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Non-random Distribution of Heterozygous Inversions in a Natural Population of *Drosophila malerkotliana*

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Abstract: ***Drosophila malerkotliana*** **is one of the members of the** ***Drosophila bipectinata*** **species complex. It has been moderately analyzed for its genetic characteristics, especially for the purpose of phylogenetic relationships with other species of this complex. It is chromosomally polymorphic due to the presence of paracentric inversions. In this article, the chromosomal polymorphism of a natural population, sampled from Bilaspur (Chhattisgarh) has been reported. More than five hundred third instar larvae were randomly selected and sacrificed to prepare their salivary gland chromosomes to observe the presence of commonly as well as rarely occurring inversions in this population. The data obtained indicate that the mean number of heterozygous inversions per individual in this population is 0.603 denoting very high level of genetic variation. The frequency of occurrence of all the observed inversions in this population has been depicted. Further, it has been observed that these inversions occur in nonrandom distribution.**

Index Terms: **Chromosomal polymorphism,** ***Drosophila malerkotliana,*** **heterozygous inversions, non-random occurrence.**

I. INTRODUCTION

The popularity of *Drosophila* in the genetical research is due to its several features, one of which is the presence of polytene chromosomes in its larval salivary glands. The distinct banding pattern on the chromosome arms helps the researchers to identify any structural change in the polytene chromosomes. Inversions, particularly paracentric inversions (that do not include centromere in the inverted area) are very common in occurrence in the natural populations of *Drosophila*, whereas, pericentric inversions (that include centromere in the inverted segment) are of very rare in appearance (Singh 2019). Spotting a heterozygous inversion in a polytene chromosome is an easy task because it forms a loop like structure in the chromosome arm. *Drosophila malerkotliana* is one of the species of the *D. bipectinata* species complex that is found to be sparsely distributed species in Indian subcontinent (Bock 1971a, Singh and Singh 2001, Kopp and Barmina 2005, Singh 2017, 2018). A detailed report on the phylogenetic relationship and population genetic approach to demonstrate the evolutionary history of the *D. bipectinata* species complex is provided by a number of researchers (Narda 1968, Bock 1971b, Bock and Wheeler 1972, Banerjee and Singh 1996, Tomimura et al 2005, Mishra and Singh 2006, Singh and Sisodia 2008, Banerjee and Singh 2017). *D. malerkotliana* is believed to be derived from a common ancestor of *D. bipectinata* (Bock 1971a, Kopp and Barmina 2005, Mishra and Singh 2006, Singh and Sisodia 2008, Banerjee and Singh 2017). There is sufficient information regarding the taxonomic and behavioral features of four species of the *D. bipectinata* species complex (Bock 1971b, Panigrahy1984, Gupta and Panigrahy1990, Banerjee and Singh 2017). Members of this species complex are sympatric, reproductively isolated and always preferred to be used for evolutionary studies (Singh and Banerjee 2016, Mishra and Singh 2006). Genetic polymorphism at the level of chromosome and protein (allozyme) has substantially been scrutinized in both the prime members of the *D. bipectinata* species complex (Bock 1971b, Jha and Rahman 1972, Naserulla and Hegde 1993, Singh and Singh 2015, Singh and Singh 2016a, Singh and Singh 2016b, Singh, Kumar and Singh 2016). Even in one of the profusely studied plant models, i.e., *Arabidopsis,* the effect of heterozygous pericentric inversion as recombination suppressor has been reported (Termolino et al 2019).

In the present study, flies from a natural population of *D. malerkotliana* was collected and chromosomally analyzed for the presence of heterozygous inversions in its different arms. The data obtained was analyzed to see whether these inversions occur randomly in this population of *D. malerkotliana.*

II. MATERIALS AND METHODS

Flies were collected from fruits and vegetable shops located in the main city of Bilaspur (Chhattisgarh state of India) by net sweeping method. Females were individually placed in the food vials to get their next generation. Food used to culture flies contained ingredients like brown (desi) sugar, maize powder, agar-agar, yeast extract, propionic acid and Nipagin-M boiled in specific amount of water. Vials having *D. malerkotliana* flies (confirmed on the basis of male morphology) were then further cultured to get their larvae for polytene chromosome squash preparation. Randomly selected third instar larvae from the culture vials were dissected for their salivary glands. Salivary glands stained in 2% lacto- aceto- orcein for half an hour were squashed in mountant (60% acetic acid and lactic acid in the same ratio) to prepare polytene chromosomes. Identification of different chromosome arms was accomplished by following the polytene chromosome map prepared by Panigrahy (1984). The presence of heterozygous loop in a chromosome arm was ascertained by its occurrence in the same arm of other chromosome clusters of the same slide.

III. RESULTS AND DISCUSSION

The heterozygotes of commonly occurring inversions were found to be in high frequency. In total, ten different types of heterozygous inversions were observed in this population, out of which, four were found to be in high frequency (6.18 - 19.30). Figure 1 show seven paracentric heterozygous inversions including the commonly occurring. We have already reported the presence of three new inversions in this population in our earlier research note (Singh and Singh 2016b). The type of inversions and their frequency in Bilaspur population is shown in Table I.

Table I. The types of heterozygous inversions and their frequency (%) in Bilaspur population.

S.N.	Type of inversions	Frequency in percentage
1.	IIL_A (Subterminal in 2L)*	19.30
2.	IIL_B (Median in 2L)*	15.30
3.	IIL_C (Basal in 2L)	0.70
4.	IIR_A (Median in 2R)	2.18
5.	IIR_C (Basal in 2R)*	6.18
6.	$IIIL_A$ (Subterminal in 3L)*	8.36
7.	$IIIL_B$ (Median in 3L)	1.63
8.	$IIIL_C$ (Basal in 3L)	2.18
9.	$IIIR_B$ (Basal in 3R)	2.18
10.	$IIIR_D$ (Median in 3R)	2.00

*commonly occurring inversions.

From the collected data, we formed a frequency distribution pertaining to the presence of these heterozygous inversions in individual larva to test whether the inversions are randomly distributed in this population. The data regarding the presence of number of heterozygous inversions and individuals bearing them is shown in the table II. To test random distribution of inversions in the population, χ^2 analysis was performed applying Poisson distribution.

Poisson distribution was done to find out expected number of individuals with respective number of inversions. By using the Poisson equation, $p = e^{-m}.m^x / x!$, the expected number of individuals carrying 0, 1, 2, or 3 heterozygous inversions were calculated (Gupta 2009). To test difference between observation and expectation, χ^2 analysis was performed. The results indicate that there is significant difference between observation and expectation ($p<0.001$). Thus these heterozygous inversions occur non-randomly in the natural population of *D. malerkotliana* at Bilaspur. Chromosomal aberrations, whether numerical or structural, are rare in occurrence in a natural population of a sexually reproducing organism. However, chromosome inversions, particularly, paracentric inversions are found to confer adaptive significance in *Drosophila* and therefore exist at a higher frequency in them (Dobzhansky and Sturtevant 1938, Da Cunha 1960, Sperlich and Pfriem 1986, Das and Singh 1991, Banerjee and Singh 1994, Singh and Banerjee 1997, Singh and Singh 2018, Singh 2019, Kapun and Flatt 2019).

A natural population of *Drosophila* may have presence of commonly as well as rarely occurring inversions. *D. malerkotliana*, like other species of genus *Drosophila* is known to harbor paracentric inversions and some of them have been reported to be polymorphic (Jha and Rahman 1972, Naserulla and Hegde 1993, Aulard et al, 2002, Singh 2001, Singh and Singh 2016b). In this study, we simply recorded the presence of heterozygous inversion/s in individual larva and collected data of 550 larvae.

The principal aim was to know whether heterozygous inversions occur randomly in this population of *D. malerkotliana*. The present observation in this species helps us to reveal that in its Bilaspur population, heterozygous inversions exist in nonrandom pattern and this may be due to natural selection of some of the inversions. Although, a population is expected to have presence of inversions, whether rarely occurring or commonly occurring, distributed in random manner but presence of certain inversion heterozygotes in larger or lesser number might have resulted this disequilibrium in this natural population of *D. malerkotliana*.

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Table II. The observed and expected numbers of larvae carrying heterozygous inversions and the mean number of heterozygous inversions per larva in natural population of Bilaspur.

S.N.	No. of heterozygous inversions (x)	Observed no. of larvae (f)	fx	Expected no. of larvae	Mean no. of heterozygous inversions per larva
1.	0	346	0	301.95	332/550 = 0.603
2.	1	104	104	182.07	
3.	2	72	144	54.89	
4.	3	28	84	11.03	
χ^2 = 71.342 ; d. f. = 2 p<0.001					

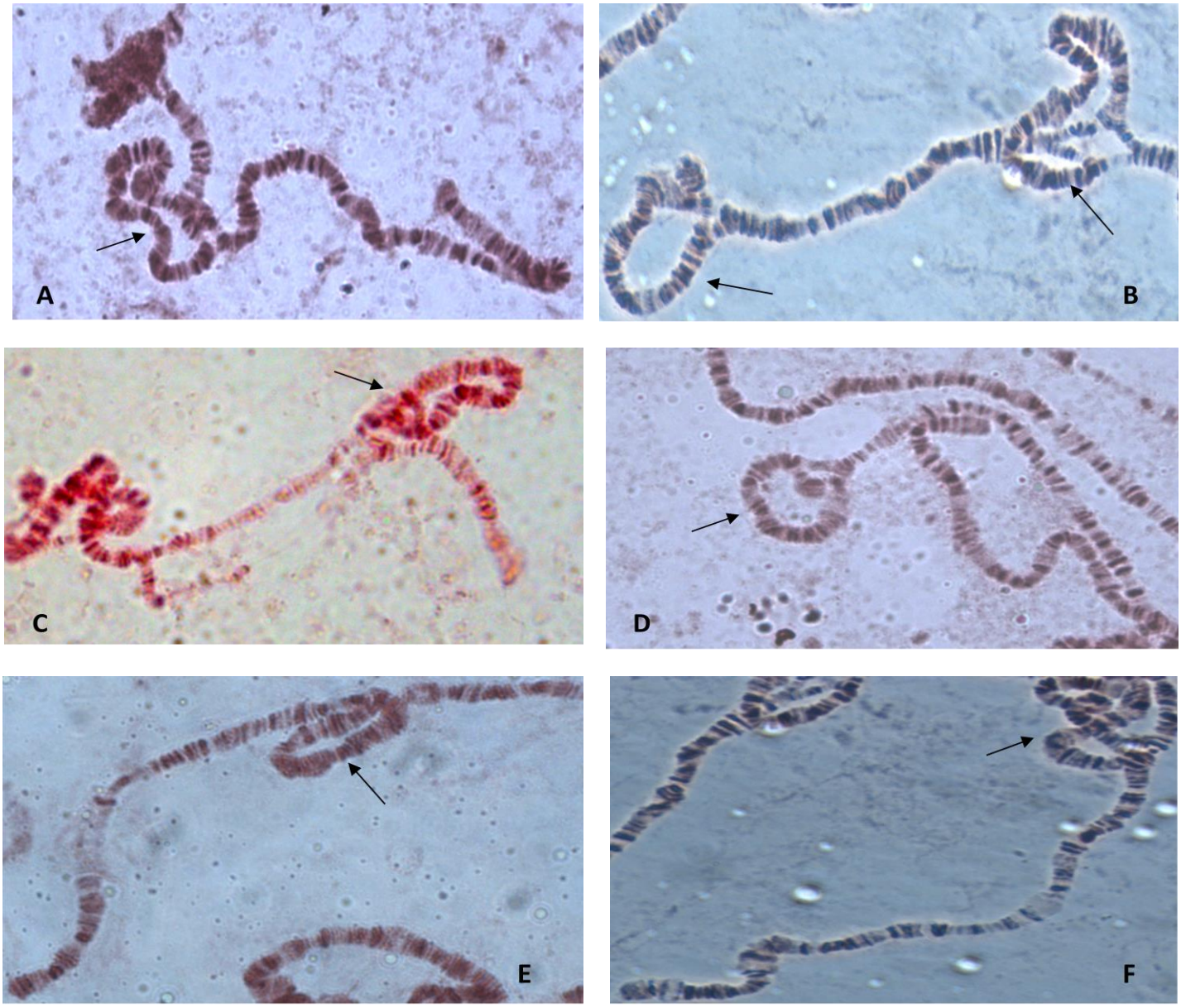

Fig. 1**. A- F**: Photomicrograph of paracentric heterozygous inversions present in different autosomal arms of *D. malerkotliana* observed in a natural population of Bilaspur: (A) IIL_C (B) IIL_A and IIL_B (C) IIR_A (D) $IIIL_A$ (E) $IIIR_D$ (F) $IIIR_B$

REFERENCES

Aulard, S., David, J. R, & Lemeunier, F. (2002). Chromosomal inversion polymorphism in Afrotropical populations of Drosophila melanogaster. Genetics Research, 79, 49-63.

Banerjee, P., & Singh, B. N. (2017). The *Drosophila bipectinata* species complex: phylogenetic relationship among different members based on chromosomal variations. J. Genetics, 96, 97-107.

Banerjee, R., & Singh, B. N. (1994). Evidence for selection and genetic drift in laboratory populations of *Drosophila bipectinata.* Proc. Zool. Soc. Calcutta, 47, 125-33.

Banerjee, R., & Singh, B. N. (1996). Inversion polymorphism in natural population of *Drosophila bipectinata* . Cytobios, 87, 31-43.

Bock, I. R., (1971a). Taxonomy of the *Drosophila bipectinata* species complex. Univ. Tex. Publ, 7103, 273–280.

Bock, I. R., (1971b). Intra- and interspecific chromosomal inversions in the *Drosophila bipectinata* species complex. Chromosoma, 34, 206–229.

Bock, I. R., & Wheeler, M. R. (1972). The *Drosophila melanogaster* species group. Univ. Texas. Publ, 7213, 1–102.

Da Cunha, A. B., (1960). Chromosomal variation and adaptation in insects. Ann. Rev. Entomol, 5, 85–110.

Das, A., & Singh, B. N. (1991). Heterosis associated with chromosome inversions in *Drosophila bipectinata.* Korean J. Genetics, 14, 173-178.

Dobzhansky, T., & Sturtevant, A. H. (1938) Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics, 23, 28-64.

Gupta, S. P. (2009). Statistical Methods. New Delhi, Sultan Chand and Sons, Educational Publishers,

Gupta, J. P., & Panigrahy, K. K. (1990). Chromosomal polymorphism in Indian populations of *Drosophila bipectinata* Duda. Genetica, 82, 45-49.

Jha, A. P., & Rahman, S. M. Z. (1972). Cytogenetics of natural populations of *Drosophila*. I. Role of chromosomal inversions in the evolution of the *bipectinata* species complex. Chromosoma, 37, 445–454.

Kapun, M., & Flatt, T. (2019). The adaptive significance of chromosomal inversion polymorphisms in *Drosophila melanogaster*. Mol Ecol., 28, 1263-1282.

Kopp, A., & Barmina, O. (2005). Evolutionary history of the *Drosophila bipectinata* species complex. Genet. Res., 85, 23-46.

Mishra, P. K., & Singh, B. N. (2006). *Drosophila bipectinata* species complex: study of phylogenetic relationship among four members through analysis of morphology of testes and seminal vesicles. J. Zool. Syst. Evol. Res., 44, 175-179.

Naseerulla, M. K., & Hegde, S. N. (1993). A new inversion in *Drosophila malerkotliana* from Varanasi, India. Dros. Inf. Serv. (USA), 72, 158-159.

Narda, R. D. (1968). Genetical and isolating mechanisms in the *ananassae* subgroup (Sophophora Drosophila)VI Cytological divergence in *Drosophila ananassae*, *Drosophila bipectinata* and *Drosophila malerkotliana.* Caryologia, 21, 293-301.

Panigrahy, K. K. (1984). Ecogeographic differentiation of species and chromosomal inversions in Indian populations of *Drosophila*. Ph D thesis, Banaras Hindu University, Varanasi, India.

Singh, A. K. (2017). Perusal of genetic variation in *Drosophila* populations through various methods of genetic polymorphisms. Recent Trends in Biological Research. Global Academy Society, 8, 112-130.

Singh, A. K. (2018). Chromosomal variability due to inversions in Indian populations of *Drosophila*. Ind. Jour. Sci. Res., 8, 169-178.

Singh, A. K., Kumar, S., & Singh, N. (2015). Electrophoretic variants of esterase in two species of *Drosophila: D. bipectinata* and *D. malerkotliana*. Dros. Inf. Serv. (USA), 98, 22-23.

Singh, B. N., & Banerjee, P. (2016). Population genetical, behavioural and evolutionary studies in the *Drosophila bipectinata* species complex. Proc. Indian Nat. Sci. Acad., 82, 99–115.

Singh, B. N., & Banerjee, R. (1997). Population and behaviour genetics of *Drosophila bipectinata*. Proc. Indian Natn. Sci. Acad., 63: 399-410.

Singh, B. N., (2001). Patterns of inversion polymorphism in three species of the *Drosophila melanogaster* species group. Indian J. Exp. Biol., 39, 611-622.

Singh, B. N. (2019). Hundred years of research on inversion polymorphism in *Drosophila*. Current Science, 117, 761-775.

Singh, B. N., & Banerjee, R. (1997). Population and behaviour genetics of *Drosophila bipectinata*. Proc. Indian Natn. Sci. Acad., B63, 399-410.

Singh, B. N., & Sisodia, S. (2008). Phylogenetic relationship among four members of the *Drosophila bipectinata* species complex. J. Sci. Res. B H U, 52, 81-97.

Singh, G., & Singh, A. K. (2015). Chromosomal translocation at the terminal end of 2L in *Drosophila malerkotliana*. Dros. Inf. Serv. (USA), 98, 43.

Singh, G., & Singh, A. K. (2016a). Electrophoretic variants of Xanthine dehydrogense enzyme in *Drosophila malerkotliana. Dros. Inf. Serv. (USA), 99, 35-36.*

Singh, G., & Singh, A. K., (2016b). Chromosomal polymorphisms in a natural population of *Drosophila malerkotliana.* Dros. Inf. Serv. (USA), 99, 49-51.

Singh, G., & Singh, A. K. (2018). Excessive occurrence of paracentric inversions in a Natural population of *Drosophila bipectinata.* J. Exp. Zool. India, 21, 29-33.

Singh, S., & Singh, B. N. (2001). *Drosophila bipectinata* species complex. Ind. J. Exp. Biol., 39 835-844.

Sperlich, D., & Pfriem, P. (1986). Chromosomal polymorphism in natural and experimental populations. In: The Genetics and Biology of *Drosophila* (ed. Ashburner M, Carson H L and Thompson J N Jr), Vol. 3e. NY, USA, Academic Press.

Termolino, P., Falque, M., Aiese Cigliano, R., Cremona, G., Paparo, R., Ederveen, A., Martin, O. C., Consiglio, F. M. & Conicella, C. (2019). Recombination suppression in heterozygotes for a pericentric inversion induces the interchromosomal effect on crossovers in Arabidopsis. Plant J., 22, doi: 10.1111/tpj.14505.

Tomimura, Y., Matsuda, M., & Tobari, Y. N. (2005). Chromosomal phylogeny and geographical divergence in *Drosophila bipectinata* complex. Genome, 48, 487–502.

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