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Cytogenetic analysis of a hybrid, *Glyptotendipes pallens* Mg. × *Glyptotendipes glaucus* Mg. (Diptera, Chironomidae): evolutionary considerations

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Abstract

The karyotype of experimentally obtained hybrids between the two closely related species *Glyptotendipes pallens* and *Glyptotendipes glaucus* is described. Hybridization was successful in one direction only (*G. pallens* ♀ × *G. glaucus* ♂). The polytene chromosomes AB and EF of the hybrid show a more or less intimate pairing throughout their length. In the chromosomes CD in which an inversion occurs the characteristic loop is formed. The homologues of chromosome G are almost completely asynaptic. The localization of centromere heterochromatin was also studied. Centromere heterochromatin as well as intercalary heterochromatin could be observed in all chromosomes. By C banding analyses it could be shown that *G. pallens* has a telomeric chromosome G while in *G. glaucus* it is acrocentric. According to karyotype similarity it can be assumed that these two species have quite recently derived from a common ancestor since they still share much of their genomic organization. On the Black Sea coast (southeast part of Bulgaria) a natural hybridization zone between the sympatric species *G. pallens* and *G. glaucus* has been detected. The idea that hybridization between the two species might finally proceed to the formation of a new species by hybrid origin and introgression is discussed.

Key words: Chironomidae – *Glyptotendipes* — *Glyptotendipes pallens* × *Glyptotendipes glaucus* hybrid — polytene chromosomes – introgression

Introduction

For a long period of time both of the species names *Glyptotendipes glaucus* and *Glyptotendipes pallens* have been considered as synonyms (Goetghebuer and Lenz 1937; Kalugina 1963). Later, a detailed analysis of all stages of metamorphosis as well as a comparison of chromosomes clearly indicated that they are two different sibling species (Michailova and Contreras-Lichtenberg 1995; Contreras-Lichtenberg 1996). The aim of this research was to trace back the path of speciation of these two sibling species on the basis of the cytogenetic characteristics of the species and their experimental hybrid. In the present paper a complex methodological approach is applied to define the reproductive relation between these two species and compare them with two other sibling species of the genus *Glyptotendipes*: *Glyptotendipes salinus* and *Glyptotendipes barbipes*. Also, the role of heterochromatin in the divergence of these two species will be demonstrated and discussed.

Materials and methods

Laboratory reared strains of the species *G. glaucus* and *G. pallens* were used and interspecies hybridization was done using a modified version of the method of Fischer (1969). The hatching hybrid larvae were counted per egg mass. In total 20 individuals (10 ♀♀ + 10 ♂♂) of the hybrid *G. pallens* ♀ × *G. glaucus* ♂ were analysed. Also, back crossing was performed (*F*₁ ♀ × *G. glaucus* ♂). The classical acet-orcein method was applied to stain salivary gland chromosomes. A differential C-band staining method was used in addition (Michailova 1987). Analysis was carried out in general on 10 hybrid individuals (5 ♀♀ + 5 ♂♂) as well as on 10 individuals (5 ♀♀ + 5 ♂♂) of *G. pallens* and on 7 individuals (3 ♀♀ + 4 ♂♂) of *G. glaucus*. Larvae, pupae and adults from egg masses collected at the Black Sea coast (Burgass region) and reared in the laboratory were also analysed cytotaxonomically. From 20 larvae (fourth instar) of this material acet-orcein salivary chromosome preparations were made. Salivary gland chromosomes of five individuals were stained by the C banding method. A detailed analysis of all stages of metamorphosis of this material was performed. Homologues between the chromosomes of *G. pallens* and *G. glaucus* were established by comparing the banding pattern of the hybrid with the standard chromosome maps of the two species (Michailova and Contreras-Lichtenberg 1995).

Results and discussion

An experimental hybrid

Under laboratory conditions only the hybrid (*G. pallens* ♀ × *G. glaucus* ♂) could be obtained. The reciprocal crossing (*G. glaucus* ♀ × *G. pallens* ♂) did not give any progeny. Egg hatchability in the offspring of the crossing *G. pallens* ♀ × *G. glaucus* ♂ was about 50%. Larval viability was also reduced so that only about 30% of eggs developed into adults. Egg hatchability of the back cross (*F*₁ ♀ × *G. glaucus* ♂) was about 20%. However, only one larva reached the fourth instar larval stage and died before forming a pupa. So, no single egg from the back cross developed to adult. This clearly demonstrates that the two species are reproductively isolated and gene flow must be assumed to be either reduced or completely absent. In the hybrid larvae some malformations were observed. The teeth of mandibles and submentum were not normally developed. Pupal female genitalia and male hypopygium resembled that of *G. pallens*.

Banding pattern of hybrid polytene chromosomes

The very close relationship in the banding pattern of the two species could be directly recognized by the tightness of the homologous hybrid polytene chromosomes throughout most of their length (Fig. 1a,b,c,d). Small areas of asynapsis could be seen very often at the telomeres of arms B, D and F. The chromosomes of the two species are distinguished in addition by a homozygous inversion in chromosome CD (Michailova and Contreras-Lichtenberg 1995), and, in arm D, a characteristic inversion loop can be observed (Fig. 1b). The puffs (Section 3ab of the standard chromosome map) in the chromosome EF appear only slightly active in the hybrid in comparison with those in the parental chromosomes. The chromosome G is almost completely asynaptic (Fig. 1d) indicating that the rearrangements which have taken place during speciation in this chromosome are considerably more substantial than those in the other chromosomes, or that so many mutations have arisen in the genes of chromosome G that synapsis no longer occurs. In conclusion, the polytene chromosomes of the hybrid between *G. glaucus* and *G. pallens* show that the chromosome

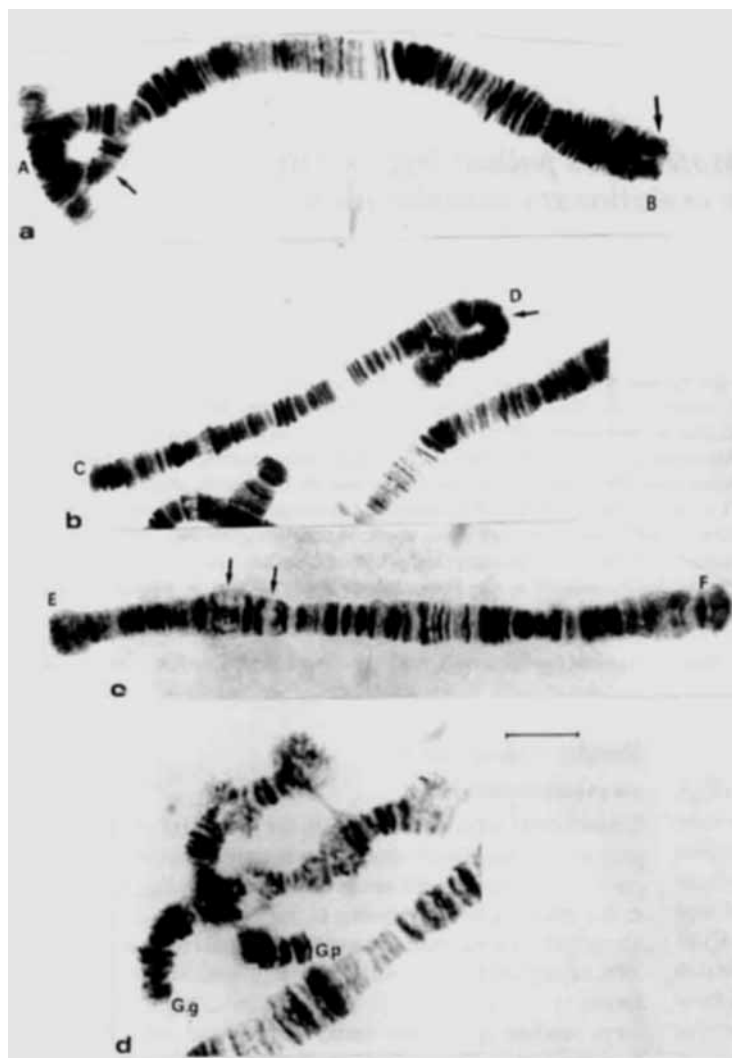


Fig. 1. Polytene chromosomes of hybrid *G. pallens* ♀ × *G. glaucus* ♂. (a) chromosome AB, with a heterozygous inversion in arm A and unpaired telomere of arm B; (b) chromosome CD, with a heterozygous inversion in arm D; (c) chromosome EF; (d) chromosome G, almost completely asynaptic

pattern is rather conserved in both species with relatively few changes.

In one set of hybrid chromosomes chromosome aberrations were observed – heterozygous inversion in arm A (Fig. 1a) and heterozygous deficiency in arm B (Fig. 2) — of probably somatic origin. Also, very often the homologues of arm B are asynaptic at the telomere (Fig. 1a).

In contrast to this species pair, the two other sibling species (*G. barbipes* and *G. salinus*) of the genus exhibit greater morphological divergence and distinct chromosomal and genetic differences. Many inversions and duplications in the centromere regions differentiate the chromosomes of these two species and an almost complete lack of synapsis occurs throughout all chromosomes (Michailova 1989). From this one can argue that more mutation has accumulated during the divergence of *G. barbipes* and *G. salinus* than between *G. glaucus* and *G. pallens* due to a longer divergence time of evolution.

In addition to chromosome rearrangements, changes in heterochromatin appearance and localization can accompany evolutionary processes too. In the sibling species *G. pallens* and *G. glaucus* heterochromatin is mainly localized in centromere regions of all chromosomes (Figs 3a,b,c, and 4a,b). However, in *G. pallens* an additional constant C band is found in chromosome EF, section 5a of the standard map, and in the telomere region of chromosome G (Fig. 3b,c). By C banding method it

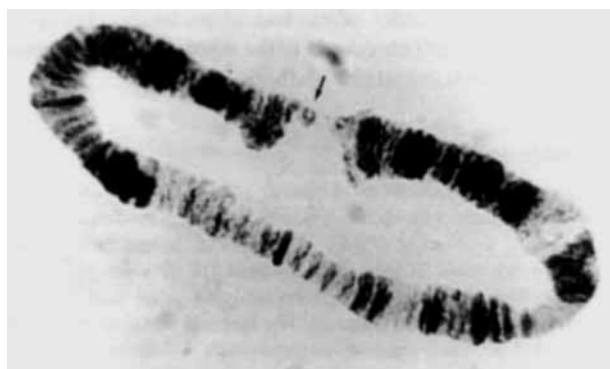


Fig. 2. Chromosome AB of a hybrid with a heterozygous deficiency

could be also established that in *G. pallens* the chromosome G is a telocentric while the same chromosome of *G. glaucus* is acrocentric. In hybrids heterochromatin is also centromeric. Further, some bands in chromosomes AB and CD and an additional C band in chromosome EF appear in heterozygous state. The hybrid chromosome G displays its differences in the morphology of C banding, too. It is possible that these differences in staining pattern reflect some chromosomal pro-



Fig. 3. C heterochromatin in chromosomes of *G. pallens*. (a) centromere heterochromatin in all chromosome; an additional C band in chromosome EF; (b) chromosome EF with a centromere heterochromatin and additional C band in arm E; (c) chromosome G

tein modification between these species or different amounts of repetitive DNA or both.

Natural hybridization

On the Black Sea coast, near the region of Burgass, we found a population of hybrid origin. Some specimens carried a hybrid chromosome G; one from *G. glaucus* and the other from *G. pallens*. Egg masses were also collected from this hybrid population and reared under laboratory conditions. The origin of these hybrids can be explained by introgression (F_1 hybrid \times *G. glaucus*). In Shabla lake (Bulgaria) and Velencea (Hungary) we (Michailova and Contreras-Lichtenberg 1995) observed a hybrid genotype produced by introgression (F_1 hybrid \times *G. pallens*). Once a hybrid (F_1) between *G. glaucus* and *G. pallens* has been produced various types of gametes can be formed giving rise, for example, to individuals with hybrid chromosome G and hybrid chromosome CD (Fig. 5a,b). Among individuals from the reared egg mass three different types of chromosome CD were established: chromosomes CD with inversion loop in arm D, or chromosomes with banding pattern of *G. pallens* or of *G. glaucus* (Fig. 5c,d). The formation of chromosome CD with a combined banding pattern of *G. pallens* or *G. glaucus* can be explained by crossing over. The homologues of arm C are paired because the banding pattern of this arm of both

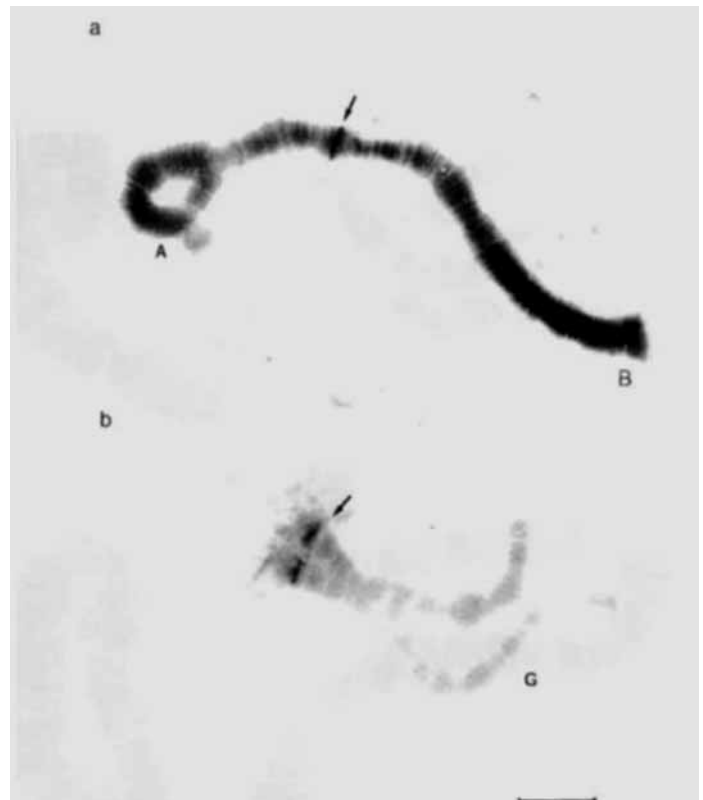


Fig. 4. C heterochromatin in chromosomes of *G. glaucus*. (a) chromosome AB; (b) chromosome G

species coincides completely. Arm D can be from *G. pallens* or from *G. glaucus*. Because chromosomes AB and chromosomes EF of both species have the same banding pattern, it is very difficult to decide whether a chromosome is derived from *G. glaucus* or from *G. pallens*. However, the pattern of heterochromatin in the chromosomes of hybrids resembles that of *G. glaucus*. In the population of Shabla, on the other hand the C banding pattern corresponded with that of *G. pallens*.

In the external morphology of larvae, pupae and adults the hybrids combine features of both species.

The hybrids show developmental stability. All individuals were normal and developed successfully and some generations of the hybrid population could be produced in the laboratory. The zones with hybrid populations are always on localities where contacts exist between the two species. Hybrid zones are natural laboratories and important for the study of adaptation and speciation (Hewitt 1988). The stability of the hybrid populations *G. pallens* \times *G. glaucus* can be probably explained by hybrid superiority (Moore 1977). According to this hypothesis the hybrids are stable because they have superior fitness within the hybrid zone. The most realistic explanation in the present case is that there is no detectable selection against hybrids. However, developmental stability is related to the amount of genetic distance between the hybridizing taxa (Graham 1992). Our data show that the species studied here are closely related species recently derived from a common ancestor and still sharing much of their genomic organization in common.

The data strongly suggests that hybridization is a potent factor in speciation in the genus *Glyptotendipes*. Through introgression new species of a hybrid origin might be formed. The introgression could either come from *G. glaucus* or from *G.*



Fig. 5. Polytene chromosomes found in populations of hybrid origin. (a) a hybrid chromosome G; (b) chromosome CD with a heterozygous inversion in arm D; (c) chromosome CD from *G. pallens*; (d) chromosome CD from *G. glaucus*

pallens (Michailova 1996). Thus, generations of these hybrids can contain different chromosome sets. When a hybrid population has been established from the back cross $F_1 \times G. pallens$ in their natural surroundings, individuals will be produced carrying different chromosome sets: individuals with chromosome sets of *G. pallens*, and those with various combinations (Michailova and Contreras-Lichtenberg 1995). When a hybrid population has been produced from the back cross $F_1 \times G. glaucus$ then individuals with chromosome sets of *G. glaucus*, or individuals with various combinations can be found in their natural surroundings. However, the existence of stable hybrid species can only be ascertained after a detailed analysis of more material from the genus *Glyptotendipes*.

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Zusammenfassung

Cytogenetische Untersuchung des Hybriden Glyptotendipes pallens Mg. \times *Glyptotendipes glaucus* Mg. (Diptera, Chironomidae): *Evolutionstbiologische Überlegungen*

Es wird der Karyotyp von experimentell erzeugten Hybriden zwischen *Glyptotendipes pallens* und *G. glaucus* beschrieben. Die Hybridisierung war nur in der Richtung (*G. pallens* ♀ \times *G. glaucus* ♂) möglich. Die Chromosomen AB und EF der Hybriden zeigen eine mehr oder weniger enge Paarung in ihrer ganzen Länge. Im Chromosom CD, in dem eine Inversion auftritt, wird eine typische Inversionsschlinge ausgebildet. Die Homologen des Chromosoms G bleiben fast vollständig asynaptisch. Auch die Lage des C-Heterochromatins wurde untersucht. In allen Chromosomen konnte sowohl centromeres als auch interstitielles Heterochromatin gefunden werden. Durch die C-Banding-Analyse konnte auch gezeigt werden, daß *G. pallens* ein telomeres Chromosom G besitzt, während das von *G. glaucus* akrozentrisch ist. Auf Grund der karyotypischen Ähnlichkeit kann angenommen werden, daß diese beiden Arten erst vor kurzem aus einem gemeinsamen Vorfahren entstanden sind und daß sie bezüglich ihrer Genomorganisation noch viele Gemeinsamkeiten haben. An der Küste des Schwarzen Meeres im Südosten von Bulgarien wurde in der Region Burgass eine Hybridisierungszone zwischen den sympatrischen Arten *G. pallens* und *G. glaucus* gefunden. Die Idee, daß die Hybridisierung zwischen den beiden Arten schließlich zu einer Artbildung durch Hybridisierung führen könnte, wird diskutiert.

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