

Meiotic Studies in Nine Species of *Erythrodiplax* (Libellulidae, Odonata). Neo-XY sex chromosome system in *E. media*

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The genus *Erythrodiplax* probably has its origin in the Amazonic basin, and nowadays is mainly distributed in the neotropical region (Borrór 1942). From the 51 species and subspecies described, 19 have been reported in Argentina: *E. anomala*, *E. atroterminata*, *E. basalis basalis*, *E. castanea*, *E. chromoptera*, *E. cleopatra*, *E. connata connata*, *E. connata fusca*, *E. corallina*, *E. famula*, *E. juliana*, *E. latimaculata*, *E. lygaea*, *E. media*, *E. melanorubra*, *E. nigricans*, *E. ochraceae*, *E. paraguayensis* and *E. umbrata* (Rodrigues Capitulo and Muzón 1990, Rodrigues Capitulo *et al.* 1991).

The chromosomal analysis of 23 species and subspecies of the genus reveals that the modal number is $n = 13$, the sex chromosome determining system is XX/XO (female/male), and a pair of *m* chromosomes is frequently present (Table 1).

Despite the great proportion of *Erythrodiplax* species present in Argentina, none had been cytogenetically studied in our country. In this work the chromosome complement and meiotic behaviour of 9 species of *Erythrodiplax* from Argentina are analyzed: *E. atroterminata*, *E. connata fusca*, *E. corallina*, *E. lygaea*, *E. media*, *E. melanorubra*, *E. nigricans*, *E. ochraceae* and *E. umbrata*.

Materials and methods

Chromosome preparations were performed by squashing a piece of testis in iron propionic haematoxylin. The number of individuals of each species and their locality of collection are the following:

Erythrodiplax atroterminata Ris: 5 males from Villa Gral. Belgrano, Córdoba Province. *E. connata fusca* (Rambur): 2 males from Otamendi, Buenos Aires Province. *E. corallina* (Brauer): 2 males from Capital Federal. *E. lygaea* Ris: 3 males from Parque Nacional El Palmar, Entre Ríos Province. *E. media* Borrór: 6 males from Parque Nacional El Palmar, Entre Ríos Province; 1 male from Garruchos, Corrientes Province; 1 male from Parque Nacional Iguazú, Misiones Province. *E. melanorubra* Borrór: 2 males from Montecarlo and 1 male from Parque Nacional Iguazú, Misiones Province. *E. nigricans* Rambur: 2 males from Tigre, 2 males from Otamendi, and 2 males from Isla Talavera, Buenos Aires Province; 2 males from Parque Nacional El Palmar, and 2 males from Villa Paranacito, Entre Ríos Province. *E. ochraceae* Burmeister: 3 males from Parque Nacional Iguazú, Misiones Province. *E. umbrata* (Linnaeus): 1 male from Parque Nacional Iguazú, Misiones Province.

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Table 1. Chromosomal data of the genus *Erythrodiplax*

Specie	n (male)	m	Procedence	References
<i>E. anomala</i>	13	+	Brazil	Souza Bueno 1982
<i>E. atroterminata</i>	2n=26 ♀	—	Uruguay	Goñi and Abenante 1982
	13	+	Argentina	this work
<i>E. attenuata</i>	13	—	Brazil	Ferreira <i>et al.</i> 1979
<i>E. basalis basalis</i>	13	—	Bolivia	Cumming 1964
	13	—	Surinam	Kiauta 1979
	13	+	Brazil	Ferreira <i>et al.</i> 1979
<i>E. berenice</i>	13	—	USA	Cruden 1968
	14	+	USA	Hung 1971
<i>E. castanea</i>	12?, 13?	—	Bolivia	Cumming 1964
	12	—	Brazil	Souza Bueno 1982
	13	+	Brazil	Souza Bueno 1982
<i>E. chromoptera</i>	11	+	Uruguay	Goñi and Abenante 1982
<i>E. cleopatra</i>	12 ♀	+	Perú	Kiauta and Boyes 1972
<i>E. connata connata</i>	13	+	Chile	Kiauta and Boyes 1972
<i>E. c. fusca</i>	13	—	Bolivia	Cumming 1964
	13	—	Guatemala	Cruden 1968
	13	+	Surinam	Kiauta 1979
	13	+	Brazil	Ferreira <i>et al.</i> 1979
	13	+	Brazil	Souza Bueno 1982
	13	+	Argentina	this work
<i>E. c. minuscula</i>	13	+	USA	Kiauta and van Brink 1978
<i>E. corallina</i>	13	+	Argentina	this work
<i>E. famula lativittata</i>	13	+	Brazil	Souza Bueno 1982
<i>E. fervida</i>	13	+	Jamaica	Cumming 1964
<i>E. juliana</i>	13	—	Brazil	Souza Bueno 1982
<i>E. justiniana</i>	13	+	Jamaica	Cumming 1964
<i>E. latimaculata</i>	13	+	Brazil	Ferreira <i>et al.</i> 1979
<i>E. lygaea</i>	13	+	Argentina	this work
<i>E. media</i>	11	+	Bolivia	Cumming 1964
	2n=22 ♀	+	Brazil	Kiauta and Boyes 1972
	11	+	Brazil	Ferreira <i>et al.</i> 1979
	11	+	Brazil	Souza Bueno 1982
	11*	+	Argentina	this work
<i>E. melanorubra</i>	13	+	Bolivia	Cumming 1964
	13	+	Venezuela	Kiauta and Boyes 1972
	13	+	Argentina	this work
<i>E. nigricans</i>	13	+	Uruguay	Goñi and Abenante 1982
	13	+	Argentina	this work
<i>E. ochraceae</i>	13	+	Argentina	this work
<i>E. pallida</i>	13	+	Brazil	Souza Bueno 1982
<i>E. paraguayensis</i>	12	+	Bolivia	Cumming 1964
	13	+	Surinam	Kiauta 1979
	12/13	—	Brazil	Souza Bueno 1982
<i>E. umbrata</i>	13	+	Bolivia	Cumming 1964
	13	+	Dominica	Cruden 1968
	13	+	Surinam	Kiauta 1979
	13	+	Brazil	Ferreira <i>et al.</i> 1979
	13	+	Argentina	this work
<i>E. unimaculata</i>	13	+	Bolivia	Cumming 1964
	2n=25 ♂	?	Surinam	Kiauta 1979

*neo-XY sex chromosomes in male.

Results

Eight out of the nine species here analyzed show a diploid number $2n=25$, $n=12+X$ (male): *Erythrodiplax atroterminata*, *E. connata fusca*, *E. corallina*, *E. lygaea*, *E. melanorubra*, *E. nigricans*, *E. ochracea* and *E. umbrata* (Figs. 1, 2, 3). They share also other mitotic and meiotic characteristics. At spermatogonial prometaphase and metaphase the chromosomes present their chromatids parallel with no primary constriction, evidencing their holokinetic nature (Figs. 1A, 2A). At this stage, a pair of very small chromosomes are readily distinguished (*m* chromosomes), while the X chromosome can't be recognized among the autosomes. All the species have a similar karyotype except for the size of the *m* bivalent and the X chromosome (see below).

At diplotene and diakinesis all the bivalents present only one chiasma at interstitial or

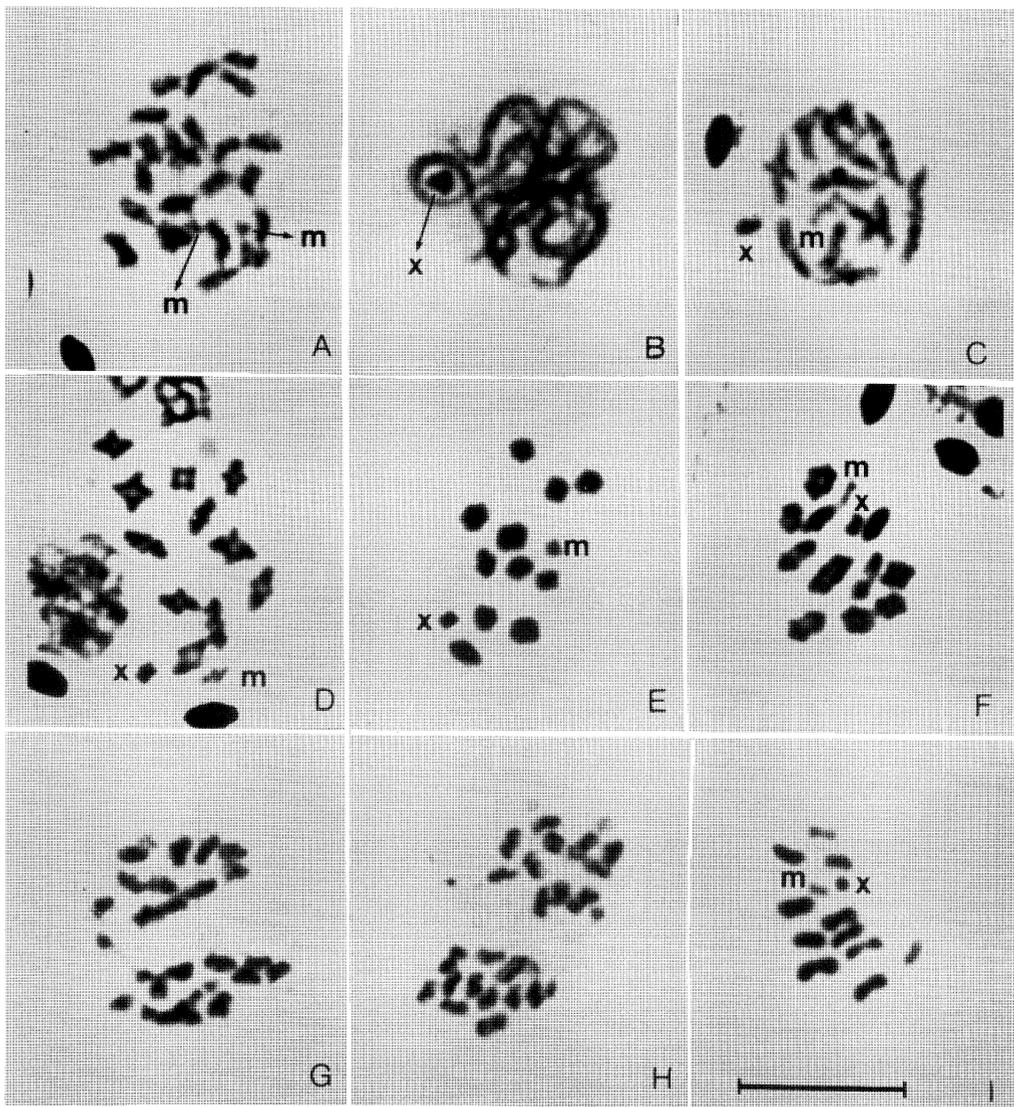


Fig. 1. *Erythrodiplax connata fusca* ($n=12+X$). A, Spermatogonial Prometaphase; B, Pachytene; C, Diplotene; D, Diakinesis; E, Metaphase I; F, Early Anaphase I; G, Middle Anaphase I; H, Late Anaphase I; I, Metaphase II. Bar: $10\ \mu\text{m}$.

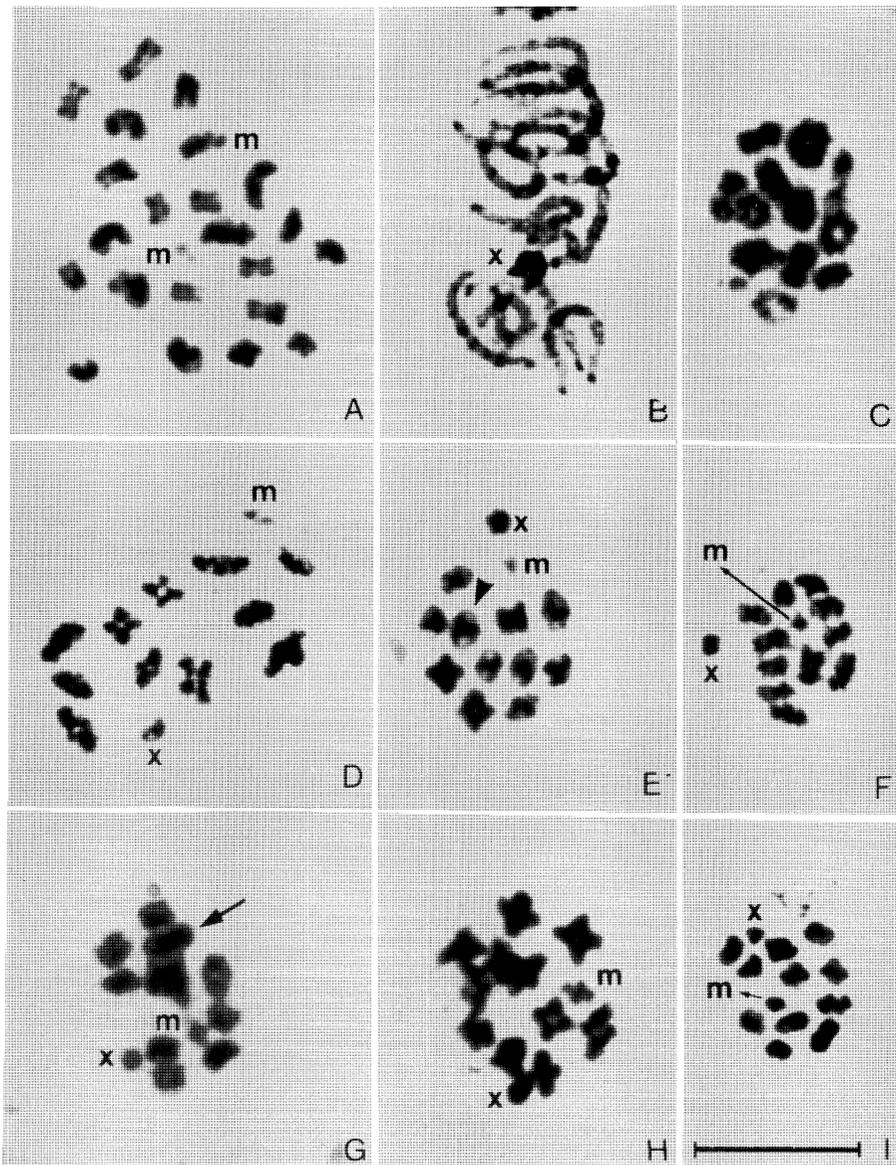


Fig. 2. *Erythrodiplax corallina* ($n=12+X$) (A-F), *E. umbrata* ($n=12+X$) (G), *E. lygaea* ($n=12+X$) (H) and *E. melanorubra* ($n=12+X$) (I). A, Spermatogonial Prometaphase; B, Pachytene (*bouquet*); C, Early Diakinesis with ring-shaped bivalents; D, Diakinesis; E, Metaphase I, arrowhead shows glasses-shaped bivalent; F, Prophase II; G, Diakinesis, arrow shows larger bivalent; H, Diakinesis; I, Diakinesis. Bar: 10 μm .

terminal position, and they decrease gradually in size, except for the *m* bivalent (Figs. 1C, D, Figs. 2D, H, I, Figs. 3B, E, H). In *E. umbrata*, however, one bivalent is a little larger (Fig. 2G). At anaphase I the X univalent divides equationally and synchronously with the autosomal bivalents (Figs. 1F, G, H). At metaphase II the X chromosome lies separated from the autosomal bivalents and near one pole (Figs. 3C, F, I), and at anaphase II it migrates precociously. In *E. connata fusca*, the X chromosome lies by the autosomes at metaphase II (Fig. 1I) and at anaphase II it migrates synchronously.

Erythrodiplax corallina shows some meiotic differences. At pachytene the bivalents

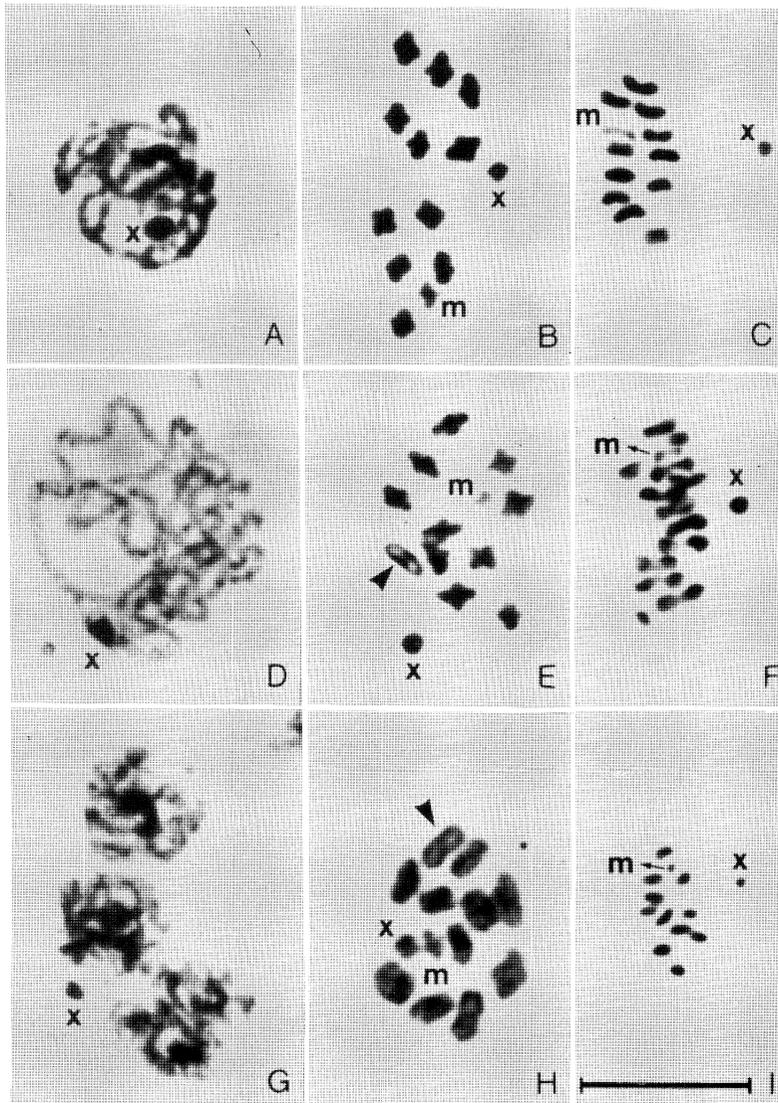


Fig. 3. *Erythrodiplax atroterminata* ($n=12+X$) (A–C), *E. nigricans* ($n=12+X$) (D–F) and *E. ochraceae* ($n=12+X$) (G–I). A, Pachytene; B, Diakinesis; C, Metaphase II; D, Pachytene; E, Diakinesis; F, Metaphase II; G, Zygotene; H, Prometaphase I; I, Metaphase II. Arrowheads show glasses-shaped bivalents. Bar: $10\ \mu\text{m}$.

arrange in a *bouquet* and some telomeric regions are positively heteropycnotic (Fig. 2B). At early diakinesis the bivalents with only one chiasma decondense and adopt a ring shape (Fig. 2C), while at middle—late diakinesis recondense and are again cross-shaped (Fig. 2D).

In *E. corallina*, *E. nigricans* and *E. ochraceae* some bivalents show a particular shape at prometaphase I and metaphase I. They are rod shaped with clear round areas at both ends (glasses-shaped) (Fig. 2E, Figs. 3E, H, arrows).

Erythrodiplax media: On the other hand, this species presents $2n=22$, $n=10+\text{neo-XY}$. At early prophase I no positively heteropycnotic body is detected (Fig. 4A). From diplotene onwards two larger bivalents and one *m* bivalent are distinguished, while the other bivalents decrease gradually in size (Figs. 4B, C, D); at diakinesis the largest bivalent (sex bivalent) is

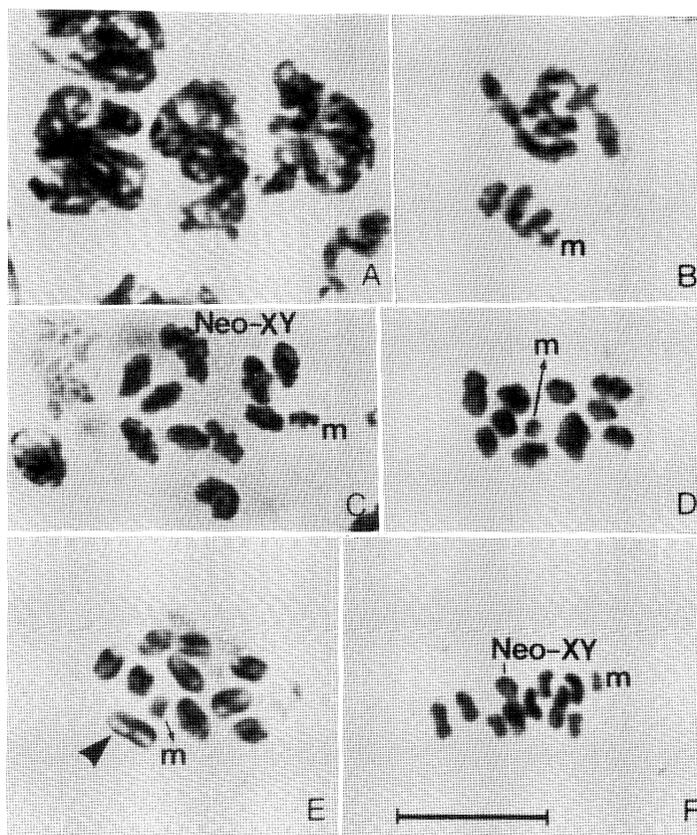


Fig. 4. *Erythrodiplax media* ($n=10+\text{neo-XY}$). A, Pachytene; B, Diplotene; C, Diakinesis; D, Late Diakinesis; E, Prometaphase I, arrowhead shows glasses-shaped bivalent; F, Metaphase II. Bar: $10\ \mu\text{m}$.

slightly heteromorphic (Fig. 4C). Like in the other species of the genus bivalents present only one chiasma; at prometaphase I and metaphase I most bivalents are “glasses-shaped” (Fig. 4E, arrow).

At all prophases II and metaphases II the sex chromosome shows its two chromatids of slightly different size, evidencing its post-reductional division (Fig. 4F). Both at anaphase I and anaphase II the sex chromosomes and the autosomes migrate synchronously.

Size and pycnotic relationships between the X chromosome and the m bivalent. During prophase I the pycnosis of the X chromosome varies among the species, while it is isopycnotic during the other meiotic stages. In *E. connata fusca* the X chromosome is isopycnotic at early prophase I and it is frequently observed out of the chromatin mass, while at pachytene and at early diakinesis it is slightly positive heteropycnotic (Figs. 1B, C). On the other hand, in *E. corallina* and *E. nigricans* the X chromosome is positively heteropycnotic until pachytene in the former and diakinesis in the latter (Fig. 2B, Figs. 3D, E). In *E. atroterminata*, *E. lygaea* and *E. ochraceae* it is isopycnotic or slightly positively heteropycnotic until diakinesis (Figs. 3A, G), while in *E. melanorubra* and *E. umbrata* it is always isopycnotic.

The *m* bivalent is generally isopycnotic, except in *E. connata fusca* in which it is negatively heteropycnotic during all meiotic stages (Figs. 1C–I) and in *E. nigricans*, in which it is slightly negative heteropycnotic until diakinesis (Fig. 3E).

The X chromosome and the *m* bivalent are the smallest elements of the complement in

meiosis, but their absolute and relative sizes vary among the species (Table 2). In *E. corallina* (Figs. 2E, F), *E. lygaea* (Fig. 2H), *E. umbrata* (Fig. 2G) and *E. nigricans* (Figs. 3E, F) the X chromosome has approximately twice the size of the X of the other four species (Figs. 1E, I, Fig. 2I, Figs. 3B, C, H, I). Nevertheless, a greater size variation of the *m* bivalent is observed among the species (Table 2).

Discussion

The taxonomic identification of the species of *Erythrodiplax* is very difficult due to the great intraspecific variation in colour and venational characters (Borror 1942). It is also very difficult to differentiate them chromosomically since circa 92% of the species share a chromosome number of $n=12+X$ (males), with a pair of *m* chromosomes and similar meiotic characteristics (Table 1). These chromosome features are present in almost all the species analyzed from Argentina: *Erythrodiplax corallina*, *E. lygaea* and *E. ochraceae* (which have not been previously analyzed), *E. melanorubra* and *E. nigricans* (with similar cytogenetic characteristics to those previously reported) (Cumming 1964, Goñi and Abenante 1982, Kiauta and Boyes 1972); and *E. atroterminata*, *E. connata fusca* and *E. umbrata* (species with polytypism for the *m* bivalent size).

Polytypism for the m bivalent size. Most karyotypic variation in populations of Odonata refer to the size of the *m* bivalent, which sometimes is absent since the size of the smallest bivalent is only slightly different from that of the second smallest autosomal bivalent (Agopian and Mola 1988, Cruden 1968, Ferreira *et al.* 1979, Kiauta 1968a, 1972, 1973, 1975, 1979, Kiauta and Kiauta 1980, 1983, Mola 1992, Mola and Agopian 1985, Souza Bueno 1982, Yadav 1979). In *E. atroterminata* Goñi and Abenante (1982) described the presence of 26 chromosomes in oogonial metaphases, without an *m* pair. In the Argentine males, the chromosome number is the same ($n=13$), but a relatively small *m* bivalent is observed. In *E. connata fusca* Cumming (1964) and Cruden (1968) reported $n=13$ without *m* chromosomes, in two Brazilian populations (Souza Bueno 1982) and in the individual from Surinam (Kiauta 1979) the *m* bivalent is a little larger than the X chromosome, while in the Brazilian populations analyzed by Ferreira *et al.* (1979), both elements have approximately the same size. In the individual here analyzed the *m* bivalent has not only half the size of the X chromosome, but it is negatively heteropycnotic during the course of meiosis. In *Erythrodiplax umbrata* all the populations present *m* chromosomes. In the sample from Surinam (Kiauta 1979), Brasil (Ferreira *et al.* 1979), and Dominica (Cruden 1968) both the X chromosome and the *m* bivalent are small and of similar size. In the Argentine individual, the size of the X chromosome and the *m* bivalent is also similar, but both are larger than in the other populations studied. Besides, the Argentine individual presents like the individuals from Surinam (Kiauta 1979) a slightly larger bivalent.

The size variation in *m* chromosomes could have their origin in unequal reciprocal translocations between one *m* chromosome and any other autosome of the complement. This rearrangement would produce a change in the relative sizes of both chromosomes implicated, but due to the karyotypic characteristics in Odonata, the change would only be detected in the *m* chromosomes. Another possibility would be that size variations are the result of duplications or deficiencies only affecting the *m* chromosomes.

Table 2. Comparison of the X chromosome and *m* bivalent sizes in the species of *Erythrodiplax* analyzed (expressed in arbitrary units)

	X Chromosome	<i>m</i> Bivalent
<i>E. corallina</i>	++++	+
<i>E. lygaea</i>	++++	+++
<i>E. nigricans</i>	++++	++
<i>E. umbrata</i>	++++	++++
<i>E. atroterminata</i>	++	++
<i>E. connata fusca</i>	++	+
<i>E. melanorubra</i>	++	++
<i>E. ochraceae</i>	++	++
<i>E. media</i>	-	+++

The polytypism for the *m* chromosome size could be associated to a speciation process, as it has been reported in *Calopteryx virgo* (Calopterygidae); in this species, different subspecies and even some populations of *C. virgo virgo* show great variation in the size of the *m* chromosomes (Kiauta 1968a, b).

Polytypism for the sex chromosome determination system in E. media. All the populations of *E. media* have 10 autosomal bivalents, but two sex chromosome determining systems have been encountered ($n=10+XO$ and $n=10+neo-XY$, in males) (Table 1). Ferreira *et al.* (1979) and Souza Bueno (1982) reported $n=10+XO$ and only one larger bivalent in males from Brazil. Kiauta and Boyes (1972) analyzed a female from Brazil with $2n=22$ and two larger pairs; at pachytene they observed a large sex bivalent ($XX?$, $neoXneoX?$) positively heteropycnotic. The Argentine males here analyzed are $n=10+neo-XY$ with two larger bivalents, being the first description of the neo-XY chromosome system in *Erythrodiplax*.

If we consider the ancestral chromosome complement of *Erythrodiplax* as $2n=26$ (females) and $2n=25$ (males), with a sex chromosome determination system XX/XO (female/male), the reduced karyotypes of *E. media* from Brazil would have originated through two autosomal fusions ($2n=21=20+XO$, males) while the karyotype of Argentine individuals is the result of one autosomal fusion, and another fusion between the X chromosome and one autosome ($2n=22=20+neo-XY$, male). Although these two karyotypes would have had an independent origin, it seems more parsimonious that one autosomal fusion is shared by both karyotypes, and the other fusion occurred between two autosomes in one instance, and between the X and an autosome in the other.

In *Crocothemis servilia* (Libellulidae) polytypism for the sex chromosome system have also been described. All populations from Japan are $n=11+neo-XY$ (males), while those from Continental Asia, Philippines and Taiwan are $n=12+X$ (males). Taking in account these differences, Kiauta (1983) considered the Japanese populations as a subspecies, *Crocothemis servilia mariannae*. The karyotypic analysis of populations of *E. media* from all its distribution will allow the detection of chromosomal races, and whether the polytypism is associated to a subspecific, or even a specific differentiation process.

Summary

Erythrodiplax is an American genus of dragonflies with a mainly neotropical distribution and with 19 species cited in Argentine. In this work 9 species have been chromosomically analyzed: *E. atroterminata*, *E. connata fusca*, *E. corallina*, *E. lygaea*, *E. media*, *E. melanorubra*, *E. nigricans*, *E. ochraceae* and *E. umbrata*. All of them, except *E. media*, have $n=12+X$ in males, with a similar karyotype and meiotic behaviour. Bivalents decrease gradually in size, except for the small *m* bivalent, whose size varies among the species. The X chromosome in *E. corallina*, *E. lygaea*, *E. nigricans* and *E. umbrata* is twice as large as in the other 4 species. On the other hand, *E. media* has $n=10+neo-XY$, *m* chromosomes, and its karyotype characterizes by the presence of two large bivalents (being the largest the heteromorphic neo-XY).

The genus *Erythrodiplax* presents a great karyotypic constancy, although polytypisms for the *m* chromosome size have been detected in *E. atroterminata*, *E. connata fusca* and *E. umbrata*, and for the sex chromosome determining system, in *E. media*. The chromosome rearrangements that probably originated these polytypisms, and their evolutionary importance are discussed.

Key words: Odonata, *Erythrodiplax*, holokinetic chromosomes, meiosis, neo-XY system, polytypisms.

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