Karyotype evolution in *Aeshna* (Aeshnidae, Odonata)

L. M. MOLA and A. G. PAPESCHI*

Lab. de Genética, Depto. Cs. Biológicas, Fac. Cs. Exactas y Naturales, Universidad de Buenos Aires, Int. Güiraldes y C. Norte. (1428) Buenos Aires, Argentina

The haploid DNA content of *Aeshna confusa* (2n = 27, n = 13 + XO, male), *A. bonariensis* (2n = 26, n = 12 + neo-XY, male) and *A. cornigera planaltica* (2n = 16, n = 7 + neo-XY, male) has been determined (2.16 ± 0.16 pg, 1.81 ± 0.17 pg, and 2.08 ± 0.08 pg, respectively). Despite the differences in chromosome size and number, differences in DNA content between species are not significant. The karyotypic analysis of *Aeshna* species leads to the conclusion that fusions between autosomes or autosome and the sex chromosome, are the only chromosome rearrangements that occurred during evolution. In the species here studied, fusions have taken place with a minimal loss of DNA; however, other species of the genus show important differences in genome size, which cannot only be justified by fusion events.

Lilianna M. Mola, Genética, Depto. Cs. Biológicas, Fac. Cs. Exactas y Naturales, Universidad de Buenos Aires, Int. Güiraldes y C. Norte, 1428, Buenos Aires, Argentina

The comparison of DNA content of related species is of great value in any analysis of karyotype evolution. This is particularly true in holokinetic systems, in which the lack of a localized centromere restricts the number of karyotypes characteristic possible to consider, and also because fusions and fragmentations are the principal chromosome rearrangements observed (Sybenega 1972; White 1973). In spite of this, DNA content has been seldom determined in insects with holokinetic chromosomes (Hughes-Schrader and Schrader 1956; Schrader and Hughes-Schrader 1956, 1958; Schreiber et al. 1972; Mello et al. 1986; Papeschi 1988, 1991) and are almost absent in Odonata (Cumming 1964; Petrov and Aljeshin 1983; Petrov et al. 1984). In the genus *Aeshna*, twenty-five species have been cytogenetically analyzed up to date, and the diploid number varies in males between 16 (14 + neo-XY) and 27 (26 + XO). The modal number in males is 2n = 27 and the most common sex chromosome system is XX/XY (female/male) (Makalovskaja 1940; Oksala 1943; Cumming 1964; Cruden 1968; Kiauta 1969; Hung 1971; Kiauta 1971, 1973; Kiauta and Kiauta 1980, 1982).

In the present work DNA content has been determined in *Aeshna confusa* (2n = 26 + XO, male), *A. bonariensis* (2n = 24 + neo-XY) and *A. cornigera planaltica* (2n = 14 + neo-XY), and the mechanisms of karyotype evolution in the genus are discussed.

**Material and methods**

The individuals analyzed in the present study are: *Aeshna confusa* Rambur, 3 males from Ciudad Universitaria (Capital Federal) and 1 male from Otamendi (Pdo. de Campana), Buenos Aires Province, Argentina; *A. bonariensis* Rambur, 5 males from Ciudad Universitaria (Capital Federal), Buenos Aires Province, Argentina, and 2 males from Real de San Carlos, Colonia Department, Uruguay; and *A. cornigera planaltica* Calvert, 2 males from Parque Nacional Iguazú, Misiones Province, Argentina. The gonads were fixed in 3.1 (absolute ethanol:glacial acetic acid) and kept at 4°C.

Cytological preparations for DNA measurements of the three species were obtained by squashing a piece of testis in 60% acetic acid; the coverslip was then removed by the dry-ice method and slides were air-dried. DNA charges were estimated by using the Feulgen reaction in conjunction with scanning densitometry. The Feulgen reaction was carried out as follows: air-dried slides were rinsed 3 times for 10 min each in distilled water, immersed in 5N HCl for 20 min at 25 ± 1°C, washed 3 times

---

* Fellow of the Argentine Research Council (CONICET).
for 10 min each in distilled water, stained with the Schiff reagent for 2 h, and washed 3 times 10 min each in SO₂ water. The optimal hydrolysis time was previously determined through the calculation of a hydrolysis curve. Although the material had different fixation times, a previous analysis has shown that differences in DNA measurements in the same species with different fixation times are statistically non-significant (Mola 1992).

Only spermatid nuclei which have just begun to elongate, were measured. The number of slides per individual (from 1 to 3) and the number of individuals per species varied according to the number of cells and individuals suitable for this study. Chicken erythrocytes were used as standard of reference for cells and individuals suitable for this study. Chicken erythrocytes in non-significant the detection and correction of any differences in chicken erythrocytes is 2.5 pg. Twenty spermatid nuclei and twenty chicken erythrocyte nuclei were measured in each slide, and slides were coded and randomized prior to scoring. The measurements were conducted with a cytospectrophotometer Zeiss MPC 64 at a wavelength of 570 nm, attached to a Kontron MOP-Videoplan computer, with the program APAMOS 99.

Results

Chromosome complement

The chromosome complement and meiotic behaviour of the three species have been previously described in detail (Mola 1992). The principal features of the karyotype of these species can be summarized as follows: Aeshna confusa (2n = 27, n = 13 + XO) has a larger bivalent and a very small one (m bivalent), while the other eleven bivalents decrease gradually in size. The X chromosome is larger than the m bivalent and of similar size to the second smallest one (Fig. 1a).

In A. bonariensis (2n = 26, n = 12 + neo-XY), the autosomal bivalents decrease gradually in size, except for the very small m bivalent; the neo-XY is the largest bivalent and is heteromorphic (Fig. 1b). A. cornigera planaltica (2n = 16, n = 7 + neo-XY) has a reduced chromosome number and larger chromosomes than the former two species. The autosomal bivalents can be grouped in five large and two small ones, while the sex chromosome bivalent is the smallest one and noticeably heteromorphic (Fig. 1c).

Table 1. Analysis of variance of DNA values in Aeshna confusa, A. bonariensis, and A. cornigera planaltica

| Item                      | df | VR  | P   |
|---------------------------|----|-----|-----|
| Between species           | 2  | 0.436 | 0.66 |
| Between individuals species | 10 | 1.517 | 0.28 |
| Between slides within individuals | 8  | 210.96 | 0.00 |
| Error                     | 399 |     |     |

DNA content

The haploid DNA content of A. confusa, A. bonariensis, and A. cornigera planaltica is 2.16 ± 0.16 pg, 1.81 ± 0.17 pg and 2.08 ± 0.08 pg, respectively (Fig. 1). The analysis of variance of the data shows that differences between the three species and between individuals within each species are non-significant (Table 1); however, differences between slides within each individual are significant.

Discussion

The modal karyotype of Aeshna (2n = 26 + XO, male) is present in 72% of the species (Fig. 2). In most of them a larger autosomal pair and a noticeably smaller one (m pair) are readily distinguished; the X chromosome is small and of similar size to the smallest bivalent (Makalovskaja 1940; Oksal 1943; Cumming 1964; Cruden 1968; Kiatuta 1969; Hung 1971; Kiatuta 1971, 1973; Kiatuta and Kiatuta 1980, 1982). The chromosome complement of A. confusa presents these characteristics. Considering this modal karyotype as the ancestral one, it can be observed that during karyotype evolution in the genus fusions have taken place, involving autosomes and/or the sex chromosome. No increase in diploid number has been reported until present. The chromosome complement of A. bonariensis (2n = 26, n = 12 + neo-XY, male) would have originated through the fusion of the original X chromosome with the largest autosomal pair, giving rise to a neo-XY system. A quite similar situation has been described in A. grandis, a species in which the neo-XY is the largest pair, heteromorphic and, hence, easily identified (Oksala 1943; Kiatuta 1969).

The chromosome complement of A. cornigera planaltica (2n = 16, n = 7 + neo-XY, male), which is much more reduced, would have originated through six fusions: five between autosomes and one between the original X chromosome and the smallest autosomal pair.
Aeshna confusa

KARYOTYPE EVOLUTION IN AESHNA

Fig. 1(a–c). DNA content in Aeshna confusa (n = 13 + XO), A. bonariensis (n = 12 + neo-XY) and A. cornigera planaltica (n = 7 + neo-XY). In a, b, and c, one cell at diakinesis from each species is shown in order to compare size and number of chromosomes; the sex univalent or sex pair is indicated. Bar = 10 μm.
The large size of the chromosomes of *A. cornigera planaltica*, when compared with those of *A. confusa* and *A. bonariensis*, suggests that all the fusions that gave rise to such a reduced chromosome complement were probably accompanied by a minimal loss of DNA. As in *A. cornigera planaltica*, OKSALA (1943) described in *A. coerulaea* that the sex bivalent was the smallest of the complement and heteromorphic. The neo-XY system is particularly frequent in *Aeshna* (28% of the species) since in the order only 5.4% of the species have the neo-system. In many species of *Aeshna*, the heteromorphism of the sex bivalent is easily recognized, a fact that is also unusual in other genera of Odonata.

DNA content is not always correlated with chromosome number and size, and particularly in insects with holokinetic chromosomes different situations have been encountered. Related species whose karyotypes differ by one or more fusions or fragmentations, can show constancy in DNA content, as in *Thyanta* and *Banasa* (Heteroptera) (SCHRADER and HUGHES-SCHRADER 1956, 1958) or significant differences in genome size, as described in some species of *Belostoma* (Heteroptera) (PAPESCHI 1988). On the other hand, there are also examples of related species with the same diploid chromosome number but significant differences in DNA content, as in *Triatoma* and other species of *Belostoma* (Heteroptera) (SCHREIBER et al. 1972; PAPESCHI 1991).

In the species of *Aeshna* here analyzed, no differences in DNA content have been detected, in spite of the great differences in diploid chromosome number; this implies that fusions have taken place with a minimal loss of DNA. CUMMING (1964) reported a similar situation in *Macrothemis musiva* (2n = 25, n = 12 + XO, male), *M. mortoni* (2n = 25, n = 12 + XO, male), and *M. hemichlora* (2n = 6, n = 2 + neo-XY) (Libellulidae, Odonata); he mentioned preliminary results suggesting that DNA contents in the three species do not differ significantly. It is interesting to point out that the scarce reports of C-banding in Odonata describe the presence of C-positive heterochromatin always at telomeric regions (AGOPIAN and MOLA 1984; FRANKOVIC and JURECIC 1987; PRASAD and THOMAS 1992). This fact suggests that fusions could be associated with a noticeable loss of DNA, not necessarily involving loss of information.

PETROV and ALESHIN (1983), and PETROV et al. (1984) estimated the haploid genome size of eleven species of Odonata belonging to 6 families, by means of DNA reassociation kinetics. They obtained values ranging from 0.37 pg to 1.7 pg. According to these authors, the DNA content of *A. coerulaea* (as *A. squamata*) (2n = 24, n = 11 + neo-XY) and *A. juncea* (2n = 26, n = 12 + neo-XY) is 1.6 pg and 1.0 pg, respectively. As both species differ only in one autosomal fusion and the species with higher chromosome number has a lower DNA content, it is evident that the DNA differences are not associated with the fusion itself; instead, they have probably occurred inde-
pendently. It can be concluded that during karyotype evolution in the genus Aeshna, although fusions are always associated with at least a minimal loss of DNA, the major changes in DNA content have to be explained by other mechanisms, e.g., heterochromatin duplications or deletions.

Acknowledgements. — We wish to thank L. Agopian for her cooperation in field collecting, Dr. S. Dunke and Dr. A. Rodrigues Capitulo for taxonomic identification of the specimens, and Lic. B. Gonzalez for advice on the statistical analyses. We express our sincere gratitude to Dr. J. H. Hunziker, Dr. L. Poggio, and Dr. C. Naranjo for their continuous encouragement. DNA measurements were performed at the CEFAPRIM (CONICET). This research was supported by grants from CONICET to Dr. J. H. Hunziker.

References

AGOPIAN, S. S. and MOLA, L. M. 1984. Bandeco C en Odonata. — Abstr. Pop. XV Congr. Soc. Arg. de Genet. Corrientes, p. 17.

CHUDEN, R. W. 1968. Chromosome numbers of some North American dragonflies (Odonata). — Can. J. Genet. Cytol. 10: 200–214.

CUMMING, R. B. 1964. Cytogenetic studies in the order Odonata. — Ph.D. thesis, Univ. Texas, Austin, USA.

FRANKOVIC, M. and JUREVIC, R. 1987. Comparative cytogenetic analysis of karyotype morphology and organization in males of species Libellula depressa L. and L. fulva Müll. (Insecta, Odonata). Proc. Abstr. Pop. 3rd Congress of Croatian Biologists, p. 292–293.

HUGHES-SCHRADER, S. and SCHRADER, F. 1956. Polytene as a factor in the chromosomal evolution of the Pentatomini (Hemiptera). — Chromosoma 8: 135–151.

HUNG, A. C. F. 1971. Cytological studies of five species of dragonflies (Odonata: Anisoptera). — Entomol. News 82: 103–106.

KIAUTA, B. 1969. Sex chromosomes and sex determining mechanisms in Odonata, with a review of the cytological conditions in the family Gomphidae, and references to the karyotypic evolution in the order. — Genetica 40: 127–157.

KIAUTA, B. 1971. Studies on the germ cell chromosomal system of some cytologically interesting or hitherto not studied Odonata from the Autonomous Region Friuli, Venezia Giulia (Northern Italy). — Atti. Mus. civ. Stor. nat. Trieste 27(2): 65–127.

KIAUTA, B. 1973. Notes on new or little known dragonfly karyotype. III. Spermatoocyte chromosomes of four nearctic Anisoptera, Aeshna subarctica (Djak and Somarchis alpestris (Scl.) from Switzerland (Anisoptera, Aeshnidae, Corduliidae). — Notul. Odonatol. 1(6): 104–105.

KIAUTA, B. and KIAUTA, M. 1982. 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. Kiatua. Report for the Kansai Research Group of Odonatology, Osaka, Mimeographed. Soc. Int. Odonatol. Utrecht. — In: Odonatological Abstracts No. 4492, Odonatologica 13(3): 484.

MAKALOVSKAJA, W. N. 1940. Comparative karyological studies of dragon-flies (Odonata). — Arch. russ. d'Anat., d'histol. et d'embryol, Leningrad 25(1): 24–39, 120–121.

MELLO, M. L. S., RANDH, M. A. F., GORZIGLIO, S., FERRAZ-FILHO, A. N., RODRIGUES, V. C. C., ROLHA, E. O., SILVA, E. and CORDEIRO, J. A. 1986. Number of chromosomes, Feulgen DNA content and nuclear phenotypes in domestic and wild specimens of Pantanangus nigritius. — Ann. Trop. Med. Parasitol. 60: 641–648.

MOLA, L. M. 1992. Estudios cromosómicos en libélulas (Orden Odonata). — Tesis de Doctorado. Fac. Cs. Exactas y Naturales, Univ. Buenos Aires, Argentina.

OKSALA, T. 1943. Zytologische Studien an Odonaten I. Chromosomenverhältnisse bei der Gattung Aeshna mit besonderer Berücksichtigung der postreduktionsellen Teilung der Bivalente. — Ann. Acad. Sci. Fenn. (Ser. A. IV) 4: 1–64.

PAPESCHI, A. G. 1988. C-banding and DNA content in three species of Belostoma (Heteroptera) with large differences in chromosome size and number. — Genetica 76: 43–51.

PAPESCHI, A. G. 1991. DNA content and heterochromatin variation in species of Belostoma (Heteroptera, Belostomatidae). — Hereditas 115: 109–114.

PETROV, N. B. and ALIESHEN, V. V. 1983. Repetitive and unique sequences in the DNA of dragonflies (Odonata, Insecta): intragenomic and interspecific divergence. — Molec. Biol. 17(2): 345–355.

PETROV, N. B., ALIESHEN, V. V. and ANTONOV, A. S. 1984. DNA sequence in two Odonata species, Culophrax splendidus (Harriss) and Aeshna coerulescens (Ström) (Zygoptera: Culophraxidae; Anisoptera: Aeshnidae). — Odonatologica 13(2): 269–279.

PRASAD, R. and THOMAS, K. 1992. C-band pattern homogeneity in dragonflies (Odonata). — Cytologia 45: 57–68.

RASCH, E. M., BARR, H. J. and RASCH, R. W. 1971. The DNA content of sperm of Drosophila melanogaster. — Chromosoma 37: 1–18.

SCHRADER, F. and HUGHES-SCHRADER, S. 1956. Polyplody and fragmentation in the chromosomal evolution of various species of Thyanta (Hemiptera). — Chromosoma 7: 469–496.

SCHRADER, F. and HUGHES-SCHRADER, S. 1958. Chromatid autonomy in Belusroma (Hemiptera: Pentatomidae). — Chromosoma 9: 193–215.

SCHREIBER, G., BOGLIOLO, A. R. and COELHO DE PINHO, A. 1972. Cytogenetics of Triatomininae: karyotype, DNA content, nuclear size and heteropycnosis of autosomes. — Rev. Brasil. Biol. 32: 255–263.

SYENGA, J. 1972. General Cytogenetics. — Elsevier, New York.

WHITE, M. J. D. 1973. Animal Cytology and Evolution (Third Edition). — Cambridge University Press, Cambridge.