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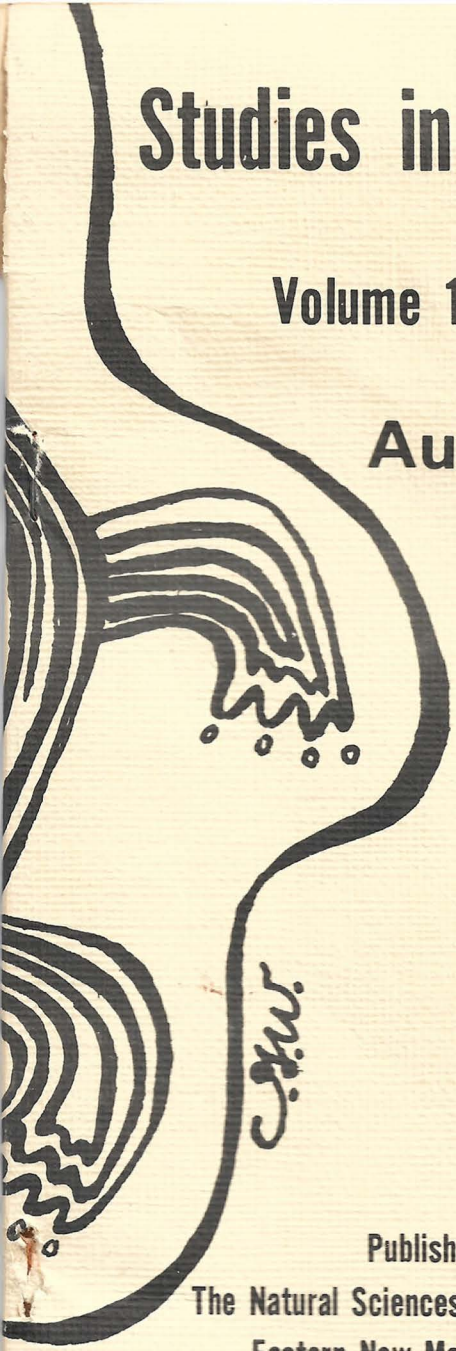

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## ABSTRACT

Two species, *Chironomus maturus* Johannsen and *Chironomus whitseli* Sublette and Sublette, having the chromosome arm combination AF, BE, CD, and G, are described. An evolutionary relationship to an ancestral group of the thummi- and pseudothummi-complexes as well as the parathummi-complex is postulated.

# A Review of the Genus *Chironomus* (Diptera, Chironomidae)
# VI. Cytology of the maturus-complex

by

Wolfgang Wülker[1] and Jon Martin[2]

## INTRODUCTION

The presence of a *Chironomus* species with the arm combination of the polytene chromosomes AF, BE, CD, and G was first reported by Wülker et al. (1967). This maturus-complex[3] of the genus *Chironomus* has only been found in North America; further investigations have revealed a second species belonging to the complex (Martin *et al.* in press).

In the system of translocation types of the genus *Chironomus* (schemes in Keyl 1962, Krieger-Wolf and Wülker 1971, Martin *et al.* in press) the complex has a unique position in that it can be connected with both the most frequent complexes of *Chironomus, i.e.*, thummi-complex and pseudothummi-complex by only one reciprocal arm translocation. Also the banding pattern of both species shows characteristic features of both the thummi- and pseudothummi-complexes indicating that all the three complexes arose at about the same evolutionary period.

Moreover, the species of the maturus-complex seem to have some relations to *Chironomus decorus* Johannsen, the single species in North America which could not clearly be homologized to the banding pattern of all other *Chironomus* species. Some specimens of *C. maturus* Johannsen at least were determined firstly as "spec. 51" or as sp. No. 1, both California *Chironomus* which are supposed to be

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3. Krieger-Wolf and Wülker (1971) used a preliminary name, paralcyon-complex.

closely related to *C. decorus* in adult morphology (Biever 1965, Bath and Anderson 1969). Therefore, the species of the maturus-complex may contribute to an explanation of the systematic position of this aberrant pattern of *C. decorus*.

## MATERIALS AND METHODS

Localities, sample data, and number of specimens used for the cytological investigation are shown in Tables 1 and 2. The cooperation of colleagues who assisted in collecting material from different places is gratefully acknowledged.

*Chironomus maturus* was detected in different areas all over the continent (California to South Dakota to Ontario); whereas, *Chironomus whitseli* Sublette and Sublette was confined to California (Tables 1 and 2). The material of *C. maturus* came from lakes, creeks, and a seepage area where the species occurred with the indicator species of polluted water, *Chironomus riparius* Meigen (=*C. thummi* Kieffer, Credland 1973). *Chironomus whitseli* was found in several localities in California.

For details of preparation of chromosome squashes see Wülker et al. (1971). The assistance of Mary F. Sublette in preparing the material has been most helpful.

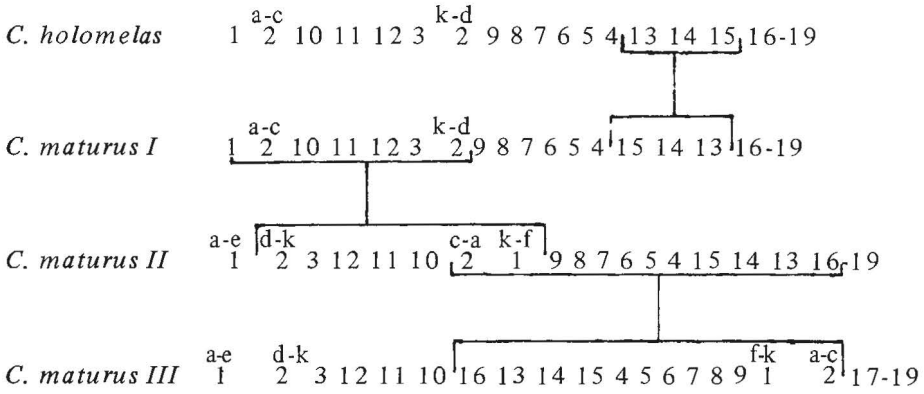
## RESULTS

### Cytology of *Chironomus maturus* Johannsen

Karotype (Fig. 1). Four salivary gland chromosomes with arm combination AF, BE, CD, and G. Centromeres distinct but without heterochromatinization.

**Arm A.** Sequence I is known only in the heterozygous condition from South Dakota (Fig. 3a). The sequence differs from the European *Chironomus holomelas* Keyl and *Chironomus melanescens* Keyl, as well as from *Chironomus staegeri* Lundbeck (Wülker *et al.* 1971) and *Chironomus yoshimatsui* Martin and Sublette (Martin and Sublette 1972) by only a short inversion 13-15, which also has some importance in the derivation of the aberrant European *Chironomus obtusidens* Goetghebuer (Keyl 1962). The more common sequence II

(Fig. 1a) was present in all collections and is characterised by an inversion 2d-1f in the distal part of the arm. A III has been found only in California and has an inversion 16-2c in the proximal part of the arm (Fig. 3b).

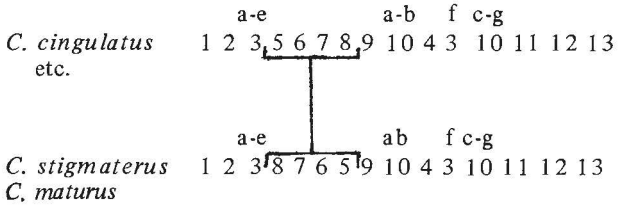



**Arm B.** In sequence I the region of light bands is situated in the proximal part of the arm. A long inversion transfers this region to the distal part. The distal break of the inversion seems to divide the typical Balbiani ring (Fig. 1b); therefore, B II sometimes shows a small Balbiani ring near the typical equidistant bands of the arm. In our material B I is prevalent in South Dakota and B II in California and Ontario. B III, which is represented only by three heterozygous specimens from Yankton, South Dakota, changes a very short region just distal to the Balbiani ring (Fig. 3c). Homozygous B III could not be found, but because of the very small segment involved, satisfactory results are limited to the very best of specimens.

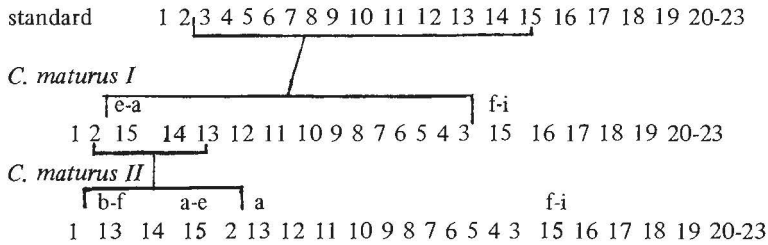
**Arm C.** Groups a1-a4 (*sensu* Keyl 1957) are in a subterminal position as in *C. riparius*, but a light region with dark distal bands which is not present in *C. riparius* is inserted between a1-a4 and the end of the arm. The typical group of a light and a dark band (! in Fig. 1c) has a proximal position. A short terminal inversion leads to C II which is common in California. In South Dakota, some specimens have a considerably longer inversion in the middle of the arm (Fig. 3e), but homozygotes for C III were not found.

**Arm D.** Three groups of dark bands and a drum-like structure lie in the distal part of the arm. An extended light area occurs near the centromere. Two specimens in Davis Lake, California are heterozygous for a relatively long inversion in the middle part of the arm (Fig. 3g), the limits of which could not be fixed with certainty.

**Arm E.** The banding pattern is apparently the same as in *Chironomus stigmaterus* Say (Martin and Wülker in press), i.e., the pattern can be derived by an inversion 5-8 from the situation of the European species *Chironomus cingulatus* Mg., *Chironomus plumosus* (L.), *Chironomus aberratus* Keyl, and *Chironomus commutatus* Keyl.




**Arm F.** Both sequences of this arm can be directly connected with the standard sequence of *Chironomus piger* Strenzke (Keyl 1962). F I differs from this sequence by a long inversion 3-15e; the more common F II has an additional short inversion 2-13b (Fig. 1f and Fig. 3f).




A large nucleolus is present in the region of group 18; also, group 11 sometimes shows a slight puffing.

**Arm G.** The arm is characterized by a subterminal nucleolus and at least two Balbiani rings, one of them near the middle of the arm. In parallel to *C. whitseli* (page 6) and *C. stigmaterus* (Martin and Wülker in press), apparently four sequences exist, although

in the case of *C. maturus* only three of them could be proved in our material. The most common sequences, G I (California) and G IV, differ by a long inversion beginning near the nucleolus and an adjacent short inversion near the other end of the arm (Fig. 1g), which transfers the Balbiani ring to a sub-terminal position. G II was present in South Dakota and Ontario only. An inversion similar to that which converts sequences II to IV would also be expected to occur with sequence I to give rise to sequence III. However, no such cases have been observed. Of the possible heterozygous combinations, only three have been found. GI/IV, in which the homologous chromosomes are mostly unpaired, occurs in California. GII/IV has been found in South Dakota and GI/II has been found once in Ontario.

## Cytology of *Chironomus whitseli* Sublette and Sublette

Karyotype (Fig. 2). Four salivary gland chromosomes with arm combination AF, BE, CD, and G. Centromeres distinct but not heterochromatinized.

**Arm A.** Identical with *C. maturus* sequence II (see page 2).

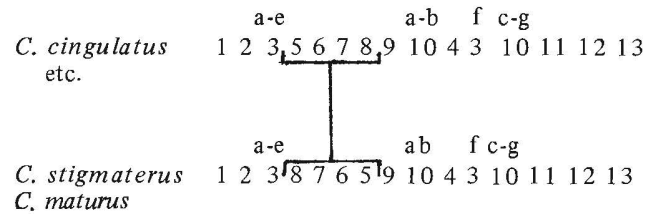
**Arm B.** B I is identical with B I of *C. maturus*. Two specimens from El Portal are heterozygous for a short inversion which includes the characteristic Balbiani ring (Fig. 2b and Fig. 3d). Homozygous B II could not be found, but its identification is difficult as in the case of the short inversion in arm B of *C. maturus* (see page 3).

**Arm C.** Banding pattern in the distal part is identical with the standard species *C. piger*, especially groups a1-a4 in the position indicated in Keyl (1957) and Keyl and Keyl (1959). In sequence II these groups are inverted due to a short inversion in the distal part of the arm. In contrast to *C. maturus*, the typical group of a light and a dark bank (! in Fig. 2c) lies about the middle of the arm.

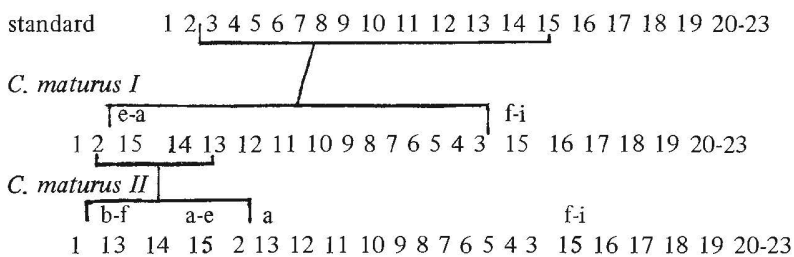
**Arm D.** One specimen from Lafayette, California shows heterozygosity in the end of the arm, one sequence of which is identical with *C. maturus* (Fig. 1d and Fig. 3h). In the other one, which should be named D II although it is present in all other specimens, the narrowing area of light bands is transferred to the end of the arm.

**Arm D.** Three groups of dark bands and a drum-like structure lie in the distal part of the arm. An extended light area occurs near the centromere. Two specimens in Davis Lake, California are heterozygous for a relatively long inversion in the middle part of the arm (Fig. 3g), the limits of which could not be fixed with certainty.

**Arm E.** The banding pattern is apparently the same as in *Chironomus stigmaterus* Say (Martin and Wülker in press), i.e., the pattern can be derived by an inversion 5-8 from the situation of the European species *Chironomus cingulatus* Mg., *Chironomus plumosus* (L.), *Chironomus aberratus* Keyl, and *Chironomus commutatus* Keyl.




**Arm F.** Both sequences of this arm can be directly connected with the standard sequence of *Chironomus piger* Strenzke (Keyl 1962). F I differs from this sequence by a long inversion 3-15e; the more common F II has an additional short inversion 2-13b (Fig. 1f and Fig. 3f).




A large nucleolus is present in the region of group 18; also, group 11 sometimes shows a slight puffing.

**Arm G.** The arm is characterized by a subterminal nucleolus and at least two Balbiani rings, one of them near the middle of the arm. In parallel to *C. whitseli* (page 6) and *C. stigmaterus* (Martin and Wülker in press), apparently four sequences exist, although

**Arm E.** Identical with the European species *Chironomus halophilus* Kieffer, *Chironomus luridus* Strenzke, *Chironomus crassimanus* Strenzke, *Chironomus acidophilus* Keyl, and *Chironomus uliginosus* Keyl, the North American *Chironomus frommeri* Atchley and Martin, and the Japanese *C. yoshimatsui* (Martin and Sublette 1972); one inversion step from standard *C. piger*.

```
      a-e ba          f c-g
1 2  3 10 9 8 7 6 5 4 3  10  11 12 13
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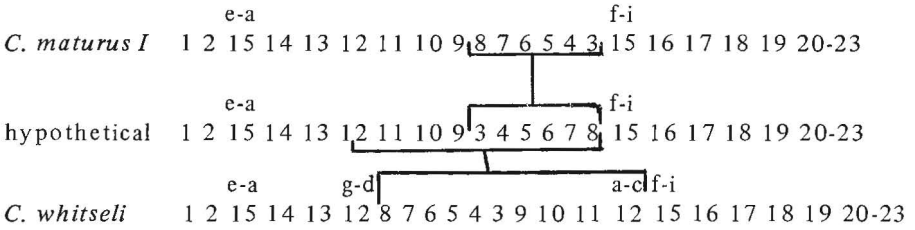
**Arm F.** The sequence of the arm can be derived from *C. maturus* I by two inversion steps, one of which is hypothetical.




Neither the nucleolus in group 18 nor the puffing in group 11 is developed as in *C. maturus*.

**Arm G.** As in *C. maturus*, the arm has a subterminal nucleolus and at least two Balbiani rings. G I is identical with G I of *C. maturus*. Also the inversion pattern of this arm has some similarities with *C. maturus*. Four sequences are present, differing by inversions near the nucleolus and at the nucleolus-free end of the arm. But contrary to *C. maturus*, the change between G I and G II, respectively, and G III and G IV implicates a kind of shift without alteration of the polarity of the regions involved. The analysis of banding patterns is obscured by changing positions of the Balbiani rings. Specimens from Stanford lands and El Portal differ in representing G I and G III or G II and G IV only.

## DISCUSSION

Both species of the maturus-complex are very closely related, with at least certain sequences of arms A, B, D, and G being identical. Moreover, in arms A, B, and D the interspecific differences are retained as intraspecific polymorphisms, a situation which has been regarded by Keyl (1962) and Wülker and Klötzli (1973) as an indication of relatively recent separation of both species.

Of particular interest is the relationship of this complex to the previously recognized complexes of the genus *Chironomus* and to the postulated common ancestor with Arm A like *C. holomelas* or *Chironomus pseudothummi* Strenzke, arm E like *C. riparius, C. halophilus* or *C. cingulatus* and arm F like *C. riparius* (Keyl 1962, Martin *et al.* in press). *Chironomus whitseli* retains the Inv10b-3f of *C. halophilus* in arm E, but Arm A and F of *C. maturus* differ from those of *C. holomelas* or *C riparius*, respectively, by a single inversion step. These species are therefore not far removed from this ancestral species. The maturus-complex arm combination AF, BE could be derived either from the thummi-complex combination AB, EF or the pseudothummi-complex combination AE, BF by a single reciprocal translocation. The sequences present in the maturus-complex which have so far been recognized, do not permit a decision as to which of these possibilities is correct. Arm F derives from the standard sequence as found in the thummi-complex; arm A derives from the Inv10-2d sequence found in the pseudothummi-complex but from which many thummi-complex species have arisen (Keyl 1962). Arm E of *C. maturus* derives from the thummi-complex sequence Inv5-10b and that of *C. whitseli* derives from Inv10b-3f of the pseudothummi-complex. Therefore, on the basis of these three arms it was not possible to determine just which complex is most closely related to the maturus-complex. Indeed it appears from these relationships that the maturus-complex is connected at the point where the thummi- and pseudothummi-complexes are related and at which the parathummi-complex also is connected (Keyl 1962). The evolutionary consequences of this have been discussed by Martin *et al.* (in press).

It is difficult to decide which of the two species in the maturus-complex may be nearer to the hypothetical ancestor. In arms A and F, *C. maturus* seems to be more primitive, but in arms C and E,

*C. whitseli* is closer to the common ancestor. It, therefore, seems unlikely that either of these species is descended directly from the other, but rather that both are descended from an intermediate which is either no longer existent or else has not yet been collected.

With regard to inversion polymorphism and geographical distribution of *C. maturus* and *C. whitseli*, the former species seems to have greater adaptibility as it is apparently distributed throughout North America and possesses inversion polymorphism in all arms with the exception of E. *Chironomus whitseli*, on the other hand, is restricted to the west coast and has inversion polymorphism only in arms B, C, D, and G, of which only the C and G polymorphisms are common. This relationship is similar to that previously recorded between *C. staegeri* and *C. frommeri* (Wülker *et al.* 1971). Before further consideration of the significance of this relationship can be given, additional material from other areas and habitats will be required. The same is true for the remarkably unequal distribution of the different sequences of certain polymorphic arms in certain areas of the continent (Tables 1 and 2). Thus, more study could be given to the relationship of arms CII, FII, and GI in the east and of CI, FI, and GII in the west in *C. maturus*, although the almost complete association of the sequence in arms C and G of *C. whitseli*, which reverses between costal populations and those about 150 miles inland, also merits further consideration.

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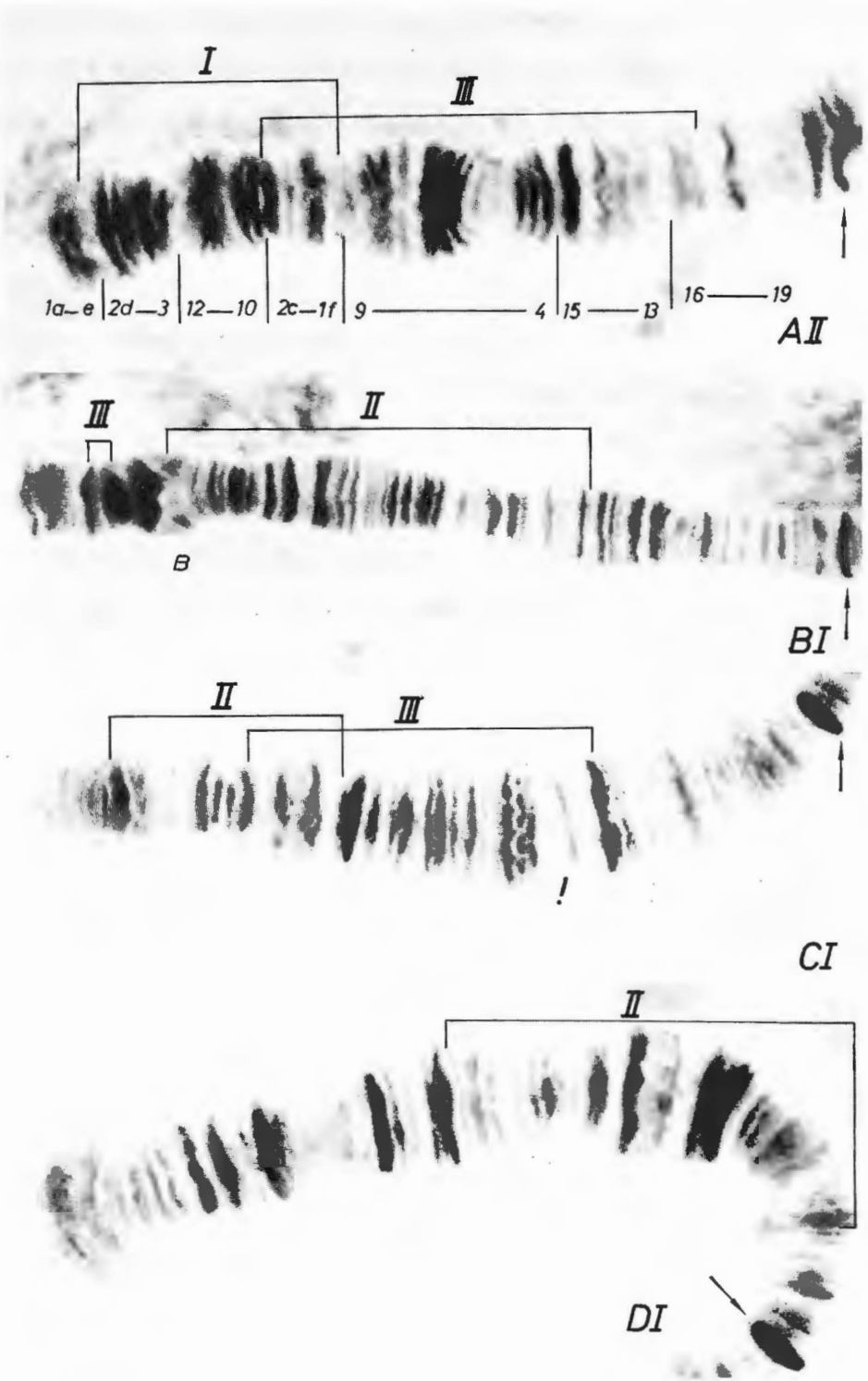
Table 1. Sampling data and distribution of sequences of *Chironomus maturus*. Homozygotes AI, BIII, CIII, DII, and GIII and some of the possible heterozygous combinations were not present in the material.

| Number of Slides | Location | Arm A | | | | Arm B | | | | Arm C | | | | Arm D | | Arm F | | | Arm G | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | II | III | I/II | II/III | I | II | I/II | I/III | I | II | I/II | I/III | I | I/II | I | II | I/II | I | II | I/II | IV | I/IV | II/IV |
| 11 | Laurel Creek, San Mateo Co., Cal., 13/10/64, R. Whitsel. | 4 | 3 | | 4 | | 11 | | | | 11 | | | 11 | | | 11 | | 2 | | | 3 | 6 | |
| 2 | Stateline, El Dorado Co., Cal., 18/7/67, G. Grodhaus. | 1 | 1 | | | 1 | 1 | | | | 2 | | | 2 | | 1 | 1 | | 1 | | | | 1 | |
| 21 | 1 mi. S Napa, Napa Co., Cal., 10/4 and 1/5/68, R.E. Doty. | 2 | 4 | | 15 | | 20 | 1 | | | 21 | | | 21 | | | 21 | | 1 | | | 13 | 7 | |
| 8 | Lake Davis, Plumas Co., Cal., 1/5/68, G. Grodhaus. | 6 | | | 2 | | 7 | 1 | | 6 | 2 | | | 6 | 2 | 5 | | 3 | 6 | | | | 2 | |
| 4 | Norco, Priester Ranch, Riverside Co., Cal., 16/3/71, S. Caton. | 4 | | | | 1 | 2 | 1 | | | 4 | | | 4 | | | 4 | | | | | 4 | | |
| 4 | Seepage area near Spring 3 mi. W Yankton, S.D., 15/4/68, P.J. Hudson. | 35 | | 6 | | 22 | 3 | 16 | | 27 | | 14 | | 41 | | | 33 | 6 | | | | 28 | | 13 |
| 17 | Yankton, S.D., May 1968, egg mass C, P.L. Hudson. | 17 | | | | 13 | | | 4 | 11 | | | 6 | 17 | | | 7 | 10 | 3 | | | 8 | | 6 |
| 5 | Mile 14.3, Highway 60 Algonquin Park, Ont., 1/6/66 and 8/6/67, J. Martin. | 4 | | 1 | | | 5 | | | 4 | | 1 | | 5 | | 5 | | | 3? | | 1 | | | 1 |
| 3 | Torbolton area, Ont. 0.59.3, Egg Mass 2 laid, 4/5/67, J. Martin. | 3 | | | | | 3 | | | 3 | | | | 3 | | 3 | | | | 3 | | | | |
| 1 | Copanspin Farm, Dunrobin, Ont. 0.23.7, 7/6/66, J. Martin. | 1 | | | | | 1 | | | 1 | | | | 1 | | 1 | | | | | | | | 1 |
| 1 | Hogs Back, Ottawa, Ont. 0.18.15, 21/11/66, J. Martin. | 1 | | | | | 1 | | | 1 | | | | 1 | | 1 | | | 1 | | | | | |

Table 2. Sampling data and distribution of sequences of *Chironomus whitseli*. Homozygotes BII, DI (=*Chironomus maturus*), GII, GIII and some of the possible heterozygous combinations of arm G were not present in the material.

| Number of Slides | Locality | Arm B | | Arm C | | | Arm D | | | Arm G | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | I/II | I | II | I/II | I | II | I/II | I | IV | I/III | I/IV | II/IV |
| 32 | 12 mi. NE El Portal, Mariposa Co., Cal., 13/4/68 Lot C, G. Grodhaus. | 30 | 2 | 32 | | | | 32 | | | 15 | | | 17 |
| 35 | Horse trough, Stanford Lands, Santa Clara Co., Cal., 28/3/68. | 35 | | | 28 | 7 | | 35 | | 30 | | 5 | | |
| 10 | Truckee River, near Tahoe City, Placer Co., Cal., 24/8/67, J. Martin. | 10 | | 10 | | | | 10 | | | 8 | | 2 | |
| 6 | Kensington, Contra Costa Co., Cal., 23/8/67, G. Grodhaus. | 6 | | | 3 | 3 | | 6 | | 6 | | | | |
| 4 (+1) | 2 mi. N Lafayette, Contra Costa Co., Cal., 20/4/68 Lot B, G. Grodhaus. | 4 | | | 4 | | | 4 | (1) | 3 | | | 1 | |
| 1 | Wildcat Creek, El Cerrito, Contra Costa Co., Cal., 9/7/67, G. Grodhaus. | 1 | | | | 1 | | 1 | | 1 | | | | |

Fig. 1. (See following pages)
Chromosome arms A-G of *Chironomus maturus* Johannsen. Centromeres (arrows) are on the right side of the photographs. Banding groups of arms A, E, and F (following Keyl 1962) are indicated below the chromosome; breakpoints of inversions are above the chromosome (for arm D only approximately). Structure type III in arm G is only hypothetical.
B=Balbiani ring, N=Nucleolus, !=Characteristic group in arm C (compare *Chironomus whitseli*)


I
III
1a—e
2d—3
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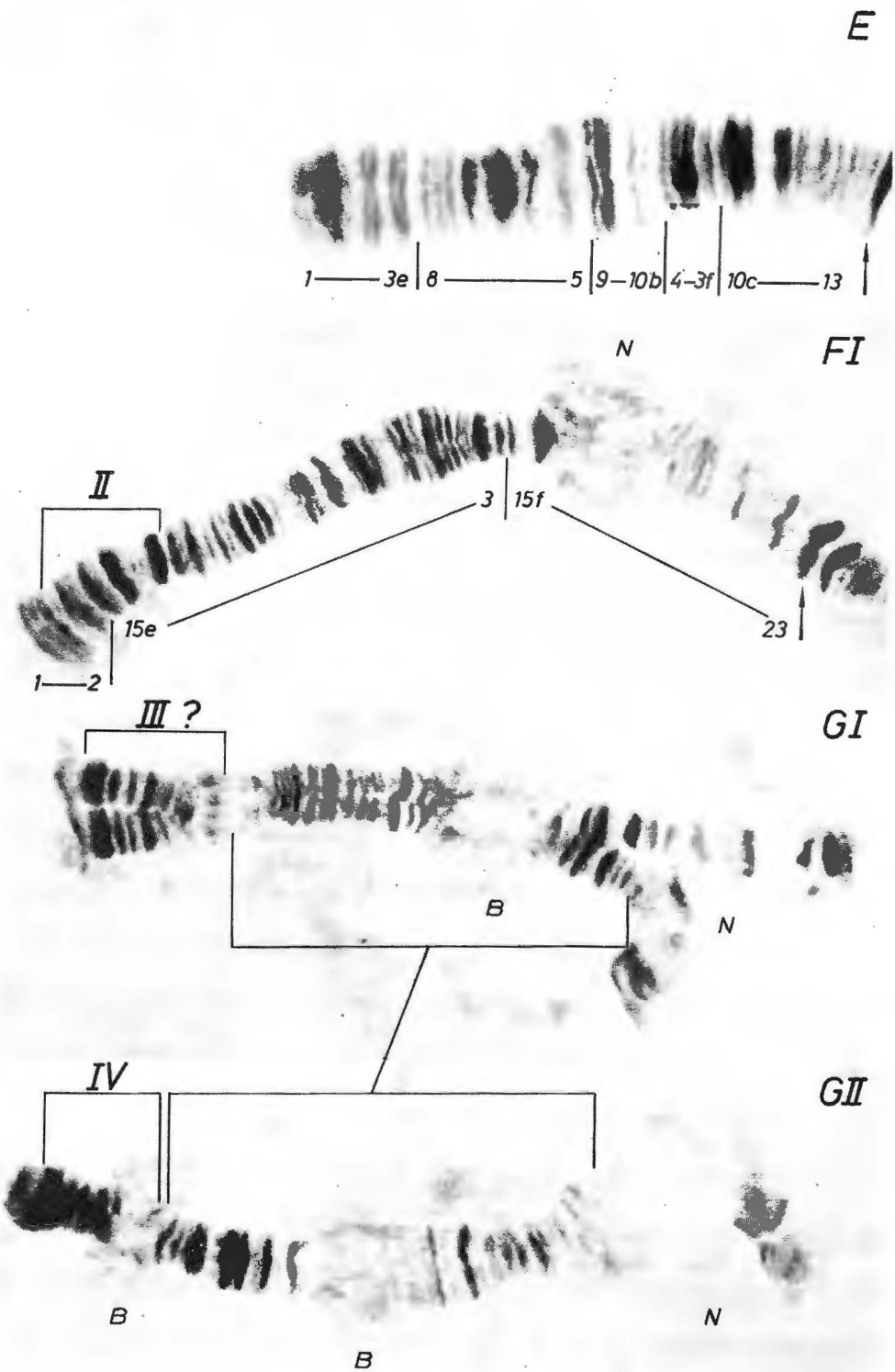
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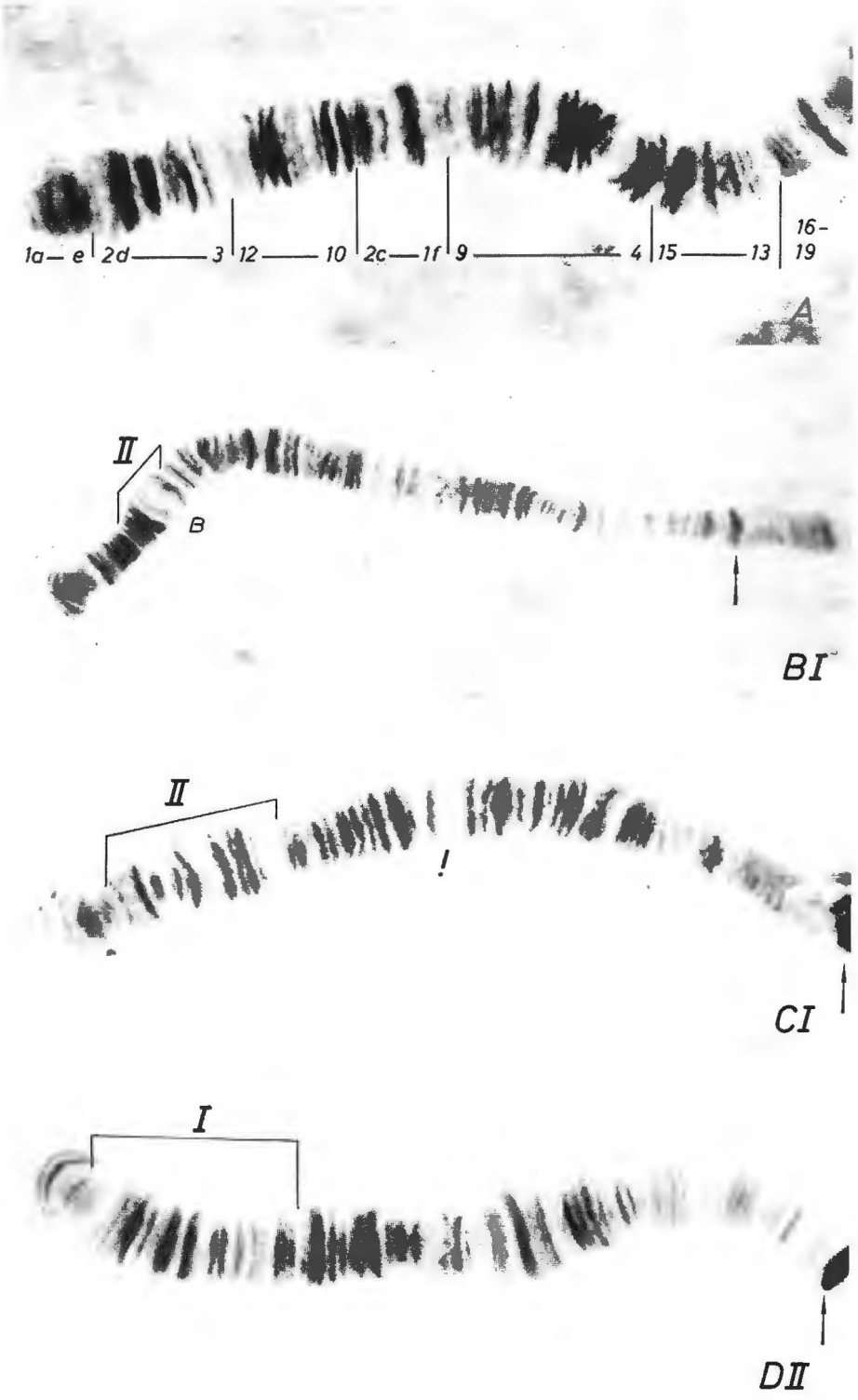
Fig. 2 (See following pages)
Chromosome arms of *Chironomus whitseli* Sublette and Sublette. Arrangement and abbreviations are as in Fig. 1.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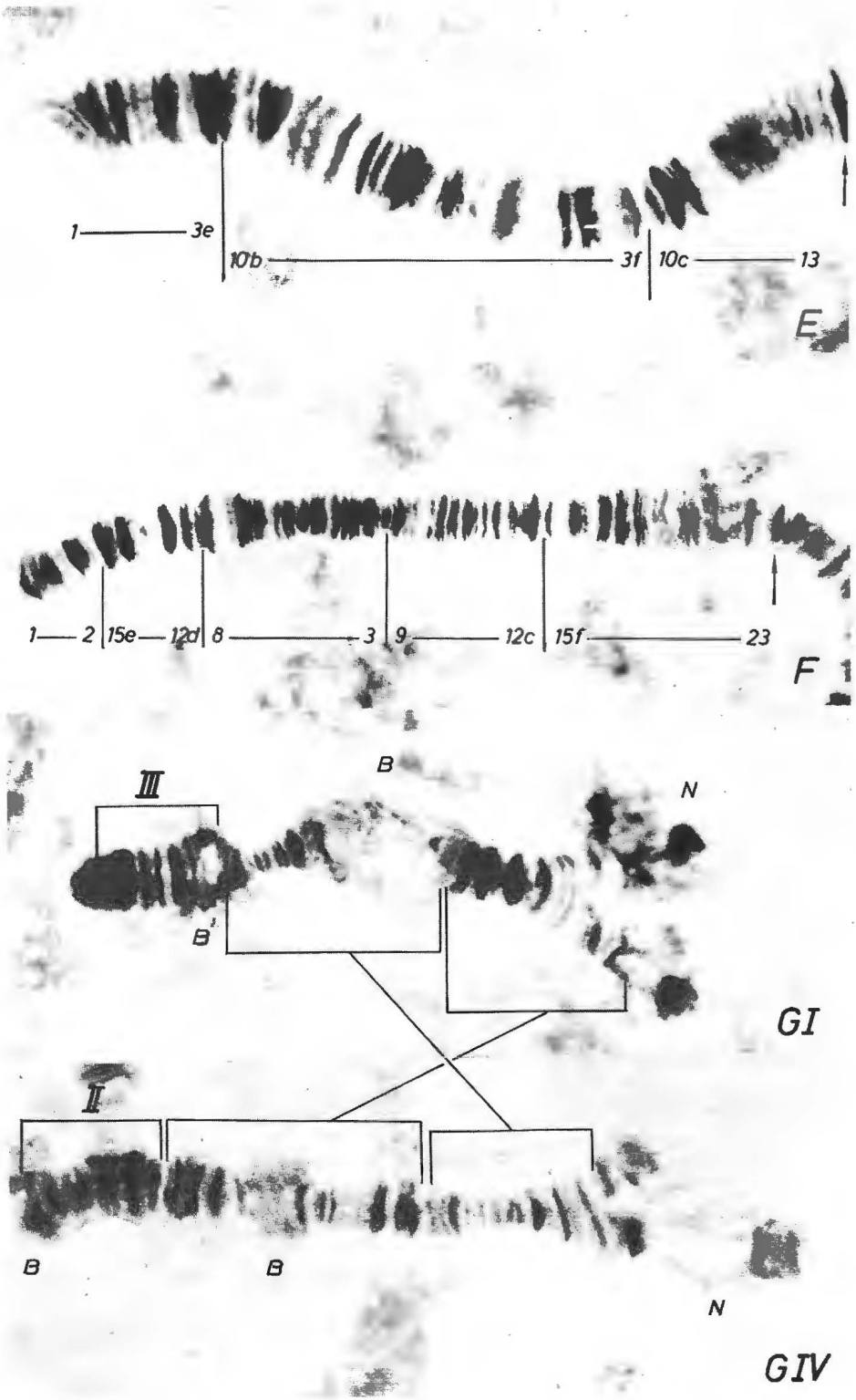
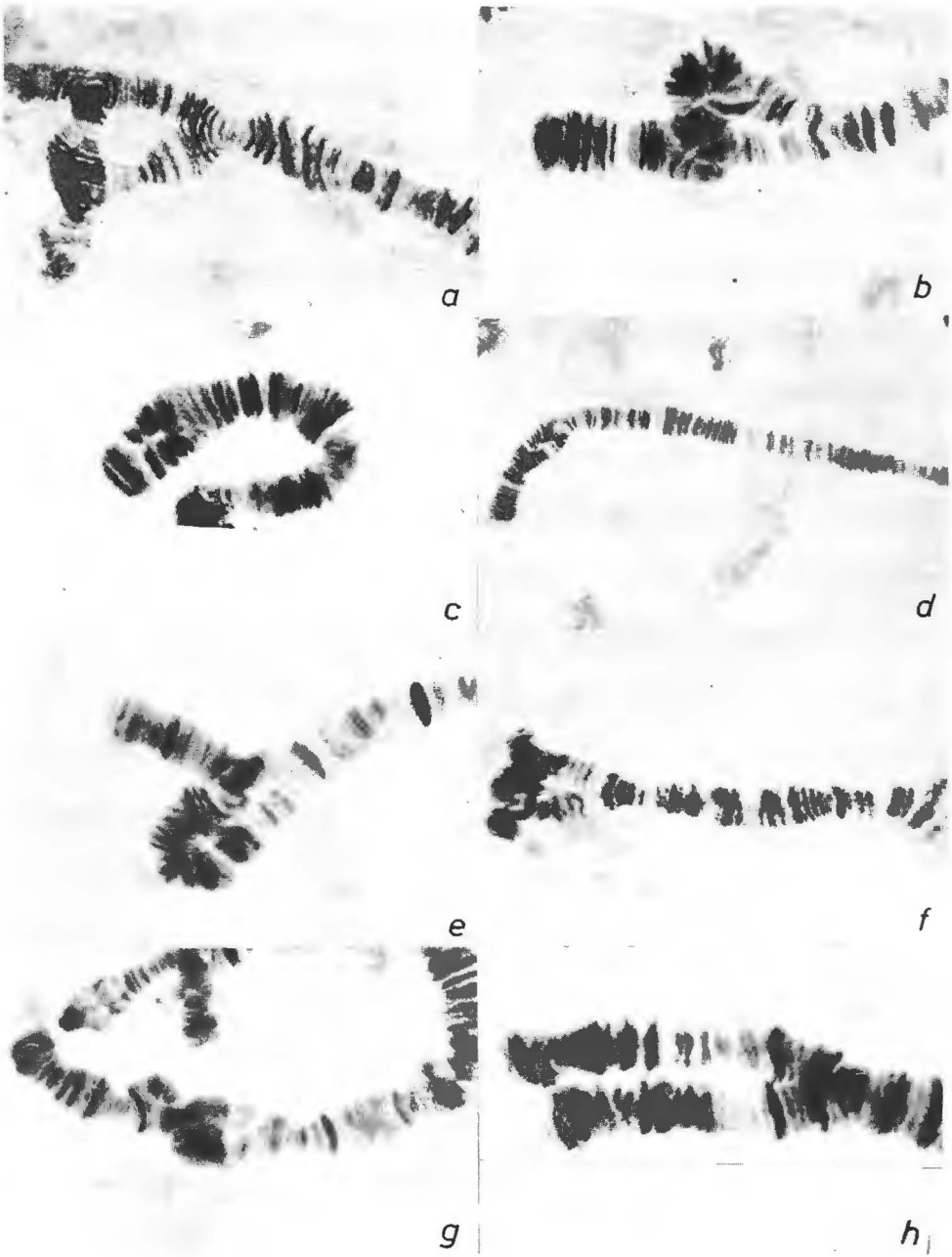

1
3e
10b
3f
10c
13
E
1
2
15e
12d
8
3
9
12c
15f
23
F
III
B
N
B'
GI
II
B
B
N
GIV

Fig. 3 Heterozygous configurations of inversion polymorphisms in *Chironomus maturus* and *Chironomus whitseli*:

a) *Chironomus maturus* AI/AII
b) *Chironomus maturus* AII/AIII
c) *Chironomus maturus* BI/BIII
d) *Chironomus whitseli* BI/BII
e) *Chironomus maturus* CI/CIII
f) *Chironomus maturus* FI/FII
g) *Chironomus maturus* DI/DII
h) *Chironomus whitseli* DI (=*Chironomus maturus*)/DII


a
b
c
d
e
f
g
h