



Diversity of *Drosophila* species in and around Amarkantak region of Madhya Pradesh during spring season

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Abstract

Permanent alterations in the *Drosophila* fauna would suggest substantial biotic changes at least at the level of plants, fungi, and hymenopterous wasps that parasite upon various life cycle stages of *Drosophila*. Since the long year times, *drosophila* has been used in genetical, cytological and evolutionary studies. In this connection fruit flies are admirable experimental animal. Hence, in the current work we studied the relative abundance of *Drosophila* species in an around Amarkantak region, Madhya Pradesh from February to April in the year 2019. 15,533 numbers of flies were collected in total comprising of seven different species of *Drosophila* i.e. *D. busckii*, *D. melanogaster*, *D. ananassae*, *D. Simulans*, *Zaprionus indianus*, *D. jambulina* and *D. immigrans*. *D. busckii* showed highest relative abundance of 23.21 % while *D. annanassae* showed the least relative abundance of 10.64 %. This study is the first of its kind to study the relative abundance of *Drosophila* species in and around Amarkantak region, Madhya Pradesh during the spring season. We also accessed the diversity of *Drosophila* species with the application of Simpson (D'), Shannon (H') and Berger-Parker (1/d) index showing the abundance, species richness and diversity of *Drosophilids*. Application of these indices to study different measures of *Drosophila* biodiversity in different months demonstrated that the month of March had a highest value of Simpson (D'), Shannon (H') index suggesting the month has highest biodiversity of *Drosophila* compared to other months.

Keywords: *Drosophila*, *Drosophila busckii*, *Drosophila melanogaster*, *Drosophila annanassae*, *Drosophila simulans*, *Zaprionus indianus*, *Drosophila jambulina*, *Drosophila immigrans*, diversity

Introduction

There are about 1500 species in the genus *Drosophila*, which is further distributed into ten subgenera. *Drosophila* is the biggest subgenus (Sturtevant 1942, Ashburner 2004) [33] which is being extensively used in the study of genetic, population, ecology, developmental biology and evolutionary biology. They have a significant contribution towards the diversity of natural habitats. Within a relatively narrow climatic range as found in the damper regions of rain forests, where there is an enormous diversity of *Drosophila* species all involved with the exploitation of microbial degradation products. Any alterations in the *Drosophila* fauna indicates substantial biotic changes at least at the level of plants (Terborgh 1986, Lachaise & Tsacas, 1983) [34, 13], fungi (Chabora *et al* 1979) [4] and hymenopterous wasps (Prince, 1976, Chabora *et al* 1979) [29, 4]. These studies indicate that they are very sensitive to small environmental change that changes its population size and structure in a that area and are considered as bioindicators (Da Mata *et al* 2008, 2010) [5, 6].

Drosophila species are more sensitive to habitat changes because of their relatively narrow physical limits for resource utilization (Parsons, 1982) [25]. They have different dietary preferences. The fungus-feeding species never get attracted towards fermenting food, similar is true is case in fermented food eating species (Dobzhansky & Pavan 1950) [7]. Apart from this, the dietary specialization in *Drosophila* species is not that strict because the same fermenting fruits are generally utilised by many species. As a result, sympatric *Drosophila* species that rely on a shared source of food compete with one another (Dobzhansky & Pavan

1950) [7]. The diversity of ecological niches available in the environment influences the intensity of competition. When there are several ecological niches accessible, a species is more likely to encounter fewer competitors than in other niches, and they may even become a monopolist. In addition, temperature and rainfall, for example, may also have an impact on the life cycle and development of *Drosophila* species, as well as their population.

The diversity and distribution pattern study of *Drosophila* in various parts of India are mainly in Mysuru (Guruprasad and Padmaja 2016, Reddy and Krishnamurthy 1974) [19, 30], Dahrwad (Srinath B S, N Shivanna, 2014) [32], Kanara district of Karnataka (Nagaraj and Krishnamurthy, 1980, Vasudev *et al* 2001) [19, 36] and the Western Ghats (Prakash and Reddy 1978, 1979, 1980, Srinath and Shivanna 2012) [26, 27, 28, 31]. Seasonal variation of studies of *Drosophila* in central India is fragmentary. As a result, the purpose of this study was to learn more about the diversity and distribution of *Drosophila* species in and around Amarkantak. Thus, we selected the Indira Gandhi National Tribal University and Lalpur, Rajendragram and Amarkantak region of Madhya Pradesh for the study of *Drosophila* population and their species diversity. Hence, the main objective was to study relative abundance, species richness and diversity of *drosophila* species in and around Indira Gandhi National Tribal University (IGNTU) and Lalpur, Rajendragram and Amarkantak region of Madhya Pradesh.

Materials and Methods

Study area

Flies were collected in three different regions of Madhya

Pradesh i.e. Indira Gandhi National Tribal University and Lalpur region, Rajendragram and Amarkantak (22.67°N 81.75°E). The location of Amarkantak is in the Anuppur district of Madhya Pradesh. It is a place where Vindhya and Satpura range meets and is the foothill of Maikal Hills. Amarkantak lies at an average height of 1048 metres (3438 ft) above sea level with 73% Humidity. The region is unique because of its natural heritage and rich variety of flora. The attractive lush green forest of Amarkantak is a hilly region covered with dense forest. Indira Gandhi National Tribal University is situated at Lalpur, 20 km. away from Amarkantak. Rajendragram is present on other side of Indira Gandhi National Tribal University which is 26 kms away.

Specimen sampling

Flies were collected done during February-April. Flies were collected from Botanical Garden, Canteen vegetable store-room, and food-bins of three different locations including Indira Gandhi National Tribal University and Lalpur region, Rajendragram region and Amarkantak region of Madhya Pradesh using two methods:

- Net Sweeping method
- Bait trap method

In the net sweeping method rotten banana and papaya fruits were spread in shades of bushes. After one day, a conical net was swiped over the rotten fruits to collect flies. Flies were transferred to food vials containing wheat-agar medium using aspirator and brought to laboratory.

Whereas in the bait trap method, plastic bottles with an opening were used with a piece of rotten bananas and papaya mixed fruit added inside the bottles. Next day flies were collected from the bottle after covering it with a dark cloth. Soon they were isolated, sexed and stored in temperature-controlled BOD and later examined under the microscope.

Species identification

Drosophila species were identified with the help of books (Markow and O'Grady, 2005) [17] and using online help: <http://flybase.org/static/Drosophilidae> based on their external morphology.

Formula for relative abundance

$$\text{Relative abundance} = \left(\frac{\text{Total no. of flies of a particular species}}{\text{Total no. of flies collected}} \right) * 100$$

Simpson (D'), Shannon index (H'), and Berger-Parker (1/d) indices were used to determine the abundance, richness, and diversity of *Drosophila* species collected, as stated by Mateus *et al.* (2006) [18]. Simpson's index (D') which measures the diversity was calculated using the following formula:

$$\text{Relative abundance} = \left(\frac{\text{Total no. of flies of a particular species}}{\text{Total no. of flies collected}} \right) * 100$$

Where, n = the total number of individuals in a certain species, and N = the total number of individuals in the whole population. D = 0 represents infinite diversity whereas D = 1 represents no diversity therefore to overcome this problem this index is calculated after subtracting from 1

(D') or represented as Simpson's reciprocal index 1/D. Shannon index which measures the value of species as a function of their frequency in the community was calculated using the formula

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

p_i = the proportion of individuals belonging to the i^{th} species in the dataset of interest. The lower H' values indicate low diversity while higher values indicate high diversity.

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

$$\frac{1}{d} = N/N_{\max}$$

Where, N = number of individuals of all species and N_{\max} = number of individuals in the most common species.

Statistical analyses

Biodiversity Pro version 2 software was used for statistical computations and visualisations. The rank abundance curve was drawn to show the relative abundance of various species during different months. Such plots are used to visually represent the species richness and species evenness (Goñi *et al.*, 2012) [8].

Results

Species identification

A total number of seven species were identified during our study belonging to 4 subgenera. Most of the species belong to *Sophophora*. They are *Drosophila melanogaster*, *Drosophila annanassae*, *Drosophila simulans*, *Drosophila jambilina*. Single species collected each from subgenus *Drosophila*, *Dorsilopha* and *Zaprionus* that are *Drosophila immigrans*, *Drosophila busckii*, *Zaprionus indianus* respectively. The description of different *Drosophila* species was done according to Bachli *et al* (2004).

▪ *Drosophila busckii*

Light yellowish fly with three dark-brown stripes across the mesonotum while the middle one bifurcates next to scutellum. Dark interrupted bands are present in the middle of abdomen of this species which are narrowed at the sides. The costal index is about 3.1 (Fig 1A).

▪ *Drosophila melanogaster*

In this species the abdominal bands are complete and the sex comb consists of 9-12 teeth on basal tarsal segment of fore-legs. Male protarsus has a sex comb formed by a single row of strong modified bristles. *D. melanogaster* females may be distinguished from *D. simulans* by having a darker abdomen band. Costal index is 2.4. (Fig 1B).

▪ *Drosophila ananassae*

Abdominal tip of this species is yellow in males and the sex comb is diffused in males. Several rows of strong black bristles are also present ventrally on proximal two tarsal segments of first leg. Male protarsus with very indistinct sex combs. Costal index is 1.5 (Fig 1C).

▪ *Drosophila simulans*

Yellowish-brown species where thorax is light reddish-brown dorsally and lighter ventrally with no distinctive

pattern. Abdomen is black or dark brown posteriorly and yellowish-brown anteriorly. Male protarsus with a sex comb formed by a single row of strong modified bristles and a costal index of 2.3. Males can be differentiated from *D. melanogaster* because of its large epandrial ventral lobe (Fig 1D).

▪ ***Zaprionus indianus***

Yellowish colored fly having four silvery-white stripes bordered by black across the head, mesotonum and scutellum. Two reduced prescutellar seta. Profemur with a row of characteristic strong setae each one arising from a small tubercle with another setula. Costal index about 2.6 (Fig 1E).

▪ ***Drosophila jambulina***

Shiny black abdominal tip and bristles are not prominent on the abdomen of this species. Sex comb is present on 1st metatarsus in 2 sets of about 24 and 25 black teeth. White spot is present on the facial carina i.e. between the antennae base and above the clypeus. Secondary clasper nearly oval in shape; larger medial seta on secondary clasper inserted dorsally (Fig 1F).

▪ ***Drosophila immigrans***

Body is comparatively larger in size than other species which is brown in color. Eyes of this species are deep- red in color. Wings with crossveins and the tips of longitudinal veins slightly shaded. Costal index is 4.4. Abdominal bands are medially interrupted with triangular marginal bands (Fig 1G).

Relative abundance of species identified

A total number of 15,731 flies were collected during our study. In the month of March, we collected the most individuals (13,614) from seven different species. Whereas 1,621 flies were collected in the month of April belonging to six different species (Table 1).

Maximum number of flies obtained during spring in 2019 was *D. buskii* (3,651) which was found only in February and March (Table 1). We didn't find any *D. buskii* in the late spring season. However, they comprised of 23.21% of the total number of flies collected during our study (Fig 2). 2,519 numbers of flies belonging to *D. jambulina* species comprising of 16.01% of the total number of flies collected and 2,161 flies from *D. melanogaster* species comprising of 13.74% (Fig 2) of the total number of flies collected were obtained in our study. Further, 2,085 numbers of flies of *D. simulans* species and 1,831 numbers of *D. immigrans* species were obtained during the present study which comprised of 13.25% and 11.64% of the total number of flies collected respectively. The least number of flies were collected from *Z. indianus* and *D. annanassae* species comprising of 1,810 and 1,674 numbers respectively. These two species both comprised of 11.51% and 10.64% of the total number of flies obtained for the present study respectively (Fig 2). We further calculated the Simpson, Shannon index and Berger-Parker index showing the abundance, species richness and diversity of Drosophilids at different locations of Amarkantak region at spring season (Table 1). In the month of February, Simpson (D') = 0.0; Shannon index (H') = 0.0 and Berger-Parker = 1.0 was obtained; in the month of March the values were as Simpson (D') = 0.846; Shannon index (H') = 1.91 and Berger-Parker = 4.32. During April, Simpson (D') = 0.824; Shannon index (H') = 1.76 and Berger-Parker = 4.52 was obtained. In the Simpson index of diversity (D'), the value 0 represents no diversity whereas 1 represents infinite diversity. It means that greater the value of D' or H' , greater be the diversity (Ludwig and Reynold, 1988; Mateus *et al.*, 2006) [15, 18]. When these indices were applied for determining the different measures of biodiversity at different months, we found that March has highest D' and H' values indicating the month has higher biodiversity of flies and was suitable for most of the species to grow as compared to other months. The rank abundance plot for each month of the species found in our study has been shown in Fig 3. Rank abundance curve could not be plotted for the month of February as only one species (*D. buskii*) was obtained during this month. The rank abundance curve for the month of March showed less even distribution with some species found more abundantly than the other. The rank abundance plot with the most even distribution was for the month of April as all the individuals obtained from different species had almost similar number of individuals although they were lesser in number as compared to the month of March. In March all seven species (*D. buskii*, *D. melanogaster*, *D. annanassae*, *D. simulans*, *Z. indianus*, *D. jambulina* and *D. immigrans*) were obtained during the collection and in April except *D. buskii* all other species were obtained.

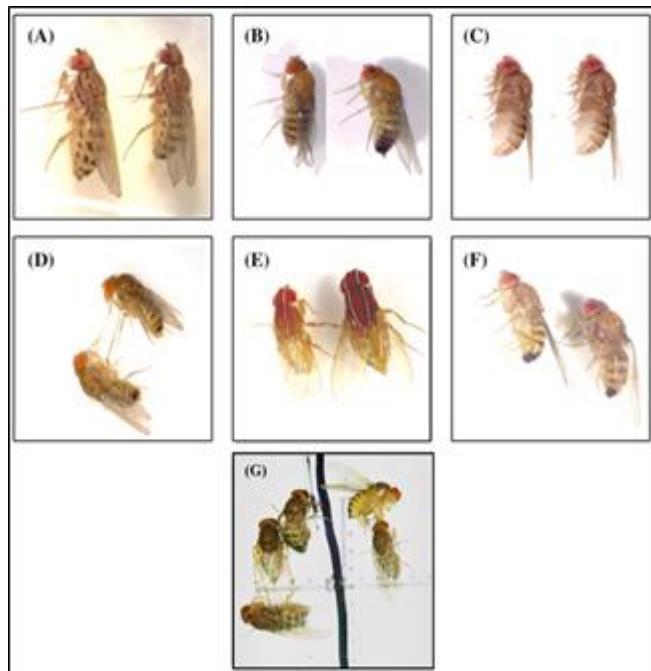


Fig 1: Different species of *Drosophila* found in our study: (A) *Drosophila buskii*, (B) *Drosophila melanogaster*, (C) *Drosophila annanassae*, (D) *Drosophila simulans*, (E) *Zaprionus indianus*, (F) *Drosophila jambulina*, (G) *Drosophila immigrans*

Table 1: Biodiversity of *Drosophila* at Indira Gandhi National Tribal University and Lalpur region, Rajendragram region and Amarkantak region of Madhya Pradesh during the spring season of 2019.

Sl. No.	Species	Feb	March	April	Total Number
1	<i>Drosophila buskii</i>	496	3155	0	3651
2	<i>Drosophila melanogaster</i>	0	1855	306	2161

3	<i>Drosophila annanassae</i>	0	1509	165	1674
4	<i>Drosophila simulans</i>	0	1764	321	2085
5	<i>Zaprionus indianus</i>	0	1451	359	1810
6	<i>Drosophila jambulina</i>	0	2277	242	2519
7	<i>Drosophila immigrans</i>	0	1603	228	1831
Grand Total		496	13614	1621	15731
Simpson's index of diversity (D')		0.00	0.846	0.824	
Shannon index (H')		0.00	1.91	1.76	
Berger - Parker index (1/d)		1.00	4.32	4.52	
Mean temperature in °C		23.591	25.806	31.786	

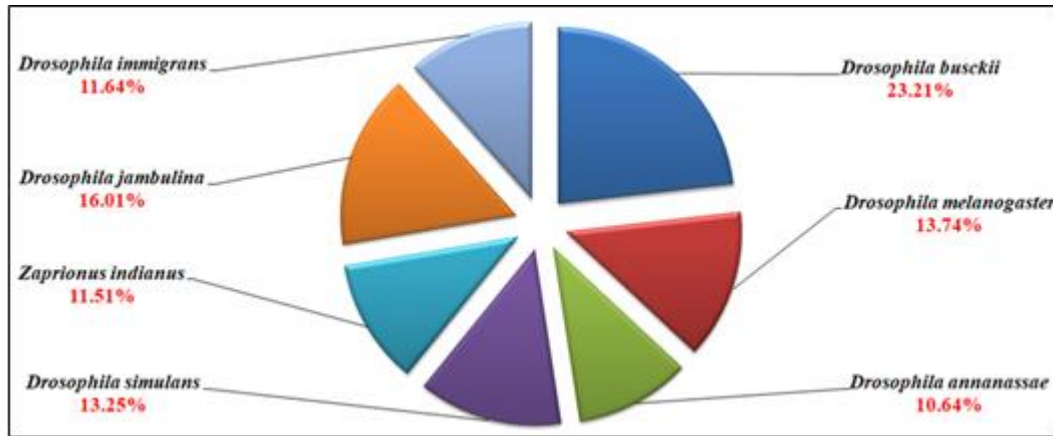


Fig 2: Relative abundance percentage of *Drosophila* species at Indira Gandhi National Tribal University, Lalpur, Rajendragram and Amarkantak regions of Madhya Pradesh during spring season of 2019.

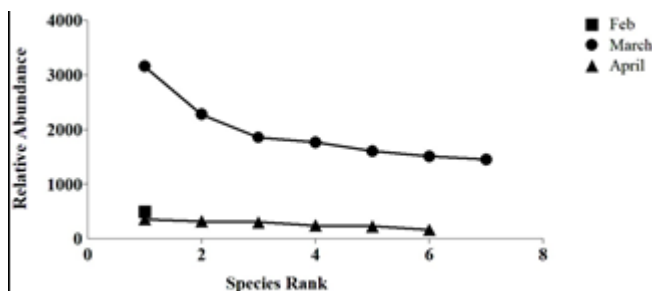


Fig 3: Rank-abundance plot showing abundance curve for different months (February, March and April) in which the drosophila flies were collected. Species richness is highest for the month of March (seven species) whereas species evenness is highest in the month of April.

Discussion

Determining the species' niche is a difficult task as there are various potentially relevant criteria which limit the species' survival and reproduction. At present, there are few evidences that may prove generalist species are more abundant over the specialists in a given population (Verberk 2011) [37]. Insufficient data related to seasonal adaptation of insects also provides a scope for many researchers to study in this aspect of insect ecology (Majerus 1990; Osawa and Nishida 1992; Aldridge *et al.* 1993; Kari and Huey 2000; Torres and Madi-Ravazzi 2006) [16, 21, 1, 12, 35]. Changing season also has a significant contribution towards the diversity and population of species found in a given area. Variation in environmental factors such as temperature, rainfall and humidity lead to change in population size of *Drosophila* species (Parshad and Paika 1964) [24]. Even though many insects around the world are well known to adapt to change in varying seasonal temperature (Lee and Denlinger 1991; Overgaard and Sorensen 2008) [14, 22], other climatic factors like humidity have been less studied (Parkash *et al.* 2009) [23].

We found seven different species of *Drosophila* in the current study namely *D. busckii*, *D. melanogaster*, *D. annanassae*, *Zaprionus indianus*, *D. jambulina* and *D. immigrans* in and around Amarkantak region. 15,533 numbers of flies in total were collected from February to April in 2019. *D. busckii* is 23.21% of the total number of species showing highest relative abundance while *D. annanassae* is 10.64% of the total number of species showing least relative abundance.

Drosophila busckii has been reported to be found mostly in the spring season when there is transition in the temperature in the ascending manner (Ochando 1980) [20]. Our study confirms this report. *D. busckii* is only the one belonging to the *Dorsilopha* subgenus (Guruprasad *et al.*, 2021) [10]. The average mean temperature of the collection months was around 23.6 °C as shown in Table 1 when only *D busckii* flies were found. *Drosophila immigrans* is a cold adapted species but in our present study it was interesting to note that this species did not occur during the February month. However, we have observed *Drosophila immigrans* flies more during our collection in the months of October and November with the start of winter season in Amarkantak (data not shown) when the mean ambient temperature is around 28 °C and 25 °C respectively (<https://www.worldweatheronline.com>). During March the mean ambient temperature was around 26 °C which is favourable for all the species to flourish. In April the rise in average temperature to around 32 °C that led to the decline in the number of individuals as these temperatures may not be favourable to most of the species. All the *Drosophila* species found in our study are cosmopolitan in nature. They can further be categorized into three subgroups according to their melanisation. *Zaprionus indianus* is a non-melanic species as they have no melanisation in their abdominal segments. *Drosophila busckii* is a fixed-melanic species and the rest of the species i.e. *Drosophila melanogaster*, *Drosophila simulans*,

Drosophila jambulina, *Drosophila immigrans* and *Drosophila annanassae* are melanic in nature (Kalra *et al.*, 2014)^[11]. These observations show that the spring season is favourable for different melanic group of *Drosophila* species to thrive in the region of our study.

This is the first of its kind to study the relative abundance of *Drosophila* species in and around Amarkantak region during the spring season. We can understand how various communities are organized through the identification, description and explanation of the common patterns that determines the structure of a community.

Environmental factor plays an important role in any living organism. The *D. busckii* species were more adapted to colder temperatures (14-25 °C) as observed in our present study in comparison to other species that are found at higher temperature (approximately 25-28 °C). The changing of environment condition changes the relative abundance, flies' structure like melanization, metabolism, behavior, colour etc. which leads to evolutionary changes. Hence, such studies on *Drosophila* species abundance across seasons over many years may provide the climatic changes occurring in a particular region as these flies also act as a bio-indicator.

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Conflict of interest

The authors do not have any conflict of interest.

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