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Ruhr-Universität Bochum, FR Germany.
Computerized EM Chromosome maps.

We have made use of the combination of three techniques which together enable us to plot computerized, semi-quantitative chromosome maps of the EM band-interband pattern from polytene chromosomes in Drosophila. These being: (1) the surface spread polytene (SSP) chromosome preparation technique, (2) a digitization technique for the cytological data of EM photos of SSP chromosomes and (3) a computer program for plotting chromosome maps.

The usability of the SSP chromosome preparation technique has already been proven for LM, TEM and SEM analyses of the band-interband patterns in Drosophila and Chironomus (Kalisch 1982a,b; Kalisch & Hägele 1981,1982; Kalisch & Jacob 1983; Kalisch & Whitmore 1983). We could show that in EM micrographs of selected SSP chromosomes all the polytene structures (i.e., individual bands and their right-handed interbands) can be depicted individually. Recently we have found a way to digitize the cytological data of the EM band-interband pattern of the SSP chromosomes from Drosophila by using a set of 'graphic elements' for the characterization of an individual polytene structure (in preparation; details will be given on request by the authors). These graphic elements are symbolized by variables: A = band diameter; B = band type; C = band thickness; D = interband length; E = outlines of interbands and puffs; R = band radius (for curved bands); W = sectioning of the chromosome map; V$ = reference numbers (for details see Table 1). The values of variables A-V$ come in part from measurements of EM micrographs of SSP chromosomes (variables A, C and D) and from standardized computer-internal and plotter-internal values (variables B, R, W, and V$). A computer had to be used to compile and to store the large amount of data.

In Table 1 we present our standard computer program, especially written for Drosophila chromosomes, by which the digitized data can be recorded, stored and plotted as a chromosome map. The program is written in EXTENDED BASIC for a SHARP PC-1500 pocket computer, which is coupled with a plotter (SHARP CE-150) and extended to ca. 11.5 Kbytes RAM with a memory module (SHARP CE-155). For the serial storage of data we used a taperecorder (SHARP CE-152). The program offers the choice of three options (in the following the first program line number concerned is given in brackets): (1) digitized data of an individual polytene structure can be recorded [50-110], (2) recorded data can be corrected [120], plotted as a chromosome map [140-895] or stored on tape [135-139]. (3) Data stored on tape can be corrected again [2000-2130] and/or plotted as chromosome map [140-895]. Data (A-V$ of each polytene structure are stored together with a code number [50] for recalling an individual data from the tape [3000]. We use the (extended) reference numbers of previously published chromosome maps as code numbers. For the plotting of chromosome maps one can select from a menu of four different possibilities: (1) only the band-interband pattern is plotted [140-895], (2) the pattern is plotted together with a (C/D) scale, which visualizes the individually measured values (in 0.2 mm units) of band thicknesses (variable C) and interband lengths (variable D) [1300-1490], (3) the pattern is plotted together with a sectioning of the chromosome (divisions, subdivisions and/or individual bands) and the reference numbers [1200-1290], (4) the pattern is plotted together with the C/D scale, the sectioning and the reference numbers [140] as to be seen in Fig. 1. For special inputs of the menu see [28-33]. Due to the limitations and specifications of the equipment used the minimum plotting unit is 0.2 mm. By the latter one, the chromosome outlines (band diameters, puff sizes) have to be calculated relative to the maximum value of 160 units.

Fig. 1 shows, as an example, the computerized EM chromosome map of region 83A-85C in D.hydei. The map (shown in its original plotting size; i.e., 3.2 mm of the map equals 1 µm of an X3,200 EM micrograph) is based on data from several EM micrographs of SSP chromosomes of salivary glands from late 3rd instar larvae. Compared with the hand-drawn LM chromosome map so far existing (Berendes 1963), the computerized EM map shows a 44% (50:72) increase in the number of bands. Besides the semi-quanitativeness of the A, C and D values the computer maps have the distinct advantage that the actual pattern can be plotted according to subseequent cytological analyses. A flow diagram of the program listed in Table 1 will be sent on request by the authors.

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References: Berendes,H.D. 1963, Chromosoma(Berl.) 14:195-206; Kalisch,W.-E. 1982a, DIS 58:85-87; ____ 1982b, Genetica 60:21-24; Kalisch,W.-E. & K.Hägele 1981, J.Cell Biol. 31:91-138; ____ 1982, In: Advances in Genetics, Development and Evolution of Drosophila, Plenum Publ. Co., New York, p.1-10; Kalisch,W.-E. & H.J.Jacob 1983, Cytobios 36:39-43; Kalisch,W.-E. & T.Whitmore 1983, Cytobios 37:37-43.

Table 1. Standard computer program for digitized cytological data from EM micrographs of SSP chromosomes in Drosophila by which data can be recorded, stored and plotted as chromosome maps. The term 'field' (line numbers 404, 504 and 604) stands for 'decondensed chromosome band'.

```
 5:REM    PROGRAM FOR SHARP PC-1500
 6:REM    + PLOTTER CE-150
 7:REM
10:REM ***************************
11:REM                           *
12:REM    "ROU/COU/TCP" =        *
13:REM    PROGRAM FOR RECORDING/ *
14:REM    CORRECTING/PLOTTING    *
15:REM    VARIABLES FOR CHROMO-  *
16:REM    SOME BANDS BY TAPE     *
17:REM                           *
18:REM ***************************
19:REM
20:REM ***************************
21:REM                           *
22:REM    INPUTS FOR VARIABLES   *
23:REM                           *
24:REM ***************************
25:REM
27:DIM E(30)
28:WAIT 150:PRINT "ROU=RECORDING OF
   VARIABLES":PRINT "MENUE = ROU+S
   +C/D"
29:PRINT "COU=CORRECTING OF VARIABL
   ES":PRINT "MENUE = COU"
30:PRINT "TCP=TAPE CONTROLLED PLOTT
   .":PRINT "MENUE = TCP+S+C/D"
31:INPUT "INPUT ROU/COU/TCP = ";Q$
32:IF Q$="COU"GOTO 2000
33:IF (Q$="TCP")OR (Q$="TCP+S")OR (
   Q$="TCP+S+C/D")GOTO 3000
37:FOR I=1TO 30
38:E(I)=0
39:NEXT I
41:A=0,B=0,C=0,D=0,R=0
42:U$=" D",M$="D",N$="D"
43:F=0,I=0,J=0,K=0,M=0,S=0
44:T=0,W=0,X=0,Y=0,Z=0,L=0
45:PRINT "NEW BAND"
50:INPUT "PROGRAM LINE, INPUT T = ";
   T
55:INPUT "BAND DIAMETER, INPUT A = "
   ;A
57:IF A>160GOTO 1510
60:INPUT "BAND TYPE, INPUT B = ";B
65:IF B<10GOTO 1520
66:IF B>69GOTO 1530
70:INPUT "BAND THICKNESS, INPUT C =
   ";C
75:INPUT "INTERBAND, INPUT D = ";D
80:IF M$="N"GOTO 90
83:IF B>=20GOTO 85
84:GOTO 100
85:IF B>=50GOTO 90
86:IF B>=40GOTO 100
90:INPUT "RADIUS, INPUT R = ";R
93:IF R=0GOTO 100
95:IF R<=A/2GOTO 1540
100:INPUT "DIVISION BORDER, INPUT W =
   ";W
103:IF M$="N"GOTO 110
104:IF W=9GOTO 113
105:IF W>36GOTO 1550
106:IF W>1GOTO 113
110:INPUT "DIVISION NO., INPUT U$ = "
   ;U$
113:IF D<0GOSUB 1600
115:WAIT 250:PRINT T;A;B;C;D;R;W;U$
116:IF D>=0GOTO 119
117:PRINT T;B;D;E(1);E(-D/2+0.5)
119:M$="Y"
120:INPUT "CORRECT VARIABLES ? Y/N=
   ";M$
121:IF M$="N"WAIT 150:PRINT "CHANGE
   DATA":GOTO 50
122:M$="Y"
123:INPUT "PLOTTING OF BAND? Y/N = "
   ;M$
124:IF M$="N"GOTO 135
125:GOSUB 140
126:N$="Y"
129:INPUT "CORRECT GRAPHING ? Y/N =
   ";N$
130:IF N$="N"PRINT "CHANGE DATA":
   GOTO 50
135:PRINT #T,A,B,C,D,R,W,U$
136:IF D>=0GOTO 139
138:PRINT #T,B,D,E(*)
139:GOTO 32
140:IF (Q$="COU")OR (Q$="ROU+S")OR (
   Q$="ROU+S+C/D")GOSUB 1200
142:IF (Q$="TCP+S")OR (Q$="TCP+S+C/D
   ")GOSUB 1200
144:IF C=0GOTO 700
145:IF B>=60GOTO 600
146:IF B>=50GOTO 500
147:IF B>=40GOTO 400
148:IF B>=30GOTO 300
149:IF B>=20GOTO 200
150:REM
152:REM ***************************
153:REM                           *
154:REM          BAND             *
155:REM                           *
156:REM ***************************
157:REM
160:Z=0
165:GRAPH
170:LINE (110-INT (A/2),-1)-(110+INT
   (A/2),-1),B-10
180:Z=Z+1
185:IF Z<CTHEN 165
190:IF (Q$="TCP+C/D")OR (Q$="TCP+S+C
   /D")GOSUB 1300
191:IF (Q$="ROU+C/D")OR (Q$="ROU+S+C
   /D")OR (Q$="COU")GOSUB 1300
195:GOTO 700
200:REM
202:REM ***************************
203:REM                           *
204:REM        CONVEX BAND        *
205:REM                           *
206:REM ***************************
207:REM
210:S=B-20,J=10,Z=0,I=0
213:IF S>0GOTO 220
215:I=2
220:K=INT (SQR (R*R-A/2*A/2))
230:GRAPH
235:GLCURSOR (110,-K):SQRGN
240:LINE -(INT (-A/2),K),9
245:FOR I=R-INT (A/2)TO R+INT (A/2)
   STEP J
250:X=INT (R-I)
255:Y=INT (SQR (R*R-X*X))
260:LINE -(-X,Y-Z),B-20
265:NEXT I
270:Z=Z+1
275:IF Z<CGOTO 240
280:GLCURSOR (0,K-C):SQRGN
290:IF (Q$="TCP+C/D")OR (Q$="TCP+S+C
   /D")GOSUB 1300
291:IF (Q$="ROU+C/D")OR (Q$="ROU+S+C
   /D")OR (Q$="COU")GOSUB 1300
295:GOTO 700
300:REM
302:REM ***************************
303:REM                           *
304:REM        CONCAVE BAND       *
305:REM                           *
306:REM ***************************
307:REM
310:S=B-30,J=10,Z=0
313:IF S>0GOTO 320
315:I=2
320:K=INT (SQR (R*R-A/2*A/2))
330:GRAPH
335:GLCURSOR (110,K):SQRGN
340:LINE -(INT (-A/2),-K),9
345:FOR I=R-INT (A/2)TO R+INT (A/2)
   STEP J
350:X=INT (R-I)
355:Y=INT (SQR (R*R-X*X))
360:LINE -(-X,-(Y+Z)),B-30
365:NEXT I
370:Z=Z+1
375:IF Z<CGOTO 340
380:GLCURSOR (0,-(K+C)):SQRGN
390:IF (Q$="TCP+C/D")OR (Q$="TCP+S+C
   /D")GOSUB 1300
391:IF (Q$="ROU+C/D")OR (Q$="ROU+S+C
   /D")OR (Q$="COU")GOSUB 1300
395:GOTO 700
400:REM
402:REM ***************************
403:REM                           *
404:REM          FIELD            *
405:REM                           *
406:REM ***************************
407:REM
410:Z=0
415:GRAPH
420:LINE (111-INT (A/2),-1)-(111+INT
   (A/2),-1),B-40
425:Z=Z+1
430:IF Z>=CTHEN 485
435:LINE (0,-2)-(0,-2),9
440:Z=Z+1
445:IF Z>=CGOTO 485
450:GRAPH
455:LINE (109-INT (A/2),-1)-(109+INT
   (A/2),-1),B-40
460:Z=Z+1
465:IF Z>=CGOTO 485
470:LINE (0,-2)-(0,-2),9
475:Z=Z+1
480:IF Z<CGOTO 415
485:IF (Q$="TCP+C/D")OR (Q$="TCP+S+C
   /D")GOSUB 1300
486:IF (Q$="ROU+C/D")OR (Q$="ROU+S+C
   /D")OR (Q$="COU")GOSUB 1300
490:GOTO 700
500:REM
502:REM ***************************
503:REM                           *
504:REM        CONVEX FIELD       *
505:REM                           *
506:REM ***************************
507:REM
510:I=10,Z=0,S=B-50
513:IF S>0GOTO 515
514:I=2
515:K=INT (SQR (R*R-A/2*A/2))
520:GRAPH
522:GLCURSOR (110,-K):SQRGN
524:LINE -(-INT (A/2)-1,K),9
526:FOR I=R-INT (A/2)-1TO R+INT (A/2
   )-1STEP J
528:X=INT (R-I)
530:Y=INT (SQR (R*R-X*X))
532:LINE -(-X,Y-Z),B-50
534:NEXT I
537:Z=Z+1
540:IF Z>=CGOTO 585
543:LINE -(0,K-2-Z),9
545:Z=Z+1
548:IF Z>=CGOTO 585
550:LINE -(-INT (A/2)+1,K),9
553:FOR I=R-INT (A/2)+1TO R+INT (A/2
   )+1STEP J
555:X=INT (R-I)
558:Y=INT (SQR (R*R-X*X))
560:LINE -(-X,Y-Z),B-50
565:NEXT I
568:Z=Z+1
570:IF Z>=CGOTO 585
575:LINE -(0,K-2-Z),9
578:Z=Z+1
580:IF Z<CGOTO 524
585:GLCURSOR (0,K-C):SQRGN
590:IF (Q$="TCP+C/D")OR (Q$="TCP+S+C
   /D")GOSUB 1300
591:IF (Q$="ROU+C/D")OR (Q$="ROU+S+C
   /D")OR (Q$="COU")GOSUB 1300
595:GOTO 700
600:REM
602:REM ***************************
603:REM                           *
604:REM        CONCAVE FIELD      *
605:REM                           *
606:REM ***************************
607:REM
610:I=10,Z=0,S=B-60
613:IF S>0GOTO 615
614:I=2
615:K=INT (SQR (R*R-A/2*A/2))
620:GRAPH
622:GLCURSOR (110,K):SQRGN
624:LINE -(-INT (A/2)-1,-K),9
626:FOR I=R-INT (A/2)-1TO R+INT (A/2
   )-1STEP J
628:X=INT (R-I)
630:Y=INT (SQR (R*R-X*X))
632:LINE -(-X,-(Y+Z)),B-60
634:NEXT I
637:Z=Z+1
640:IF Z>=CGOTO 685
643:LINE -(0,-(K+2+Z)),9
645:Z=Z+1
648:IF Z>=CGOTO 685
650:LINE -(-INT (A/2)+1,-K),9
653:FOR I=R-INT (A/2)+1TO R+INT (A/2
   )+1STEP J
655:X=INT (R-I)
658:Y=INT (SQR (R*R-X*X))
660:LINE -(-X,-(Y+Z)),B-60
665:NEXT I
668:Z=Z+1
670:IF Z>=CGOTO 685
675:LINE -(0,-(K+2+Z)),9
678:Z=Z+1
680:IF Z<CGOTO 624
685:GLCURSOR (0,-(K+C)):SQRGN
690:IF (Q$="TCP+C/D")OR (Q$="TCP+S+C
   /D")GOSUB 1300
691:IF (Q$="ROU+C/D")OR (Q$="ROU+S+C
   /D")OR (Q$="COU")GOSUB 1300
695:GOTO 700
700:REM
702:REM ***************************
703:REM                           *
704:REM        INTERBAND          *
705:REM                           *
706:REM ***************************
707:REM
710:IF D=0RETURN
720:IF D<0GOTO 800
730:GRAPH
740:LINE (0,0)-(0,-D),9
750:TEXT
760:IF (Q$="TCP+C/D")OR (Q$="TCP+S+C
   /D")GOSUB 1400
761:IF (Q$="ROU+C/D")OR (Q$="ROU+S+C
   /D")OR (Q$="COU")GOSUB 1400
770:RETURN
800:REM
802:REM ***************************
803:REM                           *
804:REM          PUFF             *
805:REM                           *
806:REM ***************************
807:REM
810:Z=0,I=0
820:FOR I=1TO (-D/2)+0.5
825:GRAPH
830:LINE (0,-2)-(0,-2),9
835:LINE (109-INT (E(I)/2),-1)-(111-
   INT (E(I)/2),-1),B
840:LINE (109+INT (E(I)/2),-1)-(111+
   INT (E(I)/2),-1),B
845:LINE (0,-1)-(0,-1),9
850:Z=Z+1
855:IF Z>=ABS (D)GOTO 880
860:LINE (0,-2)-(0,-2),9
865:Z=Z+1
870:IF Z>=ABS (D)GOTO 880
875:NEXT I
880:TEXT
895:GOTO 750
1200:REM
1202:REM ***************************
1203:REM                          *
1204:REM          LABELING        *
1205:REM                          *
1206:REM ***************************
1207:REM
1210:IF W=0RETURN
1211:GRAPH
1215:IF W=0GOTO 1225
1216:IF W=1GOTO 1235
1217:IF W>1GOTO 1245
1225:LINE (0,1)-(100-INT (A/2),1),0
1230:IF W<36GOTO 1270
1235:LINE (0,1)-(100-INT (A/2),1),1
1240:IF W<36GOTO 1270
1245:LINE (12,1)-(100-INT (A/2),1),
   3
1250:TEXT
1260:RETURN
1270:IF U$="NO"GOTO 1290
1273:CSIZE 1
1275:LINE (0,0)-(0,-3),9
1277:ROTATE 1
1280:LPRINT U$
```

Table 1 (contin.)

```
1283:ROTATE 0
1285:LINE (0,0)-(0,0),9
1287:'EXT
1290:RETURN
1300:REM
1302:REM ************************
1303:REM                        *
1304:REM     C/D SCALE          *
1305:REM                        *
1306:REM ************************
1307:REM
1310:Z=0
1315:GRAPH
1320:LINE (0,0)-(0,C),9
1325:GRAPH
1330:GLCURSOR (200,-1):SORGN
1335:LINE -(20,0),0
1340:Z=Z+1
1345:IF Z>=CRETURN
1350:GLCURSOR (0,-1):SORGN
1355:LINE -(0,0),9
1360:Z=Z+1
1365:IF Z>=CRETURN
1370:GLCURSOR (0,-1):SORGN
1375:LINE -(10,0),0
1380:Z=Z+1
1385:IF Z>=CRETURN
1390:GOTO 1350
1400:Z=0
1410:GRAPH
1420:LINE (0,0)-(0,ABS (D)),9
1425:GRAPH
1430:GLCURSOR (135,-1):SORGN
1435:LINE -(15,0),0
1440:Z=Z+1
1445:IF Z>=ABS (D)RETURN
1447:GLCURSOR (5,0):SORGN
1450:GLCURSOR (0,-1):SORGN
1455:LINE -(0,0),9
1460:Z=Z+1
1465:IF Z>=ABS (D)RETURN
1470:GLCURSOR (0,-1):SORGN
1475:LINE -(10,0),0
1480:Z=Z+1
1485:IF Z>=ABS (D)RETURN
1490:GOTO 1450
1500:REM
1502:REM ************************
1503:REM                        *
1504:REM   ORDERS FOR CORRECTING *
1505:REM   MISTAKES IN INPUTS    *
1506:REM                        *
1507:REM ************************
1508:REM
1510:BEEP ON
1511:BEEP 10,20,1000
1515:PRINT "CHANGE A . SMALLER A"
1516:GOTO 55
```

```
1520:BEEP ON
1521:BEEP 10,20,1000
1525:PRINT "CHANGE B : LARGER B"
1526:GOTO 60
1530:BEEP ON
1531:BEEP 10,20,1002
1535:PRINT "CHANGE B : SMALLER B"
1536:GOTO 60
1540:BEEP ON
1541:BEEP 10,20,1000
1545:PRINT "CHANGE R : LARGER R
1546:GOTO 30
1550:BEEP ON
1551:BEEP 10,20,1000
1555:PRINT "CHANGE W : SMALLER W"
1556:GOTO 100
1560:BEEP ON
1561:BEEP 10,20,1000
1565:PRINT "CHANGE E(*):SMALLER E(*
)
1566:GOTO 1610
1570:BEEP ON
1571:BEEP 10,20,1000
1575:PRINT "END OF PROGRAM"
1576:END
1580:BEEP ON
1581:BEEP 10,20,1000
1585:PRINT "CHANGE INF. PREVIOUS BA
ND"
1586:GOTO 2015
1600:REM
1602:REM ************************
1603:REM                        *
1604:REM      INPUTS FOR PUFFS  *
1605:REM                        *
1606:REM ************************
1607:REM
1608:REM
1610:WAIT 80:Z=0,I=0
1615:IF -D/2>INT (-D/2)GOTO 1690
1620:FOR I=1TO -D/2
1630:INPUT "A FOR PUFF-OUTLINES = "
:E(I)
1635:IF E(I)>160GOTO 1560
1640:Z=Z+2
1650:PRINT "Z = ";Z/2;"REST = ";-D/
2-Z/2
1660:NEXT I
1670:RETURN
1690:FOR I=1TO -D/2+0.5
1700:INPUT "A FOR PUFF-OUTLINES = "
:E(I)
1705:IF E(I)>150GOTO 1560
1710:Z=Z+2
1720:PRINT "Z = ";Z/2;"REST = ";-D/
2+0.5-Z/2
1730:NEXT I
1740:RETURN
```

```
2000:REM
2002:REM ************************
2003:REM                        *
2004:REM    INPUTS FOR FINDING  *
2005:REM    THE RELEVANT BAND   *
2006:REM                        *
2007:REM ************************
2008:REM
2010:PRINT "SEARCHING PREVIOUS BAND
"
2015:INPUT "PROGRAM LINE,INPUT T =
";F
2020:INPUT #T,A,B,C,D,R,W,U$
2025:IF D>=0GOTO 2040
2030:INPUT #T,B,D,E(*)
2040:IF F>1GOTO 2020
2050:WAIT 250:PRINT T;A;B;C;D;R;W;U
$
2060:IF D>=0GOTO 2070
2065:PRINT T;B;D;E(I);E(-D/2+0.5)
2070:INPUT "CORRECT VARIABLES ? Y/N
= ";M$
2075:IF M$="N"GOTO 1580
2080:M$="0"
2100:REM
2102:REM ************************
2103:REM                        *
2104:REM    INPUTS FOR          *
2105:REM    CORRECTING VARIABLES *
2106:REM                        *
2107:REM ************************
2108:REM
2110:A=0,B=0,C=0,D=0,R=0,W=0
2111:F=0,U$=" D ",Q$="O",T=0
2112:FOR I=1TO 30
2113:E(I)=0
2114:NEXT I
2120:PRINT "INPUTS FOR FOLLOWING BA
ND"
2130:GOTO 50
3000:REM
3002:REM ************************
3003:REM                        *
3004:REM    INPUTS FOR TAPE     *
3005:REM    CONTROLLED PLOTTING *
3006:REM                        *
3007:REM ************************
3008:REM
3010:T=0
3030:INPUT "FIRST BAND = ";F
3040:INPUT "LAST BAND = ";L
3050:IF F>1GOTO 3070
3060:T=T+1
3070:IF T>=L+1GOTO 1570
3080:INPUT #T,A,B,C,D,R,W,U$
3085:IF D>=0GOTO 3100
3090:INPUT #T,B,D,E(*)
3100:IF F>1GOTO 3060
3110:GOSUB 140
3120:GOTO 3060
```

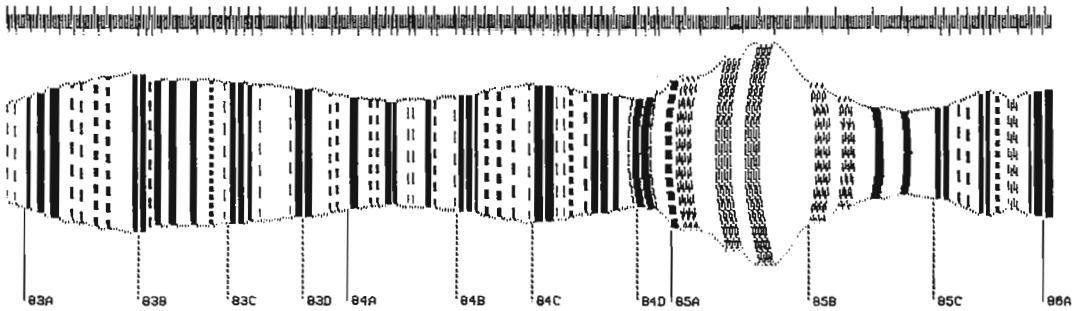

Figure 1. Computerized EM chromosome map of region 83A-85C in D.hydei. The map is based on measurements of cytological data (band diameters, band thicknesses and interband lengths) from several EM micrographs of SSP chromosomes.