The poltene chromosomes of *Nilodorum biroi* (Kieffer) (Diptera: Chironomidae)

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Abstract

The poltene chromosomes of the salivary glands of *Nilodorum biroi* (Kieffer) comprise three long metacentric or submetacentric chromosomes. It is considered that this is a derived condition from a diploid number of 8, by the tandem fusion of a small acrocentric element (C) to a metacentric chromosome (AB). A standard chromosome map for the genus is provided and the chromosomal polymorphism of Indian and Australian populations is described.

Introduction

*Nilodorum biroi* was first described morphologically from Sri Lanka by Kieffer (1918) and subsequently recorded from Assam and Bihar in India. The species has also been recorded from Australia by Freeman (1961) who noted that the Australian specimens agreed well in morphological details with those from Sri Lanka and India. The genus *Nilodorum* was originally described as a separate genus (Kieffer, 1921) but was later classed as a subgenus of the genus *Chironomus* (e.g. Freeman, 1961). Recently it has been re-allocated generic status because of differences in the immature stages (Pinder & Reiss, 1983).

The present paper describes the cytology of *N. biroi* to provide standard chromosome maps for future cytogenetic and cytotaxonomic studies on the genus. In addition, comparisons are made between the banding pattern of the poltene chromosomes and the chromosomal polymorphisms of *N. biroi* from India with those of Australian specimens to investigate whether populations on either side of the Indian Ocean have diverged from each other. Comparisons are also made with chromosomes of the genus *Chironomus* in order to determine whether the cytology provides any further evidence in support of the separation of *Nilodorum* as a full genus.

Material and methods

Indian material for cytological analysis was collected from the Yamuna River near Okhla, south east Delhi, both as larvae (30 individuals) and two egg masses. The Australian material was all from egg masses, either collected in the wild or laid by wild-caught females. A single female each was collected at both Somerset Dam and Sarina in Queensland, while nine egg masses were found attached to stems and leaves of water lily (*Nymphaea* sp.) in Goanna Lagoon on Gulungil Creek, about 5 km west of Jabiru East in the Alligator Rivers Region of the Northern Territory. The eggs were then reared in the laboratory, in the case of the Australian material in ¹× Martin's solution (Martin et al., 1980). The larvae hatch from the eggs in about 3–4 days and grow to maturity in about 5–6 weeks. The larvae are similar in gross morphology to those of *Nilodorum* described by Pinder & Reiss (1983). After about two days as pupae, the adults emerge.

Laboratory reared females, kept in cages with males, would lay egg masses but no fertile eggs were obtained under laboratory conditions.

Chromosomal preparations were made from fourth-instar larvae which were either live (Indian samples) or had been stored in fixative (Australian samples). Live larvae were dissected in insect Ringer solution, the salivary glands fixed in Carnoy's fixative, stained in 2% lacto-aceto-orcein and then squashed in 45% acetic acid. After analysis the slides were made permanent by freezing in liquid nitrogen, to enable removal of the coverglass, and mounting in euparal. Glands from pre-fixed larvae were also stained in 2% lacto-acetic orcein but squashed in the stain.

For the present maps the system of Martin (1969) has been followed. Thus the chromosomes have been numbered I to III in order of length and arbitrarily allocated left and right arms. The arms have been named A–G, such that chromosome I has the arms A and B + C (cf. Results), Chromosome II has arms D and E; and Chromosome III has arms F and G. It must be noted, however, that this system of nomenclature of the arms implies no homology with the correspondingly named arms of Chironomus (Keyl, 1962), Glyptotendipes barbipes (Martin & Porter, 1973) or Polypedilum nubifer (Porter & Martin, 1977). The whole genome has been divided into 25 major divisions, beginning from 1 in the left arm of chromosome I and continuing in order up to 25 at the right end of chromosome III.

The major divisions have been divided into three sub-divisions (a–c) each. The major advantage of this system is that any band in the complement can be specified by its number-letter combination, as in the Australian standard map for Chironomus (Martin, 1969), without the need to state the chromosome or arm. This is particularly important where pericentric inversions are involved, as in the present species.

In arms where inversion polymorphism has been observed, the most common sequence in the Okhla population has been taken as the standard sequence. The standard sequences have been termed A1, B1, C1, D1, E1, and G1 in the respective arms.

Results

The salivary chromosome complement of Nilirodorun biroi consists of three long polytene chromosomes as compared to four chromosomes in the genus Chironomus and some other species of Nilirodorum. The diploid chromosome number, as observed from the mitotic chromosomes of the supraesophageal ganglion cells, is 6 with two pairs of metacentrics and one pair of submetacentrics. This suggests that there has been a reduction in the chromosome number from a diploid number of 8 by the tandem fusion of a small acrocentric element onto one of the metacentric chromosomes. This is supported by the presence of a nucleolus and Balbiani rings, which are present on chromosome IV of N.?brevibucca (Kieffer) and N.fractilobus (Kieffer) (Martin, unpublished data), towards one end of chromosome I. Chromosomal polymorphism has been observed only in chromosomes I and III of the Indian material, and only in chromosome II of the Australian material, where it appears to be a sexual dimorphism rather than a polymorphism.

Chromosome I (Figs. 1, 2, 3)

Chromosome I is the longest chromosome of the complement and consists of three parts, corresponding to arms A, B, and C, with the functional centromere between arms A and B. Thus the left arm is A, the right arm B joined to C which appears to represent the fused chromosome IV. The centromere may be represented by the dense band in 5a1, although in Australian material the band 4a1l appears to be the darkest band and it might be the site of the centromere. The chromosome can be identified by the presence of a characteristic nucleolus at 8a2 and Balbiani rings at 9b3 and 9c5. The whole chromosome has been divided into 9 major divisions: three divisions (1–3) in arm A, four divisions (4–7) in arm B and two divisions (8–9) in arm C. A long pericentric inversion has been observed in one individual from the Indian material. The inversion extends from 2a1-7b2 (Fig. 4). In addition a paracentric inversion has been observed in arm C (see below).

Arm A (Fig. 1)

The characteristic bands in this arm are the series of bands in 1c1-8, 2c1-10, the bands in 3b1-10 and 3c1-3 and five thick dark bands, which are actually doublets, in 4a1-5. A clear constriction is formed at 4a3-4. In many preparations puffs can be seen in 2a2, 3c4, 4a5 and 4b2. Arm A in the Australian material (Fig. 8) has a basically similar banding