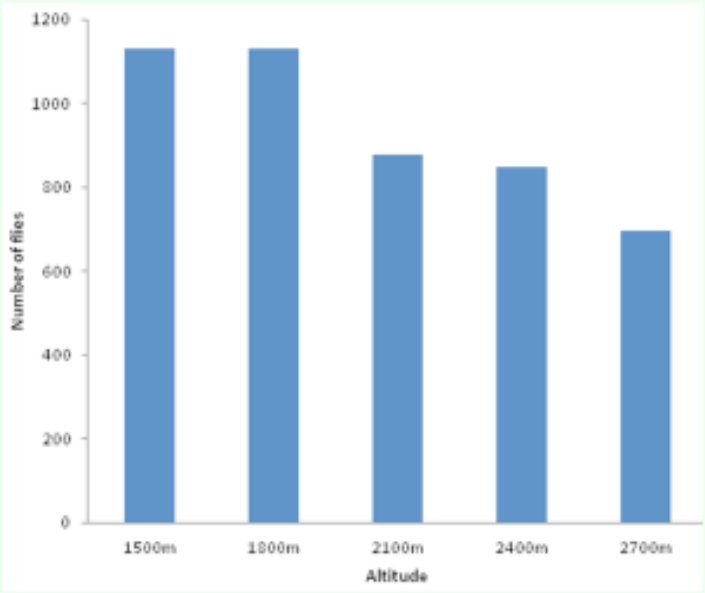

Figure 1. Altitudinal variation in the population of *Drosophila* species on Mount Japfu. High quality figures are available online.

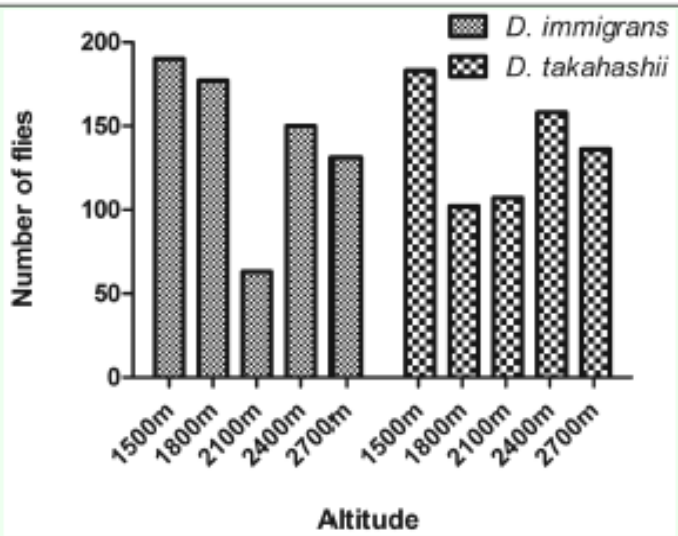

Figure 2. Altitudinal variation in the population of the most abundant *Drosophila* species on Mount Japfu. High quality figures are available online.

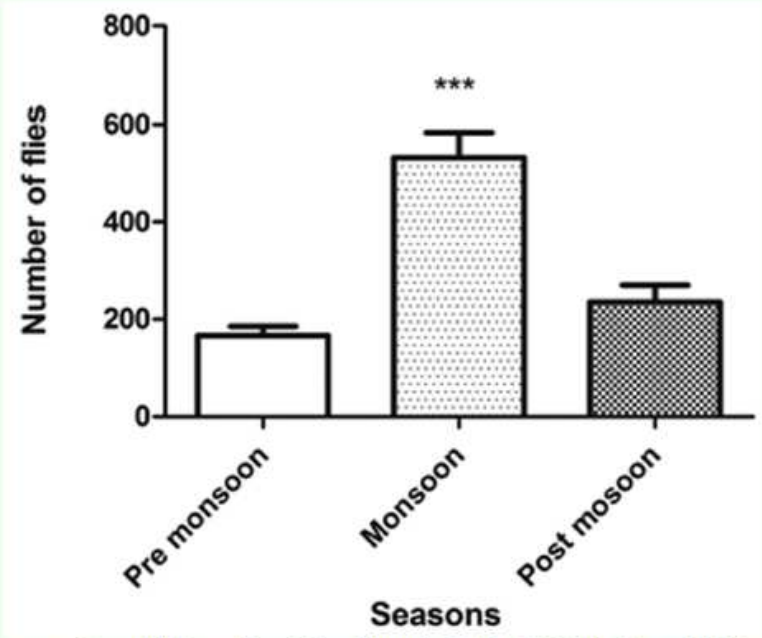

Figure 3. Seasonal variation in *Drosophila* flies collected from Mount Japfu ($F = 26.72$; $df = 2$; $p < 0.0001$). High quality figures are available online.

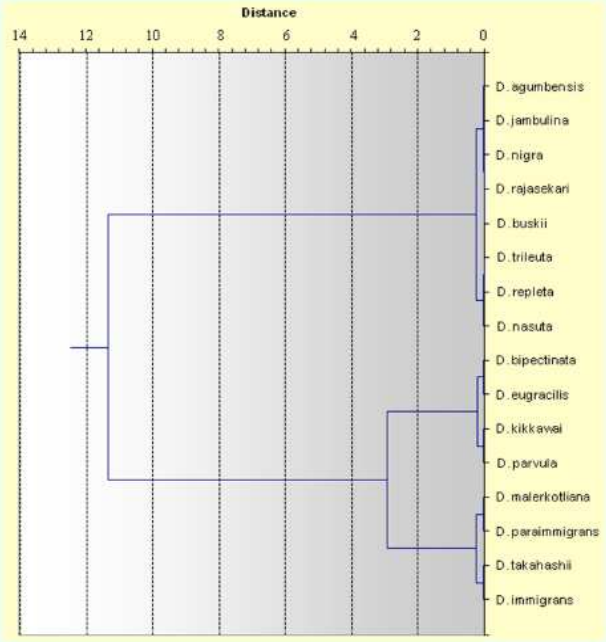

Figure 4. The cluster analysis of *Drosophila* species found on Mount Japfu (dendrogram using Ward's method). High quality figures are available online

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Drosophila hegdii, a new species of *Drosophila* (Diptera: Drosophilidae) from Lumami (Nagaland: India)

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ABSTRACT: New species *Drosophila hegdii*, member of the montium subgroup of *melanogaster* species group collected from sub-tropical, evergreen forest in Lumami, District Zunheboto, State Nagaland, India is described. The taxonomic status and relationship are discussed.

KEYWORDS: *Drosophila*, Drosophilidae, *Sophophora*, new species, Lumami, Nagaland, India

The Indian subcontinent with its vast array of vegetation and climatic conditions harbors many species of *Drosophila*. During the last few decades several investigators have surveyed the *Drosophila* fauna in various parts of South India (Reddy and Krishnamurthy, 1968, 1977; Sajjan and Krishnamurthy, 1975; Gowda, 1979; Muniyappa *et al*., 1981; Gai, 1985; Hegde *et al*., 1989). Little work is done on *Drosophila* fauna of north east India (Singh and Gupta, 1977; Dwivedi and Gupta, 1979; Gupta and Singh, 1979; Singh and Gupta, 1980).

The present survey of *Drosophila* was undertaken in Lumami of Nagaland, a sub Himalayan hilly state of north east India which is situated at 94.28° E Longitude and 26.13° N Latitude, having an altitude of 940 m above sea level. In Lumami climate is pleasant, generally cool in winter and warm in summer; temperature ranges from 17°C to 30°C and average annual rainfall is about 200 cm. The torrential monsoon rain is an integral feature of the weather. Heavy rainfall during the monsoon favours the growth of thick forest, fruit bearing trees, providing favourable natural habitats for the colonization by the members of the genus *Drosophila*. Collections were made by trapping the flies on banana baits.

A new species collected in the locality of Lumami belonging to *melanogaster* species group of the subgenus *Sophophora* is described here.

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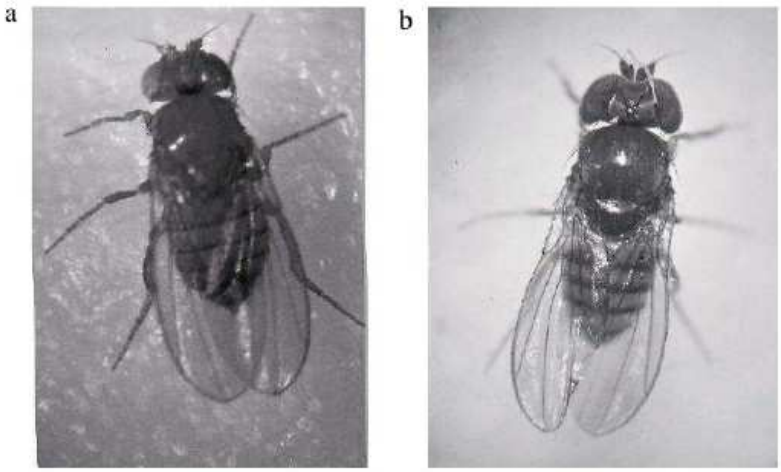

FIGURE 1. Female and male forms of *Drosophila hegdii*.

Male and female (Figure 1)

Light brown flies. Body length for female is 100 mm and for male is 97 mm.

Head

Arista with 4 branches above, 3 below, plus the terminal fork. Antenna dark, basal segment of the antenna bears a pair of dark bristles. Vibrissae with two anterior and two posterior bristles. In between the anterior and posterior bristles are 10–12 small bristles. Palp with a large and many small bristles. Anterior orbital proclinate, median orbital half the size of anterior orbital, posterior equal to anterior. Anterior verticles direct inward, posterior convergent. Ocular triangle with a pair of dark bristles, eyes red.

Thorax

Mid to dark brown, acrosticals in 8 regular rows, dorsocentrals convergent, anterior dorsocentrals are shorter than the posterior- approximately $2/3^{rd}$ the length of the posterior, anterior scutellar convergent, posterior scutellar convergent and crossed. Both anterior and posterior scutellars are of equal length. Two humerals, upper humerals half the length of the lower, posterior allars longer than anterior. Notopleurals and stenopleurals are of equal length. Notopleural and supra allars are of equal length. There are about 2–3 smaller bristles along the anterior and posterior stenopleurals. Halters translucent.

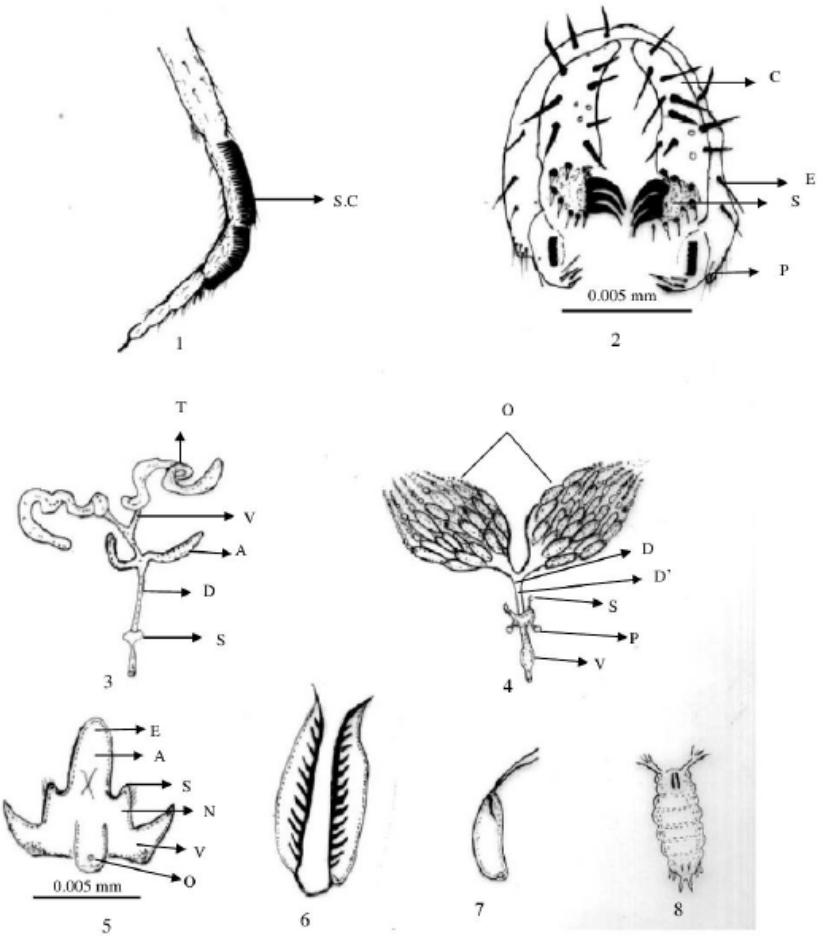

FIGURE 2. (1) S.C sex comb in first & second tarsal segment; (2) Periphallic Organ: E – Epandrium; P – Primary Claspers; S – Secondary Claspers; C – Anal Cercus; (3) Male reproductive system: T – Testis; V – Vasa deferentia; A – Paragonia; D – Anterior ejaculatory duct; S – Sperm pump; (4) Female reproductive system: O – Ovaries; D – Oviduct; D' – Common oviducts; S – Spermathecae; P – Paravaria; V – Vagina; (5) Phallic Organ: E – Edeagus; A – Anterior gonopophysis; S – Spines; N – Novasternum; O – Basal apodeme; V – Ventral fragm; (6) Egg guide; (7) Egg; (8) Pupa.

Wings

Transparent, wing length of male is 97 mm and female is 100 mm. Wing indices are calculated following the formula of Okada (1956) and presented in the Table 1.

Legs [Figure 2(1)]

Sex comb present in male on first and second tarsal segment. First tarsal consists of about 25–27 teeth and second tarsal consists of 16 teeth. Teeth are uniform and slightly curved.

Abdomen of male and female

First four tergites of male are shiny yellow-brown with broad dark apical band, last two segments are completely black. Tergites of Female are shiny dark brown with broad darker apical bands.

Internal characters

Female reproductive parts consist of ovarioles with 5–6 ventral receptacles that are transparent with 2–3 coils, spermetheca roundish colourless [Figure 2(4)]. Male testis is short showing 2–3 coils and light yellow in colour, paragonia spherical transparent [Figure 2(3)].

Periphallic organ [Figure 2(2)]

Ependrium broad, dorsally and laterally. Primary and secondary claspers present, primary claspers with a lateral row of about 5 teeth and a ventral medial cluster of teeth one elongated, toe with 3–4 bristles; secondary claspers oval, partially separated from anal plate with 3 black teeth, two are prominent and one is rudiment and about 1–8 small bristles along the ventral lateral and dorsal borders. Circus rounded on the outer side and slightly curved on inner side and with about 25 long and short bristles.

Phallic organ [Figure 2(5)]

Adeagus and anterior gonopophysis not fused. Anterior gonophysis protrude dorsally. Novasternum with prominent median convexity of variable thickness bearing a pair of spines, ventral fragma broad and concave, basal apodeme is thick and short.

Egg guide [Figure 2(6)]

Brown in colour with about 9–10 marginal and 1–2 discal teeth at the tip, teeth are dark in colour.

Egg [Figure 2(7)]

White in colour with two filaments present at the anterior.

TABLE 1. Wing indices of *Drosophila hegdii*
(Mean value of 10 flies)

Sex	Costal index	4V index	4C index	5X index
Male	2.69	1.15	2.55	2.62
Female	2.81	1.1	2.75	2.5

Pupa [Figure 2(8)]

Yellow with 9–10 spiracle filaments. At the posterior end there are 3 pairs of projections- one pair is lateral, second pair is ventral and third pair is dorsal.

Holotype

Male: India, Nagaland, Lumami, 14.xi.11 Coll. Bovito Achumi and Sarat Chandra Yenisetti.

Deposited in the *Drosophila* vivarium of Department of Zoology, University of Mysore,

Manasagangotri, Mysore 570006, India.

Allotype

Female: Same as above.

Paratype

5♂♂ and 5♀♀, India, Nagaland, Lumami; Coll. Bovito Achumi and Sarat Chandra Yenisetti.

Taxonomic status

The nature of the banding pattern of the abdominal tergites, the presence of 2 egg filaments and the puparia warrant its inclusion in the subgenus *Sophophora*. The presence of long ventral receptacle, coiled testis, convergent scutellars and two pairs of malphigian tubules qualify its inclusion in the *melanogaster* species group (Patterson and Stone, 1952). Further the presence of sex comb extending beyond the tips of the tarsal joint, the presence of primary claspers and secondary claspers with curved black teeth permit its inclusion in the montium sub group (Bock and Wheeler, 1972).

It was found that the new species resembles *D. vulcana* in the general colouration of the body, but differed in other morphological characters such as the number of teeth in sex-combs, the nature of arrangement of teeth in the sex comb, the prominent teeth, sex comb extending beyond the tips of the tarsal joints, the prominent teeth in the secondary claspers, number of rows of acrostical hairs, wing indices, periphallic and phallic organ. In addition the new species differed from other known species of montium sub group in characters such as the number of teeth in sex comb, and abdominal banding pattern.

Hence it deserved the status of a new species in the montium sub-group. This species is named as *Drosophila hegdii* in honour of Prof. S.N. Hegde for his contribution to *Drosophila* taxonomy.

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I) **List of publications:** Papers published/ to be published.

1) Bovito Achumi, Pardeshi Lal and Sarat Chanra Yenisetti. 2011. *Drosophila hegdii*, a new species of *Drosophila* (Diptera: Drosophilidae) from Lumami (Nagaland: India). *Entomon* **36(1-4):** 1-6.

2) Bovito Achumi, Shridhar N. Hegde, Pardeshi Lal and Sarat Chandra Yenisetti 2013. Altitudinal and seasonal variation in *Drosophila* species on mount Japfu of Nagaland, a sub-Himalayan hilly state of India. *Journal of Insect Science* **13**: 117. Available online: www. Insectscience.org.

3) Bovito Achumi, Shridhar N. Hegde, Pardeshi Lal, Zevelou and Sarat Chandra Yenisetti. *Drosophila* biodiversity of Nagaland, a sub-Himalayan hilly state of North-East India. (Communicated to *Journal of Bombay Natural History Society*).

4) Bovito Achumi and Sarat Chandra Yenisetti. *Drosophila hegdii* (Diptera: Drosophilidae), a new species from Lumami (Nagaland, India): Its molecular phylogeny. (Communicated to *Journal of Systematics and Evolutionary Research*).

5) Bovito Achumi, Giribabu Mahasamudram, Shridhar N. Hegde, Limamanen Phom, Pardeshi Lal and Sarat Chandra Yenisetti. Chromosomal rearrangements and its adaptive implication in Nagaland (a sub-Himalayan hilly state of North-East India) populations of *Drosophila ananassae*. (Communicated to *Genome*).

II) **Papers presented at Seminar/Symposia**.

1) Bovito Achumi and Sarat Chandra Yenisetti. 2013. Altitudinal and seasonal variation in *Drosophila* species on mount Japfu of Nagaland, a sub-Himalayan hilly state of India; National Conference on Environment and Biodiversity of India, 6th October 2013, Pune, Maharastra, India (oral presentation).

2) Bovito Achumi and Sarat Chandra Yenisetti. 2014. *Drosophila hegdii* (Diptera: Drosophilidae), a new species from Nagaland: Its molecular phylogeny; 101ST Indian Science Congress; 3rd – 7th February, 2014, Jammu, Jammu and Kashmir, India. (poster presentation).

III) **Workshop attended:**

1) “Application of Computer in Scientific Research” conducted by Bioinformatics Infrastructure (BIF) Centre, Nagaland University, Lumami; 26th - 29th March, 2012.

2) “Present Trends and Future Scope of Research in Nagaland” conducted by Nagaland University Research Scholars’ Forum, Kohima; 5th - 6th July, 2012.