16 Taylor, O. R., Bee World *58* (1977) 19.
17 Taylor, O. R., Bull. ent. Soc. Am. *31* (1985) 15.
18 Taylor, O. R., and Spivak, M., Bee World *65* (1984) 38.
19 Rinderer, T. E., Am. Bee. J. *126* (1986) 98, 128.
20 Brueckner, D., Experientia *30* (1974) 618.
21 Contel, E. P. B., Mestriner, M. A., and Martins, E., Biochem. Genet. *15* (1977) 859.
22 Del Lama, M. A., Mestriner, M. A., and Paiva, J. C. A., Rev. Brasil. Genet. *8* (1985) 17.
23 Hung, A. C. F., and Vinson, S. B., Heredity *38* (1977) 409.
24 Martins, E., Mestriner, M. A., and Contel, E. P. B., Biochem. Genet. *15* (1977) 357.
25 Mestriner, M. A., Nature *223* (1969) 188.
26 Mestriner, M. A., and Contel, E. P. B., Genetics *72* (1972) 733.
27 Sheppard, W. S., and Berlocher, S. H., J. Apic. Res. *23* (1984) 64.
28 Sheppard, W. S., and Berlocher, S. H., J. Hered. *76* (1985) 45.
29 Sheppard, W. S., and McPheron, B. A., Apidologie *17* (1986) 21.
30 Nunamaker, R. A., and Wilson, W. T., J. Kansas ent. Soc. *54* (1981) 704.
31 Nunamaker, R. A., Wilson, W. T., and Haley, B. E., J. Kansas ent. Soc. *57* (1984) 622.
32 Carlson, D. A., and Bolton, A. B., Bull. ent. Soc. Am. *30* (1984) 32.
33 Francis, B. R., Blanton, W. E., and Nunamaker, R. A., J. apicult. Res. *24* (1985) 13.
34 McDaniel, C. A., Howard, R. W., Bloomquist, C. J., and Collins, A. M., Sociobiology *8* (1983) 287.
35 Hall, H. G., Proc. natl Acad. Sci. USA *83* (1986) 4874.
36 Daly, H. V., Proc. 25th Int. Apic. Congr., Grenoble, France, p. 356. Apimondia Publishing House, Bucharest 1975.
37 Daly, H. V., and Balling, S. S., J. Kansas ent. Soc. *51* (1978) 857.
38 Daly, H. V., Hoelmer, K., Norman, P., and Allen, T., Ann. ent. Soc. Am. *75* (1928) 591.
39 Dawid, I. B., and Blackler, A. B., Devl Biol. *29* (1972) 152.
40 Giles, R. E., Blanc, H., Cann, H. M., and Wallace, D. C., Proc. natl Acad. Sci. USA *77* (1980) 6715.
41 Harrison, R. G., Rand, D. M., and Wheeler, W. C., Science *228* (1985) 1446.
42 Hutchinson, C. A. III, Newbold, J. E., Potter, S. S., and Edgell, M. H., Nature *251* (1974) 536.
43 Lansman, R. A., Avise, J. C., and Heuttel, M., Proc. natl Acad. Sci. USA *80* (1983) 1969.
44 Reilly, J. G., and Thomas, C. A. Jr, Plasmid *3* (1980) 109.
45 Avise, J. C., and Lansman, R. A., in: Evolution of Genes and Proteins, p. 147. Eds M. Nei and R. K. Koehn. Sinauer Assoc., Inc., Sunderland, Mass. 1983.
46 Brown, W. M., in: Evolution of Genes and Proteins, p. 62. Eds M. Nei and R. K. Koehn. Sinauer Assoc., Inc., Sunderland, Mass. 1983.
47 Brown, W. M., in: Monographs in Evolutionary Biology – Molecular Evolutionary Genetics p. 95 Ed. R. J. MacIntyre. Plenum Press, New York 1985.
48 Moritz, R., Hawkins, C. F., Crozier, R. H., and Mackinley, A. G., Experientia *42* (1986) 322.
49 Brown, W. M., Ann. N.Y. Acad. Sci. *361* (1981) 119.
50 Brown, W. M., Proc. natl Acad. Sci. USA *77* (1980) 3605.
51 Ferris, S. D., Wilson, A. C., and Brown, W. M., Proc. natl Acad. Sci. USA *78* (1981) 2432.
52 Nei, M., and Tajima, F., Genetics *97* (1983) 145.

0014-4754/88/030257-04$1.50 + 0.20/0

C-band differentiation between the chromosomes of two subspecies of the chironomid midge *Chironomus thummi*

K. Hägele and H. Speier

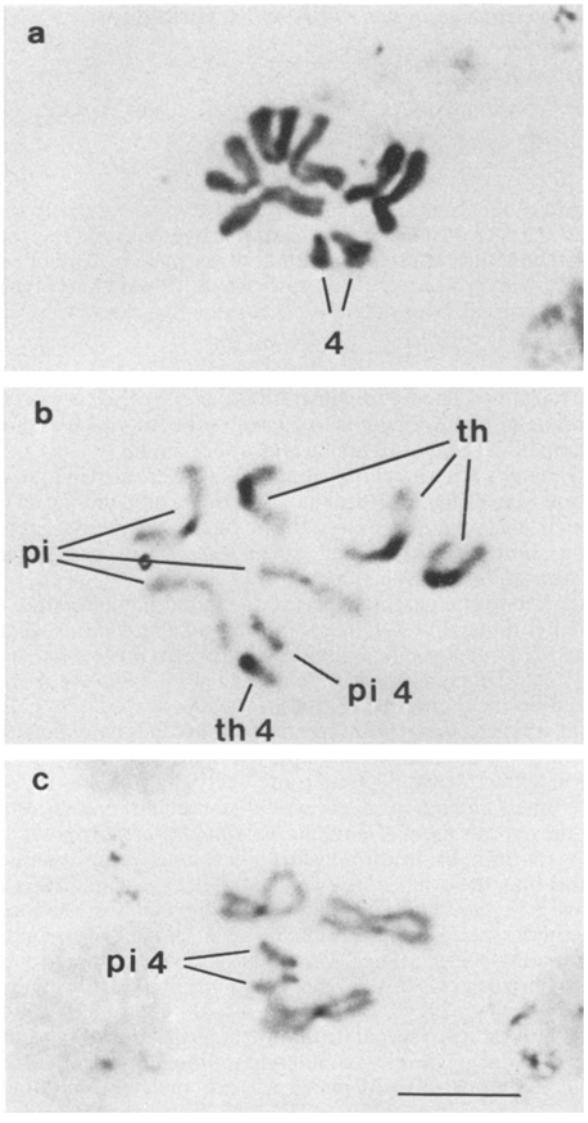
Institut für Genetik, Ruhr-Universität Bochum, P.O. Box 10 21 48, D-4630 Bochum 1 (Federal Republic of Germany)
Received 13 July 1987; accepted 10 November 1987

Summary. The metaphase chromosomes of *Chironomus th. thummi* contain approximately 17% more pericentric C-band heterochromatin than the chromosomes of *Chironomus th. piger* with 11% heterochromatin. In *Ch. th. thummi*, the proportion of heterochromatin appeared to be much larger in metaphase chromosomes than in polytene chromosomes. This discrepancy is interpreted as being due to the specific chromosome organization and not as the result of an underreplication of heterochromatin during polytenization.
Key words. Chironomus th. thummi; Ch. th. piger; metaphase chromosomes; heterochromatin.

The two subspecies of *Chironomus thummi, Ch. th. thummi* and *Ch. th. piger,* are very closely related and morphologically nearly indistinguishable [1]. Only regional differences in the banding pattern of their polytene chromosomes allow a reliable taxonomic identification [2]. There is, however, no discrimination possible between the metaphase chromosome complements of the two subspecies. This hampers the cytological analysis of the chromosome segregation in the germ line of the hybrids and their progeny during the study of the phenomenon of non-reciprocal gonadal dysgenesis [3, 4]. Here we describe differences between metaphase chromosomes of the *Ch. th. thummi* and the *Ch. th. piger* complement observed after a strong modification of the routine C-banding method.

Material and methods. Neural ganglia and testes of *Chironomus thummi piger* and *Ch. thummi piger* × *Ch. thummi thummi* hybrid larvae were treated in a 0.1% sodium citrate solution for 1 min. For C-banding, the routine fixation procedures and treatment times were modified. The tissues were fixed in 50% acetic acid, squashed and postfixed in cold methanol: glacial acetic acid (3 : 1) for 24 h. Air-dried slides were treated with 0.2 N HCl for 1 h, rinsed in distilled water and incubated in 5% $Ba(OH)_2$ for 7 min. at 50 °C. After rinsing, the slides were incubated in 2 × SSC for 1 h at 60 °C, rinsed, air-dried and stained in a 10% Giemsa phosphate buffer solution (pH 6.8) for 15 min, rinsed and air-dried. The banded chromosomes were photographed and enlarged with a projector. The banded and the total chromosome areas of the chromosomes were then measured using a Videoplan system analyzer.

Results and discussion. The karyotype of both chironomids consists of three pairs of long metacentric and one pair of short acrocentric chromosomes [2]. As shown in the figure (a), in the hybrids homologous *thummi* and *piger* metaphase chromosomes cannot be distinguished from one another after conventional orcein staining. After C-banding, however, 4 out of the 8 chromosomes of the complement show large and darkly staining pericentric regions (fig., b). The other 4 chromosomes reveal only small dark spots at the centromeric position. From C-banding results of the pure *piger* metaphase plates (fig., c) it becomes obvious that in hybrids (fig., b) those chromosomes with the small dark regions (C-



Spermatogonial metaphase of the *Ch. th. thummi* × *Ch. th. piger* hybrid *a* after orcein staining and *b* after C-banding. *c* C-banded metaphase of *Ch. th. piger*. th = *thummi*, pi = *piger*, 4 = the small acrocentric chromosome 4.

bands) must represent the chromosome complement of *piger*, whereas the other chromosomes, with the large C-banded area, are of *thummi* origin.

Measurements of the C-banded area of the metaphase chromosomes of 20 nuclei of the hybrid show that the 4 chromosomes of the *thummi* genome do not differ significantly from one another in the percentages of their C-band area ($p = 0.28$). The same is also true for the *piger* chromosomes ($p = 0.67$). The evaluation of the data also demonstrates that in the *thummi* chromosomes the pericentric C-band regions comprise 27.8% ± 3.2 of the total chromosome area and in *piger* 10.9% ± 0.8.

Since it is now widely accepted that the C-band method characterizes constitutive heterochromatin [5], the C-bands obtained in both chironomids must represent pericentric heterochromatin. Surprisingly, in metaphase chromosomes of *thummi* the proportion of pericentric heterochromatin is much larger than in polytene chromosomes. In the latter, up to 20 individual pericentric heterochromatin bands are interspersed among non-heterochromatic bands [6]. This observation suggests that the considerable differences in heterochromatin between metaphase and polytene chromosomes of *thummi* are not due to an underreplication of pericentric heterochromatin during polytenization, but are rather simulated by the different chromosome organization; those numerous interspersed heterochromatic chromosome regions which become visible as individual heterochromatic bands in polytene chromosomes, are crowded in metaphase chromosomes by the condensation process, thus simulating the large and uniform pericentric heterochromatin blocks. Another explanation for the differences in heterochromatin could be that the heterochromatin blocks in metaphase chromosomes consist of an alternating sequence of polytenizing and non-polytenizing heterochromatin types. In polytene chromosomes the polytenizing heterochromatin should then be represented by the interspersed heterochromatic bands mentioned above, whereas the not-polytenized heterochromatic regions should appear as C-banded intercalary constrictions. Since, however, such constrictions are never observed in the pericentric regions of the polytene chromosomes of *thummi*, the second explanation is unlikely.

The assumption of the non-existence of an underreplication of heterochromatin in the chironomids under study is additionally supported by the observation that in *piger*, in which only a single small heterochromatic centromere C-band is present in each polytene chromosome [6], only a small C-band occurs in the metaphase chromosomes.

1 Keyl, H.-G., and Strenzke, K., Z. Naturf. *116* (1956) 727.
2 Keyl, H.-G., Chromosoma *8* (1957) 739.
3 Hägele, K., Dev. Genet. *8* (1987) 17.
4 Hägele, K., and Speier, H., Heredity *54* (1985) 159.
5 Gatti, M., Pimpinelli, S., and Santini, G., Chromosoma *57* (1976) 377.
6 Hägele, K., Chromosoma *59* (1977) 207.

0014-4754/88/030260-02$1.50 + 0.20/0