

TWO PAIRS OF CHROMOSOMES: A NEW LOW RECORD FOR COLEOPTERA

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ABSTRACT

The known haploid modal karyotype formula for the Pyrophorini (Elateridae) is $8+X$. From this mode deviant karyotypes have arisen by repeated centric fusion and pericentric inversion. The trend reaches the extreme of $2n = 4$, in *Chalcolepidius* Esch. This number represents a new low record not only for the Coleoptera but additionally for Eukaryotes in general.

INTRODUCTION

The chromosome number $2n = 18 + XO/XX$ (karyoformula $9+X$), modal for the Elateridae, tends to be lowered in the pedaceous genera *Pyrophorus* and *Hemirrhypus* belonging to the morphologically primitive subfamily Pyrophorinae. The karyoformula is reduced to $8 + X$ in the Puerto Rican *Pyrophorus lutosus* (Virkki, 1962), to $7 + X$ in the Brazilian *P. nyctophanus* (Piza, 1960) and in the Trinidadian *P. pellucens* (Smith, 1960), to $5 + X$ and $4 + \text{neo XY}$ in Central American *P. radians* (Virkki, 1962), and to $4 + \text{neo XY}$ in the Brazilian *Hemirrhypus lineatus* (Piza, 1958). In a taxonomist's opinion (J. Bechyné, Universidad Central de Venezuela, personal

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communication), randomness of exophenotypic variation in the neotropical Pyrophorines is such that no orthogenetic lines can be defined. Do we have here one of those evolutionary cases where genotypic and karyotypic reorganization has facilitated an acceleration of the speciation process, just as teleost fishes supposedly experienced following an increased DNA content within their genomes (Hinegardner and Rosen, 1972)? With this possibility in mind, we recently started checking chromosomes of different Pyrophorines, and soon came across a representative of a predaceous genus, *Chalcolepidius*, with a new low record chromosome number not only within the Coleoptera, but also Eukaryotic animals in general.

MATERIAL AND METHODS

The three specimens studied here were collected in Rio Claro, State of São Paulo, Brazil. The testes were fixed in 3:1 ethyl alcohol-glacial acetic acid and the squashed material stained with 2% acetic orcein.

RESULTS

Spermatogenesis

The chromosome relationships are depicted in Figure 1. Early gonias show 4 large chromosomes (A). One pair has a constriction near its end, suggesting acrocentry in this chromosome.

Pachytene threads (B) show an even chromomeric structure, except for a pair of slightly larger knobs on one of the bivalents. This bivalent is assumed to be the acrocentric pair with the knobs representing pericentric heterochromatin.

Because diplotene is not diffuse, chiasmata can be easily studied (C and D). Our preliminary observations indicate the consistent presence of one chiasma in the bivalent formed by the acrocentrics. The position of this chiasma is variable, but distal locations predominate. The other bivalent is invariably a bichiasmate ring, with one chiasma terminal, and the other nearly so. This bivalent is obviously formed by a metacentric or submediocentric pair of chromosomes.

The typical forms of a cross and a ring are still recognizable at diakinesis (E), but M I shows a large rod and a ring (F).

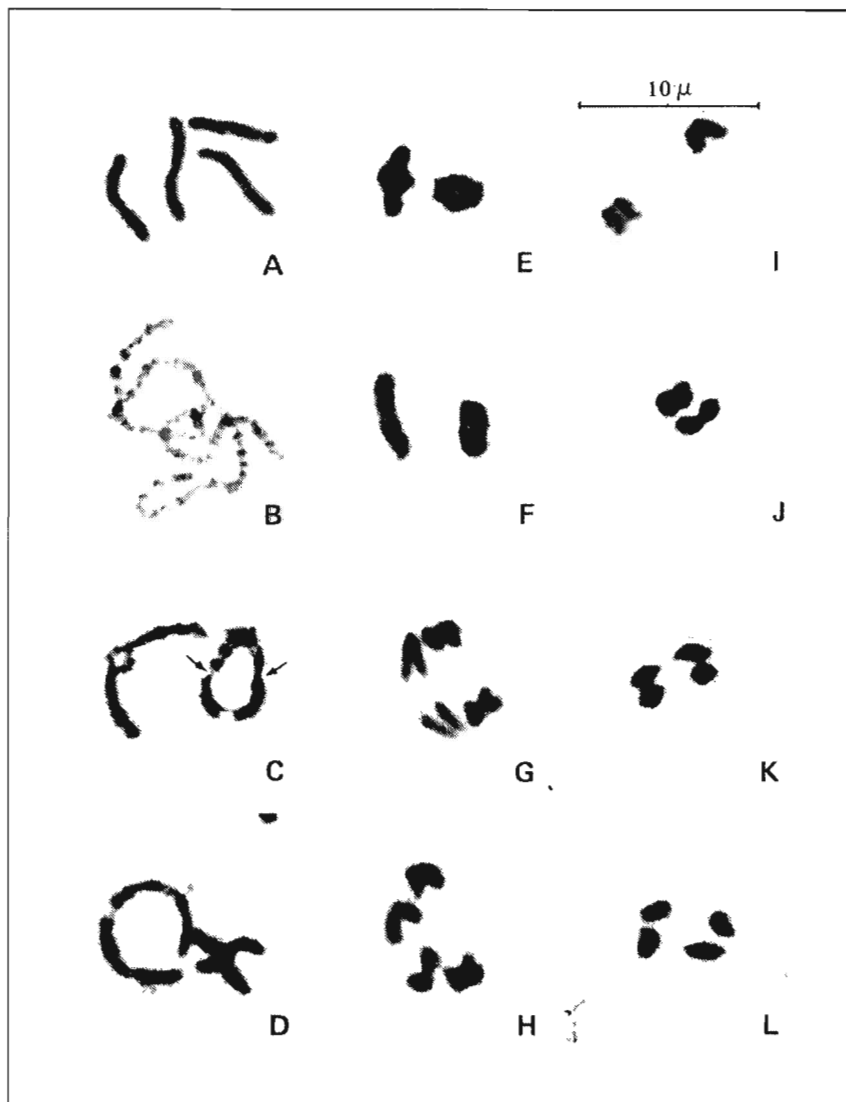


Figure 1 - Male meiosis of *Chalcolepidius zonatus*. Bar: 10 μ m. Arrows mark centromeres. The illustrations are to be read vertically starting from top left. *Left column*: A, spermatogonial metaphase, $2n = 4$; B, pachytene; C, diplotene; D, late diplotene. *Middle column*: E, diakinesis; F, M I; G, A I; H, A I. *Right column*: prophase II; I, M II; J, A II; K and L, T II.

At A I the chromatids of all chromosomes are well separated (G). The acrocentric appears to be V-shaped, while the X-shape of the other half bivalent confirms its designation as medio- or submediocentric (H and I). These shapes are still recognizable at second prophase (J). Meta and anaphase-II behavior, however, suggests that while the collochore keeping the chromatids together are proximally located up to prophase II, they are then switched to the distal ends (K-L). Apart from this, A and T II proceed in a conventional manner.

These observations establish a diploid number of $2n = 4$ for *Chalcolepidius zonatus*.

DISCUSSION

The chromosome number

Bacteria have only one or two chromosomes, and several fungi ("Mesokaryota") have $2n = 2$ (see Wakayama, 1930), but few representatives of the Eukaryota have such low numbers (Brown, 1972). The well known case of *Parascaris equorum* v. *univalens* ($2n = 2$ in the germ line) must be discounted because these chromosomes are actually compound. If, then, we also disqualify *Parascaris equorum* v. *bivalens* ($2n = 4$ in the germ line), only three Eukaryote species with $2n = 4$ remain: the composite plants *Haplopappus gracilis* (Jackson, 1957, 1959), and *Brachycombe lineariloba* (Smith White and Carte, 1970) and the present Pyrophorine beetle.

With a recombination index of 5 ($n + 3$ chiasmata), there is clearly little scope for variability in the genotype, particularly in view of the restricted location of the chiasmata. The inflexibility of the *Ch. zonatus* system is counteracted only by the relatively free distribution of the chiasma of the acrocentric bivalent. The ring bivalent, in characteristic Coleopteran fashion, permits only distal crossing-over.

Such a low genetic recombination is especially suited to a species well adapted to a relatively univariate environment which, in the case of *Chalcolepidius*, breeds under the bark of fallen trees. Here the larvae devour Cerambycid and other xylophagous larvae (Wolcott, 1948; Martorell, 1976) through the genus may be anthropochoric in regions where there are no fallen trees.

Both chromosomes of *Ch. zonatus* look genuinely euchromatic at all phases of spermatogenesis. As one pair must be sex-determining, about 50% of the genes will be sex-linked.

Evolution of the low chromosome numbers in Pyrophorini

The previous low number on record for the Coleoptera was $3+XY$, which appears to have arisen polyphyletically in the Carabid (Adephaga) *Graphipterus serratus* (Wahrman, 1966) and the flea beetle (Polyphaga) *Homoschema nigriventre* (Virkki and Purcel, 1965). The same number, $3+Xy$, has also been found in a beetle ally, *Brasixenos* near *occidentalis* (Stylopoidea) (Ferreira *et al.*, unpublished results). Since the modal (and hence presumably ancestral) karyotypes for the Adephaga and Polyphaga correspond to $8+X$ (or $8+Xy$) and $9+Xyp$, respectively (Smith, 1952; Smith and Virkki, 1978; Serrano, 1981a), we would argue against Wahrman (1966) that the low numbers must be the result of fusions, combined, presumably, with other rearrangements. Unfortunately, retracing this evolutionary sequence will not be possible until additional related karyotypes are known.

Excluding the Ctenicerini (a tribe belonging to the Elaterinae according to Arnett, 1968), $9+X$ in *Haterodeus macroderus* (Agarwal, 1962) remains the highest reported count for the Pyrophorinae: Pyrophorini and at least 12 autosomes of this karyotype are metacentric. Manha and Lahiri (1972) have determined $8+X$ ($16+XX$) for the same (?) species, without commenting on the difference. $8+X$ is modal for the Pyrophorini, and has been found in 6 genera to-date. Within *Agrypnus*, three autosomal centric fusions have lowered an $8+X$ (all autosomes acrocentric) karyotype to $5+X$ (3 large pairs of autosomes clearly metacentric) (Kacker, 1963). Fusions leading to neo XY systems have occurred polyphyletically in *Lacon* (Smith and Virkki, 1978), *Hermirrhypus* (Piza, 1958), and *Pyrophorus* (Virkki, 1962). Thus fusions certainly seem to differentiate Pyrophorine karyotypes.

An extreme karyotype such as that of *Chalcolepidius* ($n = 2$) cannot arise from $8+X$ through fusions alone. Since tandem translocation is not known to occur in the Coleoptera, we suggest that the karyotype of *Ch. zonatus* is the result of centric fusions alternating with pericentric inversions (Figure 2). If we are correct in this assumption then one of the two chromosome pairs must carry the sex-determining loci originally located in the free X, though this is not cytologically distinguishable.

The genesis of *Haplopappus gracilis* appears to be similar. This species has two karyoforms which cross freely: *dibivalens* — ($2n = 4, 2^{II}$) and *tribivalens* ($2n = 6; 3^{II}$). Their hybrid has $1^{II} + 1^{III}$ in its meiosis, which confirms the homology of the arms of the large metacentric of *dibivalens*

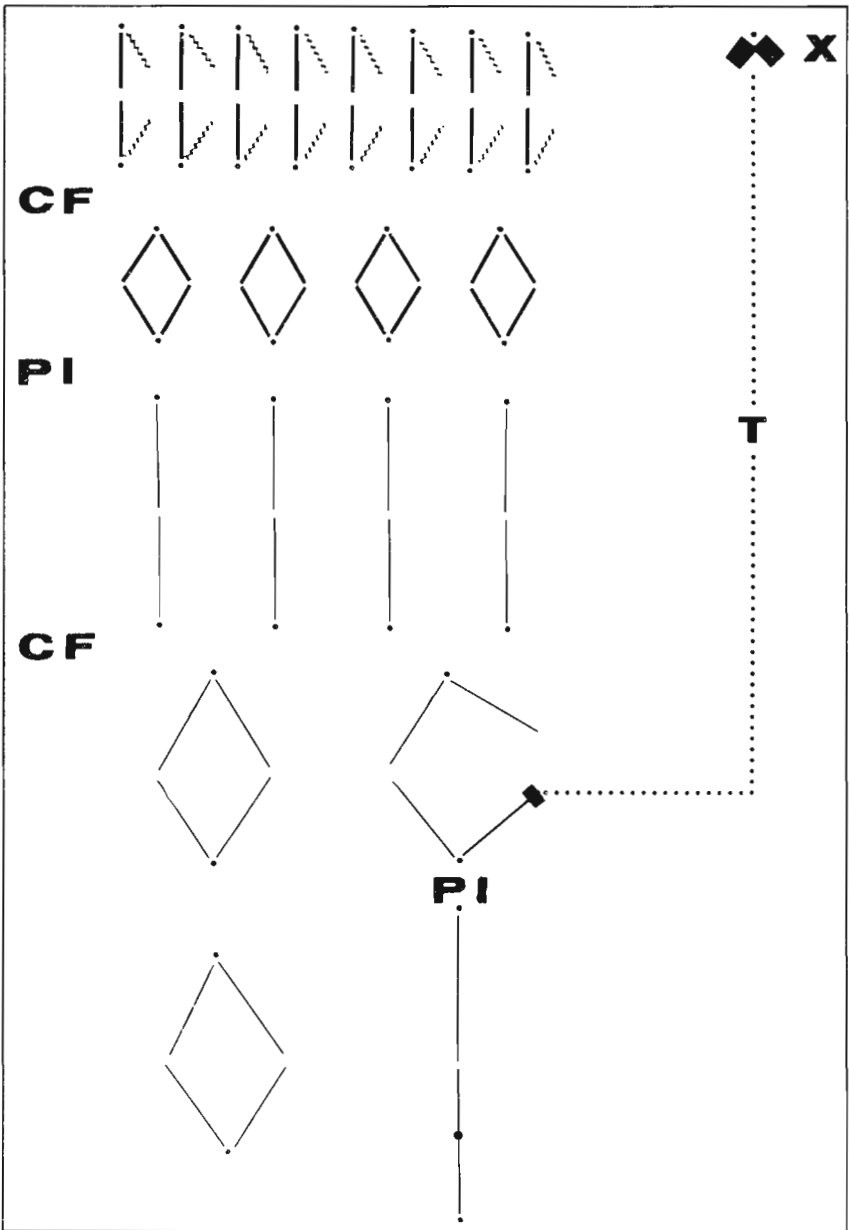


Figure 2 - A hypothetical derivation of the karyotype of *Chalcolepidius zonatus* ($2n = 4$), from the modal Pyrophorinian $8 + X$, through centric fissions (CF), pericentric inversions (PI), and incorporation into an autosome of the X segment containing the sex determinators (T). The presence of diphasic autosomes in the initial karyotype is purely hypothetical.

with two chromosomes of *tribivalens* (Jackson, 1962, 1965). *Tribivalens*, in turn, has arisen through centric fusion from a species with $2n = 8; 4\text{II}$, like *H. ravenii* (Jackson, 1959, 1964, 1965). Other chromosome numbers in *Haplopappus* are $2n = 10, = 12, = 18, = 24, = 36$ (Brown, 1972), a series partly explicable by polyploidization. Whether Robertsonian translocations, facilitated by other rearrangements, have occurred among this series, is not clear.

It is interesting to note that both records of low chromosome numbers within the Eukaryota occur in morphologically unspecialized groups. Evidently all biological characteristics do not progress step-in-step in evolution. Whereas some reach extreme values rapidly, others may remain "primitive" for prolonged periods (*Heterobathnira*: Takhtajan, 1980).

Distal collochores

Smith and Virkki (1978) applied Cooper's (1941) term *collochore* to cover the ultimate regions keeping the chromatids together, without trying to define these regions more precisely in structural terms. Normally they are situated on both sides of a localized centromere, as shown originally by Lima-de-Faria (1955). In the meiosis of certain beetles, collochores became distally located during meiosis. This includes the Bembidiinae (Serrano, 1981b), Lampyridae, Pyrophorini, Oedionychina (Smith and Virkki, 1978), Megalopodinae (Virkki, 1983), Stylopidae (Ferreira *et al.*, unpublished results). The most convincing evidence for such a switch is found in the giant sex chromosomes of the fleabeetle *Altica* (Virkki, 1970) and *Hyperaspis* (Smith and Virkki, 1978). Here the telocentric or acrocentric sex chromosomes are clearly V-shaped at first anaphase with the opening of the V pointing towards a pole and with the distal chromatid ends associated with one another. At interkinesis, however, the V's open completely, so that the chromatids lie in a straight line (see, for example, Virkki, 1970, Figures 22 to 24). We believe that Piza (1960, Figures 7 and 8) has depicted just such a process in *Pyrophorus nyctophanus*, though he refers to it erroneously as diakinesis.

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RESUMO

Dentre os Pyrophorini (Elateridae) a fórmula cariotípica modal haplóide é $8 + X$. Cariótipos secundários ($7 + X$, $5 + X$ e $4 + \text{Neo XY}$) tem surgido através de sucessivas fusões cêntricas e inversões pericêntricas ocorridas no cariótipo básico. Em *Chalcolepidius zonatus*, o número de fusões e inversões ocorridas, alcançou o máximo, reduzindo o número cromossômico básico para $2n = 4$. Este é o menor número cromossômico conhecido dentre os Coleoptera e os Eucariotas em geral.

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