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Fluorescent banding and meiotic behaviour in *Erythrodiplax nigricans* (Libellulidae) and *Coryphaeschna perrensi* (Aeschnidae) (Anisoptera, Odonata)

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Abstract — The species of Odonata are cytogenetically characterised by possessing holokinetic chromosomes, a post-reductional meiosis, an XX/X0 (female/male) sex chromosome mechanism, *m*-chromosomes, and only one chiasma per bivalent. Chromosome studies were performed on males of *Erythrodiplax nigricans* and *Coryphaeschna perrensi* from Argentina. *Erythrodiplax nigricans* has n=12+XO and lacks *m*-chromosomes, while *C. perrensi* has 2n=27, n=13+XO, *m*-chromosomes and a large autosomal pair associated with the nucleolus. The meiotic behaviour of both species follows the general pattern of the order: the X chromosome is positively heteropycnotic during early prophase I; bivalents regularly show only one chiasma; all chromosomes migrate synchronously and almost parallel to the equatorial plane at anaphase I; at metaphase II the X chromosome is present in all the cells as a consequence of the post-reductional division, lies outside the metaphasic plate, and migrates asynchronously with the autosomes at anaphase II. In *C. perrensi*, the largest bivalent exhibits two chiasmata in a large proportion of cells, which is a very rare feature among dragonflies. Heterochromatin characterisation with DAPI-CMA banding reveals that *C. perrensi* does not show fluorescent banding, except for a CMA bright band at one telomeric region of the largest bivalent, associated with the NOR region; in *E. nigricans*, autosomes have small AT-rich telomeric blocks, except for the smallest pair, which exhibits conspicuous bands in both telomeric regions, one being GC-rich and the other AT-rich. Taking into account that the *m*-chromosomes have been found in other *E. nigricans* populations, their absence in the studied population may be due to the presence of such heterochromatic blocks.

Key words: *Coryphaeschna perrensi*, DAPI–CMA banding, *Erythrodiplax nigricans,* heterochromatin, holokinetic chromosomes, meiosis, Odonata.

INTRODUCTION

Two distinctive cytogenetic features of the Odonata are the presence of holokinetic chromosomes (that is to say, without a localised centromere), and the post-reductional type of meiosis (or inverted meiosis). The most frequent sex chromosome mechanism is XX/X0, being the male the heterogametic sex; the derived neo-XY system occurs in approximately 5.5% of the species, and the $X_1X_1X_2X_2/X_1X_2Y$ multiple system is only present in *Micrathyria ungulata* (Mola *et al.* 1999). The presence of a small pair of autosomes, the m-chromosomes, is found in nearly 80% of the species; it shows a regular meiotic behaviour, and is solely characterised by its size, and in some cases by its negative heteropycnosis. The typical meiotic development in males exhibits the following features: the X chromosome is usually positively heteropycnotic during early prophase I; bivalents regularly show only one chiasma; all chromosomes migrate synchronously and almost parallel to the equatorial plane at anaphase I; at prometaphase II, the autosomal chromatids are joined by one of their telomeric regions adopting a characteristic "ε" shape, and the X chromosome is present in all the cells as a consequence of the post-reductional division, at metaphase II the sex chromosome is usually outside the metaphasic

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plane, and at anaphase II it migrates asynchronously with the autosomes, generally lying ahead (MOLA 2007)

Heterochromatin is an important component of the genome, since differences in its composition and distribution are likely to be related with the karyotypic evolution and genetic differentiation among related species. C-banding is one of the most used techniques for detecting heterochromatin because it stains almost all constitutive heterochromatin segments. Studies of the distribution of the heterochromatin in dragonflies are scarce, and the C-banding showed that autosomes usually have heterochromatic blocks in both telomeric regions, and the sex chromosome in males may be completely C-bright, may show an intermediate staining or may only exhibit C-bright terminal or interstitial bands (AGOPIAN and MOLA 1984; MEELDIJK 1984; THOMAS and PRASAD 1986; PRASAD and THOMAS 1992; SUZUKI and SAITOH 1988; FRANKOVIĆ and JUREČIĆ 1989; PEREPELOV *et al.* 1998; 2001; PEREPELOV and BURGROV 2001; NOKKALA *et al.* 2002; MOLA and PAPESCHI 2006). The heterochromatin molecular composition can be determined by using base-specific fluorochromes; among these, the most commonly employed are CMA showing a preference for GC-rich DNA regions, and DAPI showing a preference for AT-rich DNA regions (APPELS *et al.* 1998; SUMNER 2003).

In the present work, the distribution and composition of the heterochromatin (DAPI-CMA banding), and the male meiotic behaviour of *Erythrodiplax nigricans* (Libellulidae) and *Coryphaeschna perrensi* (Aeschnidae) were analysed. In addition, the chiasma distribution in the largest bivalent of *C. perrensi* was characterised using fluorescent banding.

MATERIAL AND METHODS

The present study was performed on 13 adult males of *Erythrodiplax nigricans* from Ciudad Universitaria (Ciudad Autónoma de Buenos Aires) and on 10 adult males of *Coryphaeschna perrensi* from "Eldorado" Department (Misiones Province), Argentina.

The specimens were fixed in 3:1 absolute ethanol: glacial acetic acid; then, gonads were dissected out and stored in 70% ethanol at 4ºC. For meiotic studies, slides were made by the squash method in iron propionic hematoxylin.

Fluorescent staining with CMA (chromomycin A_3) and DAPI (4'-6-diamidino-2-phenylindole) was carried out on unstained slides. A piece of gonad was squashed in 45% acetic acid; the coverslip was then removed by the dry-ice method, and the slide was air-dried. The sequential DAPI-CMA banding was performed using the technique described by REBAGLIATI *et al.* (2003)

RESULTS

Erythrodiplax nigricans (n=12+XO male).

Meiotic analysis - At early meiotic prophase the sex chromosome is condensed and positively heteropycnotic, and at pachytene it becomes isopycnotic (Fig. 1A). At diakinesis all bivalents have one chiasma at a medial position; they decrease gradually in size, with the sex chromosome being small and of similar size to the smallest bivalent (Figs. 1 B, C). This bivalent is not considered as "m" because it is just slightly smaller. At metaphase I, the autosomal bivalents and the sex chromosome lie on the equatorial plane. All metaphases II have 12 autosomes and the sex chromosome as a consequence of the post-reductional division, the latter being outside the equatorial plane (Fig. 1D). At anaphases II, the X chromosome migrates ahead of the autosomes.

DAPI – CMA banding - A small CMA-bright and DAPI-dull region is observed at early meiotic prophase (Figs. 2A, B), and the telomeric regions of the autosomes are slightly DAPI-bright at pachytene. At diakinesis, one or both pairs of telomeric regions of the bivalents are slightly DAPI-bright (Fig. 2C). In the smallest bivalent, instead, one pair of telomeric regions is DAPI-bright, while the other is CMA-bright and DAPI-dull (Figs. 2C, D). The sex chromosome is isopycnotic with the autosomes and shows homogeneous staining.

Coryphaeschna perrensi (2n=27, n=13+XO male).

Meiotic analysis - At spermatogonial prometaphase, a largest and a smallest pair (*m* pair) can be recognised, while the X chromosome cannot be identified. The largest pair is always associated with the nucleolus through one of its telomeric regions (Fig. 3A).

At early meiotic prophase, the X chromosome is positively heteropycnotic (Fig. 3B). At diakinesis the sex chromosome becomes isopycnotic with the autosomes; at diplotene and diakinesis, the *m* bivalent is negatively heteropycnotic (Fig. 3C, D). Bivalents have one chiasma in medial or terminal position, except for the largest one, which has either two terminal chiasmata in about 19% of the

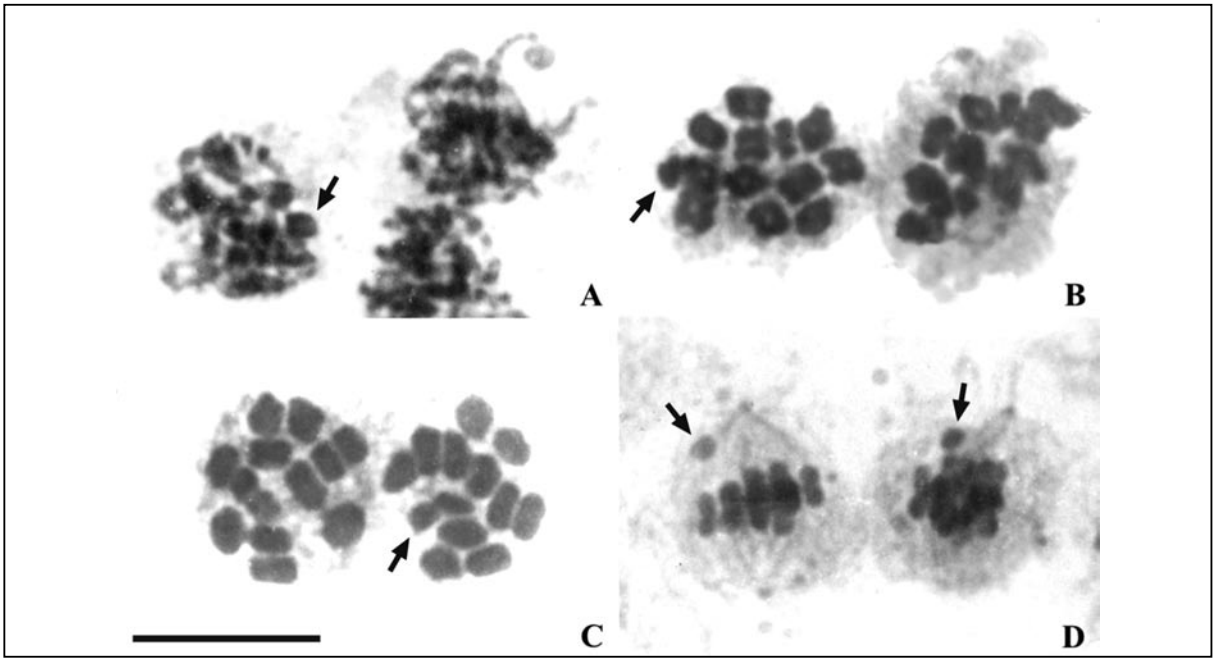

Fig. 1 — *Erythrodiplax nigricans* n=12+XO male. A) Pachytene, B) Diakinesis, C) Prometaphase I, D) Metaphase II. Arrows point to X chromosome. Bar=10μm.

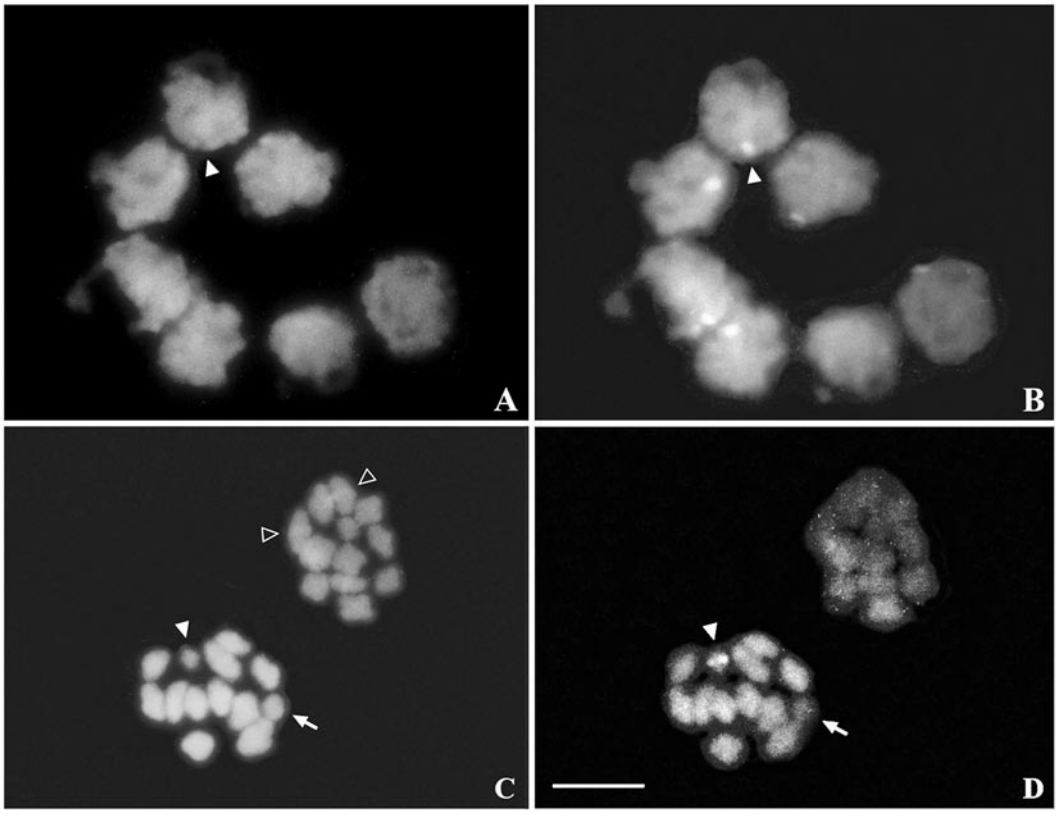

Fig. 2 — *Erythrodiplax nigricans*. n=12 + X0. DAPI-CMA banding. A-B) Leptotene, C-D) Diakinesis. Arrows point to X chromosome. Arrowheads point to DAPI dull – CMA brigth bands. Empty arrowheads point to lightly DAPI bright band. Bar=10μm.

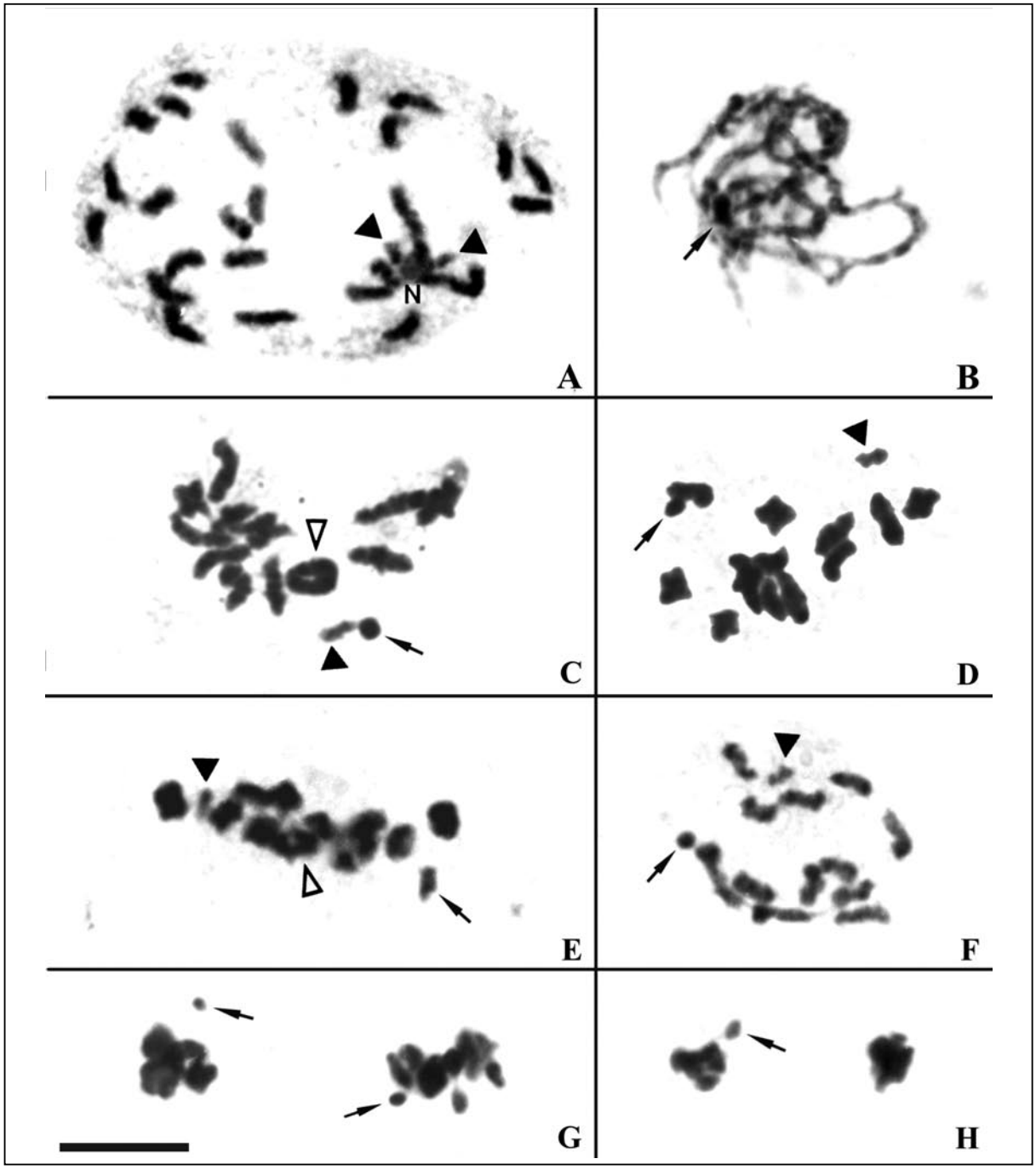

Fig. 3 — *Coryphaeschna perrensi*. n=13 + X0. A) Spermatogonial prometaphase, B) Pachytene, C) Diplotene, the largest bivalent shows two chiasmata (empty arrowhead), D) Diakinesis, E) Metaphase I, the long axis of the largest bivalent is orientated equatorial to the spindle (empty arrowhead), F) Prophase II, G) Metaphase II, H) Anaphase II. Arrows point to X chromosome. Arrowheads point to m chromosomes. N=nucleolus. Bar=10μm.

analysed cells, or one terminal or subterminal chiasma in the remainder (Figs. 3C, D). At metaphase I, the sex chromosome and the bivalents line up on the equatorial plane; in the largest bivalent the equatorial orientation is evident (Fig. 3E). All prophases II have 13 autosomes and the sex chromosome (Fig. 3F). At metaphase II, the sex chromosome is placed outside the equatorial plane (Fig. 3G), and at anaphase II it is retarded in its migration (Fig. 3H).

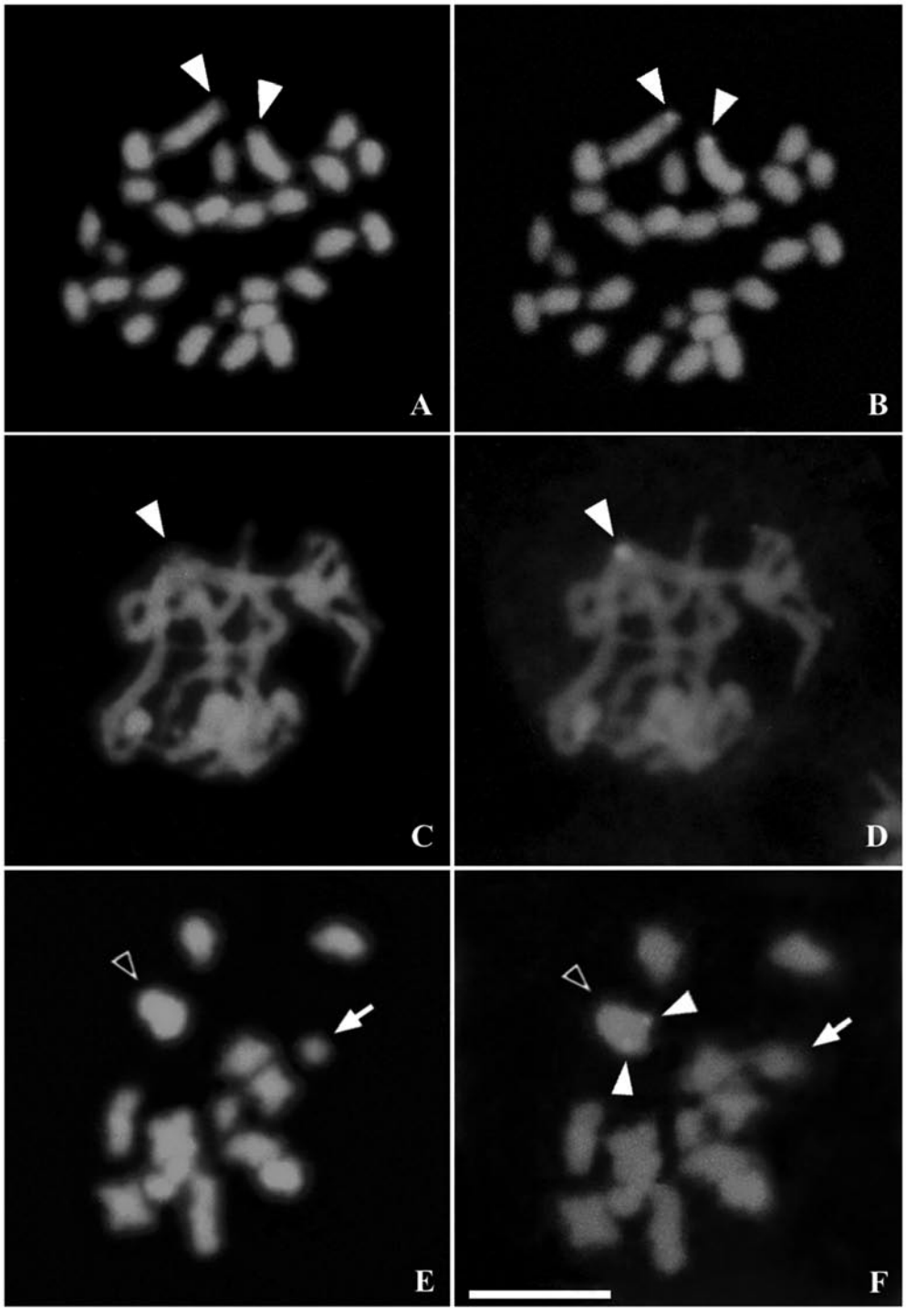

Fig. 4 — *Coryphaeschna perrensi*. n=13 + X0. DAPI-CMA banding. A-B) Spermatogonial metaphase, C-D) Pachytene, E-F) Diakinesis, the largest bivalent shows two chiasmata (empty arrowhead). Arrows point to X chromosome. Arrowheads point to DAPI dull – CMA brigth bands. Bar=10μm.

DAPI – CMA banding – At spermatogonial metaphase, the largest autosomal pair has a CMA-bright and DAPI-dull telomeric band (Fig. 4A, B). At pachytene, there is a small CMA-bright and DAPI-dull region (Fig. 4C, D), which corresponds to one pair of telomeric regions of the largest bivalent at diakinesis (Fig. 4E, F). In cells in which the largest bivalent has one chias-

ma, it is mainly located near the telomere lacking the bright-CMA band. No other DAPI- or CMA-bright bands were observed in the rest of the complement. The sex chromosome is isopycnotic with the autosomes.

DISCUSSION

Erythrodiplax is an American genus mainly distributed in the Neotropical region, with 21 species cited for Argentina (BORROR 1942; MUZÓN and VON ELLENRIEDER 1998). Cytogenetical analysis has been performed in 26 species and subspecies, of which nine are of Argentinean origin. (CUMMING 1964; CRUDEN 1968; HUNG 1971; KIAUTA and BOYES 1972; KIAUTA and VAN BRICK 1978; KIAUTA 1979; FERREIRA *et al.* 1979; GOÑI and DE ABENANTE 1982; SOUZA BUENO 1992; MOLA 1996). Chromosome analysis revealed that the genus has a modal number of n=13 (12+XO), and the most common sex chromosome determining system is XX/XO (female/male). So far, the neo-XY system has only been found in a population of *E. media* from Argentina (MOLA 1996). The *m*-chromosomes are frequent in this genus (80.8% of the analysed species). Variations in chromosome number are due to fusions between autosomes, or an autosome and a sex chromosome, while autosomal fragmentations only account for the number observed in the *E. berenice* population analysed by HUNG (1971).

Many populations of *E. nigricans* have previously been studied from the Argentinean provinces of Buenos Aires (locations of El Tigre, Otamendi and Talavera Island) and Entre Ríos (National Park "El Palmar" and Villa Paranacito) (MOLA 1996). In these individuals, the complement is n=12+XO, and the pair of *m*-chromosomes is about half of the size of the X chromosome. In contrast, no *m-chromosomes* were observed in the population from Ciudad Universitaria analysed in this work. The smallest bivalent has conspicuous heterochromatic blocks in both telomeric regions; in one of these regions, the blocks are rich in GC base pairs (CMA-bright and DAPI-dull bands), while in the other they are AT-rich (DAPI-bright bands). The rest of the autosomes have small blocks of AT-rich telomeric heterochromatin (DAPI-bright bands). With the exception of the smallest pair, all the populations so far analysed show similar meiotic behaviour and chromosome complement.

The variation in the size of the smallest bivalent may be the result of unequal reciprocal translocations between one *m*-chromosome and one of the largest chromosomes of the complement. Although such rearrangement produces changes in the size of both chromosomes, the variation can only be detected in the smallest chromosome due to its relative size. Another explanation for the size variation of the m-chromosome involves changes in the heterochromatin content. The presence of telomeric heterochromatic blocks in the population from Ciudad Universitaria supports the hypothesis of an increase in heterochromatin content. Size polytypism in the smallest bivalent has been described in other seven species of *Erythrodiplax* (*E. atroterminata*, *E. fusca*, *E. umbrata*, *E. berenice*, *E. basalis*, *E. castanea* and *E. paraguayensis*) (MOLA 1996). However, the lack of information on the content and distribution of heterochromatin in these species makes it harder to determine the origin of the variations.

Coryphaeschna is a South American genus with only two species described for Argentina, namely *C. adnexa* and *C. perrensi* (MUZÓN and VON ELLENRIEDER 1988). The species that have been cytogenetically analysed are *C. adnexa*, *C. perrensi* and *C. viriditas* (CUMMING 1964; KIAUTA 1979; MOLA *et al.* 1999). The former two species exhibit characteristics typical of the family (n= 13+XO male), while *C. viriditas* has a reduced chromosome complement (n=12+XO male).

The population of *C. perrensi* from "Eldorado" (Misiones Province) analysed in this work and that from Iguazú National Park (Misiones Province) studied previously show a similar meiotic development (MOLA *et al.* 1999). In the former population, however, the largest bivalent has two chiasmata in a high proportion of cells (19%), which is a very rare feature among dragonflies. In this species, the only heterochromatic region is located on one telomere of the largest autosomal pair, which is GC-rich (CMA-bright and DAPI-dull bands). The presence of this CMA-bright band, together with an association between the nucleolus and the largest pair, allows us to establish a correlation between the nucleolar organiser region (NOR) and the GC-rich band. The association between CMA-bright bands and NOR regions is also frequent in other insect groups with holokinetic chromosomes such as Heteroptera, Homoptera and Psocoptera, thus indicating that the rDNA is frequently rich in GC repeats (BIZZARO *et al.* 1999; MANDRIOLI *et al.* 1999; KUZTNESOVA *et al.* 2003; REBAGLIATI *et al.* 2003; CATTANI *et al.* 2004; GOLUB *et al.* 2004; GROZEVA *et al.* 2004). Nevertheless, in *Carlisis wahlbergi* NORs regions does not show to be GC rich (FOSSEY and LIEBENBERG 1995).

The fluorescent banding method also allowed to characterise chiasma distribution on the largest bivalent of *C. perrensi*. When the largest bivalent has a single chiasma, it is preferentially located in the subterminal region lacking the CMA-bright band. This may indicate that the presence of the nucleolar organiser region can interfere with the recombination process by suppressing or minimizing nearby crossovers, as previously reported for *Camptischium clavipes* and *Carlisis wahlbergi* (Heteroptera) (CATTANI *et al*. 2004).

In the few species of Odonata analysed using fluorochromes, the telomeric heterochromatin was found to be AT-rich (DAPI-bright bands), as in *Erythrodiplax nigricans, Orthemis nodiplaga,* and *Rhionaeshna planaltica*, or to have interspersed AT and GC repeats (equally localised bright DAPI and CMA bands), as in *Orthemis ambinigra*. The latter heterochromatic block composition was also described in Psocids species (Psocoptera), and Cimicidae and Miridae bugs (Heteroptera) (GOLUB *et al.* 2004; GROZEVA *et al.* 2006). Besides, a pair of bright telomeric CMA bands was observed in an autosomal pair of *Erythrodiplax nigricans* and *Coryphaeschna perrensi*, and in the neo-X of *Rhionaeshna planaltica*. Despite the limited number of species analysed, these results may indicate that the heterochromatin of dragonflies has a heterogeneous molecular composition (MOLA 2007).

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