

Studies in Natural Sciences

Volume 1, Numbers 10, 11, and 12

August 1974



Published by
The Natural Sciences Research Institute
Eastern New Mexico University
Portales, New Mexico
U.S.A.

ABSTRACT

Chironomus stigmaterus Say, although having the arm combination of the thummi-complex, does not appear to be closely related to any of the previously studied species. The banding sequences suggest some relationship to *Chironomus aberratus* Keyl.

Inversion polymorphism occurs in arms A, C, F, and G in some populations but further data are required from other areas before the extent of polymorphism in *Chironomus stigmaterus* can be fully evaluated.

The Cytology of *Chironomus stigmaterus* Say

by

Jon Martin¹ and W. Wülker²

INTRODUCTION

Chironomus stigmaterus Say is a common species in the southern United States and has a range extending as far north as New York State. At least in the southern and western United States the species commonly occurs in sewage treatment lagoons (Grodhaus 1967). Although the immature stages have been described on a number of occasions (Grodhaus 1967, Bath and Anderson 1969), there has been no published report of the polytene chromosomes.

MATERIALS AND METHODS

The materials examined came from a number of localities, mainly in the southwestern United States. The localities and the number of specimens from each are given here to provide an indication of the reliability of the data given on inversion frequencies:

-
1. Genetics Department, University of Melbourne, Parkville, Victoria, 3052, Australia.
 2. Biologisches Institut der Universität, (Zoologie) 78 Freiburg West Germany.

CALIFORNIA

Alameda Co.

71 larvae, Hayward, 16-VII-68, G. Grodhaus and E.W. Martinson of the California Department of Public Health.

Napa Co.

1 ♂ larva, Napa, 11-VII-67, G. Grodhaus. 4 larvae, 1 mi. S Napa, 10-IV-68, G. Grodhaus.

Riverside Co.

97 larvae (samples 16-1 to 16-6), Riverside, 1 to 8-XII-65.

Solano Co.

22 larvae, Dolwig Lake, Vallejo (no date), R.E. Darby.

FLORIDA

Leon Co.

1 larva, Lake Miccosuckee (no date).

NEW MEXICO

Roosevelt Co.

1♀, 22♂ and 12 unsexed larvae, from sewage treatment lagoon, 6 mi. SW of Portales, 29-X-70, M. Beard.

Guadalupe Co.

1 larva, fish hatchery at Santa Rosa, (no date).

TEXAS

Travis Co.

11 ♀ and 6 ♂ larvae, 23-VII-67; and 24 ♀ and 11 ♂ larvae, 23-X-70, Austin (both samples from wading pools used for fish breeding at the Brackenridge Experimental Station, University of Texas), J. Martin.

We wish to thank Dr. G.L. Bush for his assistance in obtaining the Austin samples. Because these populations are broken up into numerous discrete subdivisions in the separate wading pools, the differences which appeared in some inversion frequencies must be treated with caution because they could reflect sampling error if each pool were colonised by only a small number of females. The quality of the chromosomes, particularly in the second collection, was poor, with the chromosomes being exceedingly short with poor banding. Therefore, full scoring of many individuals was not possible.

Squashes were made by the usual techniques (Wülker *et al.* 1971). Many of the preparations were made by Mary F. Sublette, whose assistance is gratefully acknowledged.

CYTOLOGY

There are four salivary gland chromosomes as is usual in the genus *Chironomus*. The arm combination is the thummi-complex, i.e., AB, CD, EF, and G (see Keyl 1962). The centromeres are distinct and show some degree of heterochromatinization. The chromosomes are shown in Fig. 1.

Arm A has bands which cannot be identified with complete certainty. The bands of group 3g-6a are obvious near the centre of the arm, with groups 3-2h on one side of them, and 12-10 and 14-16 on the other side. Doubt arises as to the identity of some of the bands proximal and distal to these groups. Two sequences of this arm occur in *C. stigmaterus* but neither appears to be closely related to any of those previously described in other species.

The sequences, differing by an inversion from 2d-15, appear to be:

tw-12-4

C. stigmaterus I

1 2 ^{a-g} | 8 9 13 3 ^{f-a} ^{k-h} ^a ^{i-g} | 6 5 4 3 | 12 11 10 14 15 | 16 17 ^{a-d} ^{b-e} | 7 17 ^{e-h} | 18-19

C. stigmaterus II

1 2 ^{a-c} | 15 14 10 11 12 ^{g-i} | 4 5 6 ^a ^{h-k} ^{a-l} | 13 9 8 2 ^{g-d} | 16 17 ^{a-d} ^{b-e} | 7 17 ^{e-h} | 18-19

Other interpretations of the banding sequence are possible. A_1 can be derived from *Chironomus pseudothummi* Strenzke by four hypothetical steps, but in view of the greatly changed sequence such a relationship must be tentative. Indeed, one cannot be certain at present whether or not A_1 or A_2 is the phylogenetically older sequence.

A_2 was the most common sequence in the populations studied; A_2 is homozygous in California but polymorphic in New Mexico and Texas. At Portales, New Mexico, A_1 was present with a frequency of 6% and a single specimen from Santa Rosa, New Mexico was A_1/A_1 . The two samples from Austin, Texas differed markedly in frequency: 21% A_1 in July 1967 and 71% A_1 in October 1970. It cannot be determined whether this difference reflects a seasonal

change, long term change, or simply random drift due to the subdivision of the larval habitat (see above). The single specimen from Florida was A_2/A_2 . The heterozygous configuration is shown in Fig. 2a.

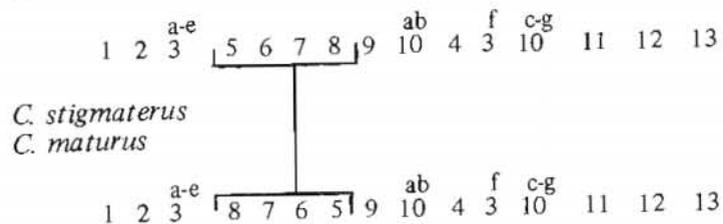
Arm B is characterised by a Balbiani ring just distal to the four characteristic bands. A puff is usually developed about one quarter of the distance from the distal end, with a group of dark bands immediately distal to the puff.

Arm C has constrictions bordered by dark bands which break the arm into three approximately equal segments. The more distal of these constrictions is bordered on the centromere side by the bands a_3 - a_4 as denoted by Keyl (1957). Bands a_1 - a_2 remain at the distal end of the arm as in *Chironomus piger* Strenzke (Fig. 1). An inversion proximal to the centre of this arm (Fig. 2b) occurs in low frequency in populations in New Mexico and Texas. The frequency of C_2 is 7% at Portales, New Mexico and perhaps as high as 16% at Austin, Texas. However, the sequence was not found in the July 1967 sample from Austin but was present at a frequency of 16% in October 1970. As with the inversion in arm A, the reason for this variation has not been determined.

Arm D has a large nucleolus just distal to the middle of the arm. No inversion polymorphism is known in this arm.

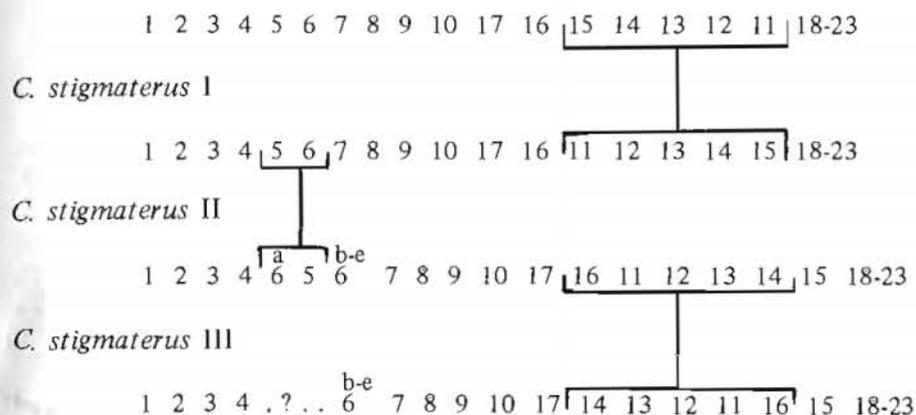
Arm E differs from that of the European species *Chironomus aberratus* Keyl, amongst others, by a short inversion 8-5. It is therefore identical in sequence with the North American species *Chironomus matusus* Johannsen (Wülker and Martin 1974), but differs from it by the thicker bands 8h-i.

C. aberratus, etc.



Arm F occurs in three sequences. F_1 differs from the European *C. aberratus* through a short inversion 11-15. F_2 then is derived from F_1 by a very short inversion 5-6a. In the heterozygotes an additional band is inserted between 4d and 6a. This band often appears heterochromatic or is somewhat puffed (Fig. 2c). The third sequence, F_3 , has only been observed once in a single individual from Ontario, California (Fig. 2d), which was also heterozygous for F_1/F_2 . The break points of F_3 could not be determined with accuracy, but they appear to be near the division of groups 16 and 17 at one end and near the division between groups 14 and 15 at the other end. It could not be determined whether or not F_3 was on the same chromosome as F_1 or F_2 . Because the two inversions are completely independent, crossing-over should be possible between them; therefore, it is theoretically possible for F_3 to occur with either sequence of the other inversion.

C. aberratus



Because of the very small segment involved in the polymorphism of F_1 and F_2 , homozygotes can only be scored in the very best specimens. It was, therefore, not practicable to attempt a complete study of the population dynamics of this inversion. However, certain features were noted, in particular that the frequency of heterozygotes was at least 50% in all populations sampled. In those

cases where larvae had been sexed, females were homozygous and males were heterozygous. This suggests that region 5-6a is either close to, or actually includes, sex determining loci. F_2 , with its additional band, would then represent a recognizable Y-chromosome similar to that reported in arm F of *Chironomus rempeli* Thienemann (Rempel *et al.* 1962). If F_2 is a Y-chromosome, F_2/F_2 homozygotes will not be expected to occur and no such individuals have been found amongst those specimens where arm F is clear enough to permit definite identification. Further sexed material is necessary to confirm these findings.

Arm G is found with four different sequences in various populations. Sequence G_1 , which is the only one present in California, has two Balbiani rings; one is about a third of the distance from the left hand end, immediately followed by a constriction and a swelling with two thick dark bands; the other, close to the right end of the arm. G_2 differs from sequence G_1 by a large inversion of the right end of the arm. In addition, the left hand Balbiani ring is not developed. G_3 , which is at the left end of arm, is a much smaller inversion than G_2 . G_3 overlaps with the end of G_2 and inverts the region around the left hand Balbiani ring. G_4 is a further inversion of G_2 . Like G_3 , G_4 is at the left hand end, but is closer to the end and smaller than G_3 . As in G_2 , the right hand Balbiani ring is not developed.

The latter three sequences were found only in the New Mexico and Texas samples. G_2 had a frequency of 57% at Portales and 54% at Austin; G_3 was 12.5% at Portales and 15% at Austin; G_4 was 18% at Portales but did not occur at Austin. An individual homozygous G_4/G_4 was found at Santa Rosa but no G_3/G_3 individual has been found. The single specimen from Florida was G_1/G_1 .

The various heterozygous configurations are shown in Fig. 3.

DISCUSSION

Chironomus stigmaterus does not appear to be closely related to any of the previously studied species of North America or Europe. Arms E and F indicate a relationship to *C. aberratus* (Fig. 4), but the very changed sequence of Arm A, which is not easily derived from *C. aberratus* suggests the relationship is somewhat distant. The possession of an arm E identical to that of *C. matusus* raises an interesting problem. If the sequence indicates a common ancestor of

these two species, then for the same sequence to occur in both the thummi- and matusus-complexes, the inversion must have occurred prior to the whole arm translocation which separates these groups. If this is correct, other species may be found carrying this sequence, which must then be of considerable antiquity. However, there are two other possible explanations *viz*: (1) an independent occurrence of a similar inversion in each of the species concerned, or (2) hybridization between the sympatric populations of *C. stigmaterus* and *C. matusus*, which could have allowed transfer of the sequence from one species to the other and its subsequent fixation in both. In the case of the latter explanation, there is no way of knowing which would have been the donor and which the recipient species.

Relatively little can be concluded with regard to the inversion polymorphism in *C. stigmaterus*. With the exception of the possible sex-linked inversion of arm F, which occurred in all populations sampled, the other inversions appear to have a limited distribution in New Mexico and Texas. However, these results simply indicate the need for further samples to fill in the gaps in the distribution of the species. In particular, the extent to which the species extends south into Mexico and Central America would be of interest. One previous record exists for Mexico (Townes 1945). Possibly, the polymorphism is more widespread through Mexico and Central America and to date only the very edges of the polymorphic populations have been sampled.

LITERATURE CITED

- Bath, J.L., and L.D. Anderson. 1969. Larvae of seventeen species of chironomid midges from Southern California (Diptera). J. Kans. Entomol. Soc. 42: 154-176.
- Grodhaus, G. 1967. Identification of chironomid midges commonly associated with waste stabilization lagoons in California. Calif. Vector Views 14: 1-12.
- Keyl, H.G. 1957. Untersuchungen am Karyotypus von *Chironomus thummi*. I. Karte der Speicheldrüsen-Chromosomen von *Ch. th. thummi*. und die cytologische Differenzierung der Subspecies *Ch. th. thummi* und *Ch. th. piger*. Chromosoma 8: 739-756.
- . 1962. Chromosomenevolution bei *Chironomus*. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. Chromosoma 13: 464-514.
- Rempel, J.G., J.M. Naylor, K. Rothfels, and B. Ottonen. 1962. The sex chromosome constitution of chironomid intersexes parasitized by nematodes. Can. J. Genet. Cytol. 4: 92-96.
- Townes, H.K. Jr. 1945. The Nearctic species of Tendipedini [Diptera, Tendipedidae (=Chironomidae)]. Amer. Midl. Nat. 34: 1-206.
- Wülker, W., and J. Martin. 1974. A review of the genus *Chironomus* (Diptera, Chironomidae). VI. Cytology of the maturus-complex. Stud. Nat. Sci. (Portales, N.M.) 1(9): 1-21.
- Wülker, W., J.E. Sublette, M.F. Sublette, and J. Martin. 1971. A review of the genus *Chironomus* (Diptera, Chironomidae). I. The *staegeri* group. Stud. Nat. Sci. (Portales, N.M.) 1(1): 1-89.

Fig. 2.a-d Heterozygous configurations of inversion polymorphisms in *Chironomus stigmaterus*.

a) A_1/A_2 b) C_1/C_2 c) F_1/F_2

Note the puff developed in F_2 (P). d) F_1/F_3 .

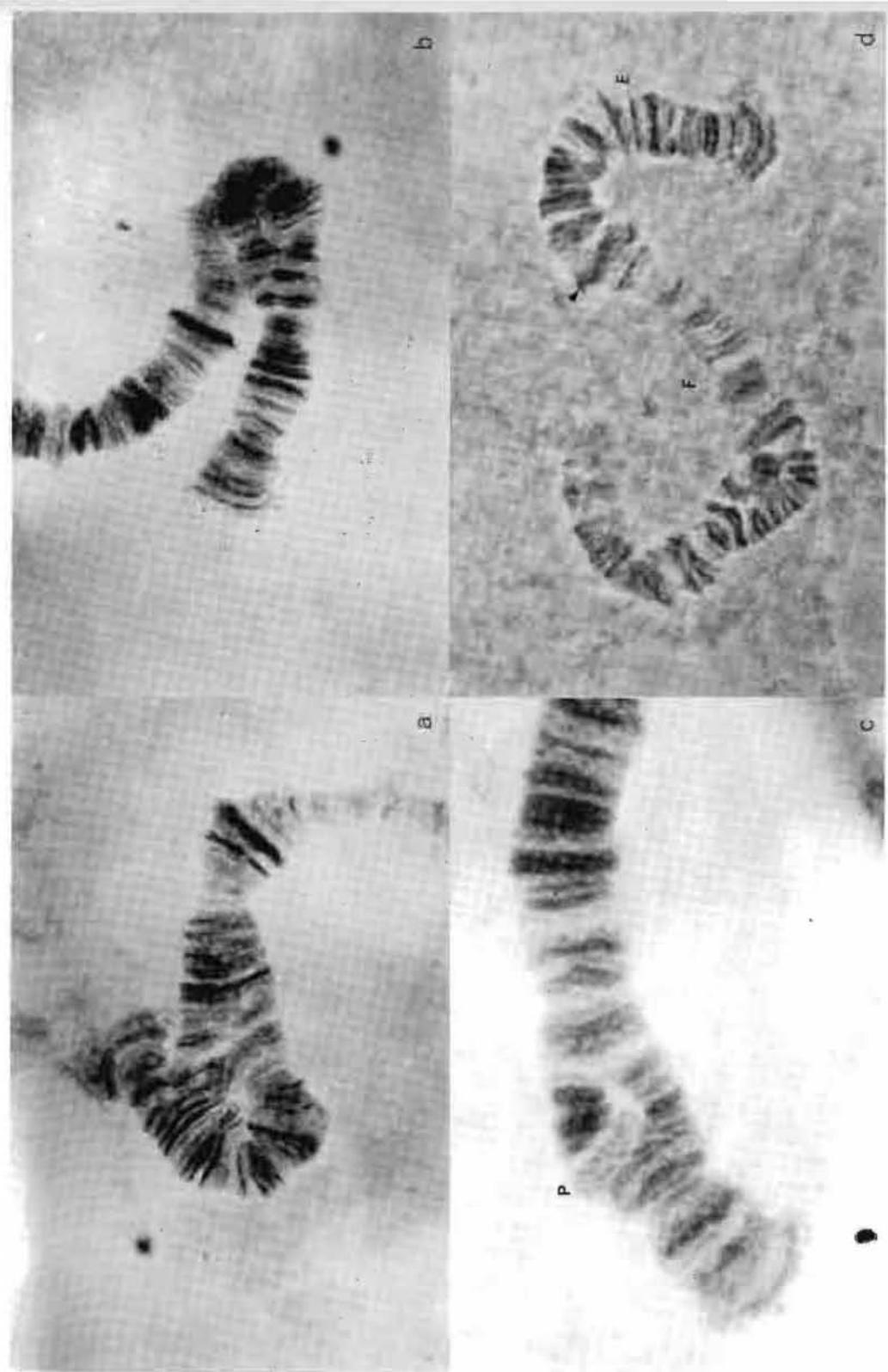


Fig. 3.a-d Heterozygous configurations of the arm G sequences in *Chironomus stigmaterus*.

a) G_1/G_2 b) G_1/G_3 c) G_2/G_3 d) G_2/G_4

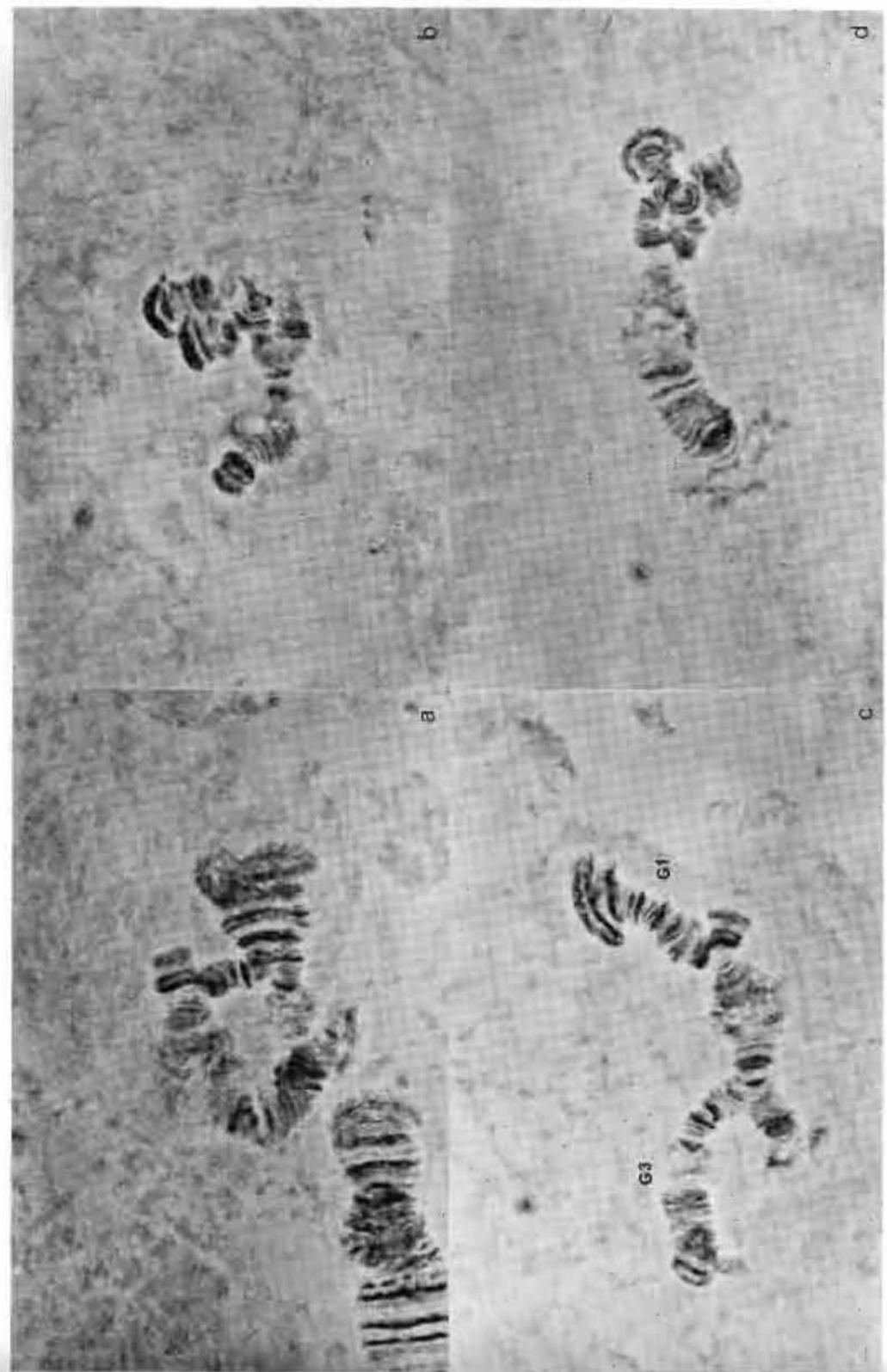


Fig. 4. Cladogram of the possible relationships between *Chironomus stigmaterus*, *Chironomus aberratus*, and *Chironomus matusus*.

- a. Arm E: Inversion 5-8, Inversion 5-10b still present.
- b. Arm F: Inversion 17-11.
- c. Arm E: fixation of Inversion 5-10b.
- d. Arm A: multiple changes. Arm E: fixation of Inversion 5-8. Arm F: Inversion 15-11. Reciprocal translocation to give AF, BE. Arm E: fixation of Inversion 5-8. Arm F: Inversion 3-15e.
- e.

