Cytogenetics of seven species of dragonflies

A novel sex chromosome determining system in *Micrathyria ungulata*

L. M. MOLA*, A. G. PAPESCHI* and E. TABOADA CARRILLO

Genética, Depto. de Cs. Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes y Costanera Norte, 1428 Buenos Aires, Argentina

Mola, L. M., Papeschi, A. G. and Taboada Carrillo, E. 1999. Cytogenetics of seven species of dragonflies. A novel sex chromosome determining system in *Micrathyria ungulata*. — *Hereditas 131*: 147–153. Lund, Sweden. ISSN 0018-0661. Received March 11, 1999. Accepted September 22, 1999

More than 80% of the taxonomically described species of Anisoptera (Odonata) belong to the families Libellulidae and Aeshnidae. Here the chromosome complement and male meiotic behaviour of seven species of dragonflies of these families are analyzed. *Anax amazili* and *Coryphaeschna perrensi* are $2n = 27$, $n = 13 + X$, which is characteristic of Aeshnidae. Within Libellulidae, *Planiplax erythropyga*, *Micrathyria spuria* and *M. hesperis* have $2n = 25$, $n = 12 + X$, which corresponds to the modal chromosome number of the family. *Oligoclada laetitia* and *M. ungulata*, on the other hand, have a reduced chromosome complement ($n = 11 + X$ and $n = 10 + X_1X_2Y$, respectively). In *Micrathyria ungulata* an X_1X_2Y sex chromosome system is described, and its origin is discussed. This represents a new sex chromosome determining system in the order Odonata.

Liliana M. Mola, Genética, Depto. de Cs. Biológicas, Fac. Cs. Exactas y Naturales, Univ. de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina. E-mail: limola@bg.fcen.uba.ar

Most species of the suborder Anisoptera (Odonata) belong to the family Libellulidae, and they differ widely in shape, size and colour (FRASER 1957). This family is the best studied from a cytogenetical point of view, and the chromosome number of more than 270 species is known (MOLA 1992; PRASAD and THOMAS 1992; SANDHU and WALIA 1994, 1995; SUZUKI et al. 1991). The modal chromosome number is $n = 13$ (84% of the species), and there are very frequently a pair of *m* chromosomes present.

Aeshnidae is another large family of Anisoptera. It includes large and vigorous dragonflies and it has representatives in almost every kind of freshwater environments (CARLE 1982). Approximately 60 species have been cytogenetically analyzed (MOLA 1992; SANDHU and MALHOTRA 1994), and the modal haploid number is $n = 14$ (70,7% of the species).

Karyotype evolution in Odonata has mainly occurred through fusions and fragmentations of holokinetic chromosomes. Fusions between autosomes and/or fusions between the X chromosome and an autosome have been described; fragmentations, on the other hand, have only been reported for autosomes (AGOPIAN and MOLA 1984; KIAUTA 1972; MOLA 1992, 1995).

The most frequent sex chromosome determining system in the order is X0/XX (male/female) and the X chromosome is generally one of the smallest elements of the complement. Until present, the only derived system described is the neo-XY; in most species the sex chromosome pair is homomorphic or slightly heteromorphic until diakinesis, and only in a few species the neo-XY is clearly heteromorphic (MOLA 1992, 1996; MOLA and PAPESCHI 1994).

Here the meiotic behaviour of five species of Libellulidae (*Oligoclada laetitia*, *Planiplax erythropyga*, *Micrathyria spuria*, *M. hesperis* and *M. ungulata*) and two of Aeshnidae (*Anax amazili* and *Coryphaeschna perrensi*) from Argentine are analyzed. Our results on *Oligoclada* and *Micrathyria* are compared with previous reports, and the origin of a new sex chromosome determining system in *M. ungulata* ($X_1X_2Y/X_1X_1X_2X_2$) is described and discussed.

MATERIALS AND METHODS

Adult males captured in the field were fixed in 3:1 (absolute ethanol:glacial acetic acid); afterwards, testes were dissected out and kept in ethanol 70% at 4°C. Slides were made by the squash method in propionic haematoxylin 2%.

Provenance and number of individuals analyzed are as follows:

Libellulidae – *Oligoclada laetitia*, 7 males from Tigre (Buenos Aires Province); *Planiplax erythropyga*, 3 males from El Palmar National Park (Entre Ríos Province); *Micrathyria spuria*, 1 male from El Palmar National Park (Entre Ríos Province) and 1 male from Garruchos (Corrientes Province); *M. ungulata*, 2 males from El Palmar National Park (Entre Ríos Province); *M. hesperis*, 4 males from Montecarlo (Misiones Province).

* Fellow of Argentine Research Council (CONICET).

Aeshnidae – *Anax amazili*, 1 male from El Palmar National Park (Entre Ríos Province); *Coryphaeschna perrensi*, 1 male from Iguazú National Park (Misiones Province).

RESULTS

Males of *Oligoclada laetitia* possess 2n = 23, n = 11 + X. At spermatogonial prometaphases and metaphases a pair of small *m* chromosomes and a pair of large autosomes (almost twice the size of the next chromosome pair) are distinguished. At meiotic prophase the X chromosome is isopycnotic (Fig. 1A and B). At diakinesis and metaphase I a large bivalent and a negatively heteropycnotic *m* bivalent, slightly larger than the X chromosome, are detected; the remaining nine bivalents decrease gradually in size. All bivalents present only one chiasma subterminally located (Fig. 1B and C). At anaphase I bivalents divide equationally, and the X migrates synchronously with the autosomes (Fig. 1D and E). At metaphase II the *m* chromosome continues negatively heteropycnotic; the X chromosome is closer to one pole (Fig. 1F) and migrates precociously at anaphase II.

Planiplax erythropyga has 2n = 25, n = 12 + X. At spermatogonial prometaphase a minute pair of *m* chromosomes and a large pair of autosomes are distinguished (Fig. 2A). At prophase I the X chromosome is isopycnotic, and all bivalents present one terminal or subterminal chiasma (Fig. 2B). A large bivalent and a minute *m* bivalent are observed from diplotene to metaphase I; the remaining ten bivalents decrease gradually in size, and the X chromosome is approximately half the size of the smallest bivalent (Fig. 2B). The meiotic behaviour of the chromosomes follows the pattern previously described (Fig. 2C).

Micrathyria spuria and *M. hesperis* are both n = 12 + X, and meiosis proceeds as in the species already described. In *M. spuria* the X chromosome is positively heteropycnotic until pachytene; at diplotene and diakinesis all bivalents present one chiasma and decrease gradually in size, except the *m* bivalent that is clearly smaller and negatively heteropycnotic; the X chromosome is small, but larger than the *m* bivalent (Fig. 2D). In *M. hesperis* the X chromosome is

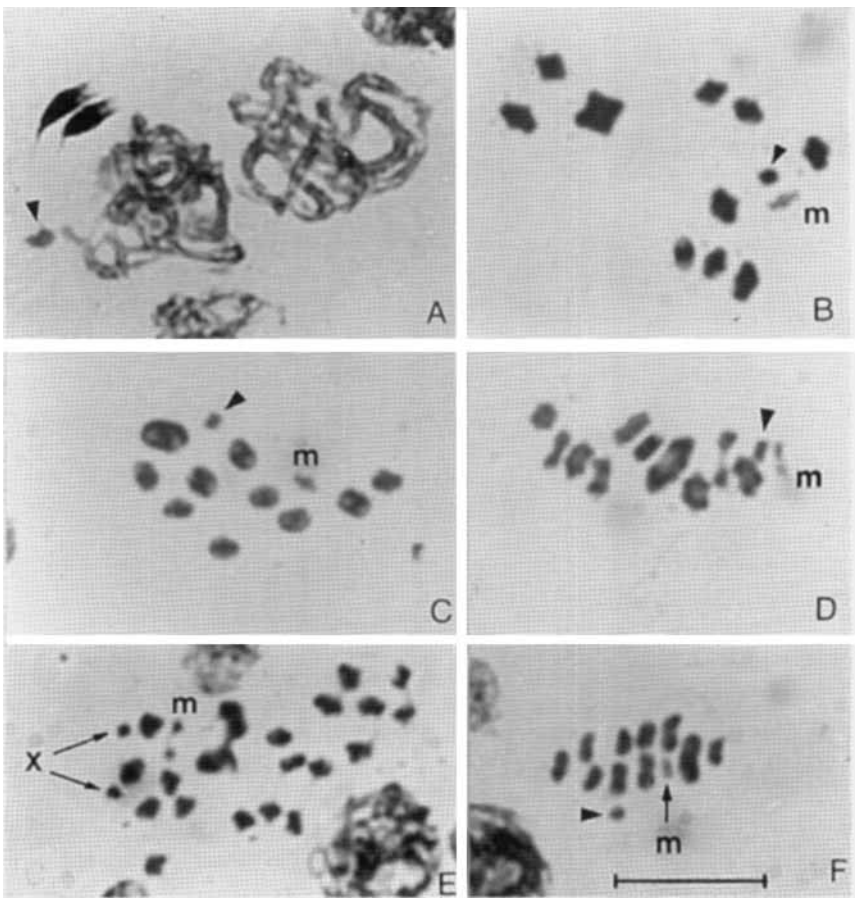

Fig. 1. A–F. Meiosis in *Oligoclada laetitia* (n = 11 + X). **A** Pachytene. **B** Diakinesis. **C** Prometaphase I. **D** Early Anaphase I. **E** Anaphase I. **F** Metaphase II. Arrowheads point the X chromosome, and m marks the m chromosomes. Bar = 10 μm.

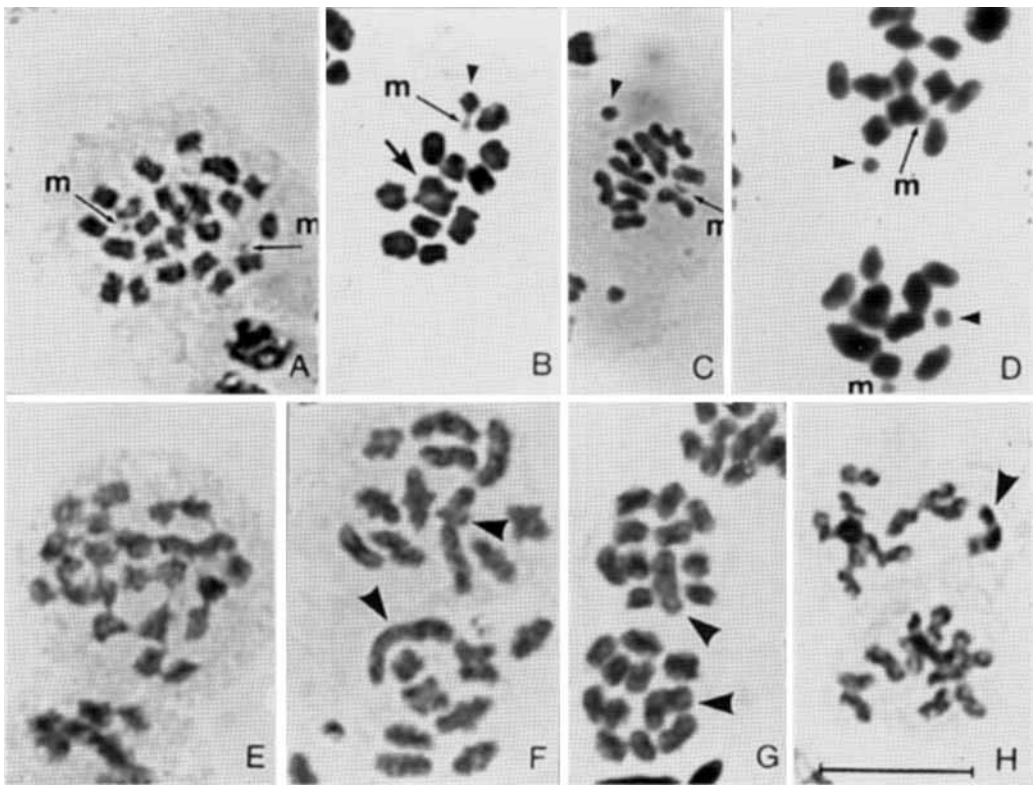

Fig. 2. A–H. Meiosis in *Planiplax erythropyga* (n = 12 + X) (**A–C**), *Micrathyria spuria* (n = 12 + X) (**D**) and *M. ungulata* (n = 10 + X_1X_2Y) (**E–H**). **A** Spermatogonial prometaphase. **B** Diakinesis, arrow points the largest bivalent. **C** Metaphase II. **D** Diakinesis. In (**B–D**) small arrowheads point the X chromosome. **E** Spermatogonial prometaphase. **F** Diakinesis. **G** Prometaphase I. **H** Prophase II. In (**F–H**) arrowheads point the sex trivalent. Bar = 10 μm.

always isopycnotic; bivalents decrease gradually in size, except the *m* bivalent and the X chromosome which are the smallest elements of the complement.

Micrathyria ungulata, on the other hand, has 2n = 23 (Fig. 2E). No positively heteropycnotic body is distinguished at early prophase, and at diakinesis and metaphase I ten homomorphic bivalents (with only one chiasma) and one lineal trivalent are observed (Fig. 2F and G); neither an *m* bivalent nor an X chromosome are present. The trivalent always presents two chiasmata, one generally terminal and the other subterminal (Fig. 2F, 4E). The three chromosomes of the trivalent are of different size; the larger one is always placed between the other two, and the medium sized chromosome presents a positively heteropycnotic region at the free telomeric end (not involved in the chiasma); this region represents the original X chromosome (Fig. 2F, 4E). Among bivalents one is slightly larger, and the remaining decrease gradually in size. At anaphase I all chromosomes divide synchronously and equationally, and in all cells at prophase II ten semibivalents and one semitrivalent are observed (Fig. 2H).

Anax amazili and *Coryphaeschna perrensi* (Aeshnidae) present 2n = 27, n = 13 + X. In the former the X chromosome is positively heteropycnotic until pachytene (Fig. 3A), while in *C. perrensi* it is isopycnotic or slightly positive heteropycnotic from leptotene to pachytene (Fig. 3D). In both species the X and the *m* bivalent are the smallest elements of the complement (Fig. 3B and E); the remaining bivalents decrease gradually in size and present always one chiasma. In both species chromosomes divide equationally and synchronously at anaphase I, giving rise to cells at meiosis II with 14 chromosomes (Fig. 3C and F).

DISCUSSION

The American genera *Oligoclada* and *Planiplax* (Libellulidae) are represented in Argentine by *Oligoclada laetitia*, *O. haywardii* and *Planiplax erythropyga* (Carle 1982, Rodrigues Capítulo 1992, Muzón and Von Ellenrieder, 1998). All the species of these genera cytogenetically analyzed share a sex chromosome determining system X0/XX (male/female) (Table 1).

The population of *O. laetitia* analyzed here (n = 11 + X) differs karyotypically from the Brazilian specimen studied by Souza Bueno (1982). She re-

ported a mosaicism in the specimen with most cells having n = 10 + X, and a few cells with n = 11 + X due to the presence of two *m* bivalents. Both the male from Brazil and our specimens possess a large autosomal bivalent and a small *m* bivalent. With the present information it is not possible to explain the origin of the different diploid number between the two samples. The reduced chromosome complement of *O. laetitia* with respect to the family modal number (n = 12 + X) probably originated in an autosomal fusion, which gave rise to the large autosomal pair.

Planiplax erithropyga as well as *P. sanguiniventris*, the only species of the genus cytogenetically analyzed before this work, present the modal number of Libellulidae (n = 12 + X) (Table 1).

From the South American genera *Anax* and *Coryphaeschna*, *Anax amazili*, *A. longipes*, *Coryphaeschna adnexa*, *C. luteipennis luteipennis* and *C. perrensi* have been cited in Argentine (CARLE 1982, RODRIGUES CAPÍTULO 1992, MUZÓN and VON ELLENRIEDER, 1998). With our results on *Anax amazili* and *Coryphaeschna perrensi*, all these taxa have been cytogenetically analyzed. Almost all the species of these genera present n = 13 + X; exceptions are *A. guttatus* (n = 7 + X) and *C. viriditas* (n = 12 + X) (Table 2).

A novel sex chromosome system in Micrathyria ungulata

The American genus *Micrathyria* is represented in our country by 16 species (RODRIGUES CAPÍTULO 1992, MUZÓN and VON ELLENRIEDER, 1998), ten of which have been previously studied from a cytogenetical point of view. Our results on *Micrathyria hesperis* and *M. spuria* (2n = 25, n = 12 + X) agree with previous reports on these species (FERREIRA et al. 1979, CUMMING 1964).

The two individuals of *M. ungulata* here studied present a reduced chromosome number (2n = 23) and a derived sex chromosome system; the original X chromosome is fused to an autosome and is involved in the trivalent observed at meiosis. These males present a sex chromosome system X_1X_2Y, which originated through two fusion events very probably in the following sequence (Fig. 4). First, the free small X chromosome became fused to an autosome giving rise to a neo-XY system (2n = 24, n = 11 + neo-XY). Later, the "neo-Y" became fused to one member of another autosomal pair originating a new large chromosome, which we refer to as "Y" chromosome. This large "Y" chromosome is always placed in a middle position in the trivalent, and after its

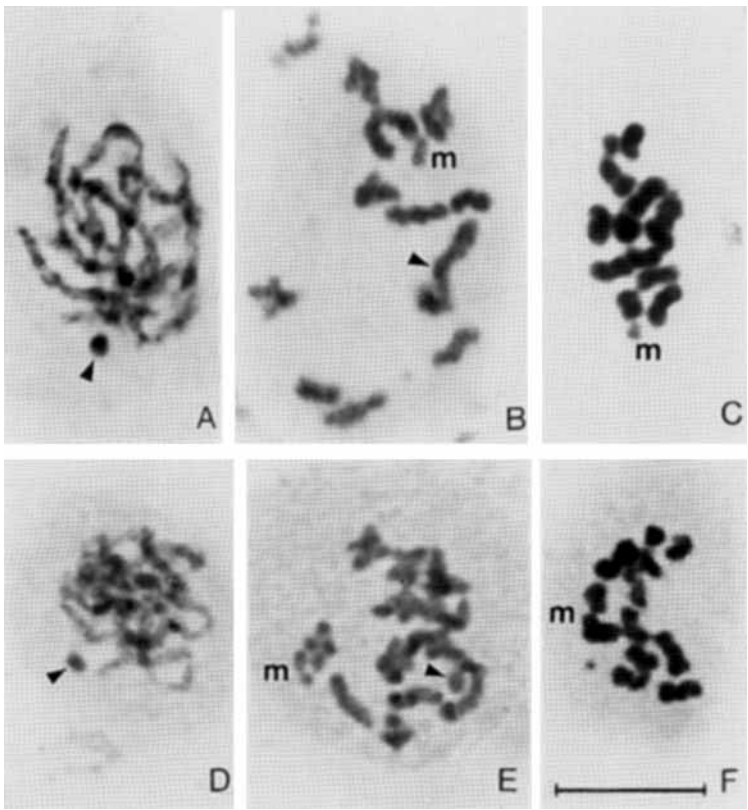

Fig. 3. A–F. Meiosis in *Anax amazili* (n = 13 + X) (**A–C**) and *Coryphaeschna perrensi* (n = 13 + X) (**D–F**). **A** and **D** Pachytene. **B** and **E** Diakinesis. **C** and **F** Prometaphase II. Arrowheads point the X chromosome. Bar = 10 μm.

Table 1. *Karyotypic characteristics and provenance of the species of Oligoclada, Planiplax and Micrathyria (Libellulidae) cytogenetically analyzed*

Species	n (male)	m	Provenance	References
Oligoclada amphinome	12+X0	+	Surinam	KIAUTA, 1979
*O. laetitia**	10+X0	+	Brazil	SOUZA BUENO, 1982
	11+X0	+	Argentine	this work
O. monosticha	11+X0	+	Brazil	FERREIRA et al., 1979
O. pachystigma	11+X0	–	Brazil	SOUZA BUENO, 1982
*Planiplax erythropyga**	12+X0	+	Argentine	this work
P. sanguiniventris	13	+	Guatemala	CRUDEN, 1968
*Micrathyria artemis**	12+X0	+	Brazil	FERREIRA et al., 1979; SOUZA BUENO, 1982
*M. atra**	13	+	Bolivia	CUMMING, 1964
*M. catenata**	12+X0	+	Brazil	SOUZA BUENO, 1982
*M. didyma**	13	+	Jamaica	CUMMING, 1964
*M. eximia**	12+X0	+	Surinam	KIAUTA, 1979
Micrathyria sp. nr. eximia	11	–	Bolivia	CUMMING, 1964
M. hageni	13	+	Jamaica	CUMMING, 1964
*M. hesperis**	12+X0	+	Brazil	FERREIRA et al., 1979
	12+X0	+	Argentine	this work
*M. hypodidyma**	11+X0	+	Brazil	SOUZA BUENO, 1982
	12+X0	+	Argentine	AGOPIAN and MOLA, 1988
M. iheringi	12	+	Bolivia	CUMMING, 1964
M laevigata	13	+	Bolivia	CUMMING, 1964
	12+X0	+	Brazil	KIAUTA and BOYES, 1972
*M. longifasciata**	11+neo-XY	+	Argentine	AGOPIAN and MOLA, 1988
*M. ocellata dentiens**	13	+	Bolivia	CUMMING, 1964
	12+X0	–	Brazil	SOUZA BUENO, 1982
*M. spuria**	13	+	Bolivia	CUMMING, 1964
	12+X0	+	Argentine	this work
M. starwisky	2n = 23	–	Brazil	SOUZA BUENO, 1982
Micrathyria sp. ungulata group	12	–	Bolivia	CUMMING, 1964
*M. ungulata**	$10+X_1X_2Y$	–	Argentine	this work

* species reported for Argentine.

Table 2. *Karyotypic characteristics and provenance of the species of Anax and Coryphaeschna (Aeshnidae) cytogenetically analyzed*

Species	n (male)	m	Provenance	References
*Anax amazili**	13+X0	+	Argentine	This work
A. concolor	13+X0	+	Surinam	KIAUTA, 1979
A. guttatus	7+X0	+	Nepal	KIAUTA and KIAUTA, 1982a
A. inmaculifrons	13+X0	+	India	SANGAL and TYAGI, 1982
A. imperator	13+X0	+	France	KIAUTA, 1965
	13+X0	–	Kenya	WASSCHER, 1985
A. junius	14	+	USA	CRUDEN, 1968; KIAUTA, 1972
	14	–	USA	CRUDEN, 1968
*A. longipes**	14	+	USA	CRUDEN, 1968
A. nigrofasciatus nigrolineatus	13+X0	+	Nepal	KIAUTA, 1975
A. parthenope julius	13+X0	+	Japan	KIAUTA and KIAUTA, 1982b; SUZUKI and SAITOH, 1990
	13+X0	+	China	ZHU and WU, 1986
*Coryphaeschna adnexa**	14	–	Bolivia	CUMMING, 1964
*C. l. luteipennis**	13+X0	+	Brazil	FERREIRA et al., 1979
*C. perrensi**	13+X0	+	Argentine	this work
C. viriditas	12+X0	–	Surinam	KIAUTA, 1979

* species reported for Argentine.

correct segregation (to give balanced gametes) this "Y" chromosome will always be inherited to the male progeny. The "neo-X" is the medium sized chromosome of the trivalent, and we refer to it as X_1 since it bears the original X. Finally, the autosome not directly involved in the fusion events co-segregates with the X_1, and hence we consider it as X_2; both X_1 and X_2 are always inherited to the female progeny.

Micrathyria is a genus cytogenetically heterogeneous; approximately 60% of the analyzed species present the modal karyotype of the family (n = 12 + X). All the other species present a reduced chromosome number. The Brazilian male of *M. hypodidyma* presents an autosomal fusion in homozygous condition (n = 11 + X); in *M. longifasciata* an X- autosome fusion originated the neo-XY system (n = 11 + neo-XY), and in *M. ungulata*, both an X- autosome fusion and an autosome- autosome fusion have occurred (n = 10 + X_1X_2Y). In the remaining four species with a reduced chromosome complement, nothing can be said with respect to the chromosomes involved in the fusion events (Table 1).

Since only two individuals of *M. ungulata* have been analyzed, it can not be ascertained whether the rearrangement is fixed at the population or species level. In any of these circumstances, it constitutes a new sex chromosome determining system in the order Odonata.

The X_1X_2Y sex chromosome determining system is unusual in organisms with holokinetic chromosomes. This sex chromosome mechanism has been previously reported in two populations of *Cacopsylla sorbi* (2n = 18 + neo X_1X_2Y) and *C. mali* (2n = 20 + neo X_1X_2Y) (Homoptera, Psyllidae). In both species, populations with a neo-XY sex chromosome system have also been encountered (GROZEVA and MARYANSKA-NADACHOWSKA 1995). Evidently this case has also been derived from the neo-XY system. The neo-XY system has evolutionary potential to develop further and we may expect to find it in other insect groups as well.

ACKNOWLEDGEMENTS

The authors wish to thank to Lic. Susana Agopian for her contribution in the collection of the material and laboratory work, to Dr. Arturo Wulff for critical reading of the manuscript, and to Dr. J. H. Hunziker and Dr. Lidia Poggio for their continuous encouragement. We are also

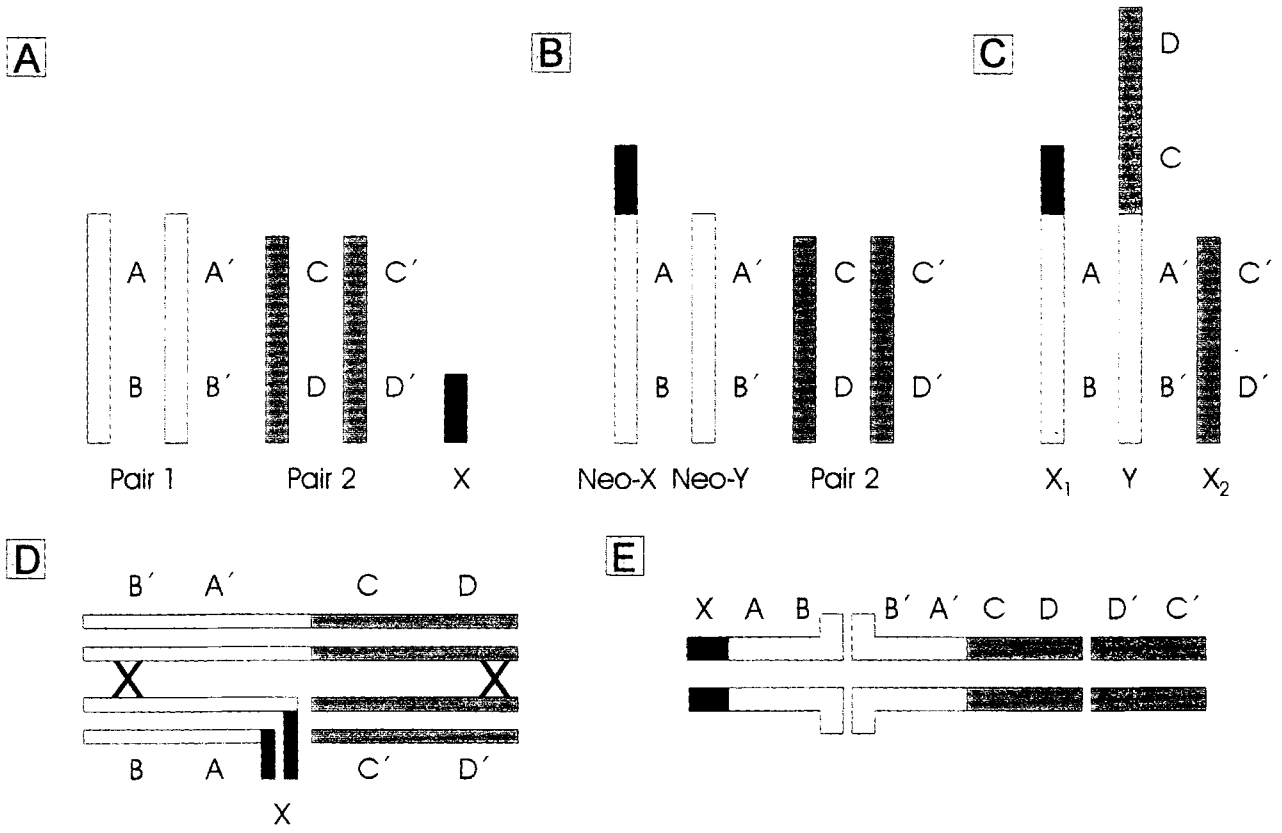

Fig. 4. A–E. Origin of the X_1X_2Y sex chromosome system in *Micrathyria ungulata*. **A** Ancestral chromosomes. **B** First fusion event (X- autosome fusion), giving rise to a neo- XY system. **C** Second fusion event (autosome- autosome fusion), giving rise to the X_1X_2Y system. **D** Pachytene pairing of the sex trivalent. **E** Diakinesis configuration of the sex trivalent.

indebted to Dr. J. Muzón, Dr. A. Rodrigues Capítulo and Dr. G. Jurzitza for taxonomic determination of the specimens. We wish to thank the anonymous referees and Dr. A. Saura for their suggestions to improve the manuscript. This work was supported by grants from UBA (TW01) and CONICET (PIP 4217) to Dr. L. Poggio and Dr. L. Mola, and Fundación Antorchas to Dr. Alba G. Papeschi.

REFERENCES

Agopian SS and Mola LM, (1984). An exceptionally high chromosome number in Orthemis nodiplaga Karsh (Anisoptera: Libellulidae). Notul. Odonatol. 2: 45.

Agopian SS and Mola LM, (1988). Intra and interspecific karyotype variability in five species of Libellulidae (Anisoptera, Odonata). Caryologia 41: 69–78.

Carle FL, (1982). A contribution to the knowledge of the Odonata. Ph.D. thesis, Virginia Polytechn. Inst. and St. Univ.

Cruden RW, (1968). Chromosome numbers of some North American dragonflies (Odonata). Can. J. Genet. Cytol. 10: 200–214.

Cumming RB, (1964). Cytogenetic studies in the order Odonata. Ph.D. thesis, Univ. Texas, Austin.

Ferreira A, Kiauta B and Zaha A, (1979). Male germ cell chromosomes of thirty-two Brazilian dragonflies. Odonatologica 8: 5–22.

Fraser FC, (1957). A reclassification of the Order Odonata. Roy. Zool. Soc., New South Wales, Sydney.

Grozeva SM and Maryanska-Nadachowska A, (1995). Meiosis of two species of Cacopsylla with polymorphic sex chromosomes in males (Homoptera, Psyllidae). Folia biologica 43: 93–98.

Kiauta B, (1965). The chromosome behaviour in spermatogenetic meiosis of Anax imperator Leach (Aeshnidae). Tombo 7: 18–20.

Kiauta B, (1972). Synopsis of the main cytotaxonomic data in the Order Odonata. Odonatologica 1: 73–102.

Kiauta B, (1975). Cytotaxonomy of dragonflies, with special reference to the Nepalese fauna. Nepal Research Center, Kathmandu.

Kiauta B, (1979). The karyotypes of some Anisoptera from Surinam. Odonatologica 8: 267–283.

Kiauta B and Boyes JW, (1972). Cytology of ten South American Libellulidae with cytophylogenetic considerations on the genera Orthemis Hagen and Erythrodiplax Brauer (Odonata, Anisoptera). Genetica 43: 407–421.

Kiauta B and Kiauta M, (1982a). The chromosome numbers of sixteen dragonfly species from Arun Valley, Eastern Nepal. Notul. Odonatol. 1: 143–145.

Kiauta B and Kiauta M, (1982b). List of species, with chromosome numbers and preliminary notes on the karyotypes of the Odonata, collected in May, 1979 and August, 1980 by the members of the Kansai Research Group of Odonatology, and examined by B. and M. Kiauta. Report for the Kansai Research Group of Odonatology, Osaka, Mimeographed. Soc. Int. Odonatol. Utrecht.

Mola LM, (1992). Estudios cromosómicos en libélulas (Orden Odonata). Tesis de Doctorado, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires.

Mola LM, (1995). Post-reductional meiosis in Aeshna (Aeschnidae, Odonata). Hereditas 122: 47–55.

Mola LM, (1996). Meiotic studies in nine species of Erythrodiplax (Libellulidae, Odonata): neo-XY sex chromosome system in E. media. Cytologia 61: 349–357.

Mola LM and Papeschi AG, (1994). Karyotype evolution of Aeshna (Aeschnidae, Odonata). Hereditas 121: 185–189.

Muzón J and Von Ellenrieder N, (1998). Odonata. In Biodiversidad de Artrópodos argentinos, una perspectiva biotaxonómica (eds JJ Morrone and S Coscarón), Ediciones Sur, La Plata, Argentina, p. 14–25.

Prasad R and Thomas KI, (1992). C-band pattern homogeneity in dragonflies (Odonata). Caryologia 45: 57–68.

Rodrigues Capítulo A, (1992). Los Odonata de la República Argentina (Insecta). In: Fauna de Agua dulce de la República Argentina, Vol. 34, Fascículo 1 (ed. ZA de Castellanos), PROFADU-CONICET, La Plata, Argentina.

Sandhu R and Malhotra I, (1994). Karyological studies of four Aeschnid dragonflies from states Jammu and Kashmir and Himachal Pradesh (India). Adv. Oriental Odonatology (Ed. V. K. Srivastava): 111–115.

Sandhu R and Walia GK, (1994). Karyological study on four female libellulids from Assam (India). Fraseria (NS) 1: 11–15.

Sandhu R and Walia GK, (1995). A note on the karyotype of Potamarcha conger (Anisoptera, Libellulidae). Chr. Inf. Serv. 58: 24–25.

Sangal SK and Tyagi BK, (1982). The spermatocyte chromosomes of Anax inmaculifrons Rambur from India (Anisoptera, Aeschnidae). Notul. Odonatol. 1: 154–155.

Souza Bueno AM de, (1982). Estudos cromossômicos na ordem Odonata. M. Sc. thesis, Univ. Estad. Paulista, Rio Claro.

Suzuki KJ and Saitoh K, (1990). A revised chromosome study of Japanese Odonates (I). Chromosomes of 14 species belonging to nine families. Sci. Rep. Hirosaki Univ. 37: 38–49.

Suzuki KJ, Saitoh K and Sawano J, (1991). Male germ-line chromosomes of Orthetrum poecilops miyajimaensis Yuki et Doi, 1938 (Libellulidae: Odonata). Tombo 34: 29–30.

Wasscher M, (1985). The karyotypes of some dragonflies from Kenya and Sudan. Notul. Odonatol. 2: 105–106.

Zhu H-Q and Wu J-I, (1986). Notes on the male germ cell karyotypes of some Odonata from the Shanxi Province, China. Notul. Odonatol. 2: 118–120.