Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

JEZS Journal of Entomology and Zoology Studies

ISSN 2320-7078
JEZS 2014; 2 (4): 35-41

Received: 14-06-2014
Accepted: 23-06-2014

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Seasonal variation in natural populations of *Drosophila* in Dharwad, India

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ABSTRACT
Drosophila flies were collected in and around Dharwad district during different seasons of 2011 and 2012. A total of 13853 individual flies comprising of 20 different species were collected by bottle trapping and net sweeping method. Maximum number (19) of species was recorded during monsoon 2012 and minimum number of species (5) during winter 2012 Statistical analysis revealed a positive correlation between abundance and average rainfall. No correlation was observed between abundance and temperature. The diversity of species was more during post-monsoon 2012 and less during winter 2012. Their abundance cluster analysis revealed three clusters where *D. malerkotliana*, *D. bipectinata*, *D. nasuta nasuta* and *Phorticella striata* are predominant species. *D. eugracilis*, *D. jambulina*, *D. rajasekari*, *D. sulfurigaster neonasuta* and *D. ananassae* are intermediate species whereas remaining 11 species are less dominant species.

Keywords: Biodiversity, *Drosophila*, Dharwad, seasonal variation, diversity indices, cluster analysis.

1. Introduction
Drosophila has been considered as a model system in the studies of genetics, evolution and ecology. There are about 65 genera classified under Drosophilidae family which is composed of more than 3,500 described species that occur in diverse ecosystems all over the world [15]. Some species are endemic to certain regions and few are cosmopolitan. These insects are highly sensitive to environmental variations which lead to fluctuation in their size of natural population. Studies on the population structure and ecology of *Drosophila* species have provided important information in understanding their evolutionary process [2].
Studies on seasonal variation of *Drosophila* in different parts of the world have been carried out by earlier workers like Dobzhansky and Pavan [5] from Brazil; Paik [18] from South Korea and Wakahama [31] from Japan. Torres and Madi Ravazzi [29] have studied seasonal variation of *Drosophila* from Sao Paulo, Brazil. In India Parshad and Paika [19] have studied the effect of seasonal variation on *Drosophila* population from Punjab. Hegde *et al.* [11] have reviewed biodiversity, eco distributional pattern and seasonal variation of *Drosophila* species in South India. According to them out of 140 species in India, South India has 50 species. Most of the *Drosophila* diversity studies were concentrated on surrounding areas of Mysore in South Karnataka [25] and North Kanara district in northern part of Karnataka [17, 30] and the Western Ghats [22, 23, 24]. Most of the studies have revolved around altitudinal variation in relation to rainfall and temperature [7, 21, 8, 9, 10]. Recently Srinath and Shivanna [27] reported a variant of rare species *D. daruma* belonging to *Polychaeta* subgroup of the genus *Drosophila* from Kalghatgi forests of Western Ghats of South India. Seasonal variation studies on *Drosophila* of South India are fragmentary and poorly understood. In view of this, the present work has been undertaken to study the effect of seasonal variation on *Drosophila* species of Dharwad district.

2. Material and methods
2.1 Study area
Dharwad district is situated in the western sector of the northern half of Karnataka state. It lies between the latitudinal parallels of 15° 15' and 15° 35' North and longitudes of 75° 00' and 75° 20' East with an area of 4, 27,329 ha out of which 8.24% of land area is under forest. The district lies approximately about 800 meters above sea level, which is the reason for a moderate and healthy climate. The annual rainfall in the district ranges from 998.2 to 594.30 mm. Dharwad district is divided into 5 taluks Dharwad, Hubli, Kalghatgi, Kundagol and Navalgund. Physiographical status of Dharwad falls within the macro region of Deccan Plateau.

The district is classified into two prominent landscapes: the Dharwad and the black soil plains. The Dharwad landscape which features Sahyadrian region of the western belt of the district covers the Kalghatgi taluk. The landscape is marked by chain of low hill ranges. The landscape of black soil plains on this Sahyadrian region constitute a transitional belt where the hilly landscape of the west passes into the undulating plain of black soil. The forest land is concentrated in Kalghatgi taluk (28.39%), Dharwad (12.23%) and Hubli (2.75%) taluks. There is no forest land in Kundagol and Navalgund taluk. Monsoon deciduous forests are found in the Malnad zones which include Kalghatgi and Dharwad (Part of it) taluks [16]. On an average the western region of the district receives more rainfall than the eastern plains.

2.2 Collection and Sampling method

Flies were collected using both bottle trapping and net sweeping methods as mentioned by Hegde *et al*. [11]. Bottle trapping was done using 200 ml milk bottles filled with banana smash tied to trees at least two and half feet above the ground and left open for 24 to 48 hours. It was plugged with cotton before collection. The bottles were tied to trees where there was minimum exposure to sunlight especially during summer and monsoon. Care was taken to avoid rain entering the bottles as they get filled with water and flies collected from such bottles get stuck in the fruit media and are dead by the time they are brought to the laboratory. Net sweeping method was done by spreading the fruits on the ground under cool shaded areas. Sweeping was done after 24 to 48 hours of spreading. Collection of flies was done during morning hours and the temperature was recorded.

A total of 8 bottles were tied per site for collection during each season. The trapped flies were brought to the laboratory and segregated according to their sex. Individual females were cultured in vials containing wheat cream agar medium [26]. The male flies were identified and recorded according to Hsu [12], Bock and Wheeler [3], Grimaldi [6] and Markow and O' Grady [14]. Collection of flies was done from different localities such as the Western Ghat forests of Kalghatgi and Dharwad, fruit markets of Hubli and Dharwad, Mango and Guava orchards, residential areas and agricultural fields of all the 5 taluks. *Drosophila* flies were collected during different seasons of 2011 and 2012. The seasons were divided according to meteorological department of India as winter (January to February), summer (March to May), monsoon (June to September) and post-monsoon (October to December). The rainfall data was obtained from the meteorological department of India (available online http://www.imd.gov.in).

The relationship between the abundance of individual species, average rainfall and temperature was analysed for different seasons by means of the Spearman correlation coefficient (r) [29]. Diversity of the flies during different seasons and of different taluks was calculated using Simpson Index (1-D), Shannon index (H) and Equitability index (J) using PAST 3.X software [15, 9]. Simpson Index was calculated to analyze the probability that two individuals randomly selected from a sample belong to the same species. Formula to calculate Simpson index is $D = 1- \sum n_i (n_i-1)/N (N-1)$ where n_i = the total number of organisms of particular species; N= the total number of organisms of all population. Shannon index was calculated using the formula $H' = -\ln \sum p_i \ln p_i$ where p_i = proportion of individuals belonging to the same species in a given sample. The equitability index was also calculated using the formula $J = - \ln \sum p_i \ln p_i / \ln N$. This is the Shannon Weiner diversity Index divided by ln N. This index measures the uniformity or equity of the species distribution [13].

SPSS 16.0 statistical package was used to determine the level of similarity of the 20 species of Drosophilids obtained and hierarchical cluster analysis (Euclidean distances, Ward's method) was used to construct a single linkage dendrogram. Cluster analysis is a technique used to classify cases into groups that are relatively homogeneous within themselves and heterogeneous between each other.

3. Results

Table 1 shows the number of *Drosophila* species collected in and around Dharwad district during different seasons of 2011 and 2012. It revealed that 13853 individual flies were collected consisting of a total of 20 species; among which, 11 species belonged to subgenus *Sophophora*, 4 belonged to subgenus *Drosophila*, and 2 belonged to genus *Scaptodrosophila*, while one species each belonged to genus *Zaprionus* and *Phorticella* respectively. Maximum flies were obtained during monsoon 2012 whereas minimum flies were obtained during winter 2012. *D. malerkotliana* and *D. bipectinata* were found to be abundant during monsoon, followed by *D. n. nasuta* and *P. striata*. During summer, *P. striata* was abundantly found. During post-monsoon, *D. n. nasuta* was found dominant followed by *D. malerkotliana* and *D. bipectinata*. During winter *D. ananassae* was dominant species. *D. melanogaster*, *D. ananassae*, *D. malerkotliana* and *P. striata* were found in all the seasons. Rare species such as *D. punjabiensis*, *D. bhagamandalensis*, and *D. daruma* were found only during monsoon and post-monsoon season whereas *D. latifshahi* was found during summer 2012 season. The abundance of *Drosophila* population varied for different seasons. *Drosophila* population size was found to be maximum during monsoon and minimum during winter.

Table 1: Seasonal variation of *Drosophila* in Dharwad District collected during 2011-12 season

Species	Winter 2011	Summer 2011	Monsoon 2011	Post-monsoon 2011	Winter 2012	Summer 2012	Monsoon 2012	Post-monsoon 2012	Total
Genus *Drosophila* Subgenus *Sophophora* *melanogaster* subgroup									
Drosophila melanogaster	12	15	65	11	14	8	82	24	231
***ananassae* subgroup**									
D. ananassae	121	273	206	41	86	211	148	25	1111
D. malerkotliana	18	64	1186	196	8	46	1405	155	3078
D. bipectinata	3	53	1314	167	--	35	1398	117	3087

***suzukii* subgroup**									
D. rajasekarii	--	42	187	30	--	17	234	10	520
***eugracilis* subgroup**									
D. eugracilis	--	2	82	136	--	7	67	112	406
***montium* subgroup**									
D. kikkawai	6	7	18	25	--	3	34	15	108
D. jambulina	--	12	114	65	--	16	178	34	419
D. punjabiensis	--	--	2	--	--	--	3	--	5
D. bhagamandalensis	--	--	4	5	--	--	3	4	16
***takahashii* subgroup**									
D. takahashii	--	--	5	--	--	--	6	2	13
Subgenus *Drosophila*									
***immigrans* group**									
D. nasuta	20	39	447	152	12	76	762	178	1686
D. neonasuta	--	3	289	48	--	14	133	88	575
***repleta* group**									
D. repleta	8	13	17	2	--	21	21	13	95
***Polychaeta* group**									
*D. daruma**	--	--	--	--	--	--	3	--	3
*D. latifshahi**	--	--	--	--	--	3	--	--	3
Genus *Scaptodrosophila*									
Scaptodrosophila nigra	--	12	34	--	--	4	24	--	74
S. krishnamurthyii	--	4	7	--	--	15	4	--	30
Genus *Zaprionus*									
Zaprionus bogoriensis	18	10	48	2	--	13	73	13	177
Genus *Phorticella*									
Phorticella striata	13	237	776	78	9	337	681	85	2216
Total	219	786	4801	958	129	826	5259	875	13853
Average rainfall (in mm)	3.85	37.4	112	56.9	0.0	22.8	70.9	40.6	
Average temperature (^{0}C)	23.5	27.5	24	25	23.5	27	24.5	26.5	

Source: Classification according to Hsu (1949); Bock and Wheeler (1972); Grimaldi (1990) and Markow and O' Grady (2006)
* Rare species collected for first time from South India

Figure 1 revealed that there was a positive correlation between average rainfall and abundance of the species (r = 0.952).

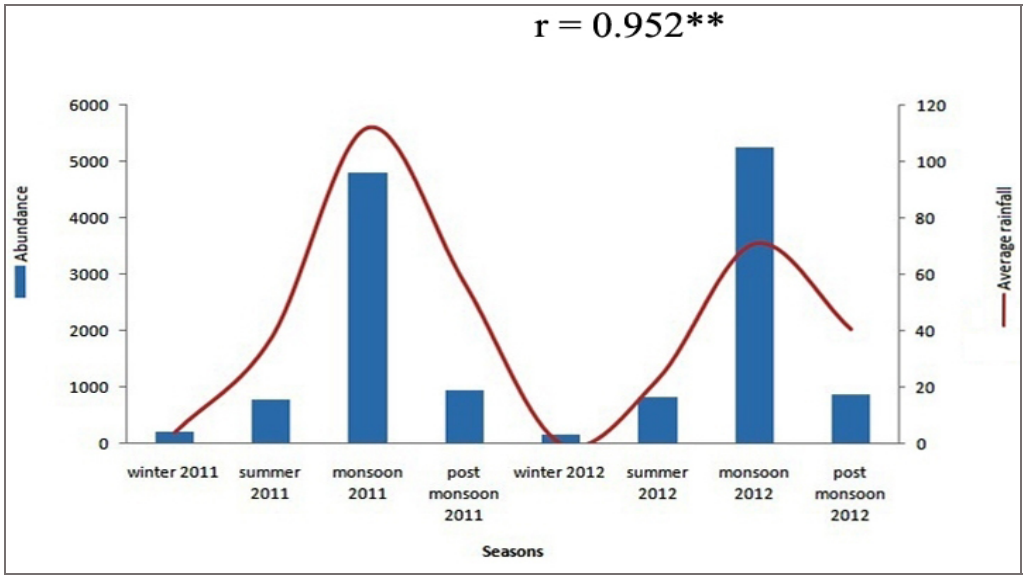

Fig 1: Correlation between average rainfall and population abundance of *Drosophila* during 2011-12 seasons.

The correlation coefficient analysis for individual species revealed that out of 20 species about 12 species showed positive correlation with rainfall (Table 2).

Table 2: Correlation coefficient (r) value of individual species of *Drosophila* against average rainfall for 2011-12 season

S. No.	Species	r value
1	*Drosophila melanogaster*	0.595
2	*D. ananassae*	0.048
3	*D. malerkotliana*	0.976**
4	*D. bipectinata*	0.976**
5	*D. rajasekari*	0.850**
6	*D. eugracilis*	0.778*
7	*D. kikkawai*	0.905**
8	*D. jambulina*	0.946**
9	*D. punjabiensis*	0.733*
10	*D. bhagamandalensis*	0.779*
11	*D. takahashii*	0.764*
12	*D. nasuta*	0.929**
13	*D.s. neonasuta*	0.946**
14	*D. repleta*	0.494
15	*D. daruma*	0.412
16	*D. latifshahi*	-0.247
17	*Scaptodrosophila nigra*	0.634
18	*S. krishnamurthyii*	0.345
19	*Zaprionus bogoriensis*	0.551
20	*Phorticella striata*	0.762*

* Significant at 0.05 level, ** Significant at 0.01level

The correlation coefficient between temperature and abundance of species was not found significant (Fig. 2)

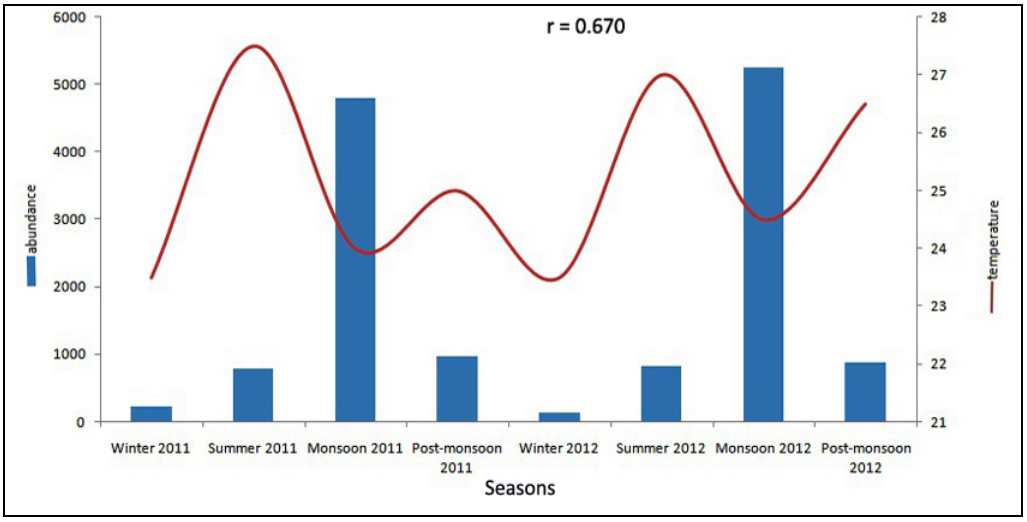

Fig 2: Correlation between average temperature and population abundance of *Drosophila* during 2011-12 seasons

The Simpson index of diversity and Shannon index calculated for different seasons revealed that post-monsoon 2012 were more diverse whereas winter 2012 was less diverse. Post-monsoon 2011 had more values for equitability index whereas summer 2012 revealed less values (Table 3).

Table 3: Diversity indices values calculated for Seasonal variation of *Drosophila* during 2011-12

Seasons	No. of species recorded	Simpson index	Shannon index	Equitability index
Winter 2011	9	0.6641	1.562	0.7109
Summer 2011	15	0.7706	1.852	0.684
Monsoon 2011	18	0.8155	2.011	0.6957
Post-monsoon 2011	14	0.865	2.178	0.8253
Winter 2012	5	0.6454	1.09	0.6775
Summer 2012	16	0.7524	1.838	0.6628
Monsoon 2012	19	0.815	1.981	0.6728
Post-monsoon 2012	15	0.8695	2.231	0.8239

Cluster analysis on the basis of abundance of different species showed two main clusters (Fig. 3). First cluster consists of about 16 species whereas second cluster consists of 4species. First cluster is further divided into 2 sub clusters consisting of 11 and 5 species respectively. Out of 11 species 5 belong to the subgenus *Sophophora* whereas 3 species belong to subgenus *Drosophila*, 2 species belong to Genus *Scaptodrosophila* and one species belongs to Genus *Zaprionus*. Out of 5 species present in the second sub cluster 4 belong to subgenus *Sophophora* while one species belong to subgenus *Drosophila*. The second cluster also consists of two sub clusters consisting of sympatric species *D. malerkotliana* and *D. bipectinata* belonging to subgenus *Sophophora*. *D. n. nasuta* belonging to subgenus *Drosophila* and *P. striata* belonging to Genus *Phorticella* are in the second sub cluster.

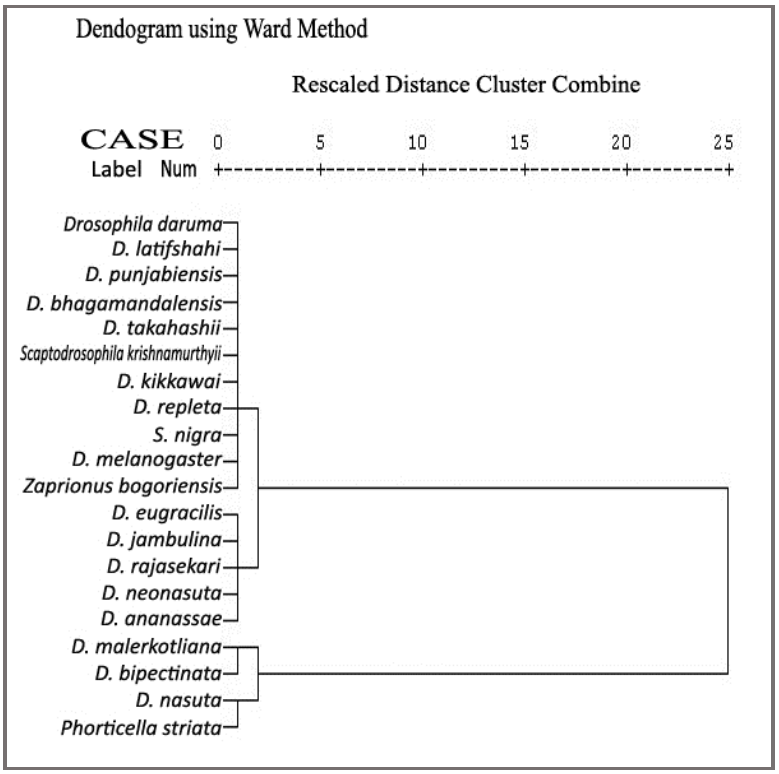

Fig 3: Cluster analysis of *Drosophila* species

4. Discussion

There is an effect of seasons on the population of *Drosophila* of Dharwad. They were more abundant during monsoon, less abundant during winter and moderate during summer and post-monsoon season. Few species which were found during monsoon season were rare and endemic. During monsoon, *D. bipectinata*, *D. malerkotliana*, *D. n. nasuta* and *P. striata* were more dominant than other species. During post-monsoon and winter seasons, the population of these species decreased but they maintained their dominancy compared to other species. Present study agrees with the reports of Prakash and Reddy [22, 23]; Guruprasad *et al*. [8] and Achumi *et al*. [1] where majority of species collected belonged to *melanogaster* species group of the subgenus *Sophophora* and *immigrans* species group of the subgenus *Drosophila* indicating their sympatric association and ecological dominance [11]. During the months of summer, *P. striata* emerged as most successful species in summer as it is the mango fruit harvesting season in this area (Table 1). It is also reported that *P. striata* species is dependent on mango fruiting season during summer and decreases its abundance during other seasons. This fruit provides an excellent source of feeding and breeding site for the Drosophilids [28].

Fluctuations in tropical population sizes of *Drosophila* during different seasons are due to variations in the environmental factors such as rainfall, temperature and humidity [19]. Present study revealed that fluctuation in population densities of various species of *Drosophila* can be attributed to rainfall with more number of individual flies collected in monsoon compared to other seasons and the size of the population decreases slowly by post-monsoon and reaches its lowest during dry winter season and again increases during summer. Torres and Madi – Ravazzi [29] reported that there is a positive correlation between population abundance and rainfall of three populations of Brazil. They have reported that populations of *D. sturtevanti* and *D. malerkotliana* were strongly influenced by rainfall. During the dry season, the number of individuals of both species was significantly reduced. Present study shows positive correlation between total abundance of species and rainfall (Fig. 1) and also among 12 individual species. *D. melanogaster* and *D. ananassae* are considered as cosmopolitan species [20]. But these species showed no positive correlation with rainfall. Sympatric species *D. bipectinata* and *D. malerkotliana* coexisted during all the seasons in equal numbers and hence, justifies their adaptability to share the same habitat and maintain a successful reproductive isolation (Table 2).

Dobzhansky and Pavan [5] showed that rainfall has greater influence on the abundance of species than temperature. An increase in rainfall has direct influence on flowering and fruiting of plants, thus increasing the number of oviposition and feeding sites for the species [4]. Torres and Madi – Ravazzi [29] have reported no correlation between the total number of individuals captured and temperature among two out of three populations of Brazil. Present study also revealed that temperature has no relation with abundance of *Drosophila* population (Fig. 2).

Species richness alone has no effect on diversity indices [29]. Achumi *et al*. [1] have calculated Simpson's index during the study

of seasonal variation of *Drosophila* from Mount Japfu. Their results revealed that species richness has no significant effect on diversity. Similar results were reported by Guruprasad *et al*. [8] where a lower altitude region (680 m) with less number of species (18) was more diverse than higher altitude region (980 m) with more number of species (19). The Simpsons Index of diversity (1-D), Shannon Index revealed a greater diversity in the post-monsoon 2012 season even though monsoon 2012 season recorded maximum number of species (19). Minimum diversity was found in winter 2012 (Table 3). This may be more easily understood if we observe the quantity and dominance of each species collected during different seasons, Most of the flies collected during monsoon seasons were concentrated in just two species, *D. malerkotliana* and *D. bipectinata*, which together represent 52.7% of the whole sample collected. Whereas in post-monsoon season their population was stable and evenness maintained. Simpson's Index is heavily dependent towards evenness and most abundant species in the sample and it is less sensitive to the species richness whereas Shannon index takes into account both species richness and abundance [13].

Eco-distributional analysis of *Drosophila* revealed that the distributional pattern of species or related group of species is uneven [1, 8]. Cluster analysis revealed that there are 2 major clusters of species, first cluster consisting of rare, endemic and cosmopolitan species which were found in less number where as the other sub cluster consists of intermediate species whose abundance is in between first sub cluster and second cluster. In the first cluster out of 16 species, 9 belonged to subgenus *Sophophora* which are morphologically related to each other thus indicating the coexistence of species having similar ecological preferences. Second cluster is further divided into two sub clusters where one sub cluster consists of dominant sympatric species *D. malerkotliana* and *D. bipectinata*. The other sub cluster consists of *D. n. nasuta* and *P. striata* which were also dominantly found species (Fig. 3).

5. Conclusion

The study revealed that Dharwad District is rich in *Drosophila* fauna endowed with rare and endemic species and further studies on species specific habitat/ microhabitat preferences will be very much helpful in understanding their ecological pattern and speciation.

6. Acknowledgement

The authors are thankful to the chairperson, Dept. of Zoology, Karnatak University, Dharwad for providing necessary facilities. S.B.S. is thankful to UGC for award of Rajiv Gandhi National Fellowship and N.S to UGC MRP F. NO. 37/-241/2009 (SR) for financial assistance

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