ZOOLOGICAL SCIENCE 8: 959–965 (1991)

Chromosomal Characterization of *Chironomus striatipennis* Kieffer (Diptera : Chironomidae)

JAI PAL GUPTA and AJAI KUMAR

Genetics Laboratory, Centre of Advanced Study in Zoology, Banaras Hindu University, Varanasi 221 005, India

ABSTRACT—Chromosomal studies pertaining to the mitotic and polytene chromosomes, nucleolus, C-band positive heterochromatin and naturally occurring chromosomal polymorphism have been undertaken for the first time in *Chironomus striatipennis*. Our present analysis of larval samples from six different Indian natural populations has yielded four paracentric inversions in this species. The distribution of inversions in relation to the interspecific competition and environmental conditions has been discussed.

INTRODUCTION

The flies of the family Chironomidae, particularly those of the genus *Chironomus*, have contributed remarkably in elucidating several important phenomena of cytogenetics. Despite this, Indian species of *Chironomus* have been largely neglected until recently. It is, however, only very recent that the importance of these flies has been appreciated by various workers in India [1–3].

The present paper deals with the results of some chromosomal studies undertaken for the first time in *Chironomus striatipennis*, a commonest and wide-spread Indian species.

MATERIALS AND METHODS

The larvae for the present study were collected from six different isolated aquatic localities in the vicinity of Varanasi. All localities were of semi-permanent nature. Out of them, three localities namely Zoology Department, Central Office and Hostel Campus were situated within the area of the Banaras Hindu University (BHU), whereas the remaining localities, Saket Nagar, Naharia and Bhagwanpur placed approximately within a radius of 5 km from BHU campus. At Saket Nagar, Naharia and Zoology Department *Chironomus striatipennis* was found to coexist with a closely related species, *Ch. circumdatus*, while at other three localities the former species lived solitary. The culture of this species was also established in our laboratory following the procedure of Hägele [4].

The mitotic chromosomes, polytene chromosomes and their photomap were prepared from mid 4th instar larvae by the methods adopted by Kumar and Gupta [3]. The numbering of polytene chromosome was done in decreasing order of size, chromosome I being the largest and chromosome IV the shortest. For marking centromeric position in each chromosome, the BSG method of Sumner [5] was used with minor modification as suggested by Lentzios *et al.* [6] for polytene chromosomes. The left and right arms of a polytene chromosome corresponding to a mitotic metacentric chromosome were marked arbitrarily after determining the position of the centromere. Some important landmarks in each chromosome were also detected in order to facilitate its quick identification.

For naturally occurring chromosomal polymorphism a considerable number of wild caught larvae from six foresaid localities were examined through their polytene chromosomes. The frequency of each inversion heterozygote and the mean number of heterozygous inversions per individual in each population were also inspected.

Accepted April 13, 1991
Received November 15, 1990

RESULTS

Mitotic chromosomes

The karyotype of this species as revealed by the mitotic metaphase chromosomes from the larval brain ganglion consists of 3 pairs of metacentrics and 1 pair of small rod-like chromosomes (Fig. 1a). The X and Y chromosomes are not distinguished from each other.

Polytene chromosomes

The salivary gland nuclei of this species comprise four unequal polytene chromosomes which lie freely within the nucleus in the absence of an organized common chromocenter. These chromosomes have been designated from I to IV in the order of decreasing length. Each chromosome has been further characterized by the presence of certain constantly occurring landmarks (Fig. 2).

Chromosome I

This chromosome, besides being the longest, can be distinguished also by its flared tip, a large puff in 6E, a spindle-shaped structure with a medianly placed thick dark band corresponding to the centromere in 3D-4A and a puff-like swelling in 2C region.

Chromosome II

This chromosome is smaller in its size than the chromosome I. Besides this, its fan-shaped free tip in 11D, two closely placed thick dark bands in 11B, two small bulb-like swelling containing several fine bands in 8D and 9D and a characteristic swollen segment in 7B were also found to be of great diagnostic importance.

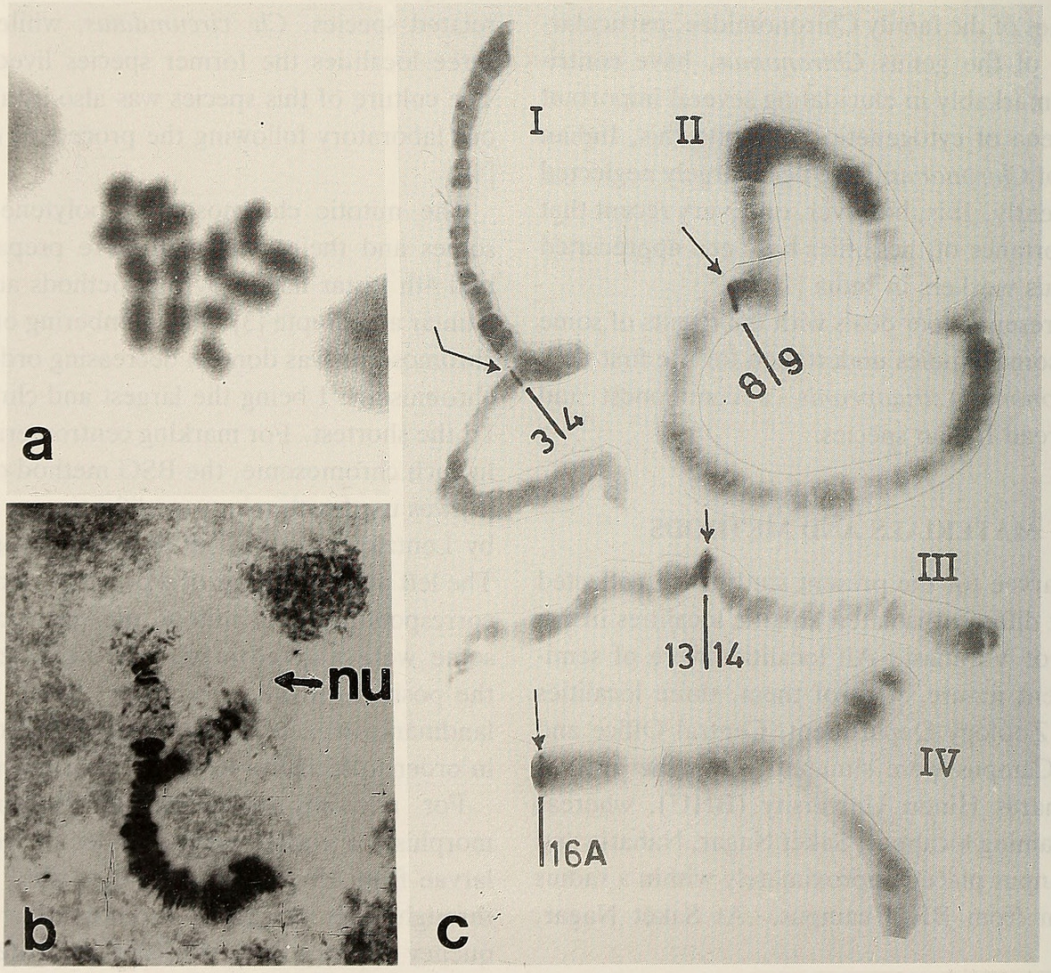

FIG. 1. *Chironomus striatipennis* a) Mitotic metaphase chromosomes, b) showing association of nucleolus with IV chromosome, c) arrows: centromeric C-band. nu: nucleolus.

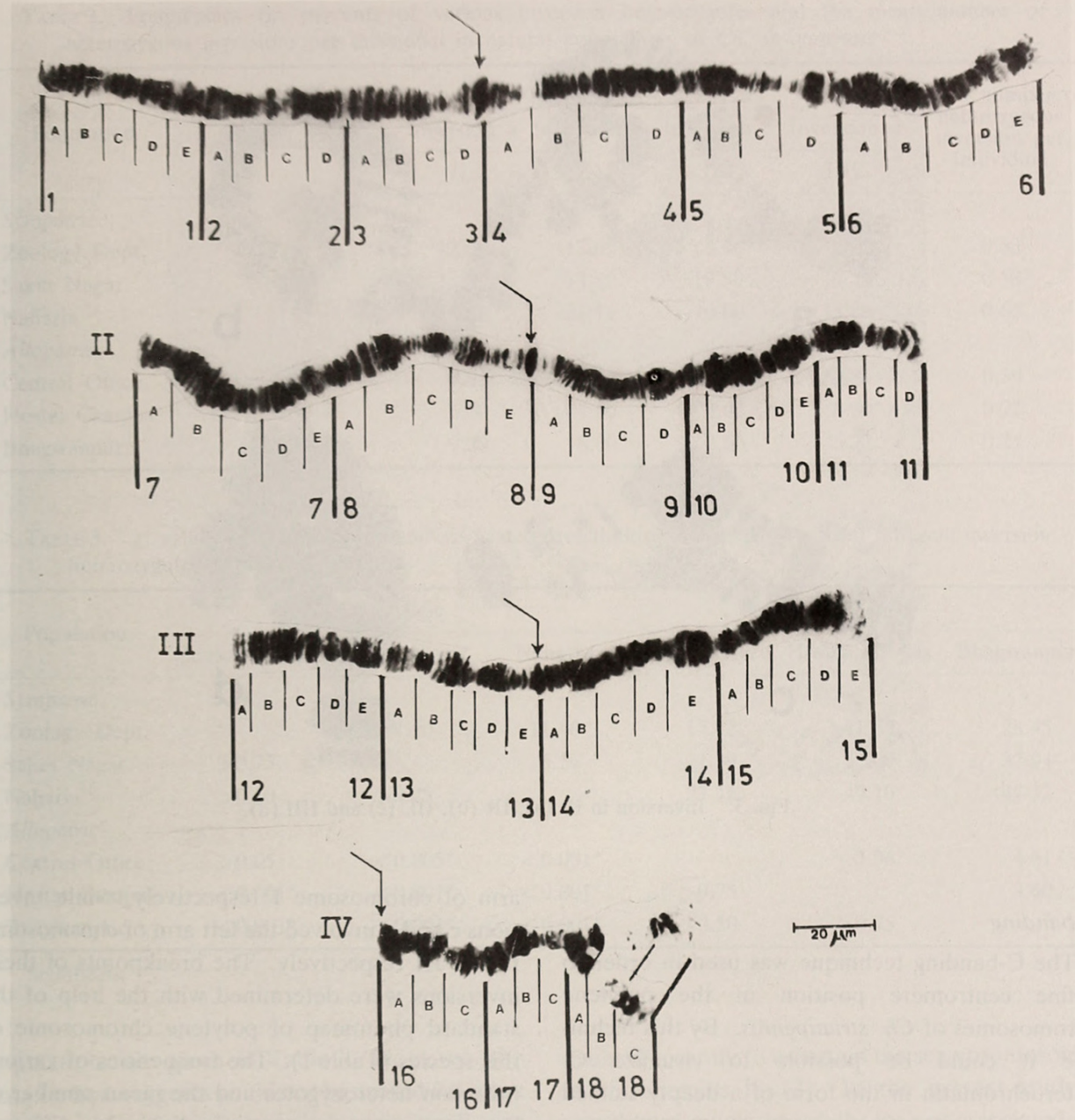

FIG. 2. Photomap of the polytene chromosome of *Chironomus striatipennis*. Arrows: Centromere position.

Chromosome III

This chromosome is found to be much smaller than the foregoing two chromosomes. However, its highly expanded free tip in 15E, two consecutively placed spindle-like structures in 13B-E and a large bulb with several dark bands in 14E also proved to be of great importance.

Chromosome IV

This chromosome, besides being the shortest, is further characterized by its terminal splitting and also bearing the nucleolus in 18C segment.

Nucleolus

The association of nucleolus with the salivary gland chromosomes of *Ch. striatipennis* was observed. Detailed chromosome analysis revealed that the 18C segment of the chromosome IV was associated with nucleolus (Fig. 1b).

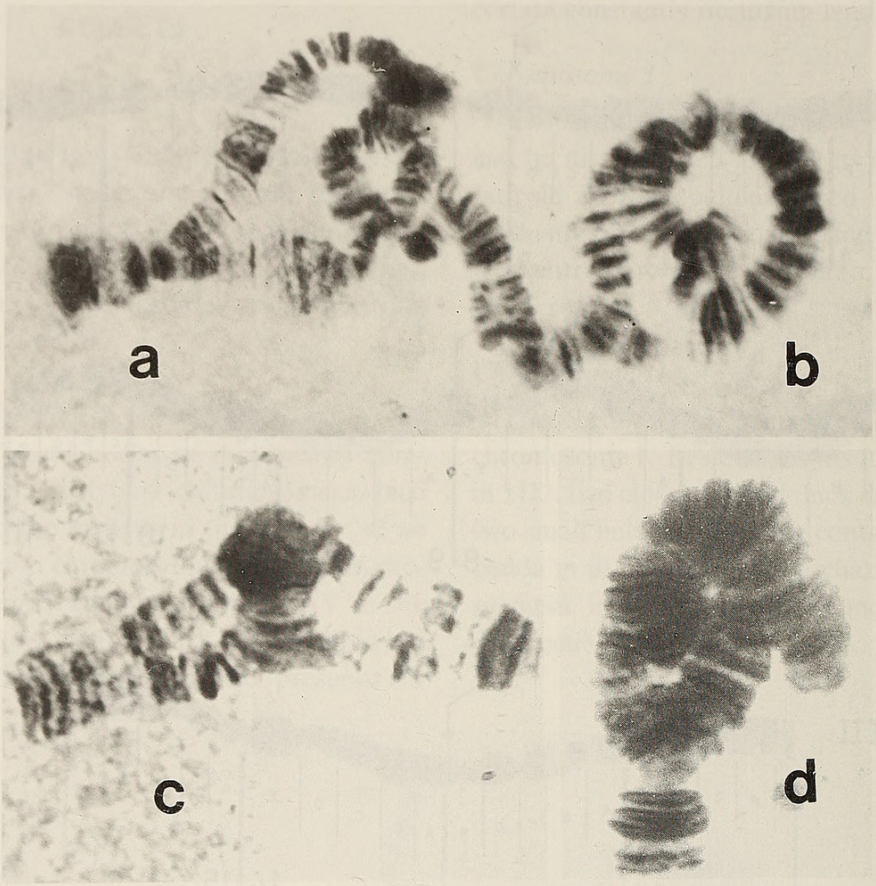

Fig. 3. Inversion in IL (a), IR (b), IIL (c) and IIIL(d).

C-banding

The C-banding technique was used in order to define centromere position in the polytene chromosomes of *Ch. striatipennis*. By this technique it could be possible to visualize C-heterochromatin in the form of a deeply stained band at the site of centromere in each chromosome: the centromeres of chromosomes I, II, III, and IV were identified on 3/4, 8/9, 13/14 and 16A segments respectively (Fig. 1c).

Chromosomal Polymorphism in Indian Natural Populations

A little over 1200 larvae of *Ch. striatipennis* from six different localities mentioned above were analysed for naturally occurring chromosomal polymorphism. In this species four paracentric inversions were found, and they were arbitrarily designated as a, b, c and d (Fig. 3a-d). The inversions a and b were found in the left and right arm of chromosome I respectively, while inversions c and d involved the left arm of chromosome II and III respectively. The breakpoints of these inversions were determined with the help of the standard photomap of polytene chromosome of this species (Table 1). The frequencies of various inversion heterozygotes and the mean number of heterozygous inversions per individual in different populations were also calculated (Table 2). The χ^2 values and associated probabilities between

Table 1. Details of various paracentric inversions in *Ch. striatipennis*, their positions, breakpoints and types of inversions

Inversion	Chromosome arm involved	Position	Breakpoints
a	IL	Submedian	1D ; 3C
b	IR	Subterminal	4D ; 6C
c	IIL	Submedian	7C ; 8B
d	IIIL	Subterminal	12B ; 13A

TABLE 2. Frequencies (in percent) of various inversion heterozygotes and the mean number of heterozygous inversions per individual in natural populations of *Ch. striatipennis*

Population	No. of Larvae examined	Inversion heterozygotes				Mean number of heterozygous inversion per individual
		Inversion a	Inversion b	Inversion c	Inversion d	
		IL	IR	IIL	IIIL	
Sympatric						
Zoology Dept.	260	12.30	17.69	8.46	14.61	0.53
Saket Nagar	200	18.00	14.50	9.50	16.50	0.58
Naharia	180	15.55	21.11	10.00	18.89	0.65
Allopatric						
Central Office	175	6.85	9.14	3.42	10.85	0.30
Hostel Campus	200	6.50	4.50	3.00	8.50	0.22
Bhagwanpur	225	9.30	8.00	2.66	5.77	0.25

TABLE 3. χ^2 values (above diagonal) and associated probabilities (below diagonal) for different inversion heterozygotes between populations

Population	Sympatric			Allopatric		
	Zoology Dept.	Saket Nagar	Naharia	Central Office	Hostel campus	Bhagwanpur
Sympatric						
Zoology Dept.		4.20	11.48	15.42	41.55	28.45
Saket Nagar	>0.75		6.56	21.39	37.00	32.94
Naharia	>0.10	>0.25		27.16	49.10	44.12
Allopatric						
Central Office	>0.05	<0.005*	<0.001*		3.36	4.61
Hostel Campus	<0.001*	<0.001*	<0.001*	>0.75		3.60
Bhagwanpur	<0.001*	<0.001*	<0.001*	>0.50	>0.75	

* Significant

populations are given in Table 3. However, the data on homozygotes could not be scored for some of these inversions because of their small size which prevented us to analyse Hardy-Weinberg frequencies during present study.

DISCUSSION

The chromosomal complement in the genus *Chironomus* ranges from the standard number of four pairs (2n=8) to three pairs (2n=6) or, in one case to two pairs (2n=4) [7]. The karyotype of *Ch. striatipennis* comprises 4 pairs of chromosomes (2n=8) wherein the sex-chromosomes, like other *Chironomus* species, are not distinguished from the autosomes. However, several methods have evolved recently to ascertain the sex-chromosomes in chironomids [8–12]. In the present study a search was made especially for sex-associated inversions, but none of the inversions exhibited such association with any particular sex.

The nucleolus organizer region, containing parts of the genetic code for ribosomes, has also proved to be an excellent cytological marker for species identification even in the species with strict karyotypic similarity. Our study in *Ch. striatipennis* revealed that only 18C segment of the chromosome IV is associated with nucleolus. This cytological marker proved to be of great help in separating the wild-caught larvae of *Ch. striatipennis* and *Ch. circumdatus* which are often found together in natural habitats. In *Ch. circum-*

datus both chromosome I and II exhibit association with the nucleolus though having karyotype similar to that of *Ch. striatipennis* [3].

Unlike *Drosophila* species, centromeric position in the polytene chromosomes of *Chironomus* is not clearly defined because of the absence of an organized common chromocenter. However, C-banding analyses have revealed that C-band positive segment tend to show in general preferential localization at regions designated as centromeres, telomeres and nucleolar organizers and at some interstitial regions of chromosomes. This has been noticed in several species of Chironomidae and Simuliidae [6, 13, 14]. However, in a number of chironomid species C-heterochromatin (constitutive heterochromatin) was not observed in all the regions mentioned above and this situation is not unique, but seems to wide-spread both in plant [15, 16] and animal species [17]. Despite this, the varying degree of amount and pattern of C-heterochromatin has been attributed to be of great evolutionary significance in *Chironomus* species [6].

In the present study C-banding technique was used in order to define centromeric position in the polytene chromosomes of *Ch. striatipennis*. By this technique it could be possible to visualize C-heterochromatin in the form of a deeply stained band at the site of centromere in each chromosome (Fig. 1c). This technique was found to be of great help particularly in case of metacentrics to designate their right and left arms. This was necessary for distinguishing a pericentric inversion from a paracentric inversion.

It is well documented that inversion polymorphism is adaptive and this condition is maintained in nature owing to the superiority of structural heterozygotes [18–20]. In the present study a little over 1200 larvae of *Ch. striatipennis* from six different localities in India were examined for naturally occurring chromosomal polymorphism. This study has detected four paracentric inversions in this species (Fig. 3a-d). Our present data on geographic distribution of these inversions show that though all the populations were found to be polymorphic for the same inversions, the mean number of heterozygous inversions per individual varied in different populations ranging between 0.22 (Hostel campus) to 0.65 (Naharia). Among the inversions, a, b and d were found with higher frequencies in all localities than inversion c (Table 2). The inversion a and b were also found coupled together only in 5 larvae obtained from Saket Nagar. Moreover, the wide occurrence of these inversions suggests that they have not originated in the recent past. Further, the variation in the frequencies of inversion heterozygotes observed in different geographic populations seems to be in accordance with the assumption that different gene arrangements react in different ways to the different conditions of the environments [21, 22].

Perhaps one of the most striking features of the present study is the significant variation observed in the frequencies of inversion heterozygotes in two types of populations. For instance, all four inversion heterozygotes appeared relatively in much higher frequencies in the sample obtained from the localities where the larvae of *Ch. striatipennis* competed with those of *Ch. circumdatus* than from the localities where the *Striatipennis* larvae inhabited solitary (Table 2). The statistical analyses also show that the difference in frequencies of inversion heterozygotes between sympatric and allopatric populations is significant (Table 3). Several studies have demonstrated that more than one selection component can be implicated in the maintenance of the polymorphism [23–25]. However, the fact that genetic variation for competitive ability exists in response to interspecific competition has also been reported by many workers [26–29].

No attempts was made to determine the effect of several other factors such as temperature, seasons, altitude and depth and therefore it seems premature at present to conclude that interspecific competition alone has been largely responsible for the variations in the frequencies of inversion heterozygotes in these populations. Hence a more detailed investigation of various factors through laboratory experiments as well as field studies seems to be desirable.

ACKNOWLEDGMENTS

The authors are grateful to Dr. P. K. Chaudhuri, Burdwan University for his help in the identification of the species. One of us (AK) is also thankful to the

University Grants Commission, New Delhi for awarding him a Teacher Fellowship under the Faculty Improvement Programme.

REFERENCES

1 Chaudhuri, P. K. and Guha, D. K. (1987) A conspectus of chironomid midges (Diptera : Chironomidae) of India and Bhutan. Ent. Scand. Suppl., **29**: 23–33.

2 Nath, B. B. and Lakhotia, S. C. (1989) Heat-shock responses in a tropical *Chironomus*: Seasonal variation in response and the effect of developmental stage and tissue type on heat shock protein synthesis. Genome, **32**: 676–686.

3 Kumar, A. and Gupta, J. P. (1990) Cytogenetic studies of *Chironomus circumdatus* from India (Diptera: Chironomidae). Genetica, **82**: 157–163.

4 Hägele, K. (1975) *Chironomus* in "Handbook of Genetics" Ed. by R. C. King, Vol. 3, Plenum Press, New York/London, pp. 269–278.

5 Sumner, T. A. (1972) A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res., **75**: 305–306.

6 Lentzios, G., Stocker, A. J. and Martin, J. (1980) C-banding and chromosome evolution in some related species of Australian Chironomidae. Genetica, **54**: 51–68.

7 Martin, J., Wulker, W. and Sublette, J. E. (1974) Evolutionary cytology in the genus *Chironomus* Meigen, Stud. Nat. Sci. (Portales, N. M.), **1**: 1–12.

8 Martin, J. (1981) Location of a sex determining regions in *Chironomus tepperi* Skuse (Diptera : Chironomidae) using irradiation induced chromosomal rearrangements. Genetica, **57**: 113–117.

9 Beermann, W. (1955) Geschlechtsbestimmung und Evolution der genetischen Y-chromosomen bei *Chironomus,* Biol. Zentbl., **74**: 525–544.

10 Thompson, P. E. (1971) Male and female heterogamety in populations of *Chironomus tentans* (Diptera : Chironomidae). Canad. Entomologist, **103**: 369–372.

11 Martin, J., Kuvangkadilok, C., Peart, D. H. and Lee, B. T. O. (1980) Multiple sex determining regions in a group of related *Chironomus* species (Diptera: Chironomidae). Heredity, **44**: 367–382.

12 Hägele, K. (1986) Localization of male sex determining chromosome region in *Chironomus thummi piger*. Genetica, **70**: 187–190.

13 Bedo, D. G. (1975a) C-banding in polytene chromosomes of *Simulium ornatipes* and *Simulium melatum* (Diptera: Simuliidae). Chromosoma (Berl.), **51**: 291–300.

14 Hägele, K. (1977) Differential staining of polytene chromosome bands in *Chironomus* by Giemsa banding methods. Chromosoma (Berl.), **59**: 207–216.

15 Vosa, C. G. and Marchi, P. (1972) Quinacrine flourescence and Giemsa staining in plants. Nature, **237**: 191–192.

16 Sharma, N. P. and Natrajan, A. T. (1973) Identification of heterochromatin in region in the chromosome of rye. Hereditas, **74**: 233–238.

17 Hsu, T. C. (1975) A possible function of constitutive heterochromatin: The bodyguard hypothesis. Genetica, **79**: 137–150.

18 Dobzhansky, Th. (1951) "Genetics and the origin of species". 3rd ed. New York: Columbia University Press.

19 Sperlich, D. (1967) Populations genetik (Teil. 1. *Drosophila*). Fortschr. Zool., **18**: 223–278.

20 Wallace, B. (1968) "Topics in population Genetics". W. W. Narton and Co. Inc. New York.

21 Freeman, G. H. and Perkins, J. M. (1971) Environmental and genotype environmental components of variability. VII. Relations between genotypes grown in different environments and measures of these environments. Heredity, **27**: 15–24.

22 Lewontin, R. C. (1974) "The genetic basis of evolutionary change". Columbia Univ. Press, New York.

23 Bungaard, J. and Christiansen, F. B. (1972) Dynamics of polymorphism: I. Selection components in an experimental populations of *D. melanogaster.* Genetics, **71**: 439–460.

24 Christiansen, F. B. and Frydenberg, O. (1973) Selection component analysis of natural polymorphisms using population samples including mother-child combinations. Theor. Pop. Biol., **4**: 425–445.

25 Christiansen, F. B. and Frydenberg, O. (1976) Selection component analysis of natural polymorphism using mother-offspring samples of successive cohorts. In: "Population Genetics and Ecology" Ed. by S. Karlin and E. Nevo, Academic Press, New York, pp. 277–301.

26 Ayala, F. J. (1969) Evolution of fitness IV. Genetic evolution of interspecific competitive ability in *Drosophila.* Genetics, **61**: 737–747.

27 Futuyma, D. J. (1970) Variation in genetic response to interspecific competition in laboratory population of *Drosophila.* Amer. Naturalist, **104**: 239–252.

28 Barker, J. S. F. (1973) Adult population density, fecundity and productivity in *D. melanogaster.* Oecologia, **11**: 85–92.

29 Clark, A. (1979) The effect of interspecific competition on the dynamics of polymorphism in an experimental population of *D. melanogaster.* Genetics, **92**: 1315–1328.