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Post-reductional meiosis in *Aeshna* (Aeshnidae, Odonata)

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In many groups of insects with holokinetic chromosomes the meiotic process is, without doubt, either pre-reductional or post-reductional. In Odonata, however, the mode of orientation (axial or equatorial) and type of meiosis (pre- or post-reductional) of bivalents is still controversial.

A careful analysis of the meiotic behaviour of autosomal and sexual bivalents and univalents in *Aeshna confusa*, *A. bonariensis*, and *A. cornigera planaltica* demonstrates that, in these species, autosomal bivalents divide post-reductionally. Bibliographic evidence also supports this type of meiosis in other species of Odonata, suggesting that it is characteristic of the order.

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In many groups of insects with holokinetic chromosomes the meiotic process is either pre-reductional or post-reductional (WHITE 1973). In Odonata, however, the type of meiosis has been controversial, and different authors have described it as pre-reductional (SESHACHAR and BAGGA 1962; HANDA et al. 1984; MITTAL and GANDHI 1984) or post-reductional (OKSALA 1943; CUMMING 1964; CRUDEN 1968). This uncertainty may be a consequence of the difficulty to determine the mode of orientation (axial or equatorial) of bivalents at metaphase I because of high degree of condensation, close aggregation at the equatorial plate, and, in many species, the high number and small size of bivalents.

On the other hand, the post-reductional division of the sex chromosome in XO/XX systems has been largely recognized (KIAUTA 1969, 1975; SOUZA BUENO 1982; TYAGI 1986).

In the present work the meiotic behaviour of autosomal and sexual bivalents, as well as sex and autosomal univalents of *Aeshna confusa*, *A. bonariensis*, and *A. cornigera planaltica* are described and carefully analyzed, in order to determine the type of meiosis in these species.

Material and methods

The number and locality of the individuals analyzed in the present study are the following:

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Aeshna confusa. — 6 males from Ciudad Universitaria (Capital Federal) and 1 male from Otamendi (Campana), Buenos Aires Province, Argentina; 3 males from Real de San Carlos, Colonia Department, Uruguay.

A. cornigera planaltica. — 2 males from Parque Nacional Iguazú, Misiones Province, Argentina.

A. bonariensis. — 8 males from Ciudad Universitaria (Capital Federal) (No. 1 to 8), 1 male from Talavera Island (Campana) (No. 9), Buenos Aires Province, Argentina; 7 males from Real de San Carlos (No. 10 to 16), Colonia Department, Uruguay.

Individuals were caught at flight; some of them were immediately fixed in 3:1 (absolute ethanol: glacial acetic acid) while other specimens were kept alive, etherized, dissected, and only the gonads fixed in 3:1. All the material was kept at 4°C. Cytogenetic preparations for meiotic analysis were obtained by squashing a piece of gonad in iron-acetic haematoxylin.

Results

Aeshna confusa (2n = 27, n = 13 + XO male)

At spermatogonial prometaphases and metaphases, a strikingly small pair of autosomes are observed (*m* chromosomes), while the X chromosome cannot be identified because it is isopyknotic and of similar size to the second smaller autosomes

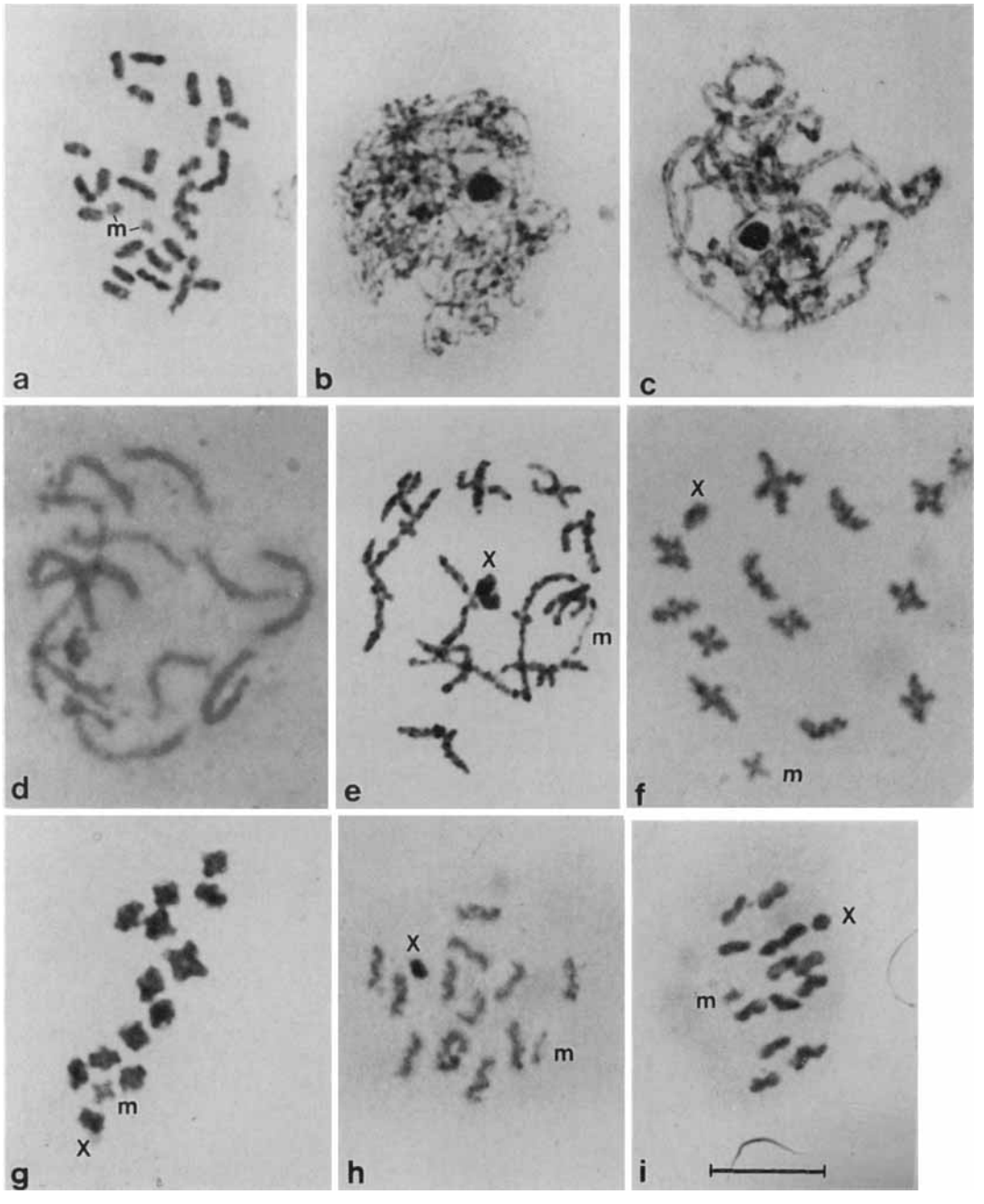

Fig. 1a–i. *Aeshna confusa* (n = 13 + X0). **a** Spermatogonial prometaphase. **b** Zygotene. **c** Middle pachytene. **d** Late pachytene. **e** Diplotene. **f** Diakinesis. **g** Metaphase I. **h** Prophase II, showing the characteristic ε shape of autosomes. **i** Metaphase II. Bar = 10 μm.

(Fig. 1a). The X chromosome is positively heteropyknotic during early meiotic prophase until diplotene (Fig. 1b,c,d,e). At diakinesis and metaphase I all bivalents have only one chiasma at medial or terminal position (Fig. 1f,g). A larger bivalent and the negatively heteropyknotic *m* bivalent are detected, while the other eleven bivalents decrease gradually in size; the X chromosome is isopyknotic and larger than the *m* bivalent (Fig. 1f,g). At metaphase I many bivalents are clearly orientated with their long axis on the equatorial plane (Fig. 1g); the X chromosome also orientates equatorially and divides equationally at anaphase I, migrating synchronously with the autosomes. All cells at prophase II and metaphase II have 14 chromosomes (Fig. 1h,i); at prophase II the autosomes adopt the characteristic ε shape, while the X is rod-shaped and positively heteropyknotic (Fig. 1h). At metaphase II (Fig. 1i) the X lies ahead and migrates precociously at anaphase II.

A. bonariensis (2n = 26, n = 12 + neo-XY male)

From leptotene to pachytene the original X chromosome of the neo-X is positively heteropyknotic (Fig. 2a). At diakinesis all bivalents have only one chiasma at a medial or terminal position. The autosomal bivalents decrease gradually in size, the noticeably smaller *m* bivalent excepted; the neo-XY is the largest bivalent and heteromorphic (Fig. 2b,c). At metaphase I this heteromorphism is no longer observed due to the high degree of chromatin condensation. In all cells at prophase II and metaphase II the largest chromosome is heteromorphic (Fig. 2f,g,h,j).

Sex and autosomal univalents. — In all individuals from Buenos Aires and in two individuals from Colonia (11 and 13), the neo-XY pair appears as univalents in a variable number of cells (Table 1). The presence of univalents can only be detected from early diakinesis onwards, because chromosomes are difficult to recognize individually and analyze at early meiotic prophase. At diakinesis these univalents are generally observed lying apart (Fig. 2d), but in three cells they were connected by a thin chromatin thread (Fig. 2e). At metaphase I these univalents are always completely separated. At prophase II and metaphase II a variable number of cells with 12 autosomes with two chromatids each and 2 independent sex chromosomes of different sizes with only one chromatid each are observed (Fig. 2i,k).

There are no significant differences between the percentage of cells with univalents at meiosis I and meiosis II within each individual (Fisher's exact test for independence, P varying between 0.141 and 1) (SOKAL and ROHLF 1981). In this analysis individual 9 was not included because of the low number of cells observed.

Autosomal univalents in a low frequency were also observed at meiosis I and II in individuals 6, 7 and 11 (Fig. 2j) (Table 1). As most bivalents decrease gradually in size, it is not possible to assure whether it is always the same chromosome pair that is present as univalents. No cell with 4 univalents has been detected. In this case, too,

Table 1. Number of cells with different chromosome configurations observed in *A. bonariensis*

Ind. No.	Diakinesis–M I			Prophase II–M II			Total
	13II	12II + X + Y	12II + 2I	13Chr.	12 + X + Y	12 + 2I	
1	69	3	0	50	1	0	123
2	37	3	0	25	0	0	65
3	92	7	0	70	1	0	170
4	54	2	0	33	2	0	91
5	14	3	0	12	4	0	33
6	83	4	2	100	6	3	198
7	65	3	1	50	0	1	120
8	52	1	0	54	0	0	107
9	0	0	0	9	5	0	14
10	50	0	0	9	0	0	59
11	70	1	0	81	1	1	154
12	57	0	0	58	0	0	115
13	8	0	0	81	1	0	90
14	62	0	0	61	0	0	123
15	55	0	0	20	0	0	75
16	216	0	0	179	0	0	395

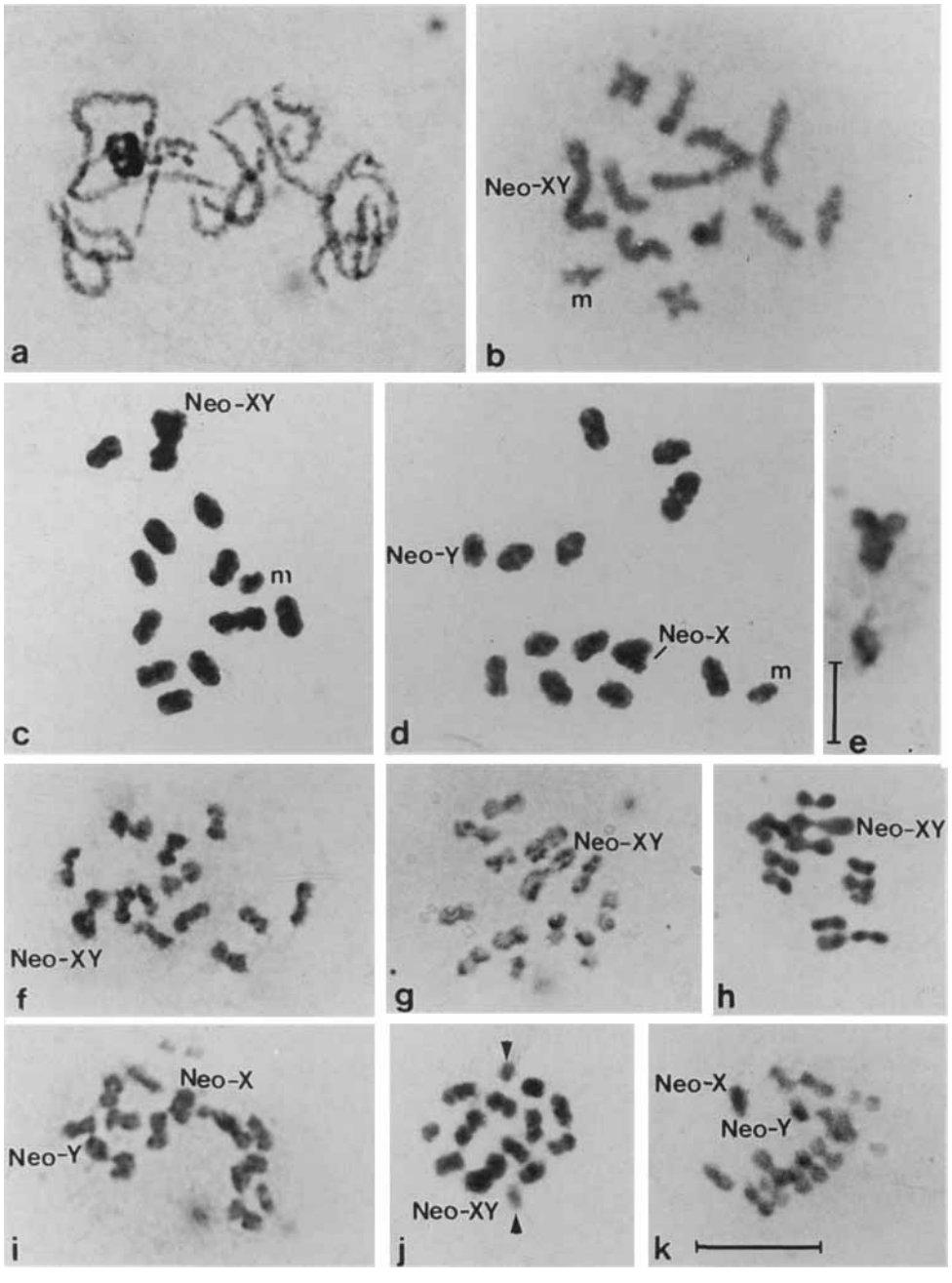

Fig. 2a–k. *Aeshna bonariensis* (n = 12 + neo-XY). **a** Pachytene. **b** Early diakinesis. **c** Diakinesis. **d** Diakinesis with sex univalents. **e** Detail of sex chromosomes at diakinesis (Bar = 5 μm). **f** Prophase II. **g** Prometaphase II. **h** Metaphase II. **i** Prometaphase II with the neo-XY chromatids separated. **j** Prometaphase II with autosomal univalents (arrowheads). **k** Metaphase II with the neo-XY chromatids separated. Bar = 10 μm.

there are no significant differences between the percentage of cells with univalents at meiosis I and II within each individual (Fisher's exact test for independence, $P = 1$).

Since at metaphase II the univalents do not associate and at anaphase II–telophase II the number of chromosomes in each pole cannot be ascertained, it is not possible to know whether the sex or autosomal univalents segregate normally. Lagging chromosomes or any other meiotic abnormality has not, however, been observed, and neither micro- nor macrospermatids have been detected.

Whether the univalents of *A. bonariensis* are the result of asynapsis or desynapsis cannot be determined since cells at zygotene and pachytene are difficult to analyze. As sex univalents appear close to one another in some cells at diakinesis and appear sometimes connected by a thin chromatin thread, we may suppose that univalents are of desynaptic origin.

A. cornigera planaltica (2n = 16, n = 7 + neo-XY male)

This species has a reduced diploid chromosome number and larger chromosomes than the former. At spermatogonial prometaphases and metaphases the chromosomes can be grouped according to their size: ten chromosomes are large, five (four autosomes and the neo-X) are smaller, and the neo-Y is the smallest one (Fig. 3a).

At zygotene and pachytene the original X of the neo-X is positively heteropycnotic (Fig. 3b). At diakinesis autosomal bivalents have only one chiasma, which in most of the five larger bivalents is at terminal position; in the smaller bivalents chiasmata are generally medial (Fig. 3c). The sex bivalent is the smallest one and noticeably heteromorphic, since the original X chromosome is fused with one *m* chromosome (Fig. 3c,d,e); it always shows one chiasma at terminal position (Fig. 3c,d). In only one of 228 diakinesis cells analyzed, the smallest autosomal pair was present as univalents (Fig. 3d). At metaphase I the sex bivalent orientates with its long axis perpendicular to the spindle fibers, the type of orientation that is also observed for the larger autosomal bivalents with terminal chiasmata (Fig. 3e). At anaphase I the sex bivalent divides synchronously with the autosomes.

All the cells at prophase II–metaphase II have a small heteromorphic sex chromosome (Fig. 3f,h); at anaphase II the sex chromosome divides reductionally and synchronously with the autosomes. One cell at prophase II–metaphase II (among 102) showed the sex chromatids separated (Fig. 3g).

Discussion

Most cytogenetic studies in Odonata make no reference to the type of division (pre- or post-reductional) of autosomal bivalents. Some authors have claimed that meiosis is pre-reductional, but no evidence has been given (SESHACHAR and BAGGA 1962; MITTAL and GANDHI 1984; HANDA et al. 1984). On the other hand, the few authors that stated it as post-reductional presented some evidence supporting this hypothesis (OKSALA 1943; CUMMING 1964; CRUDEN 1968) (see below).

Fig. 4 shows the behaviour of autosomal bivalents and sex bivalents and univalents in post- and pre-reductional meiosis. In both types of meiosis, autosomes will be present at prometaphase II as a chromosome with two chromatids of equal size, giving no information about the type of meiosis. However, the result will be different when considering heteromorphic bivalents (such as sex bivalents). At post-reductional meiosis, these bivalents orientate at metaphase I with their long axis on the equatorial plane (Fig. 4a) and at prometaphase II a chromosome with two chromatids of unequal size migrates to each pole (Fig. 4b). At pre-reductional meiosis, heteromorphic bivalents orientate axially (with their long axis perpendicular to the equatorial plane) (Fig. 4e) and at prometaphase II one cell receives a larger chromosome (neo-X) with two equally sized chromatids, while the smaller chromosome (neo-Y) migrates to the other pole (Fig. 4f). The sex univalents equatorially orientated (Fig. 4c), divide equationally at anaphase I and all cells at metaphase II have 14 chromosomes, 12 with two chromatids and 2 chromosomes of unequal size with only one chromatid each (Fig. 4d). On the other hand, if the univalents are axially orientated (Fig. 4g), then the neo-X could migrate with its two chromatids to one pole and the neo-Y to the other (Fig. 4h), resulting in two daughter cells with 13 chromosomes with two chromatids each; or else could both the neo-X and neo-Y migrate to the same pole (Fig. 4i), resulting in two daughter cells with 14 and 12 chromosomes with two chromatids respectively.

OKSALA (1943) made a thorough analysis of the meiosis in species of *Aeshna*, and observed that bivalents orientated equatorially at metaphase I

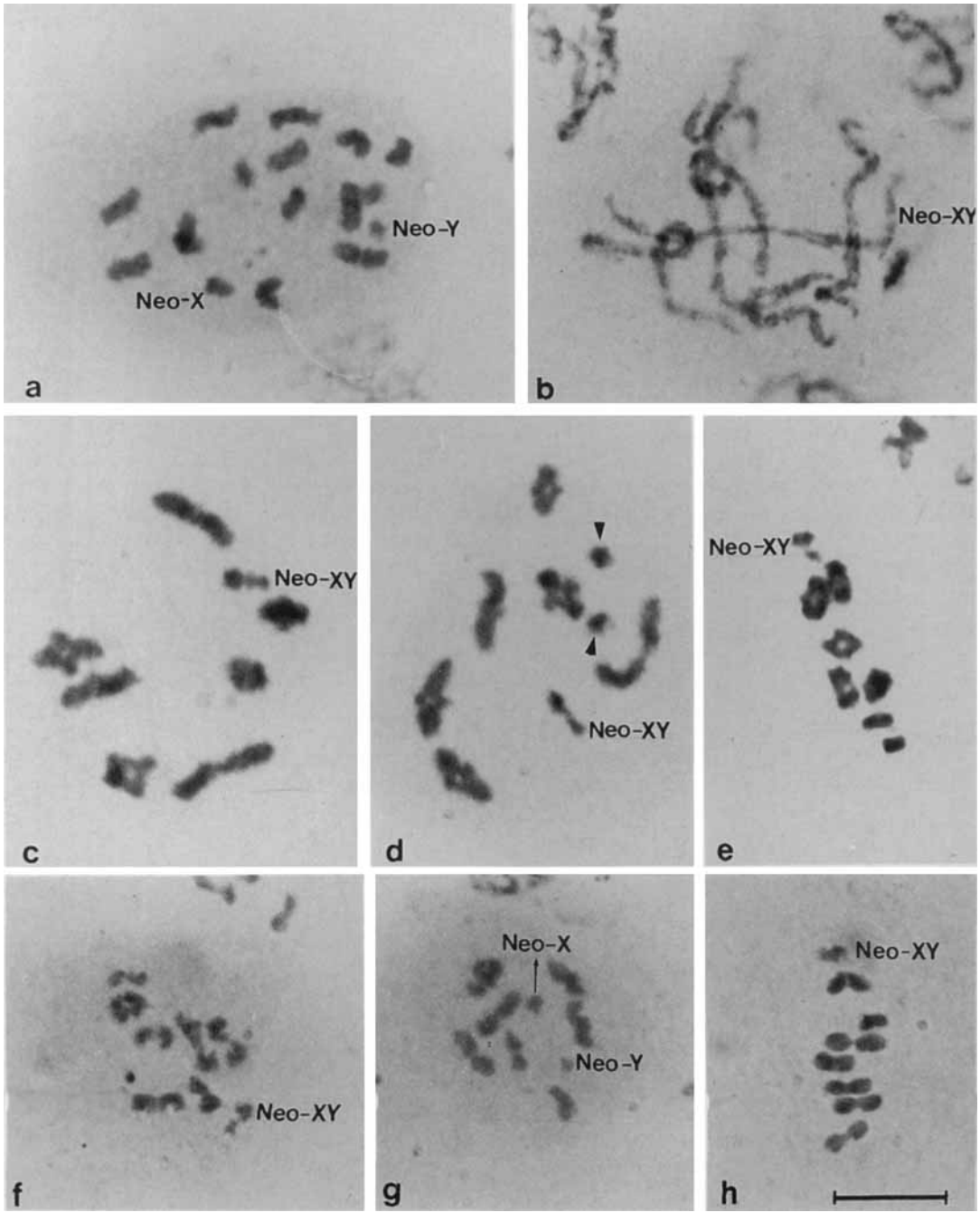

Fig. 3a–h. *Aeshna cornigera planaltica* (n = 7 + neo-XY). **a** Spermatogonial prometaphase. **b** Pachytene. **c** Diakinesis. **d** Diakinesis with autosomal univalents (arrowheads). **e** Metaphase I. **f** Prophase II. **g** Prophase II with sex chromosome chromatids separated. **h** Metaphase II. Bar = 10 μm.

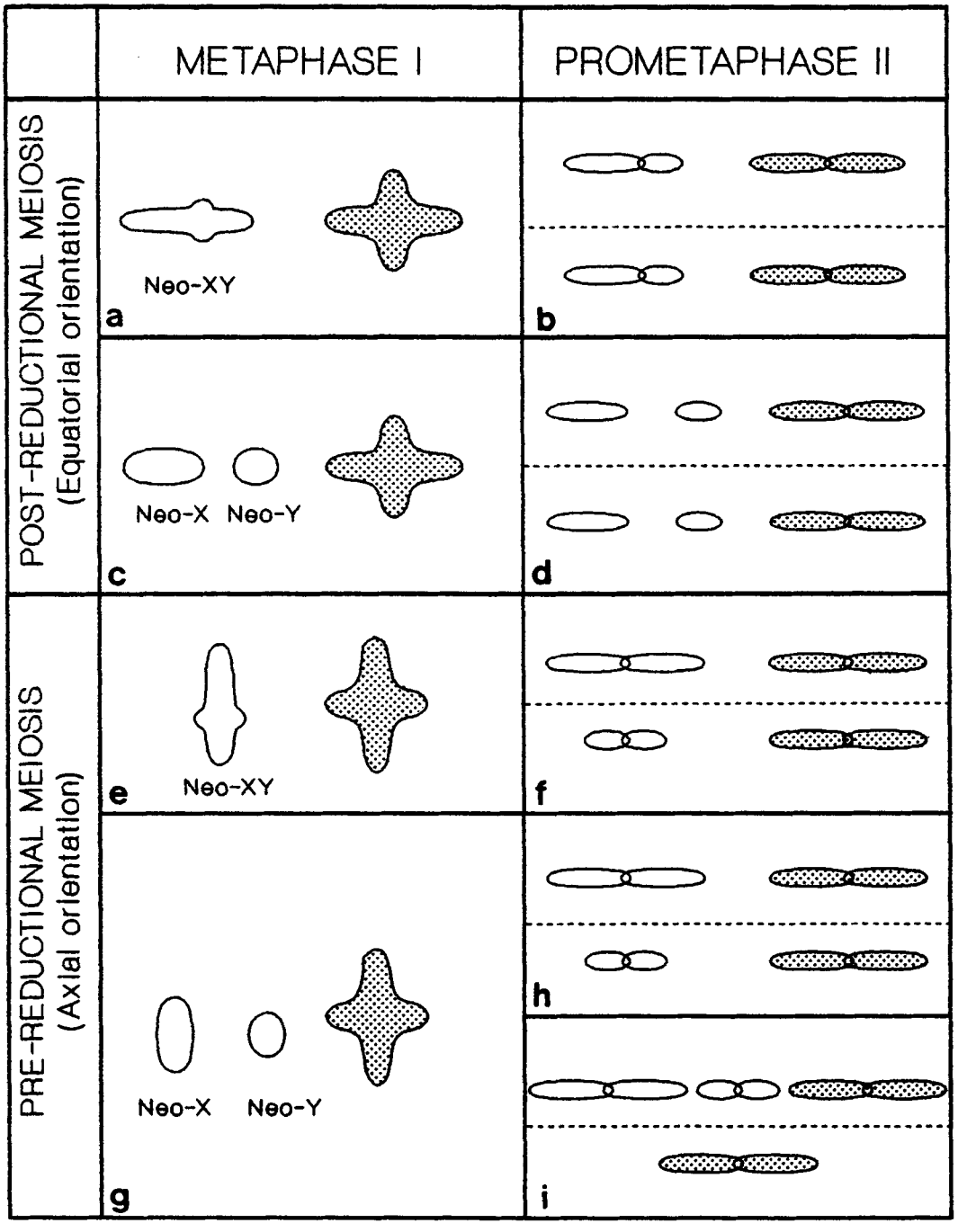

Fig. 4a–i. Different prometaphases II resulting from the post-reductional (**a–d**) or pre-reductional (**e–i**) meiosis of sex bivalent and univalents (see explanation in text).

(Fig. 4a). In the present work, the equatorial orientation of the bivalents has been observed in *Aeshna confusa* and in *A. cornigera planaltica*. In the latter, the low diploid number, the relatively large size of the chromosomes and the high frequency of terminal chiasmata in the larger bivalents allowed the clear observation of the equatorial orientation of both the autosomal and sex bivalents.

CUMMING (1964) described the course of meiosis in two individuals of *Orthemis levis* (Libellulidae) heterozygotes for a fusion (2n = 5 + neo-XY), and he observed that at metaphase I the fusion trivalent ("tripartite bivalent") orientated equatorially. All cells at meiosis II carried a "triple chromatid", as the result of the equational division of the trivalent; at metaphase II it adopted an "U shape" and divided reductionally at anaphase II.

Although in *A. bonariensis* the mode of orientation of bivalents and univalents at metaphase I could not be ascertained, the behaviour of univalents and the heteromorphic sex bivalent has been very valuable in determining the type of division. All normal cells at meiosis II had a heteromorphic chromosome, indicating that the sex pair divides equationally at anaphase I (Fig. 4b). The cells at metaphase II with sex univalents always showed 14 chromosomes (12 with two chromatids and the two unequally sized sex univalents). Taking in account this observation and the similarity between the percentage of cells with sex univalents at meiosis I and II, it can be said that these univalents divide regularly and equationally at first meiotic division. The same can be concluded for autosomal univalents since their frequencies at both meiotic divisions do not differ significantly.

The presence of a heteromorphic sex chromosome at meiosis II has also been reported in *Aeshna coerulea* and *A. grandis* (OKSALA 1943; KIAUTA 1969) and in the Libellulidae species *Erythrodiplax media* and *Pseudothemis zonata* (OMURA 1955; MOLA 1992). The heteromorphic autosomal bivalent of *Brachymesia furcata* (Libellulidae) has also been observed as a heteromorphic chromosome at meiosis II (AGOPIAN and MOLA 1988). This can be taken as evidence for an equational division of heteromorphic bivalents at anaphase I in all these species.

Post-reductional meiosis has been demonstrated in Coccids (Homoptera) (HUGHES-SCHRADER 1948; BATTAGLIA and BOYES 1955) and *Luzula* (Juncaceae) (NORDENSKIÖLD 1962), among other organisms with holokinetic chromosomes. In these groups at anaphase I homologous chromatids migrate parallel to the equatorial plane, very close or even associated by their telomeric regions. In the few Odonata species in which cells at anaphase I have been observed (OKSALA 1943; MOLA and AGOPIAN 1985; AGOPIAN and MOLA 1988; MOLA 1992), the migrating chromatids show an ε shape, i.e., both chromatids are joined by one telomeric region and they migrate almost parallel to the equatorial plane; this particular shape is retained until prometaphase II stage, which is usually observed.

Finally, the evidence supporting a post-reductional meiosis in Odonata can be summarized as follows:

a. — Equatorial orientation of large bivalents and heteromorphic neo-XY at metaphase I in many *Aeshna* species, and of the fusion trivalent in *Orthemis levis*.

b. — Metaphases II with heteromorphic autosomal or sex chromosomes (or unequally sized sex univalents) in *Brachymesia furcata* and in species of *Aeshna*; "triple chromatid" in *Orthemis levis*.

c. — ε shape of chromosomes at anaphase I, prophase II and prometaphase II in many species of the order.

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