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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

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The morphological and kinetical features of the odonate sex chromosomes are reviewed and trends of the evolution of different modes of sex determination in the order are considered. The cytological conditions in the family *Gomphidae* are of particular importance for the understanding of the evolution of sex determination in dragonflies and are discussed in detail.

The original mode of sex determination in Odonata is of the XO/XX type, the male being the heterogametic sex, and is observed in all primary complements (cf. KIAUTA, 1967) regardless of the chromosome number viz. the degree of phylogenetic advancement and specialisation achieved by the taxons concerned.

The mean length of the sex chromosome and the ratio between the longest autosomal bivalent and X are not characteristic at superspecific taxonomic levels. In the male the sex chromosome is usually positively heteropycnotic at all spermatocyte stages, save metaphase and anaphase, whereas in the female it is nearly without exception isocyclic. The first maturation division is equational for X, the second is reductional.

In secondary complements a neo-XY sex determination occurs, in those cases where the original X was involved in a fusion with an autosome. Its occurrence is not related to phylogeny.

The neo-XY condition is often reversible: it occurs in some cells (or stages) while not in others of the same individual. If stabilised, it tends to evolve further, as it was demonstrated in *Gomphidae*, into a secondary XO type. The process has three stages, with an intermediate neo-X neo-neo-Y sex determination and a final numeric reduction of the diploid complement by two elements.

It was demonstrated, that the present type number of *Gomphidae* is of secondary rather than of primary origin.

Introduction

So far the chromosome complements of nearly 400 dragonfly species

belonging to 17 families of the three living suborders have been examined. Though incomplete, the reviews by CUMMING (1964a) and CRUDEN (1968) include most of the hitherto published evidence on the odonate chromosome numbers. Sex chromosomes are recognizable in species assigned to all families studied, but no special attention has ever been payed either to the morphological and kinetical features of the sex chromosomes, or to the general aspects of the sex determination in the order. The sole exceptions are the publications by OGUMA (1930), OKSALA (1943), SESHACHAR & BAGGA (1962), KIAUTA (1967, 1968b) and KIAUTA & VAN BRINK (1968).

The more primitive type of sex determining mechanism in organisms is the XY-XX mode, from which it is supposed that the other mechanisms have been derived (WHITE, 1954; SAEZ, 1963; DARLINGTON, 1965). In dragonflies, however, the combination XY-XX has secondarily originated from the XO-XX type. The latter is met with in most of the odonate species examined. The male is the heterogametic sex.

MCGILL (1904) was the first to note the sex element ("accessory chromosome") in *Anax junius* (Drury). She did not yet recognize its proper sexual nature; nevertheless, she described and figured correctly its postreductional conduct during meiosis. The latter has been confirmed by numerous subsequent workers, but two exceptions to the general rule were published by SMITH (1916) and MAKALOWSKAJA (1940).

LEFEVRE & MCGILL (1908) were the first to suggest a possible sexual character of the "accessory chromosome" in *Anax junius* (Drury). But it was SMITH (1916) who ultimately recognized it as the sex element in *Sympetrum semicinctum* (Say).

The neo-XY-XX mode of sex determination was discovered in dragonflies by MAKALOWSKAJA (1940). Although she properly described and figured the situation in *Aeshna grandis* (L.) and *A. juncea* (L.), she apparently did not understand the phenomenon. OKSALA (1943) dealt in extenso with the behaviour of the sex chromosomes in neo-XY complements of several species of the same genus. Several more cases of this mode of sex determination were brought on record by RAY CHAUDHURI & DAS GUPTA (1949), OMURA (1955), SESHACHAR & BAGGA (1962) and CRUDEN (1968).

Materials and Methods

In the material studied seven species are included the cytology of which has not been studied previously. The mean cytological data for these are given in Table 1. The general locality data of other species used are partially listed in Acknowledgements and in other tables and figures.

TABLE 1

MEAN CYTOLOGICAL DATA ON SPECIES NOT PREVIOUSLY STUDIED CYTOLOGICALLY

Species (and family)	Origin	Chromosome number 2n	Chromosome number n	m	Remarks and figures
Lestes virens (Charp.) (Lestidae)	Netherlands		13 ♂	yes	m very large; Figs. 11a, 11b
Lestes viridis (v. d. Lind.) (Lestidae)	Netherlands		13 ♂	yes	m very large
Gomphus graslini (Ramb.) (Gomphidae)	France (Dépt. Lot)	23 ♂		yes	Figs. 19, 20
Onychogomphus forcipatus (L.) (Gomphidae)	Austria (Carinthia)	24 ♂	12 ♂	no	Figs. 23–33
Aeshna mixta (Latr.) (Aeshnidae)	Netherlands	27 ♂	14 ♂	yes	Fig. 3
Planaeschna milnei (Sel.) (Aeshnidae)	Japan (Osakawa)		14 ♂	yes	
Hemianax papuensis (Burm.) (Aeshnidae)	Australia (Perth)		14 ♂	yes	

All figures are based on lacto-acetic-orcein squash preparations, photographed with Zeiss phase contrast equipment (100 × oil immersion, 8 × oculars, n.a. 1.25, green filter, Agepan FF panchromatic film). The positives were printed originally at 2250 × and 4000 × and are reduced in this paper to 1500 × and 2900 × respectively.

The measurements for the statistical treatment were carried out on 2250 × photographs with the accuracy of 0.5 mm (1 mm = 0.44 μ). The μ values were rounded to one decimal, square values to two.

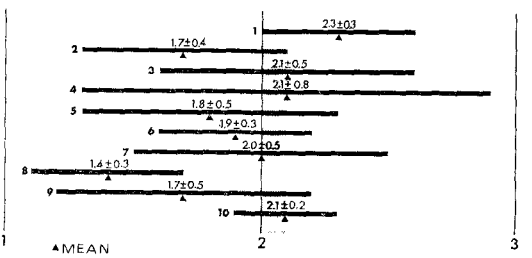
Morphological and Kinetical Features of the Sex Element in the XO-XX Complement

The sex element, in the XO-XX complement, has a number of

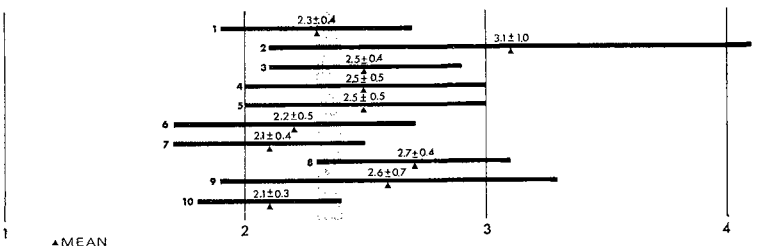
morphological and kinetical features, by which it is distinguished clearly from the autosomes. These are reflected in its size, shape, and in its behaviour in the course of the maturation cycles.

SIZE. At mitotic metaphase the sex chromosome usually is one of the smallest elements of the set. In the great majority of species thus far studied it is superior in size only to the m-chromosomes (Figs. 1–4). The sole exceptions known are met with in some species of the family *Gomphidae* (Figs. 18–20). At primary spermatocyte metaphase the sex element is equal in size to the m-bivalent, occasionally it is longer, but often shorter.

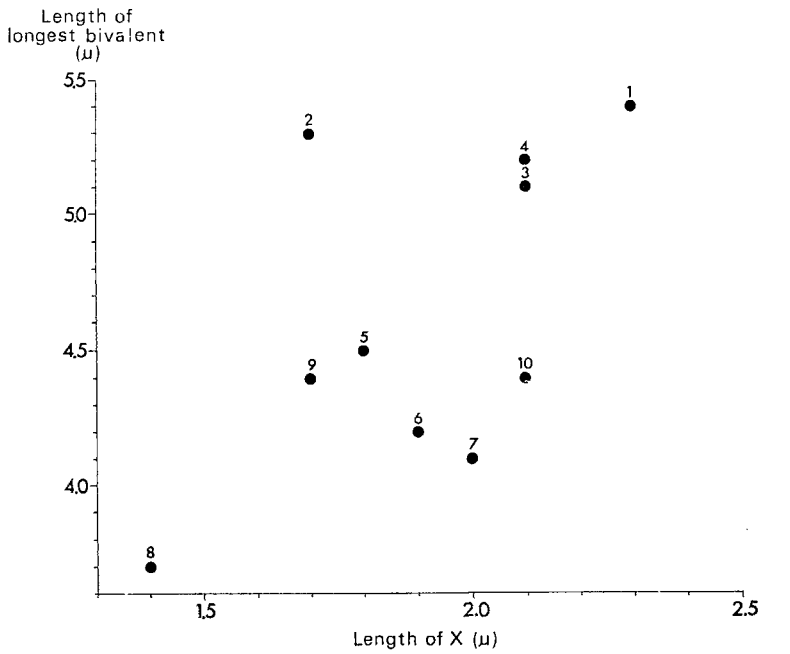
Table 2 presents the absolute (minimal, maximal and mean) lengths of the sex element and ratios, longest bivalent: X, as observed at primary spermatocyte metaphase of ten randomly chosen species of various families belonging to the two major living suborders. The confidence intervals account for 95 per cent of cases (cf. DIXON & MASSEY, p. 73) and are plotted, for the two values, in Textfigs. 1 and 2 respectively. The relation of the absolute lengths of the X and the longest spermatocyte metaphase I bivalent is given graphically, for the same ten species, in Textfig. 3.



Textfig. 1. Mean values and confidence intervals (95%) for the length (in μ) of the sex element in primary spermatocyte metaphase of ten randomly chosen dragonflies. Explanation of numbers: (1) *Coenagrion pulchellum* (v. d. Lind.), *Coenagrionidae* (De Bilt, Netherlands); (2) *Enallagma cyathigerum* (Charp.), *Coenagrionidae* (Wasmeer, Hilversum, Netherlands); (3) *Ischnura elegans* (v. d. Lind.), *Coenagrionidae* (De Bilt, Netherlands); (4) *Aeshna cyanea* (Müll.), *Aeshnidae* (De Bilt, Netherlands); (5) *Planaeschna milnei* (Sel.), *Aeshnidae* (Asakawa Experiment Forest, Japan); (6) *Hemianax papuensis* (Burm.), *Aeshnidae* (Lake Bibra, Perth, Australia); (7) *Cordulegaster bolloni* (Don.), *Cordulegasteridae* (Karawanken Mts., Austria); (8) *Orthetrum pruinosum neglectum* (Ramb.), *Libellulidae* (Yung Nin San Park, Taipei, Formosa); (9) *Orthetrum triangulare* Sel., *Libellulidae* (Yung Nin San Park, Taipei, Formosa); (10) *Sympetrum corruptum* (Hag.), *Libellulidae* (Ledona, Tucson, Arizona, U.S.A.). Further explanation in text.



Textfig. 2. Mean values and confidence intervals (95%) for the ratio, longest bivalent: X, in primary spermatocyte metaphase of ten randomly chosen dragonflies. For explanation of numbers cf. Textfig. 1. Further explanation in text.



Textfig. 3. Relation between the mean absolute length of the longest bivalent and that of the sex element in primary spermatocyte metaphase of ten randomly chosen dragonflies. For explanation of numbers cf. Textfig. 1. Further explanation in text.

From this evidence, leaving aside the exceptional situation in the *Gomphidae* (to be discussed later in this paper), the following conclusions can be made:

TABLE 2

VARIATION IN LENGTH OF THE SEX ELEMENT AND IN THE RATIO, LONGEST BIVALENT: X, IN THE PRIMARY SPERMATOCYTE METAPHASE OF TEN DRAGONFLY SPECIES

Species (and family)	Origin	Length of X (in μ) Number of figures measured	Length of X (in μ) Range and mean with confidence limits	Ratio, longest bivalent: X Range and mean with confidence limits
Coenagrion pulchellum	Netherlands	16	2.0—2.6	2.0—2.7
(v. d. Lind.) (Coenagrionidae)			2.3±0.3	2.3±0.4
Enallagma cyathigerum	Netherlands	30	1.3—2.2	2.1—4.2
(Charp.) (Coenagrionidae)			1.7±0.4	3.1±1.0
Ischnura elegans	Netherlands	16	1.5—2.4	2.2—3.1
(v. d. Lind.) (Coenagrionidae)			2.1±0.5	2.5±0.4
Aeshna cyanea	Netherlands	22	1.5—3.3	2.1—3.1
(Müll.) (Aeshnidae)			2.1±0.8	2.5±0.5
Planaeschna milnei	Japan	27	1.3—2.2	2.1—3.1
(Sel.) (Aeshnidae)			1.8±0.5	2.5±0.5
Hemianax papuensis	Australia	25	1.5—2.2	1.6—2.7
(Burm.) (Aeshnidae)			1.9±0.3	2.2±0.5
Cordulegaster boltoni	Austria	26	1.5—2.4	1.7—2.4
(Don.) (Cordulegasteridae)			2.0±0.5	2.1±0.4
Orthetrum pruinosum neglectum	Taiwan	16	1.1—1.8	2.5—3.2
(Ramb.) (Libellulidae)			1.4±0.3	2.7±0.4
Orthetrum triangulare	Taiwan	18	1.2—2.0	2.2—3.3
Sel. (Libellulidae)			1.7±0.5	2.6±0.7
Sympetrum corruptum	U.S.A.	10	2.0—2.2	1.9—2.4
(Hag.) (Libellulidae)			2.1±0.2	2.1±0.3

(1) The mean length of the dragonfly sex chromosome at primary spermatocyte metaphase amounts to about 1.9 μ. Nine out of ten species in the sample, overlap at the 2.0–2.1 μ level (Textfig. 1: stippled zone);
(2) The mean length of the sex chromosome is not characteristic at the superspecific taxonomic levels. Whether or not it has any significance on the species level can not be ascertained on the basis of the limited material studied. It differs considerably in *Orthetrum pruinosum neglectum* (1.4 μ) and *O. triangulare melania* (1.7 μ) (Textfig. 1);
(3) The same is true if the total variation in length (confidence intervals included) is considered. Only in the pair *Orthetrum pruinosum neglectum* (1.1–1.7 μ) and *Sympetrum corruptum* (1.9–2.3 μ) (Textfig. 1) of the given sample are these values completely non-overlapping;
(4) The mean ratio between the longest spermatocyte metaphase I bivalent and the X, in dragonflies under study, is 2.5. As is shown in Textfig. 2, the ten species overlap in the range 2.3–2.4 μ. Roughly, bigger X-es are met with in the complements with longer autosomes, though there are exceptions to the general rule (e.g. *Orthetrum pruinosum neglectum;* Textfig. 3);
(5) The taxonomic value of the ratio is limited to the incidental species level. In *Calopteryx virgo* (L.) the ratio, X:m, is peculiar for certain infraspecific forms (distant populations) (KIAUTA, 1968a).

SHAPE. In mitotic and first meiotic metaphase the sex element usually has a round or oval shape. Save for the large X-es in some *Gomphidae,* it is seldom slightly elongate. The shape depends much on its size; the small X-es are for all practical purposes regularly spheric.

ALLOCYCLY. The allocyclic behaviour of sex chromosomes is a regular feature of dragonfly spermatogenesis. It is only seldom observed in the oogenetic cycle.

In the male, the sex chromosome is positively heteropycnotic at all spermatocyte stages with the exception of metaphase and anaphase in which the autosomes following the standard cycle are also maximally condensed.

Negative heteropycnosis is less frequent. In part of the figures of

late spermatocyte diakinesis of *Ischnura elegans* and *Cordulegaster boltoni* a despiralised sex element was seen. It is positively heteropycnotic in the great majority of diakinetic figures of the same individual (Figs. 5–8). The phenomenon has not been observed so far in any other XO dragonfly.

In oogenesis the sex element is nearly without exception isocyclic (Fig. 9). A positively heteropycnotic X was observed so far only in some pachytene figures of *Enallagma cyathigerum* and *Cordulia aenea* (L.) (Fig. 10).

ANAPHASE BEHAVIOUR. The sex element divides equationally and simultaneously with the autosomes at first meiotic anaphase, whereas at anaphase II it lies at a certain distance (usually in a poleward position) from the dividing autosomes and precedes undivided to a pole in the post-reductional way (Figs. 11–17). Two exceptions to this rule were so far published: in the anisopteran *Libellula luctuosa* (Burm.) (syn.: *L. basilaris* /Mc. Lach./) (SMITH, 1916) and in the zygopteran *Enallagma cyathigerum* (MAKALOWSKAJA, 1940). In the latter case the observation was definitely erroneous as demonstrated by VAN BRINK & KIAUTA (1964). *Libellula luctuosa* has not been reexamined.

TABLE 3

MEAN LENGTHS (IN μ) OF THE SEX ELEMENT AT PRIMARY AND SECONDARY SPERMATOCYTE METAPHASE IN REPRESENTATIVES OF SIX MAJOR FAMILIES

Species (and family)	Origin	Metaphase I	Metaphase II
Enallagma cyathigerum (Charp.) (Coenagrionidae)	Netherlands	1.7	1.3
Lestes viridis (v. d. Lind.) (Lestidae)	Netherlands	2.0	1.5
Calopteryx virgo padana Conci (Calopterygidae)	Slovenia (Yugoslavia)	2.3	1.6
Anax imperator Leach (Aeshnidae)	France	1.8	1.5
Anotogaster sieboldi (Sel.) (Cordulegasteridae)	Japan	2.0	1.5
Orthetrum cancellatum (L.) (Libellulidae)	Netherlands	1.8	1.3

Due to the lack of any growth in the interkinetic period, the volume of a secondary spermatocyte is approximately half that of a primary

spermatocyte. The same is true for the size of the nuclei and the volumes of the chromosomal elements. A comparison of the lengths of the sex element in the primary and secondary spermatocyte metaphase of some species is given in Table 3.

Sex chromosomes and sex determination in the family *Gomphidae*

Due to its phylogenetic primitivity and the relatively large number of species that have been cytologically examined and the peculiar features of the sex chromosomes in some of them, the *Gomphidae* are of particular interest for the study of sex determination in Odonata. Though we had the opportunity to examine the original material of no more than three members of the family, we believe it worthwhile to discuss the situation in the group in some detail.

REVIEW OF THE GENERAL CYTOLOGICAL SITUATION IN THE FAMILY

So far the cytological conditions of 41 gomphidan dragonflies belonging to all five living subfamilies recognized by FRASER (1957) have become known. The type number, n = 12, was found in 36 species, whereas in others the haploid numbers 12–13 (2 species), 11 (1 species) and 10 (2 species) occur. In Table 4 a review of the cytologically studied gomphids is given.

TABLE 4

REVIEW OF THE CHROMOSOME NUMBERS IN THE FAMILY GOMPHIDAE

SUBFAMILY Species	Locality	n	m	Reference [1]
GOMPHINAE				
Anisogomphus bivittatus (Sel.)	India	12	yes	DAS, 1956
Davidius nanus (Sel.)	Japan	12	no	KICHIJO, 1939
Dromogomphus spinosus Sel.	U.S.A.	12	yes	CRUDEN, 1968
Dromogomphus spoliatus (Hag.)	U.S.A.	12	yes	CRUDEN, 1968
Erpetogomphus designatus Hag.	U.S.A.	12	yes	CUMMING, 1964a
Erpetogomphus diadophis Calv.	U.S.A.	12	no	CUMMING, 1964a
Gomphus confraternus Sel.	U.S.A.	12	yes	CRUDEN, 1968
Gomphus exilis Sel. [2]	U.S.A.	12	yes	CRUDEN, 1968
Gomphus graslini Ramb.	France	12	yes	this paper

[1] Reference is made only to the first original paper.

[2] For the Canadian material see text.

Table 4 (continued)

SUBFAMILY Species	Locality	n	m	Reference [1])
Gomphus lentulus Needh.	U.S.A.	12	no	CRUDEN, 1968
Gomphus lividus Sel.	U.S.A.	12	yes	CRUDEN, 1968
Gomphus melaenops Sel. [3])	Japan	12	?	OMURA, 1957
Gomphus militaris Hag.	U.S.A.	12	no	CRUDEN, 1968
Gomphus pallidus Ramb.	U.S.A.	12	no	CUMMING, 1964a
Gomphus plagiatus Sel.	U.S.A.	12	yes	CRUDEN, 1968
Gomphus postocularis Sel.	Japan	12	?	OMURA, 1957
Gomphus scudderi Sel.	U.S.A.	12	no	CRUDEN, 1968
Gomphus spicatus Hag.	U.S.A.	12	yes	CRUDEN, 1968
Gomphus submedianus Will.	U.S.A.	12	no	CRUDEN, 1968
Nihonogomphus viridis Oguma	Japan	12	?	OMURA, 1957
Octogomphus specularis (Hag.)	U.S.A.	12	no	CRUDEN, 1968
Onychogomphus forcipatus L.	Austria	12, 13	no	this paper
Ophiogomphus bison Sel.	U.S.A.	12, 13	no	CRUDEN, 1968
Ophiogomphus colubrinus Sel.	U.S.A.	12	no	CRUDEN, 1968
Ophiogomphus occidentalis Hag.	U.S.A.	12	no	CRUDEN, 1968
Ophiogomphus rupinsulensis (Walsh)	U.S.A.	12	no	CRUDEN, 1968
Ophiogomphus serpentinus (Charp.)	Finland	12	no	OKSALA, 1945
Stylogomphus suzukii (Oguma)	Japan	12	yes	OGUMA, 1930
Trigomphus citimus tabei (Asah.)	Japan	11	yes	TOYOSHIMA & HIRAI, 1953
Trigomphus melampus (Sel.)	Japan	10	yes	OGUMA, 1930
Trigomphus melampus bifasciatus (Asah.) [3])	Japan	10	?	OMURA, 1957
EPIGOMPHINAE				
Epigomphus llama (Calv.)	Bolivia	10	no	CUMMING, 1964a
ICTINOGOMPHINAE				
Ictinogomphus rapax (Ramb.)	India	12	yes	ASANA & MAKINO, 1935
GOMPHOIDINAE				
Aphylla edentata Sel.	Bolivia	12	no	CUMMING, 1964a
Aphylla producta Sel.	Bolivia	12	no	CUMMING, 1964a
Gomphoides sp.	Bolivia	12	no	CUMMING, 1964a
Phyllocycla sp.	Bolivia	12	no	CUMMING, 1964a
Progomphus borealis McLach.	U.S.A.	12	no	CRUDEN, 1968
Progomphus intricatus Hag.	Bolivia	12	no	CUMMING, 1964a
Progomphus obscurus (Ramb.)	U.S.A.	12	no	CRUDEN, 1968
Progomphus phyllochromus Ris	Bolivia	12	no	CUMMING, 1964a
HAGENINAE				
Sieboldius albardae Sel.	Japan	12	?	OMURA, 1957

[3]) See text.

There was some controversy as to the chromosome numbers of *Gomphus melaenops* Selys and *Trigomphus melampus bifasciatus* (Asahina) (CRUDEN, 1968). The following numbers were reported for two Japanese populations of these species:

Gomphus melaenops: n ♂ = 10 (I) (TOYOSHIMA & HIRAI, 1953; HIRAI, 1956; – both publications obviously refer to the same material and are illustrated with the same figures); 2n ♂ = 23, n ♂ = 12 (I, II) (OMURA, 1957).

Trigomphus melampus bifasciatus: n ♂ = 12 (I) (TOYOSHIMA & HIRAI, 1953; HIRAI, 1956; – see the note above); n ♂ = 10 (I, II) (OMURA, 1957). For the Japanese "*Gomphus melampus*" (obviously synonym of *Trigomphus melampus bifasciatus* /Asah./) OGUMA (1930) also reported 10 elements in the primary and secondary spermatocytes.

We cannot but regard either the taxonomic identification or the chromosome counts given by TOYOSHIMA & HIRAI (1953) and HIRAI (1956) as erroneous.

For 19 of the species studied only the chromosome numbers were published. In 14 other species the sex chromosome picture is either "normal" (XO sex determination, X being one of the smallest elements) or no details of importance are recognizable in the documentation published. These 33 species will, therefore, not be further considered. In 8 species, on the other hand, peculiar features as to the sex chromosomes and the sex determination were found. These are: *Gomphus exilis, G. graslini, Onychogomphus forcipatus, Ophiogomphus bison, O. serpentinus, Trigomphus citimus tabei, T. melampus* and *Ictinogomphus rapax.*

OBSERVATIONS ON SEX CHROMOSOMES AND SEX DETERMINATION IN "ABNORMAL" COMPLEMENTS

In *Gomphus graslini* (Figs. 19–20), *Ophiogomphus serpentinus, Trigomphus melampus, Ictinogomphus rapax* and presumably in *Trigomphus citimus tabei* the sex element is by far the largest chromosome of the karyotype (for references cf. Table 4). So it is in the n = 12 complement of *Onychogomphus forcipatus* (to be discussed later).

The sex chromosome is positively heteropycnotic at spermatocyte pachytene, but at oocyte pachytene it is isocyclic (*O. serpentinus;* OKSALA, 1945). Undivided it precedes the dividing autosomal bi-

PLATE 1

Figs. 1–4. Spermatogonial metaphase and karyograms. (1500 ×): (1) *Enallagma cyathigerum* (Charp.) (Wasmeer, Hilversum, Netherlands). – (2) *Erythromma najas* (Hans.) (Nardermeer, Netherlands). – (3) *Aeshna mixta* Latr. (De Bilt, Netherlands). – (4) *Libellula pulchella* Drury (Ottawa, Canada) (m-chromosomes indicated by the arrows).

PLATE 2

Figs. 5–10. Allocycly of the sex element. (1500 ×): (5–6) *Ischnura elegans* (v. d. Lind.) (De Bilt, Netherlands). Heterocyclic (Fig. 5) and isocyclic (Fig. 6) sex element in two primary spermatocytes of approximately the same stage of the same individual. – (7–8) *Cordulegaster boltoni* (Don.) (Karawanken Mts., Carinthia, Austria). Heterocyclic (Fig. 7) and isocyclic (Fig. 8) sex element in two primary spermatocytes of approximately the same stage of the same individual. – (9) *Enallagma cyathigerum* (Charp.) (Wasmeer, Hilversum, Netherlands). Oocyte pachytene. (Note the isocyclic behaviour of all elements). – (10) *Cordulia aenea* (L.) (Hatertse Vennen, Nijmegen, Netherlands). Oocyte pachytene with positively heteropycnotic sex bivalent.

PLATE 3

Figs. 11–17. Lateral views of primary (a) and secondary (b) spermatocyte metaphase. (The arrows indicate the sex element). (1500 ×): (11) *Coenagrion pulchellum* (v. d. Lind.) (De Bilt, Netherlands). – (12) *Lestes virens* (Charp.) (De Bilt, Netherlands). – (13) *Calopteryx splendens splendens* (Harr.) (Karlsruhe, Western Germany). – (14) *Anax imperator* Leach (Montpellier, France). – (15) *Antogaster sieboldi* Sel. (Mt. Kurama, Kyoto, Japan). – (16) *Orthetrum cancellatum* (L.) (Oranjezon, Walcheren, Netherlands). – (17) *Sympetrum striolatum* (Charp.) (Gypsweiher, Bridel, Luxembourg).

PLATE 4

Figs. 18–22. Chromosomes of *Gomphidae*. (1500 × unless stated otherwise): (18) *Gomphus graslini* Ramb. (between Cabrerets and Sauliac on the Celé, Dépt. Lot, France). Spermatogonial metaphase. (Note the large sex element indicated by the arrow). – (19) Karyogram from Fig. 18. (2900 ×). – (20) The same specimen. Spermatocyte pachytene. (Note the large heteropycnotic sex element). – (21) *Gomphus exilis* Sel. (Montreal, Canada). Oogonial metaphase; 2n = 24. – (22) The same specimen. Oogonial metaphase with two pairs associated; 2n = 22.

PLATE 5

Figs. 23–25. Chromosomes of *Gomphidae*. (1500 ×). Spermatogonial metaphase and karyograms of *Onychogomphus forcipatus* (L.) (Warmbad Villach, Carinthia, Austria). (The dotted lines indicate the cell and chromosome borders). The association of two autosomes in each cell is understood as the first stage in the formation of the neo-neo-Y element. Further explanation in text (cf. also Textfig. 4).

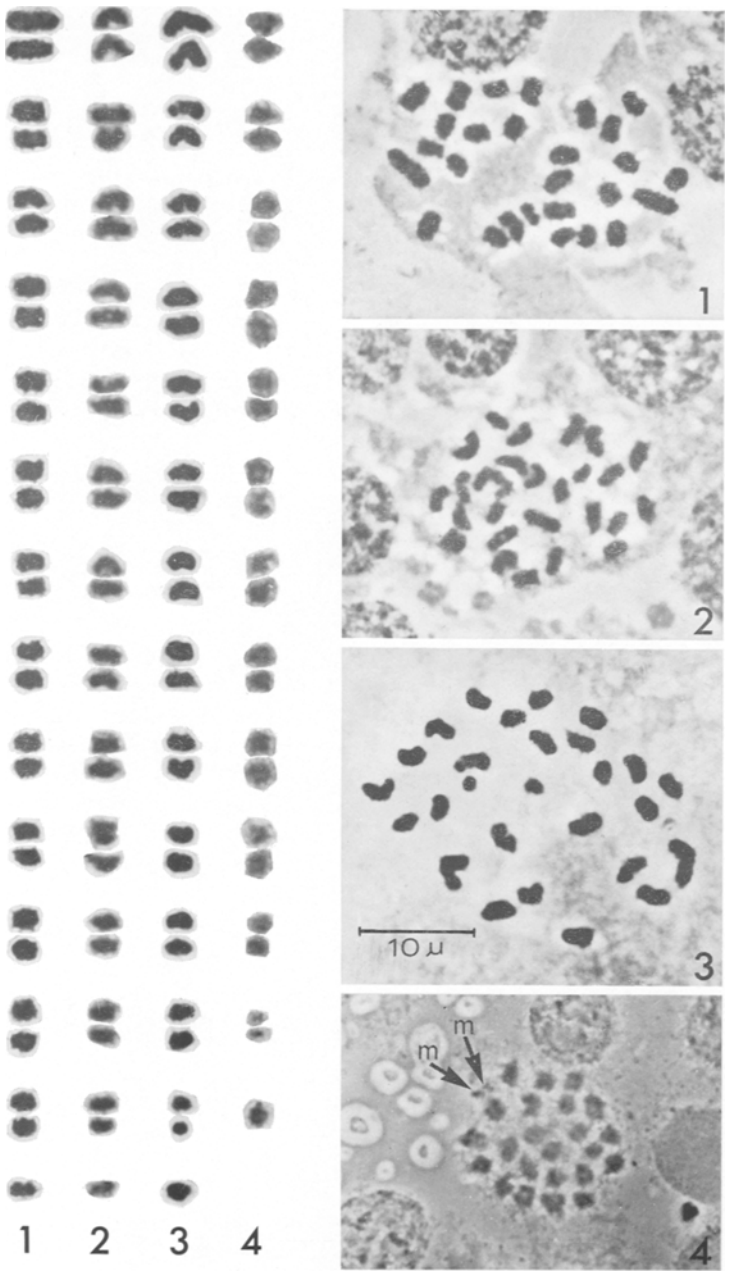
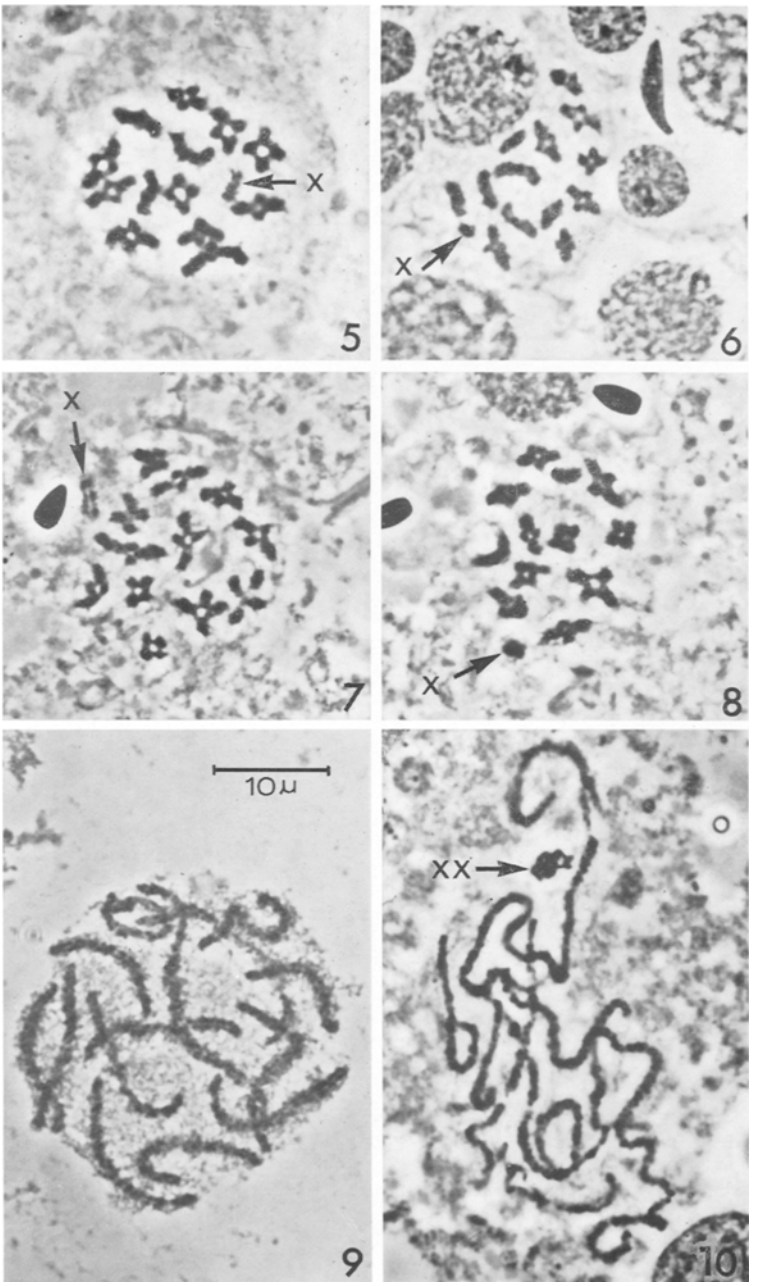


Plate 1.



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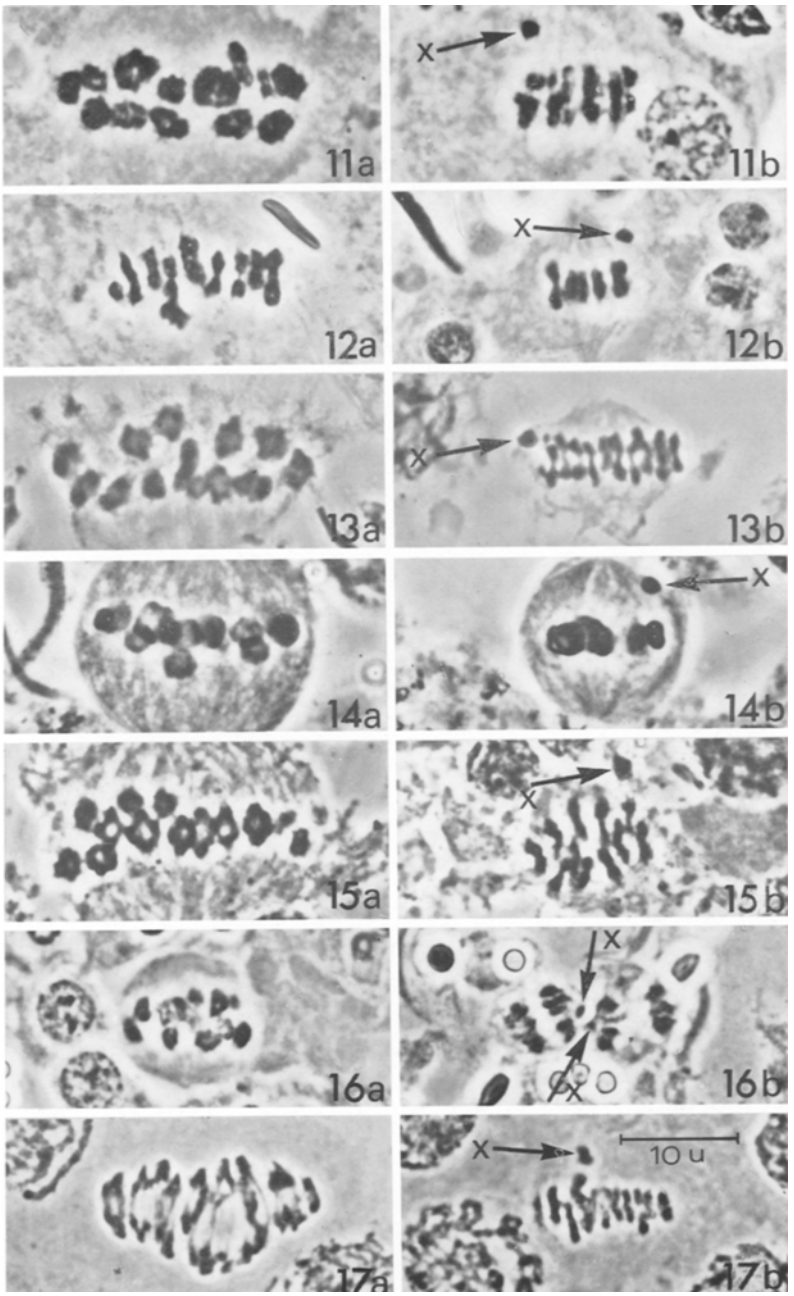
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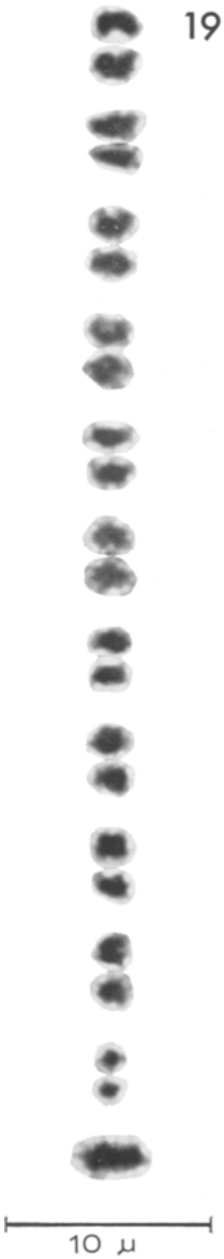
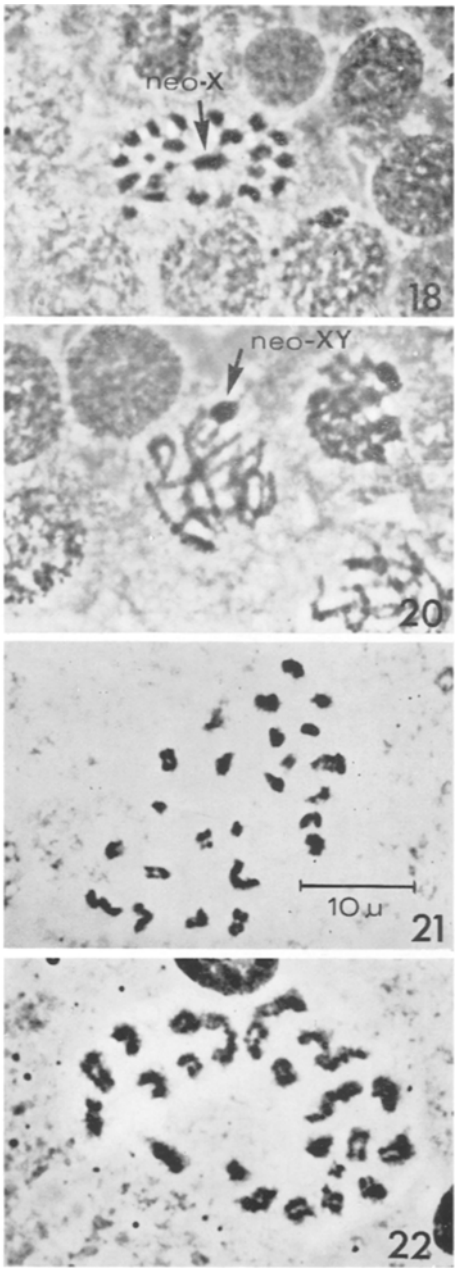
Plate 3.





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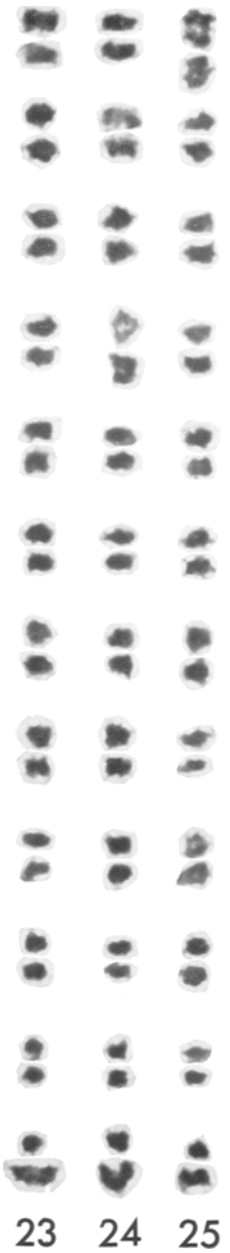

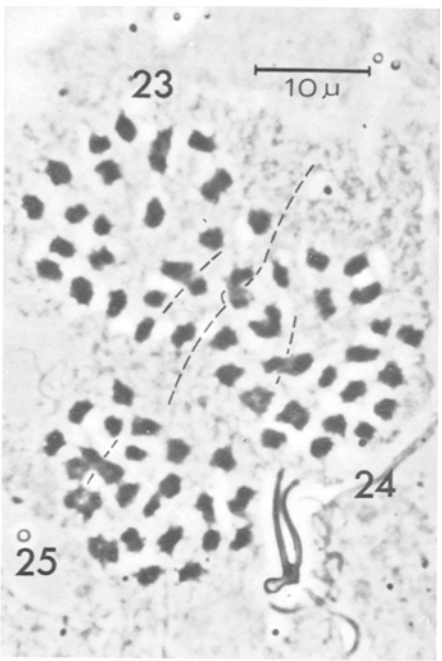

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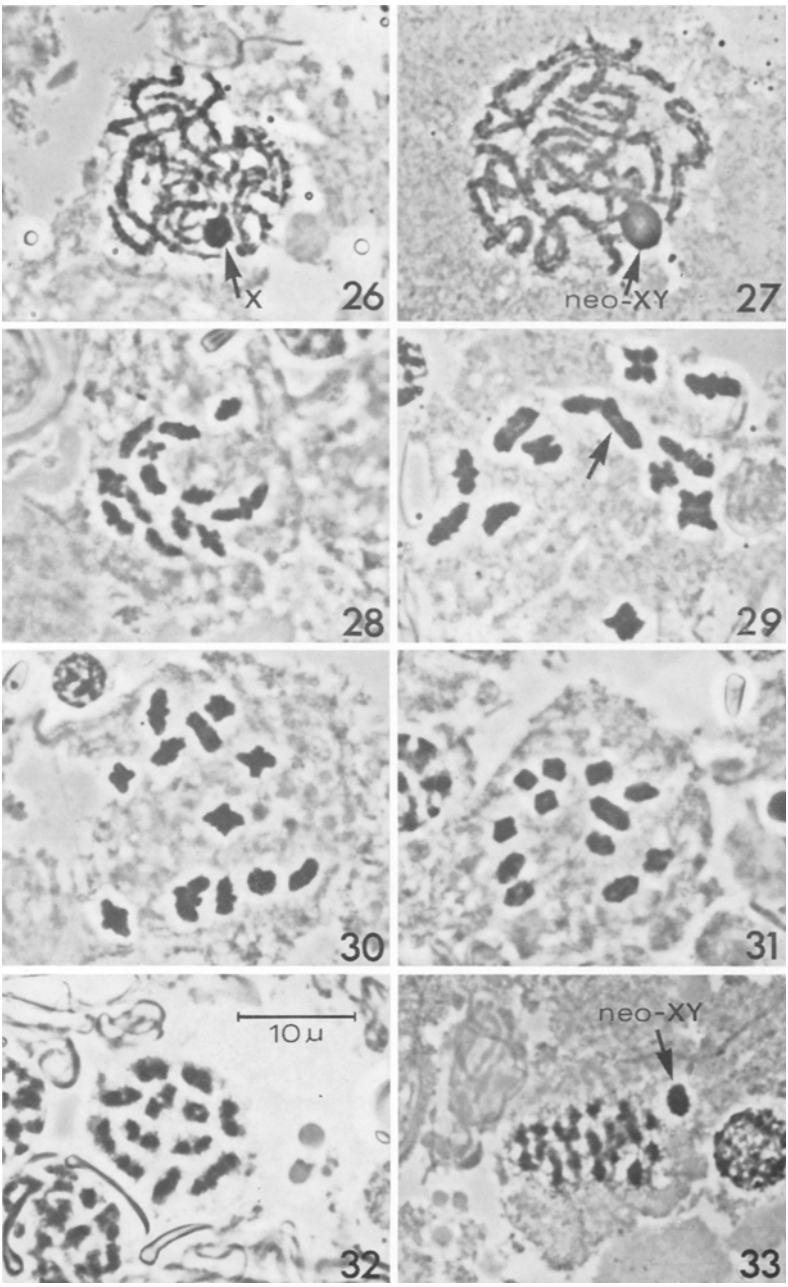

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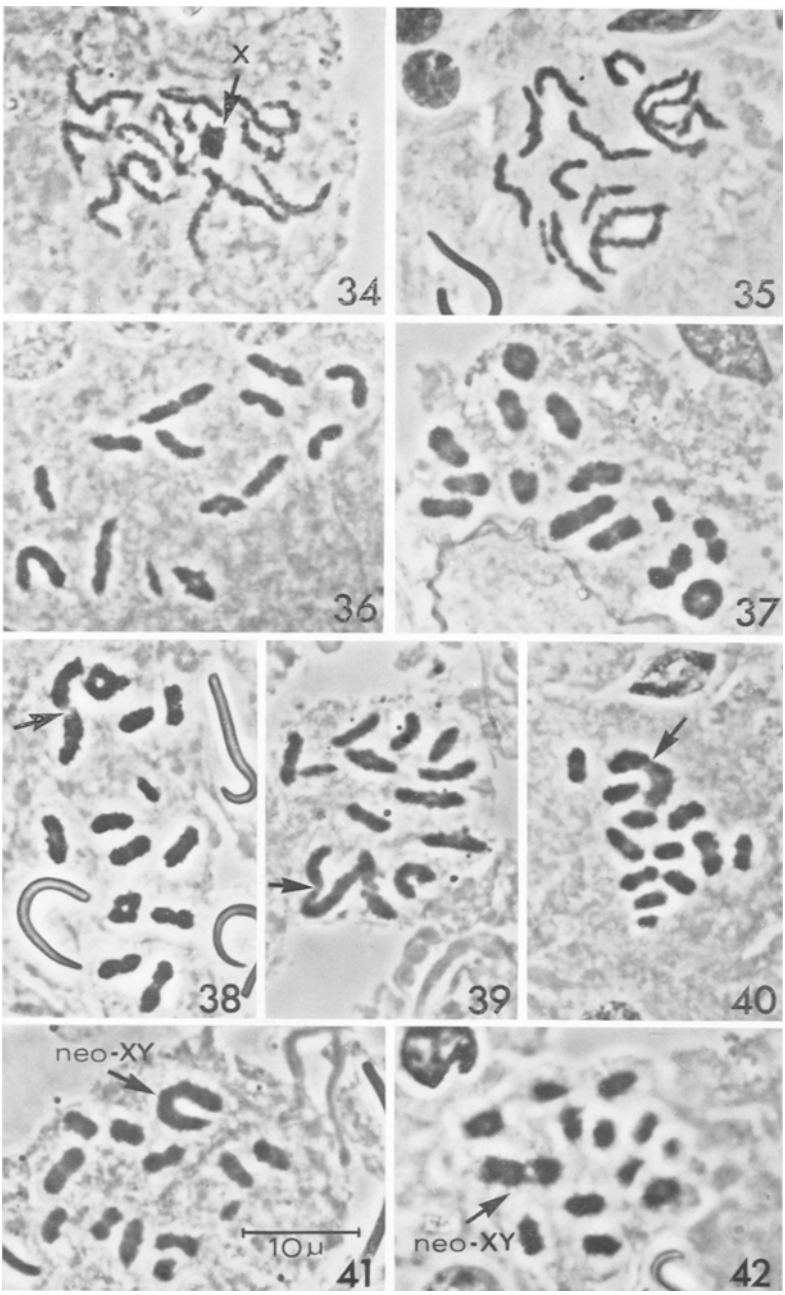

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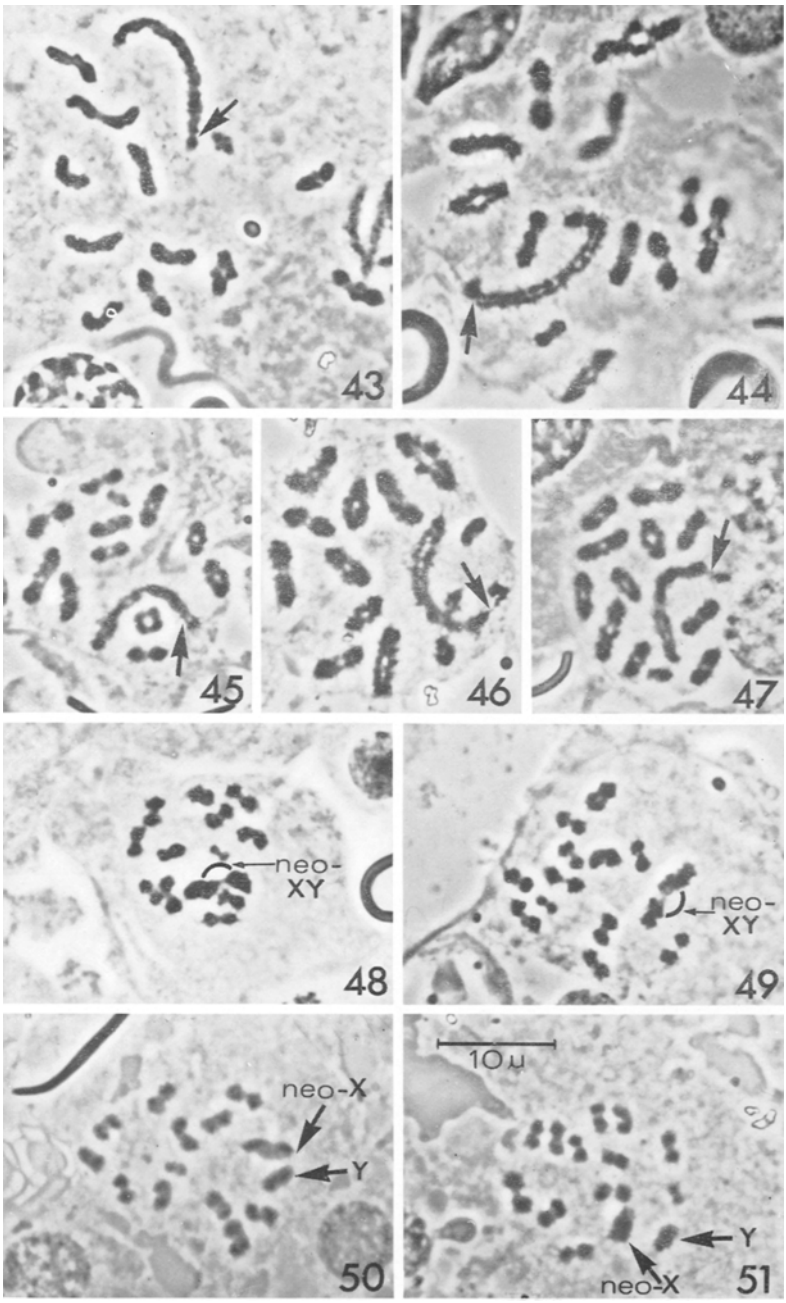

Plate 8.

PLATE 6

Figs. 26–33. Chromosomes of *Gomphidae*. (1500 ×). Spermatocyte stages of *Onychogomphus forcipatus* (L.) (Warmbad Villach, Carinthia, Austria): (26) Pachytene. (Note the relatively small heteropycnotic sex element, belonging probably to an XO complement). – (27) Pachytene. (Note the large heteropycnotic sex element, belonging presumably to a neo-XY complement). – (28) Primary spermatocyte metaphase of an n = 13 complement (XO sex determination). – (29) Primary spermatocyte metaphase. End-to-end association of two elements (one of these presumably represents the sex chromosome). – (30) Early primary spermatocyte metaphase of an n = 12 complement (neo-XY sex determination). – (31) Primary spermatocyte metaphase of an n = 12 complement. – (32) Secondary spermatocyte metaphase of an n = 12 complement. – (33) Secondary spermatocyte anaphase of an n = 12 complement (lateral view). (Note the heterokinesis of the neo-XY element).

PLATE 7

Figs. 34–42. Spermatocyte stages of *Aeshna grandis* (L.) (De Bilt, Netherlands). (1500 ×): (34) Pachytene. – (35) Late pachytene of an n = 14 complement (XO sex determination). (Note the isocyclic behaviour of all elements). – (36) Early primary spermatocyte metaphase of an n = 14 complement. – (37) Primary spermatocyte metaphase of an n = 14 complement. – (38–40) Primary spermatocyte metaphases of an n = 14 complement. (Note the increasing grade of attachment of X to an autosomal bivalent). – (41–42) Primary spermatocyte metaphase of an n = 13 complement. (Note the big size of the neo-X trivalent).

PLATE 8

Figs. 43–51. Spermatocyte stages of *Aeshna grandis* (L.) (De Bilt, Netherlands). (1500 ×): (43–47) Early primary spermatocyte metaphase with a subterminal constriction on the neo-X trivalent (indicated by the arrows). In Figs. 46–47 the terminal section is nearly entirely separated from the trivalent. – (48–49) Secondary spermatocyte anaphase (polar views) (Note the advanced division of the sex element in two unequal parts, indicated by the arrows). – (50–51) Secondary spermatocyte anaphase (polar views). The division of the sex element (indicated by the arrows) is completed. (Note the difference in size of the two parts, Y and neo-X).

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valents at anaphase II (*T. melampus;* OGUMA, 1930; *I. rapax;* ASANA & MAKINO, 1935).

In the figure of spermatocyte diakinesis of *Gomphus exilis* published by CRUDEN (1968, fig. 39) the sex element is the second smallest element of the set (an m-bivalent is present). In our figures (material from Montreal, Canada) of female mitotic metaphase the sex pair is, of course, not recognizable. In most of the figures available 24 elements

can be counted (Fig. 21), but in one of them two smaller elements seem to be attached to the longest two chromosomes (Fig. 22). If this were so, and provided that the sex elements are involved, a tentative explanation of the origin of the unusually large sex chromosome as seen in some representatives of the family, would lie at hand.

Another curious situation is met with in *Onychogomphus forcipatus* and *Ophiogomphus bison.*

In spermatogonial metaphase of Austrian specimens (Warmbad Villach, Carinthia) of *O. forcipatus* 24 elements were counted. The longest element apparently represents the neo-X, whereas the Y is one of the smallest chromosomes of the set (Figs. 23–25). Thus the original chromosome number in spermatogenesis must have amounted to $2n = 25$.

At spermatocyte pachytene the heteropycnotic neo-X is unusually large (Fig. 27). At metaphase I cells with 13 and with 12 elements occur (Figs. 28–31). The X of the $n = 13$ complement is one of the smaller chromosomes, but cannot always easily be distinguished from the autosomal bivalents (Fig. 28). In metaphase I figures of the $n = 12$ set, the sex element is the biggest element (Figs. 30–31). In figures of metaphase II 12 elements were always counted (although cells with 13 elements are to be expected, in view of the $n = 13$ primary spermatocytes), but the sex chromosome cannot be readily distinguished from the other elements (Figs. 32–33). At secondary spermatocyte anaphase the X is by far the largest of the 12 elements. Its kinetic behaviour is normal (Fig. 33).

Of particular interest are the figures of late spermatocyte diakinesis, showing very clearly the association of two longer elements. The structure of the latter is not clear (Fig. 29).

In view of the presence of the numerical variation within the same specimen, fusion seems to be reversible. Cells with 12 elements are more numerous than the 13-chromosomes sets.

A similar situation has been found in *Ophiogomphus bison* of which three different Californian populations have been studied. Here too, the complements $n = 12$ and $n = 13$ occur. CRUDEN (1968; figs. 45–46), who studied the original material, is of the opinion that the $n = 13$ complement is "apparently due to a 'breaking' of a chromosome". We are inclined to believe, however, on the basis of the figures published by CRUDEN, that not a split in two of a chromosome in the

n = 12 complement, but a fusion of two elements in the n = 13 set is cause of the karyotypic variation in this species. The suggestion is supported by the presence of a very big bivalent in the n = 12 set, which is unusual for the *Gomphidae*. Whether or not only the autosomes are involved in fusion can not be ascertained solely on the basis of CRUDEN's figures. In view of the situation in *Onychogomphus forcipatus* the assumption that the sex element is taking part in the fusion is certainly tentative.

Summarizing our knowledge on the sex chromosomes in the *Gomphidae*, the following points should be stressed:

(1) Next to the *Polythoridae* (of which the chromosome numbers of only two species have become known, but no figures were ever published; CUMMING, 1964a), the *Gomphidae* are the only dragonfly family with a family type number of 12. At the same time, this is the only family known where the sex chromosome is often by far the largest or among the largest elements of the mitotic and meiotic complement.

(2) Unusually large X-es are seen in the genera *Gomphus*, *Onychogomphus*, *Ophiogomphus*, *Trigomphus* and *Ictinogomphus*. The size of the sex element, however, is not taxonomically characteristic either on the generic or the subfamily level.

(3) The fusion of X with an autosome (neo-XY sex determination mechanism) occurs at least in *Onychogomphus forcipatus*, probably in *Ophiogomphus bison* and, less certainly, in *Gomphus exilis*. The haploid number after fusion amounts to 12 elements in the former two species and to 11 in the latter.

(4) It is likely that fusion is reversible in the species mentioned and occurs in some cells of one individual, while not in others. The phenomenon is specifically characteristic at least in *Ophiogomphus bison*, of which three different populations were studied.

(5) In view of the fact that the fusions in the *Gomphidae* bring the chromosome number to n = 12 or lower, it seems likely that the present type number of the family (n = 12) is of secondary rather than of primary origin (cf. KIAUTA, 1967). The problem will be considered below in some detail.

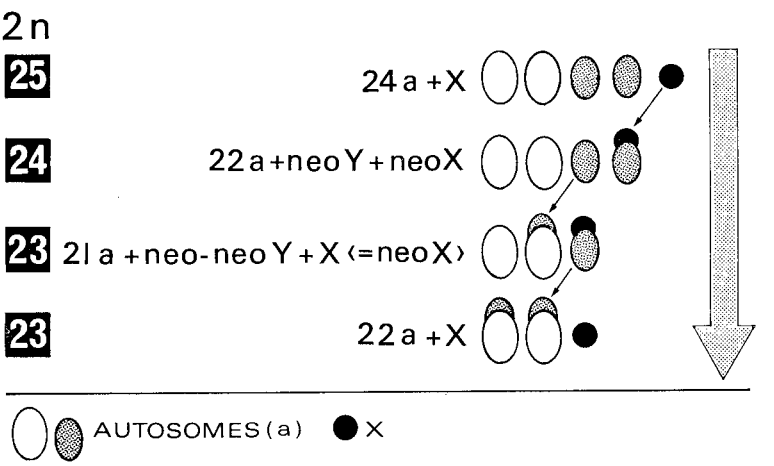
KARYOTYPIC EVOLUTION IN THE GOMPHIDAE, WITH SPECIAL REFERENCE TO SEX CHROMOSOMES AND SEX DETERMINING MECHANISMS IN THE FAMILY

The highest diploid number assumed to occur in the family is 25. The suggestion is based on the occurrence of the haploid number, n = 13, in primary spermatocytes of *Onychogomphus forcipatus* (Fig. 28) and *Ophiogomphus bison*. In the former species, at least in some spermatogonial cells also 24 elements are present: 22 autosomes, a big neo-X and a small neo-Y (Figs. 23–25). In *Gomphus graslini* (Figs. 18–20), *Onychogomphus serpentinus*, *Trigomphus melampus* and *Ictinogomphus rapax* the diploid number is 23: 22 autosomes and a large X. In most other *Gomphidae* 23 elements are present at mitosis in the male, but the X is one of the smallest chromosomes of the set, like in all other dragonflies studied.

The problem as to the evolutionary sequence of the four karyotypes described above lies at hand. No definite proof can be provided for either direction, but, for the time being, we are inclined to suggest an evolutionary trend in the direction of the numeric reduction of the complement. Our supposition is illustrated in Textfig. 4. The sequence of the rearrangements in the karyotype is supposed to follow the following pattern:

(1) In a 2n = 25 complement, with an XO-XX sex determining mechanism and a small sex element, the latter fuses with an autosome. The fusion gives rise to a 2n = 24 complement and a neo-XY sex determination. This situation is met with in *Onychogomphus forcipatus* (Figs. 23–25) and *Ophiogomphus bison*.

(2) The neo-Y of the 2n = 24 set becomes attached to an autosome. The result is a 2n = 23 complement, including a big neo-neo-Y and a neo-X of similar size. This step seems to be recognizable in figures of spermatogonial metaphase of *Onychogomphus forcipatus*, where two autosomes indeed appear more or less associated, though the original 24 elements are still recognizable (Figs. 23–25). An advanced stage of this step is met with in *Gomphus graslini* (Fig. 19), *Onychogomphus serpentinus*, *Trigomphus melampus* and *Ictinogomphus rapax*. In these species the double structure of the unusually large X is not recognizable; nevertheless, we assume it to be of a true neo-X nature by origin. Likewise, the neo-Y can not be distinguished from other autosomes. This phenomenon can tentatively be ascribed to the minute morphologic variation in



Textfig. 4. Schematic interpretation of the evolution of sex determination and reduction of the chromosome number in the family *Gomphidae*. Explanation in text.

the chromosomes, which makes identification difficult and uncertain.

(3) The last step is the fusion of the autosomal component of the large neo-X of the former karyotype with an autosome. The result is a "normal" karyotype, 2n = 23, with the sex element being again one of the smallest elements – exactly as it is in the original, 2n = 25, sets. The process of this fusion can not be actually demonstrated on our material. The assumed result of it are the complements of most of the so far studied gomphidan dragonflies.

If one applies the same evolutionary pattern to *Trigomphus citimus tabei*, with the haploid number of 11 and a large sex chromosome, the original diploid number of this species has been either 23, or the process of numeric reduction evolved for a cycle further than in the preceding species.

There are firm grounds to assume a general trend for the karyotype in insect orders processing holokinetic chromosomes to evolve from numerically low (primitive forms) to numerically high (advanced and specialised forms) complements (KIAUTA, 1967, with bibliography; KIAUTA, 1968c). The *Gomphidae* do not make any exception to this

rule. The present type number of the family, however, is of secondary and not of primary origin. The family has reached, in the course of evolution, a higher chromosome number, but the latter has been reduced by secondary fusions. The following are the reasons for the suggestion that secondary fusions rather than primary fragmentations are responsible for the curious numeric situation in the karyotypes of the present-day gomphids:

(1) The highest haploid number met with in the family is 13. It appears in a "normal" complement, whereas in the lower complements the sex chromosome is often unusually large; therefore, its size can only be explained by fusion of two elements.

(2) The formation of the neo-XY sex determination came about in dragonflies, without a single exception, by fusion of the original X with an autosome. The result is a big neo-X and a numeric reduction of the primary complement by one.

(3) If the evolution under discussion were numerically progressive, simultaneous occurrence of fragmentations and fusions would have to take place. There is not any evidence of such a phenomenon in the material so far studied. On the other hand, there is a conclusive cytological evidence for the following evolutionary stages and their sequence reflected in the intermediate stages (cf. Textfig. 4): $2n = 24a + X$; process of reduction to $2n = 22a +$ neo-Y + neo-X; $2n = 22a +$ neo-Y + neo-X; process of reduction to $2n = 21a +$ neo-neo-Y + neo-X; and $2n = 22a +$ X. The process of reduction from $2n = 21a +$ neo-neo-Y + neo-X to $2n = 22a + X$ is the only step which can not be demonstrated on our material.

Sex Chromosomes in the Neo-XY-XX Complement

The neo-XY-XX sex determining mechanism has been found so far in 15 species of the families *Gomphidae, Aeshnidae, Corduliidae* and *Libellulidae*. The figure represents approximately 4% of the dragonflies studied cytologically, but not even in all of these is the nature of sex determination absolutely clear (cf. Table 5). In Table 5 the known cases of the occurrence of the neo-XY-XX sex determination in dragonflies are listed. However, a number of species have not been included, in which a neo-XY sex determining mechanism could not be

demonstrated with certainty (cf. CUMMING, 1964a). The neo-X nature of the large X-es met with in various *Gomphidae* was discussed in the preceding chapter.

Though the neo-XY-XX mode of sex determination seems peculiar for some species, it is not characteristic on any higher taxonomic level.

ORIGIN OF THE NEO-XY-XX SEX DETERMINING MECHANISM IN DRAGONFLIES

In most cases where the neo-XY mode of sex determination can be demonstrated, it originates in the fusion (or end-to-end association) of the original sex chromosome with a single autosome. In the same species the sex chromosome and one autosome are the only fused elements of the karyotype. The fusion results in the numeric reduction of the original complement by one. The sole exception is *Hemianax ephippiger*, where all elements of the original complement are fused two by two, save for the two m-chromosomes which remain unfused, and the neo-X which is composed of two autosomes and the original sex element. The original diploid number is thus reduced, in this species, by n – 1 element (from 27 ♂ into 14 ♂, ♀ (cf. SESHACHAR & BAGGA, 1962).

So far only three cases were reliably described, where a fusion takes place only between some autosomes, whereas the original X element remains free. These are: *Aeshna diffinis diffinis* Ramb. (2n ♂ = 21, n ♂ = 11), *A. intricata* Martin (2n ♂ = 19, n ♂ = 10) and *Perithemis lais* (Perty) (2n ♂ = 17, n ♂ = 9) (CUMMING, 1964a). This evidence leads to the assumption that the sex chromosome is more liable to fusion than the other elements of the odonate karyotype.

In the same species the original sex element in the neo-XY-XX complement is always fused with or attached to the same autosome (cf. also KIAUTA, 1967) and differs, in this feature, essentially from the situation met with in some Orthoptera, where in different specimens of the same species it can be attached to different autosomes (MCCLUNG, 1917).

In diakinetic and metaphase I figures of *Onychogomphus forcipatus* and *Aeshna grandis* a series of intermediate stages occur next to primary complements (n = 13; Fig. 28 and n = 14; Figs. 36–37

TABLE 5

SUMMARY OF THE DATA ON THE NEO-XY-XX SEX DETERMINATION IN DRAGONFLIES

Species (and family)	Locality	Chromosome number original 2n	original n	after fusion 2n	after fusion n	Character of fusion *Remarks*	References
Gomphus exilis Sel. (Gomphidae)	Canada	24 ♀		22 ♀		in some figures of oogonial metaphase two small elements attached to the two longest chromosomes; fusion uncertain. *original sex chromosomes not identified; no fusion noted by* CRUDEN (*1968, U.S.A.*).	this paper (see above)
Onychogomphus forcipatus (L.) (Gomphidae)	Austria		13 ♂	24 ♂	12 ♂	original X one of the smaller elements; neo-X the biggest, neo-Y the smallest; fused and unfused complements in the same individual; fusion reversible (?).	this paper
Ophiogomphus bison Sel. (Gomphidae)	U.S.A.		13 ♂		12 ♂	fused and unfused complements in the same individual; fusion reversible (?); the same situation found in three different populations. *original author believed the numeric variation being due to fragmentation.*	CRUDEN 1968
Aeshna coerulea Ström (Aeshnidae)	Finland				12 ♂	original X small and attached to the m-bivalent at metaphase I.	OKSALA 1943
Aeshna grandis (L.) (Aeshnidae)	U.S.S.R.		14 ♂	25 ♂	13 ♂	*neo-X referred to as "gigantic bivalent"; no fusion noted by* FUCHSÓWNA & SAWCZYŃSKA (*1928, U.S.S.R.*).	MAKALOWSKAJA 1940
	Finland	28 ♀		26 ♂ 26 ♀	13 ♂ 13 ♀	original X big and attached to the biggest autosome.	OKSALA 1943, 1945
	Netherlands		14 ♂		13 ♂	original X of medium size and attached to the biggest bivalent; fused and unfused complements in the same individual; fusion reversible (?).	KIAUTA 1967, 1968b
Aeshna juncea (L.) (Aeshnidae)	U.S.S.R.				13 ♂	—	MAKALOWSKAJA 1940
	Finland	27 ♂	14 ♂	26 ♂ 26 ♀	13 ♂	original X very small; not known with which element fused; fusion not obligatory (in six ♂♂	OKSALA 1943

					2n = 26, in one ♂ 2n = 27). 2n = {26 *referred to as "gewöhnlich".*	
Aeshna osiliensis fennica Valle (Aeshnidae)	Finland			13 ♂	original X very small; not known with which element fused.	OKSALA 1943
Aeshna viridis Eversm. (Aeshnidae)	Finland		26 ♀	13 ♂	original X very small; not known with which element fused; fusion not obligatory. *number referred to as "gewöhnlich".*	OKSALA 1943
Hemianax ephippiger (Burm.) (Aeshnidae)	India		14 ♂ 14 ♀	7 ♂	—	SESHACHAR & BAGGA 1962
Epitheca cynosura (Say) (Corduliidae)	U.S.A.	11 ♂		10 ♂	whether or not X takes part in fusion is uncertain; fused and unfused complements in the same population. *according to the original author only autosomes are involved in fusion.*	CRUDEN 1968
Crocothemis servilia (Drury) (Libellulidae)	Japan			12 ♂	"dot-shaped X end-to-end associated with an autosome"; in one case X free. *no fusion noted (2n = 25, n = 13 complements described and figured) by* ASANA & MAKINO *(1935, India),* RAY CHAUDHURI & DAS GUPTA *(1949, India).*	OMURA 1955
Neurothemis tullia tullia (Drury) (Libellulidae)	India		28 ♂	14 ♂	originates in fragmentation of two autosomal pairs and subsequent fusion of the original X with an autosomal fragment. *delayed pairing of neo sex elements in primary spermatocytes.*	RAY CHAUDHURY & DAS GUPTA 1949
Pseudothemis zonata Burm. (Libellulidae)	Japan			12 ♂	"dot-shaped X end-to-end associated with an autosome"; fusion appears permanent.	OMURA 1955
Rhyothemis fuliginosa Sel. (Libellulidae)	Japan			12 ♂	one element very big; whether or not X takes part in fusion is not clear. *n = 13 (elements decreasing in size) described by* OMURA *(1955, Japan).*	TOYOSHIMA & HIRAI, 1953; HIRAI 1956
Leucorrhinia frigida Hagen (Libellulidae)	U.S.A.	12 ♂		11 ♂	whether or not X takes part in fusion is not clear. *according to the original author only autosomes are involved in fusion.*	CRUDEN 1968

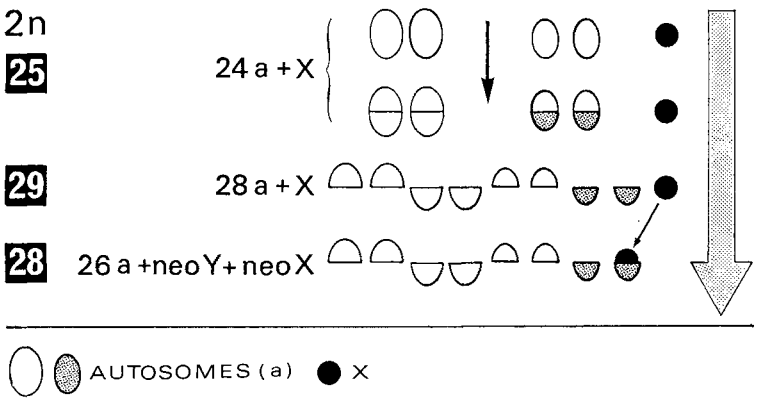
respectively) and secondary complements (n = 12; Figs. 30–31 and n = 13; Figs. 36–37 respectively). However, in these stages, an element, considered to represent the original isocyclic X-chromosome, and an autosomal bivalent appear to be associated end-to-end (Figs. 29 and 38–40 respectively). Apparently, the association has reached a different degree in different cells. This evidence suggests that, at least in some cells, the secondary fusion does not take place before the first maturation division.

OKSALA (1943) stated that in those species of the genus *Aeshna* where the neo-XY system occurs, the fusion of the original sex element is obligatory ("ganz unbedingt") in spermatocytes and oocytes, whereas it is not so in spermatogonia and in somatic cells. An obligatory fusion of the sex chromosome was supposed by OMURA (1955) for *Pseudothemis zonata* as well, but in the same paper he reported on the neo-XY and XO karyotypes in *Crocothemis servilia* (cf. Table 5).

Our observations on *Onychogomphus forcipatus* and *Aeshna grandis* do not confirm OKSALA's opinion. On the contrary, the neo-XY system, though characteristic for some species, is not obligatory for even all meiotic cells of one individual. This suggestion is supported by observations on *Ophiogomphus bison, Epitheca cynosura* and *Leucorrhinia frigida* (CRUDEN, 1968; cf. Table 5).

In *Neurothemis tullia tullia*, studied by RAY CHAUDHURI & DAS GUPTA (1949), the neo-XY sex determination originated in a unique way. There are 28 chromosomes at spermatogonial metaphase, whereas 13 bivalents (including the m-bivalent) and an unpaired neo-X and neo-Y occur in primary spermatocytes. The latter do not pair at diplotene and diakinesis. The first maturation division is equational for the sex elements, whereas they lie in a forward position on either side of the equatorial plane in the second anaphase.

The type number of the family *Libellulidae* is 13 (2n = 25) and *Neurothemis tullia tullia* is the only species with a higher number. The numerical increase can be due only to fragmentation of two autosomal pairs and subsequent fusion of the original X with a fragment of an autosome. The process is illustrated in Textfig. 5. The fragmentation results in a diploid complement, 2n = 29, in which the XO sex determination is still retained (this stage has been reached in an unidentified species of the pseudostigmatidan genus *Mecistogaster;* CUMMING, 1964a). The subsequent fusion of the original X with an



Textfig. 5. Schematic interpretation of the evolution of sex determination and numeric increase of the chromosome complement in *Neurothemis tullia tullia* (Drury). Explanation in text.

autosomal fragment reduces the chromosome number by one and gives rise to the neo-XY sex determination.

Neurothemis tullia tullia is thus the only so far known dragonfly in which the neo-XY condition does originate in a fusion of the unpaired X, not with an entire autosome, but only with a fragment.

BEHAVIOUR OF THE NEO-X ELEMENT

The sex component of the neo-X retains, in general, all features of the original X and transmits some of them also to the autosomal part. The latter exercises only occasionally a limited influence on the original sex component. The mutual influences result in a behavioural pattern characterised by the following features:

(1) In species (or individuals) characterized by facultative neo-XY sex determination, *unfused* X chromosomes at spermatocyte prophase are isopycnotic in about 50% of the cells. It seems likely, therefore, that isocycly might represent one of the conditions for fusion between two elements (Figs. 34–35).

(2) In part of the spermatocyte prophase figures of these species the positively heteropycnotic sex element corresponds in size with the original X, whereas in others it is approximately twice as big. The former are assumed to represent the primary, and the latter the

secondary complements (Figs. 26–27). Consequently, at spermatocyte prophase both the original X and the autosomal component of the neo-X are positively heteropycnotic.

(3) At secondary spermatocyte anaphase the neo-X either is heterokinetic and lies in a forward position with regard to the dividing autosomes (Fig. 33), or it is lined up in the equatorial plane with the autosomes, only its autosomal part dividing simultaneously with them (*Aeshna grandis*, Figs. 48–51, and OKSALA, 1943, figs. 76, 81; *A. coerulea*, OKSALA, 1943, figs. 77–78). The difference in anaphase II behaviour does not depend on the relative sizes of the original sex element and the autosome. (In *Onychogomphus forcipatus* and *Aeshna grandis* both parts are similar in size, while in *A. coerulea* the original X is fused with an m-element; cf. Figs. 48–49).

According to observations of SESHACHAR & BAGGA (1962) in *Hemianax ephippiger*, primary spermatocyte anaphase in this species, gives rise to two types of daughter nuclei: one with a neo-X chromosome and the other with a neo-Y, whereas anaphase II is reported to be equational for all elements. If this were true, the species could be regarded as an exceptional case of prereduction for all elements. The fact, however, that the present complement of *Hemianax ephippiger* originates in simultaneous fusion two-by-two of the elements of the primary complement, in which only the m-chromosomes remain unaffected (cf. figs. in SESHACHAR & BAGGA, 1962), demonstrates clearly the neo-XY nature of sex determination in this species. Since the two neo sex elements are of almost equal size, the second maturation division is only seemingly equational for the neo-X. Actually the situation is the same as in *Aeshna grandis*. In this species too, only the autosomal part of the neo-X element divides.

In some spermatocyte I figures of *Aeshna grandis* a secondary subterminal constriction was observed on the neo-X trivalent (Figs. 43–47). It is particularly pronounced in some figures of early metaphase I (Figs. 46–47). The constriction is not identical with the point of fusion of the original X. It occurs at the other end of the trivalent and forms a segment essentially shorter than the X part. Whether or not it leads to fragmentation and thus to a secondary increase of the chromosome number, cannot be ascertained on our material.

THE NEO-Y ELEMENT

The homologue of the autosomal component of the neo-X plays the role of the neo-Y and is confined to the male line (Figs. 23–25).

The gonial behaviour of the neo-Y does not differ in any respect from that of the other autosomes. The sole exception may be its positive heteropycnosis at spermatogonial interphase in *Hemianax ephippiger*, as recorded by SESHACHAR & BAGGA (1962, fig. 5). No similar case has ever been observed in dragonflies, though occasional heteropycnosis of autosomal segments is a not uncommon feature in various mitotic and meiotic stages of Odonata (cf. SRIVASTAVA & DAS, 1953); therefore a further examination would be useful.

In the first maturation division the neo-Y pairs with the homologous part of the neo-X, forming in this way a sex trivalent.

The neo-Y is supposed to play an important role in the process of numeric reduction of the complement in *Gomphidae*. The latter has been discussed in the preceding chapter (cf. Textfig. 4).

SEX DETERMINATION IN SPECIES WITH STRONGLY REDUCED CHROMOSOME COMPLEMENTS

Hemianax ephippiger is the only dragonfly with a strongly reduced complement in which the neo-XY-XX sex determining mechanism can be demonstrated cytologically.

CUMMING (1964a) reported on the cytological conditions in four other dragonflies of the families *Pseudostigmatidae* and *Libellulidae* in which a simultanous fusion of all elements has taken place. In none of these is the sex determining mechanism recognizable, nor could any sex chromosomes be demonstrated cytologically. The same holds for *Dorocordulia libera* studied by CRUDEN (1968), though in one population of it, one of the smaller elements of the original set appears to remain free (cf. CRUDEN, 1968, figs. 55–57). The main cytological data on these species are given in Table 6.

It seems that a neo-XY sex determination can safely be assumed for species studied by Cumming and at least for the n = 6 complements of *Dorocordulia*.

TABLE 6

CYTOLOGICAL DATA ON SPECIES WITH STRONGLY REDUCED CHROMOSOME COMPLEMENTS

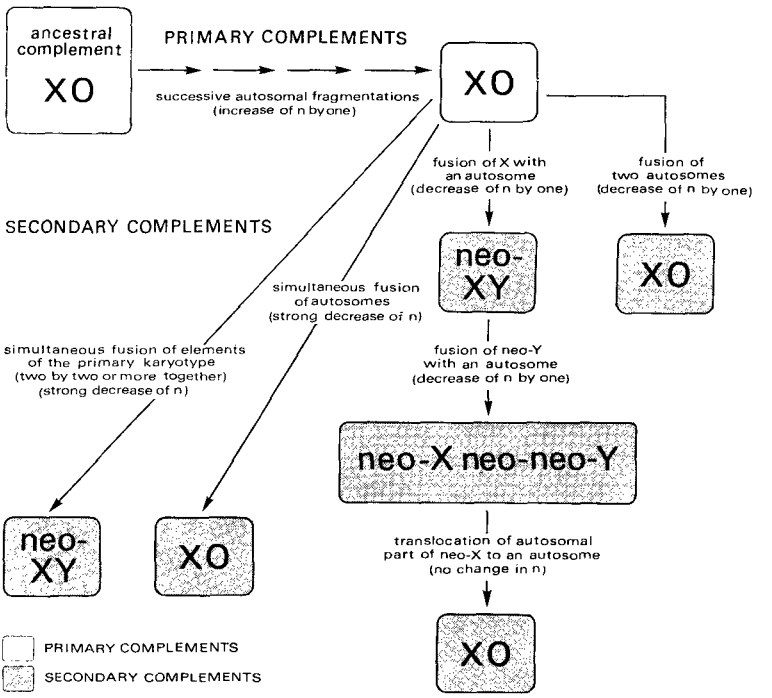
Species (and family)	Locality	Chromosome number 2n	n	Remarks	Reference
Mecistogaster sp. (Pseudostigmatidae)	Bolivia		6 ♂		CUMMING, 1964a
Dorocordulia libera (Sel.) (Corduliidae)	U.S.A.	13 ♂	6 ♂ 7 ♂	two populations studied; in one of these only n = 7.	CRUDEN, 1968
Orthemis ferruginea (Fab.) (Libellulidae)	Bolivia	10 ♂	5 ♂	in specimens from U.S.A. (Texas) 2n ♂ = 23, n ♂ = 12 and XO sex determination.	CUMMING, 1964a
Orthemis levis Calv. (Libellulidae)	Bolivia	6 ♂ 7 ♂ 8 ♂	3 ♂ 4 ♂		CUMMING, 1964a, 1964b (partim)
Macrothemis hemichlora (Burm.) (Libellulidae)	Bolivia	6 ♂	3 ♂		CUMMING, 1964a

Trends in the Evolution of Sex Determination in Odonata

The XY/XX sex determining mechanism which is considered to be most primitive in organisms and from which all other modes of sex determination are supposed to be derived (DARLINGTON, 1965), is not present in dragonflies. In the complements considered to be most primitive (KIAUTA, 1967) the XO/XX sex determining mechanism occurs. The latter can be considered, therefore, as the most primitive condition in the order.

A graphical interpretation of the paths of evolution of sex determining mechanisms in dragonflies is given in Textfig. 6.

The increase in advancement and specialisation was accompanied by the numeric increase of the chromosome complement, which came about exclusively by successive autosomal fragmentations. The original XO condition remained unaffected in this process. Not a single case of fragmentation of the original sex element has ever been reported in dragonflies. In the primary complements, therefore, the XO/XX



Textfig. 6. Diagram of the trends in the evolution of the sex determining mechanism in Odonata (cf. also KIAUTA, 1967, fig. 4). Explanation in text.

system is the only mode of sex determination met with. It is interesting to note, in this connection, that in Heteroptera (which are also characterised by diffuse condition of kinetochore) fragmentation of the original sex element has been reported in numerous species, belonging to at least ten different families (TROEDSSON, 1944; SCHRADER, 1947; WHITE, 1954, with bibliography).

In two advanced species (*Mecistogaster* sp. and *Neurothemis tullia tullia*) a simultaneous fragmentation of two autosomal pairs is supposed to have occurred. It was followed, in the latter species, by a fusion of the original X with an autosomal fragment, resulting in a numeric decrease of the complement and establishment of the neo-XY sex determination (cf. Textfig. 5). In spite of the high chromosome number (2n ♂ = 28) *Neurothemis* thus has a secondary chromosome complement (sensu KIAUTA, 1967), whereas the karyotype of *Mecisto-*

gaster sp., in which no fusion has occurred (2n ♂ = 29), represents a typical primary complement.

The numerical reduction of the primary complements is either stepwise or simultaneous. In both, the sex chromosome does or does not play a role. The first case is by far the most common of the two and it is the only one to be dealt with here.

The process of the stepwise numerical reduction, as far as the sex element is involved, is a cycle of three successive stages (cf. Textfig. 4). At the first stage one autosome of the primary complement fuses with the original X. The result is a neo-XY karyotype and numeric reduction of the primary complement by one. The second step in the process is represented by fusion of neo-Y with an autosome, which reduces the chromosome number for another element and gives rise to a neo-X neo-neo-Y sex determining mechanism. The final stage is achieved by translocation of the autosomal part of the neo-X to an autosome. Its result is the reappearance of the XO system, which is not accompanied by a numerical reduction. All three stages were demonstrated in the family *Gomphidae*, but the relatively common occurrence of the neo-XY complements in most advanced families suggests the universality of the process.

If the reduction of the primary chromosome number is achieved by simultaneous fusion of all elements of the karyotype (two by two or more together) including the original X, a neo-XY system arises. Thus far, this special case could be demonstrated cytologically only in a single species (*Hemianax ephippiger*).

From the foregoing the following conclusions can be drawn:

(1) The XO sex determination is the original mode of sex determination in dragonflies and is the only type found in all primary complements, regardless of the chromosome number viz. the degree of phylogenetic advancement and specialisation achieved by the forms concerned.

(2) In secondary complements which originate exclusively by fusion of the elements of the primary karyotypes and do not have any relationship with the phylogenetic position of the species concerned, both the XO and the neo-XY sex determination occur. The former is either original and inherited from the primary complement if the sex element was not involved in fusion, or it is

of a secondary origin and has evolved through the intermediate neo-X neo-neo-Y stage.

(3) The neo-XY condition is characterised by (*a*) reversibility at the first step of the stepwise reduction process (it is usually not present in all cells of one individual), (*b*) by the tendency to evolve through the neo-X neo-neo-Y system into an XO sex determination again, and (*c*) by the occurrence in forms which have achieved different degrees of advancement and specialisation.

(4) The neo-XY condition, therefore, cannot always be regarded as a character of cytological specialisation. Nevertheless, it appears as such in the families *Gomphidae, Aeshnidae,* and perhaps also in the libellulidan genus *Orthemis* (cf. the chromosome numbers in CUMMING, 1964a).

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