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RESEARCH ARTICLE

BIODIVERSITY OF *DROSOPHILA* IN THREE DIFFERENT ALTITUDES OF BILIGIRIRANGA HILLS WILDLIFE SANCTUARY

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ABSTRACT

The genus *Drosophila* represents an unprecedented model system, it has been initially used to understand basic genetics i.e.., Pattern of inheritance, speciation and evolution, comparative experimental research and to study human diseases. Further, many of the mechanisms from the cellular level to molecular levels were very well conserved both in *Drosophila* and human beings. Therefore, information on the biodiversity of genus *Drosophila* is limited. Many species of the genus *Drosophila* are endemic to certain regions and a few are cosmopolitan, dispersed vastly in association with human activities. Hence the present investigation has been undertaken biodiversity of *Drosophila* in Biligirirangana Hills. This study revealed that altitudinal variation in *Drosophila* species of B.R. hills in Karnataka, which is present in between eastern ghats and western ghats. Post monsoon studies of this area revealed that a total of 1739 *Drosophila* flies belonging to 10 species of 3 sub genera were collected at altitudes of 300m, 700m, and 1200m. Further there are about 8 different species belongs to sub genus Sophophora where as in subgenus *Drosphila* and *Scapto Drosophila* represent one species each. The population density varied in all the altitudes and its sequence is as fallows: 300m>1200m>700m. The least population density was found in 700m. This shows effect evolution on population density. The diversity of the *Drosophila* community was also assessed by applying the *Simpson, Shannon-wiener, and Berger-parker index* and cluster analysis to asses the Biodiversity in B. R. hills.

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INTRODUCTION

The family *Drosophilidae* (*Diptera*) is composed of more than 3,500 described species that occur in a number of ecosystems all over the world (Bachli, 1998). Most genera are found in tropical regions. The genus *Drosophila* is the most abundant and comprises around 53% of the total species. Many of them are endemic to certain regions and a few are cosmopolitan, dispersed mostly in association with human activity. The distribution of *Drosophila* species is known to affect by both physical and biological factors. It is known that changes in temperature and rainfall affect viability, fertility, developmental time and other factors that influence the rate of population growth and survival (Torres and Madi-Ravazzi, 2006).Rainfall and light intensity also have an influence on the supply of resources, principally in relation to the periods of flowering and fruiting of various vegetable resources that provide most of the sites for oviposition and feeding (Brncic *et al.*, 1985). In addition to above physical factors, biotic factors also influence the diversity and abundance of natural populations of *Drosophila* including intra–inter specific relationships, such as population density, population age, distribution, competition and relationship between *Drosophilidae* and their hosts and predators. Elevation is one important aspect of topography and one has to look at the animal distribution from that perspective. A few attempts have been made to collect *Drosophila* at different altitudes, but these data are not analyzed with an ecological perspective (Reddy and Krishnamurthy, 1977). Reddy and Krishnamurthy (1977) have also said that physical and biotic factors are the sole determinants of animal communities. If that is so elevation and season should not have any influence on animal distribution. Thus more information is required from different ecological habitats to understand the effect of altitude variation on the biodiversity of *Drosophila*. Furthermore, in the competitive exclusion theory, Gause suggested that two related species competing for the same resources could not co-exist together in the same ecological niche. Laboratory experiments have questioned the validity of the Gause Principle (Ayala, 1969).

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The presence of taxonomically or phylogenetically related species in an ecological niche indicates their coexistence and absence of such related species suggests competitive exclusion. The aim of the present study is to investigate whether taxonomically or phylogenetically related *Drosophila* species co-exist in nature or not. Biligiriranga Hills commonly called B. R. hills, Is a hill range situated in south eastern Karnatak Yelandure and Kollegal Taluk of Chamarajanagar District of Karnataka. At its border with Tamilnadu (erode district) in south India The area is called BRT wildlife sanctuary it is protected reserved under the wildlife protection act of 1972 being at the confluence of the Western ghats and the Eastern ghats the site was declared a Tiger reserve in December 2010 it is one of such place where collection studies of *Drosophila* has not been made the sanctuary has a varying altitude up to 1800m The forests are mainly evergreen and semi evergreen in nature, Shola vegetation is dominant in higher altitudes of the sanctuary. Further in the lower regions of the sanctuary, deciduous forests are seen, which are an abode for rich faunal diversity of Drosophilidae. Therefore, the present study has been undertaken in this sanctuary to understand the biodiversity of *Drosophila* in relation to microhabitat variations.

MATERIALS AND METHODS

Using sweeping and bottle trapping method, *Drosophila* collections were made during post monsoon period (October to December 2014) in the Biligiriranga hills commonly called B.R.hills, is a hill range situated in south eastern Karnatak Yelandure and Kollegal Taluks of Chamarajanagar District of Karnataka at its border with Tamilnadu (erode district) in south india to account for the biodiversity of *Drosophila* fauna.
The collections were made in three different altitudes (300, 700 and 1200m) of Biligirirangana hills. In net sweeping methods, various rotting fruits, such as, *Vitas vanifera* (grape), *Musca paradisca* (banana), *Manilkara zapota (sapodilla)*, *Citrus sinensis* (orange), *Malus domestica* (apple), *Carica papaya* (papaya) and *Ananas comuses* (pineapple), were mixed and spread under shaded areas to attract flies. After a day of spreading, the flies were collected by sweeping using fine net. The flies were then transferred to the bottles containing wheat cream-agar medium and brought to the laboratory for identification.

The bottle trapping method was also followed for collection, in this technique, culturing bottles containing smashed banana sprayed with live yeast were tied to the twigs of bushes under shaded areas. The following day, bottles with attracted flies were collected by plugging the bottles and later transferring to culture bottles containing wheat cream-agar medium and brought to the laboratory for identification. The collected males were identified using taxonomical markers such as body pigmentation, sex comb and genital plate. Since there are no such taxonomical markers in females of *Drosophila* species, therefore the collected females were subjected to isofemale lines. The male flies obtained from the progenies of isofemale lines were used for species identification. Uniformity was maintained by using the techniques and in the number of baits used in the collection sites. The sanctuary is rich in floral diversity. Vegetation at the collected sites included *Acacia catechu*, *Albizzia amara*, *Artocarpus*, *Bauhinia species*, *Bombax*, *Caryota*, *Calophyllum, Carea, Cinnamomum species, Clementis trifolia, Eucalyptus grndis, Ficus bengalensis, Garcinis gummi-gutta, Gymnima sylvestres, Hibiscus malva, Lantana camera, Litsea species, Mesua, Pongamia glabra, Vitex negundo*, *Holorrhina* and *Strobilanthes* which are the main vegetation growing in the sanctuary. The abundance, richness and diversity relationship of flies collected were assessed by Simpson (D), Shannon-Wiener (H) and Berger-Parker (1/d) indices (Mateus *et al*., 2006). The Simpson index (D) that measures the probability that two individuals are randomly selected from a sample that belong to the same species, was calculated using the formula:

$$D=\sum n(n-1)/N(N-1)$$

Where, n = the total number of organisms of a particular species and N = the total number of organisms of all populations. Shannon-Wiener measures the value of species as a function of their frequency in the community and was calculated using the formula:

$$H' = - \sum p_i \ln p_i$$

pi = the proportion of individuals belonging to the ith species in the dataset of interest.

Berger- Parker index (1/d) which shows the relative abundance was calculated using the formula:

$$1/d=N/NMax,$$

Where, N = number of individuals of all species and Nmax = number of individuals in the most common species.

RESULTS

The list of *Drosophila* species collected at different altitude of Biligiriranga Hills from October-November 2015 and their taxonomic position given in Table 1. A total of 10 species of *Drosophila* were collected belonging to 3 sub-genera (*Subgenus Sophophora*, *Drosophila*, *Scaptogrosophila*). Pooled data collection of *Drosophila* yielded a total of 1739 individuals. Out of these 8 species of the *subgenus Sophophora* 1476 (84.87%). Individuals belonged to 1 species of subgenus *Drosophila* 188 (10.81%). Individuals belonged to 1 species of *subgenus scaptogrosophila* 75 (4.31%). The *Simpson's index* value of the *Simpson's index* indicating the abundance, richness and diversity of *Drosophila* flies at different altitudes (300m a.s.l). The was 0.11 *(Shannon-Wiener index* was 2.2, *Berger-parker index* was 0.19), and at the highest altitude (1200m) *Simpson's index* was 0.33 (*Shannon-Wiener index* was 1.03, *Berger-parker index* was 0.39). Altitudinal variation of the *Drasophila* population is shown in Figure 1. The number of *Drosophila* flies was higher in low altitude and low in 700m. The dominant species in the all the true altitudes are depicted in Figure 2. There were three common species namely *Drosophila anannassae, Drosophila anamolani and Drosophila takahashii* were found to be dominant in all the three altitude studied. Among the three, *Drosophila anannassae* was found to be dominant over other two species.

Table 1. Frequency distribution of the various species of *Drosophila* in the Chamaraja nagara District south eastern Karnataka at its border with tamil nadu (Erode District) During post monsoon season(October to December 2014)

S/n	Species	300m	700m	1200m	Total number
	Subgenus sophophora				
1	*D. anannassae*	148	40	178	366
2	*D.anamolani*	108	83	132	323
3	*D. bipectinata*	50	50		100
4	*D. kikkawai*	72	31		103
5	*D. malarkotliana*	132	78		210
6	*D. sampangiensis*	70			70
7	*D. takahashi*	75	40	138	253
8	*D. variens*	51			51
	Total	706	322	448	1476
	Subgenus Drosophila				
9	*D. neonasuta*	188			188
	Total	188			188
	Subgenus scaptogrosophila				
10	*D. nigra*	75			75
	Total	75			75
	Grand total	969	322	448	1739
	Simpson index	0.11	0.18	0.33	
	Shannon-wiener index	2.2	1.72	1.09	
	Berger-parker index	0.19	o.25	0.39	
	Mean temperature in °c	18	14	10	

Table 2. The absolute (A), relative abundance (R) and constancy value (c) *Drosophila* collected at the different altitudes of biligirirangana hills 2014-2015

Species	300m			700m			1200m		
	A	r	c	A	R	c	A	R	C
Subgenus sophophora									
D. anannassae	148	0.15	100	40	0.12	100	178	0.39	100
D.anamolani	108	0.11	100	83	0.25	100	132	0.29	100
D. bipectinata	50	0.05	66.66	50	0.15	66.66	-	-	66.66
D. kikkawai	72	0.07	66.66	31	0.09	66.66	-	-	66.66
D. malarkotliana	132	0.13	66.66	78	0.24	66.66	-	-	66.66
D. sampangiensis	70	0.07	33.33	-	-	33.33	-	-	33.33
D. takahashi	75	0.07	100	40	0.12	100	138	0.3	100
D. variens	51	0.05	33.33	-	-	33.33	-	-	33.33
Total-	706	-	-	322	-	-	448	-	-
Subgenus Drosophila									
D. neonasuta	188	0.19	33.33			33.33			33.33
Total	188	-	-	-	-	-	-	-	-
Subgenus scaptoDrosophila									
D. nigra	75	0.07	33.33	-	-	-	-	-	33.33
Total	75	-	-	-	-	-	-	-	-

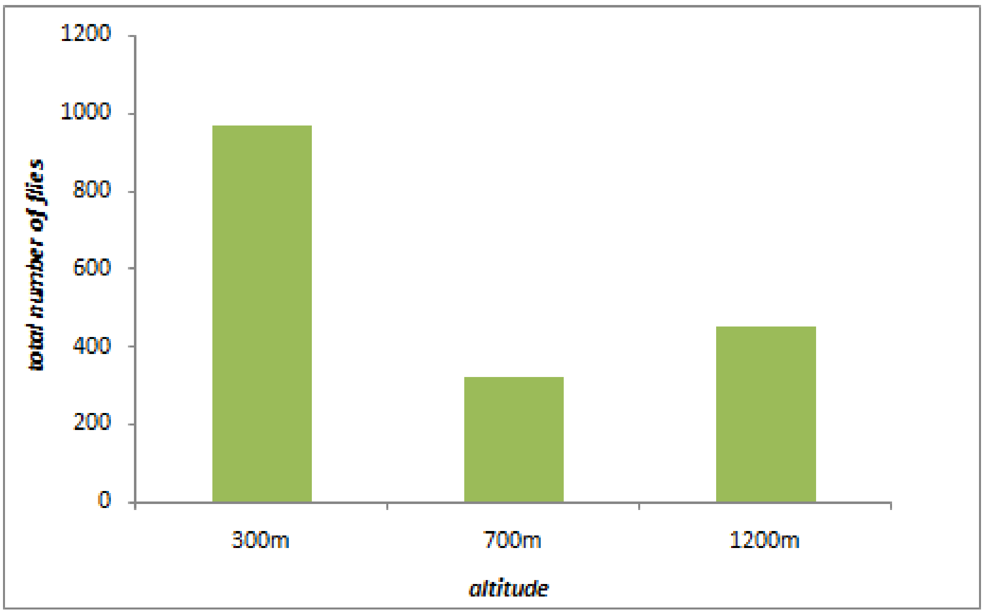

Fig. 1. Total number of species collected from different altitude

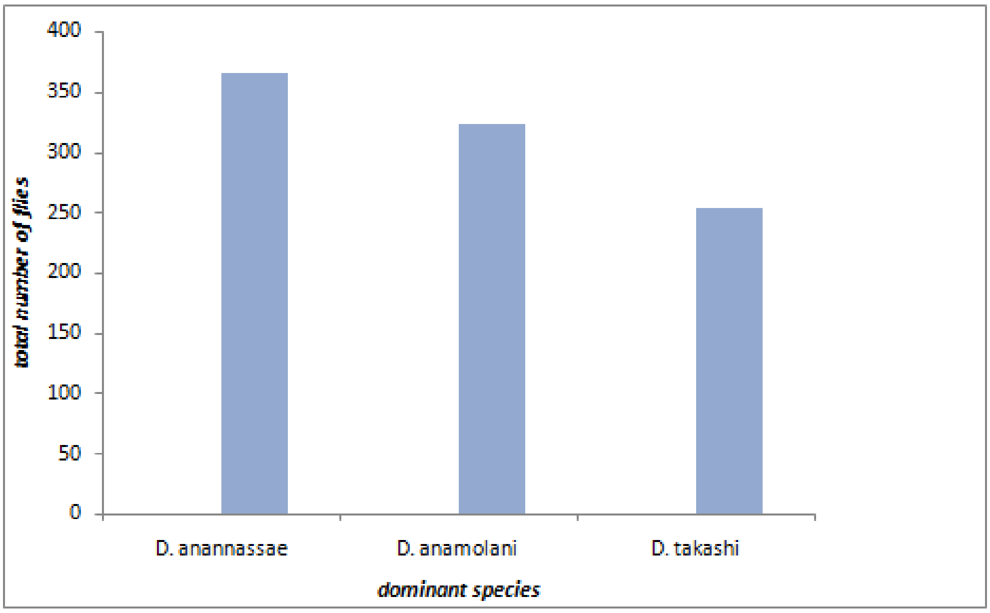

Fig. 2. Dominant species present in three altitudes

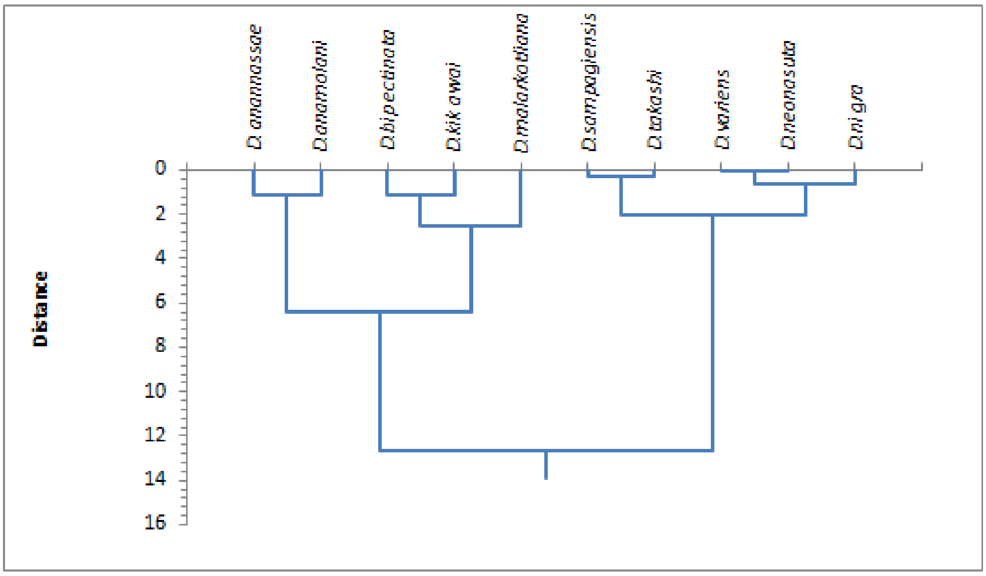

Fig. 3. Cluster analysis

Table 2 revealed that constancy value (C) of all +ve species at altitudes along with absolute numbers and relative abundance constancy species (C≥50) represented, *D.ananassae, D. anamolani, D. bipectinata, D. kikkawai, D.malerkotliana, D.neonasuta, D. sampangiensis, D. takahashii, D. varians and D. nigra* . Total collected species 10 out of 10. Accessory species represented (at 10 out of 10). At 700m 6 out of 10, *D. anamolani, D.ananassae, D. bipectinata, D. kikkawai, D.malerkotliana, D. takahashi*. At 1200m 3 out of 10, *D. anamolani D. ananassae, D. takahashii.* Constant species were *D. anamolani, D. ananassae, D. takahashii*. In the cluster analysis Figure 3. The accidental species stand first in the cluster, followed by the accessory species and the bottom is occupied by constant species. The species and genus of the *Drosophila* belongs to first cluster are*, D. bipectinata, D. kikkawai, D. malerkotliana, D. sampangiensis, D. takahashii. Here the constant species was D. takahashii*. The species and genus of the *Drosophila* belongs to second cluster are, *D. anamolani, D. bipectinata, D. kikkawai, D. takahashii, D. varians, D.neonasuta*. The species and genus of the *Drosophila* belongs to third cluster are*, D. kikkawai, D. malerkotliana, D. neonasuta, D. nigra*. Species and genus of the *Drosophila* belongs to fourth cluster are*, D. anamolani, D. ananassae, D. bipectinata, D. kikkawai, D. takahashi, D. neonasuta , D. sampangiensis, D. varians*.

DISCUSSION

The number of flies at three different altitudes of Biligiriranga hill Wildlife Sanctuary collected during the post monsoon season is shown in able 1. At 300 m, the number of flies collected was the highest with 969 flies which comprised of 10 different species. At 700 m, a total of 322 flies belonging to 6 different species were recorded. But at 1200 m, a total of 448 flies of only 3 different species were collected. *Drosophila anannassae, Drosophila anamolani* and *Drosophila takahashii*, species were seen as common species in all altitudes. The collected data of *Drosophila* at different altitudes of Biligiriranga hill Wildlife Sanctuary show that highest *Drosophila* density is in the lowest altitude of 300 m, after which it drastically falls at 700 m. Following this, at 1200m once again the *Drosophila* density increased to 448flies. This shows that *Drosophila* community is affected by elevation. Studies of Guruprasad *et al*. (2011) in Chamundi hill and Wakahama (1962) have also reported the influence of elevation on the distribution of *Drosophila* flies.

They have found that the density of *Drosophila* decreased with an increase in elevation. Greater density of *Drosophila* flies in the lowest altitude found in the present study could be due to the increased floral diversity. The value of Simpson, Shannon-Weiner and Berger-Parker indices that indicate the abundance, richness and diversity of *Drosophila* flies in different altitudes of the hill are shown in Table 1. At the lowest altitude of 300 m, Simpson = 0.11; Shannon-Weiner = 2.2 and Berger-Parker = 0.19; at 700 m Simpson = 0.18; Shannon-weiner = 1.72 and Berger-Parker = 0.25. At the higher altitude of 1200m, Simpson = 0.33; Shannon-weiner = 1.09 and Berger-Parker = 0.39 In the Simpson index (D), 0 represents infinite diversity and 1, no diversity, that is, the greater the value of D, the lower the diversity but the reverse is true in the case of Berger-Parker and Shannon-Wiener indices (Ludwig and Reynold, 1988; Mateus *et al*., 2006). Applying these indices to understand the measures of biodiversity of flies at different altitudes demonstrates that the lower altitude of 300 m has a higher value (D) and lower value of 1/indicating more biodiversity as compared to the higher altitude of 700 and 1200 m (Table 1).

The density or richness of species also depends on the number of biotic and abiotic factors encountered in the seasons. A change in the relative frequency of different species from season to season due to changes in the natural environment was reported by Guruprasad *et al*. (2010). Dobzhansky and Pavan (1950) showed that rainfall appears to have a greater influence on the abundance of *Drosophila* than temperature. In studies of *Drosophila* biodiversity showed the effect of temperature and altitude on the density of *Drosophila* population (Carson, 1956: Reddy and Krishnamurthy, 1977). In the present study it was noticed that temperature varied in different altitudes (at 300m 18ºc, 700m 14ºc, 1200m 10ºc). Even in rainfall also varied between different altitudes (at 300m 600mm-1200mm, 700m 600mm-2000mm, and 1200m 600mm-3000mm). This suggests that difference in the biodiversity of *Drosophila* at different altitudes of Biligiriranga Hills wild life sanctuary could be due to variation in the observed temperature and rainfall. Our study also confirms the work of Guruprasad *et al.* Regarding biodiversity of *Drosophila* in chamundi hills. They also found influence of temperature and rainfall on the *Drosophila* community, thus the ecological condition change with changing altitude in Biligiriranga Hills. The lower altitude is comparatively cooler with lesser rain and dryness. Further Hegde *et al.* (2000), have also pointed out that the growth and size of the population depend on several environmental factors in addition to genetic structure. 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 Hod kinson (2005), the above-mentioned changes are often strongly interactive and together create an environmental envelope within which insect species survive and reproduce. Hodkin son (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 a population depend 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*. So our results in B. R. hills are in agreement with the work of Cooper and Dobzhansky (1956), Reddy and Krishnamurthy (1977), Hegde *et al.* (2001) and Bovito *et al*. (2013), all these studies have shown that influence of microclimatic conditions on the diversity of *Drosophila*. According to constant, accessories and accedentale species as well as cluster analysis indicates several species that co-existed had similar ecological preference. It is clear from our study that the distributional pattern of a species or related group of species is uneven in space and time. The *Drosophila* community of B.R.hills was highly diverse and dependent on several environmental factors in addition to the general structure of the species present in it.

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