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AUTOSOMAL FRAGMENTATIONS AND FUSIONS IN ODONATA AND THEIR EVOLUTIONARY IMPLICATIONS

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The fragmentation of autosomes in the karyotypes of *Enallagma cyathigerum* (Charp.) (n = 14 → 15), *Mecistogaster sp.* (n = 15), *Hetaerina rosea* Sel. (n = 14), *Libellula depressa* L. (n = 12 → 13), *Orthetrum coerulescens* (Fabr.) (n = 12 → 13), *Diplacodes bipunctata* (Br.) (n = 13 → 15) and *D. haematodes* (Burm.) (n = 12 → 13) is discussed. Original material of *Mecistogaster* and *Hetaerina* was not available.

Fragmentations were found to be the only way in which the recombination index is obligatorily changed (increased) in dragonflies. In at least five out of the seven cases considered the chromosome number is not stabilised: cells in which fragmentation occurred and those in which it did not are found in the same individual (*Enallagma, Libellula, Orthetrum, Diplacodes*). Fragmentation results in an increase of chromosome number (a) up to the family type number level (*Libellula, Orthetrum, D. haematodes*), (b) above the latter (*Hetaerina*) and (c) above any chromosome number ever reported in dragonflies (*Mecistogaster, D. bipunctata*). In our material the element or elements formed by fragmentation have always the size and usually also the heterochromatic features of the m-chromosomes, irrespective of the presence or absence of the latter in the original complement.

A review of species in which fusions of autosomes were recorded or can be assumed is given. *Orthetrum brachiale* (Beauv.) (n = 11) is added to the list. The situation in this species and in *Sympetrum eroticum* Sel. is discussed in detail.

The most essential differences between the fusion of (an) autosome(s) with the sex element on one hand (cf. KIAUTA, 1969), and the fusion of two or more autosomes on the other, lies in the observations: (a) that in the former case, fused and unfused complements occur in one individual, whereas the latter is specifically characteristic and occurs in all cells, all individuals and all populations of the species, and (b) that autosomal fusion results in an increase of chiasma frequency, due to which the recombination index in secondarily low-n complements remains the same as it was in the primary high-n sets, or becomes even higher (*O. brachiale, S. eroticum*).

Autosomal fragmentation (found so far in some advanced forms only) is considered as a character of phylogenetic advancement. Autosomal fusion, on the other hand, does not have any relation with phylogeny.

Introduction

The recombination index, i.e. the sum of the number of bivalents and the average number of chiasmata per nucleus, is one of the most important characters of the genetic system of a species (DARLINGTON, 1939). The evolutionary patterns are quite different in species with low recombination index and in those with high chromosome numbers and/or high chiasma frequency. A high recombination index promotes flexibility, i.e. the ability of a genotype to vary and adapt itself to changing conditions. A low recombination index promotes fitness, i.e. the survival value and the reproductive capability of a genotype as compared with the average of the population or the other genotypes in it (RIEGER et al., 1968). Since a balance must be achieved between genetic variability and biological efficiency and stability, the organisms certainly evolve a recombination index which suits their evolutionary requirements optimally.

OKSALA (1943, 1952) and DASGUPTA (1957) have shown that in male dragonflies approximately one chiasma occurs per bivalent, whereas there are two in the female (OKSALA, 1945). The recombination index in (XO) male dragonflies thus amounts to approximately $2n - 1$, whereas in females it equals approximately the 3n number. Since it is evident, that the chiasma frequency is thus rather well fixed in the order, a change in the chromosome number is the only way of changing the species recombination index. The small numerical variation of the complement within odonate families (cf. KIAUTA, 1967) would suggest (1) that recombination indices have been stabilized at selectively adaptive levels of the families concerned, and (2) that relatively few species of a family have had a need to deviate from the general pattern adapted by the family. The latter applies both to the primary and to the secondary complements (e.g. *Gomphidae*, KIAUTA, 1969).

Due to lack of any visible structural differentiations in dragonfly chromosomes, only those rearrangements of the odonate karyotypes can be studied, which at the same time produced a numerical increase or decrease of the chromosome complement. These led to alteration in gene sequence and most probably to subsequent position effects. This is probably another reason for the fundamental role which is played by the fragmentations and fusions in the evolution and phylogeny of the order.

General considerations on this problem were given by KIAUTA (1967) and CRUDEN (1968), whereas the evolutionary implications of fusions of the sex element with (an) autosome(s) were dealt with by KIAUTA (1969). In the present report, therefore, only the autosomal fragmentations and fusions will be discussed.

Material and Methods

In the material studied, four species are included which were not previously examined cytologically (*Orthetrum brachiale* /Beauv./, *O. coerulescens* /Fabr./, *Diplacodes bipunctata* /Br./, *D. haematodes* /Burm./). The locality data are given at the respective places of treatment.

All observations are based on lacto-acetic-orcein squash preparations. The equipment has been described in the preceding paper of this series (KIAUTA, 1969). Figures 18–26, 28 and 30–34 were taken without phase contrast optics, the other with it. The positives were printed originally at 2250 × and have been reduced in this paper to 1500 ×.

Autosomal Fragmentation

Chromosomal fragmentations are considered as the normal mode of increase of chromosome numbers in the course of the evolution of Odonata. The phenomenon is supposed to be responsible for the present numeric situation in all karyotypes consisting of more than 9 elements at the haploid stage (KIAUTA, 1967).

The selective adaptive level of the recombination index is apparently rather uniform in a family, therefore only very few species deviate from the family type number. In those in which the chromosome number exceeds the type number of the family, the fragmentations are supposed to have taken place only very recently. This suggestion is strongly supported by the fact, that in five out of seven dragonflies in which such a numeric situation was so far found, the course of the fragmentation could be actually followed up through some stages of the process. In all of these both unfragmented as well as fragmented complements occur.

Due to insufficient documentation published for *Hetaerina rosea* (CUMMING, 1964) the fragmentation cannot be proved in this species. Nevertheless, its high chromosome number strongly suggests a relatively recent fragmentation.

The main cytological data for species in which autosomal fragmentations have or are supposed to have taken place, are given in Table 1.

The situation in *Neurothemis tullia tullia* (Drury) where the autosomal fragmentation was followed by a fusion of the sex element with a fragment, has been dealt with in another paper (KIAUTA, 1969).

DESCRIPTION OF MATERIAL

Enallagma cyathigerum (Charp.) (Figs. 1–6)

The cytology of this species has been studied originally by OKSALA (1939, 1945) and MAKALOWSKAJA (1940) on Finnish and Russian material respectively. They did not note any irregularity in the male and female haploid complement. VAN BRINK & KIAUTA (1964) have drawn attention to a peculiar constriction occurring occasionally in a pair of smaller autosomes at mitotic metaphase in the male, but only 14 elements were always found in metaphase figures of the first maturation division in both sexes. It was CRUDEN (1968) who first reported on n = 15 complements found in spermatocytes of Californian material (U.S.A.). In material from Lake Co. only 14 elements were counted in the haploid set, but 14 and 15 occurred in the spermatocytes of insects from San Bernardino and Tuolomne. In specimen(s) (?) from Lassen 15 elements seem to occur in all spermatocytes. CRUDEN did not report on the numerical situation in spermatogonial cells and in the oogenetic cycle.

These observations stimulated the present author to reinspect the figures of the Dutch material, including a more recently prepared series. In all, 346 larval and adult specimens of both sexes were examined. All material originates from Wasmeer lake, Hilversum, Netherlands and was collected between January, 1964 and June, 1965.

In the diploid set there are 26 autosomes of decreasing magnitude. The m-chromosomes do not occur in the normal, 2n ♂ (♀) = 27 (28), complement.

No peculiarities were observed at any stage of the oogenetic cycle. In about 60% of figures of spermatogonial metaphase the chromosome

TABLE 1

MAIN CYTOLOGICAL DATA ON SPECIES IN WHICH AUTOSOMAL *FRAGMENTATION* CAN BE DEMONSTRATED CYTOLOGICALLY OR MAY BE SUPPOSED TO HAVE TAKEN PLACE

Species (and family)	Locality	Chromosome number original 2n	Chromosome number original n	Chromosome number after fragmentation 2n	Chromosome number after fragmentation n	m in original complement	References
Enallagma cyathigerum	U.S.A.	27 ♂	14 ♂		15 ♂	no	CRUDEN, 1968
(Charp.) (Coenagrionidae)	Netherlands	27 ♂, 28 ♀	14 ♂, 14 ♀		15 ♂	no	this paper
		in Russian and Finnish material no fragmentation observed (MAKALOWSKAJA, 1940 and OKSALA, 1945 respectively): n ♂ = 14 and n ♀ = 14 respectively.					
Mecistogaster sp. (Pseudostigmatidae)	Bolivia			29 ♂	15 ♂		CUMMING, 1964
Hetaerina rosea Sel. (Calopterygidae)	Bolivia				14 ♂		CUMMING, 1964
		fragmentation is probable, but no figures and/or description of karyotype were published.					
Libellula depressa L. (Libellulidae)	Austria		12 ♂		13 ♂	no	KIAUTA, 1968; this paper
		in English material no fragmentation observed: 2n ♂ = 23, 2n ♀ = 24; n ♂ = 12 (HOGBEN, 1921).					
Orthetrum coerulescens (Fabr.) (Libellulidae)	Austria		12 ♂	25 ♂	13 ♂	no	this paper
Diplacodes bipunctata (Br.) (Libellulidae)	Australia		13 ♂		15 ♂	yes	this paper
		neo-elements of unequal size; their structure is not clear.					
Diplacodes haematodes (Burm.) (Libellulidae)	Australia		12 ♂		13 ♂	no	this paper
		m extremely minute.					

morphology is also normal (cf. KIAUTA, 1969, fig. 1 and karyogram), but in the remaining figures of this stage two of the smaller elements show a subterminal constriction. The latter is in some figures less, and in others more pronounced, though in no case do the chromosomes seem to be actually broken (Figs. 1–2).

In 315 figures of primary spermatocyte metaphase the usual 13 bivalents and an unpaired sex element were counted (Figs. 3–5), whereas in five figures of this stage an additional m-bivalent was observed. The latter is approximately half the size of the unpaired sex chromosome and thus corresponds to the expected size of a bivalent formed by the terminal portions of the two constricted elements observed in spermatogonial metaphase (Fig. 6). The absorption of stain of the additional m-bivalent is the same as that of the other bivalents, whereas, according to CRUDEN (1968), the m-bivalent is negatively heterochromatic in Californian specimens. It pairs simultaneously with the other autosomes.

In all other spermatocyte stages the usual 14 elements were counted.

In view of the large material examined two points in the above observations are of particular interest: (1) the relatively high percentage (40%) of spermatogonial cells with a constriction in a pair of the smaller autosomes and the total lack of any figures with the break completed, and (2) the relatively small percentage (1.5%) of the spermatocyte figures with the additional m-bivalent.

The phylogenetically very modern *E. cyathigerum* has a circumpolar distribution and has entered Eurasia only recently (KENNEDY, 1922b). It is the only European representative of the genus, which contains more than seventy living species found in all continents except Australia (and Papua). About two thirds of them are from the Americas, most of them from North America.

So far six other species of the genus *Enallagma* Charp., all from North America (U.S.A.), have been studied cytologically viz. *aspersum* (Hag.), *boreale* Sel., *carunculatum* Morse, *civile* (Hag.), *ebrium* (Hag.) and *praevarum* (Hag.). All have 14 elements in primary spermatocytes, and no m-chromosomes (CRUDEN, 1968). The cytological situation in *E. cyathigerum* thus clearly deviates from the picture met with in all other representatives of the genus so far studied. It is unfortunate that no representatives of the genera split out from *Enallagma* by KENNEDY (1920) have ever been examined cytologically.

Mecistogaster sp.

CUMMING (1964) reported on the cytology of an unidentified species of this genus. A single male was taken in the surroundings of Carinavi, Nor Yungas, La Paz, Bolivia. The chromosome number is 2n ♂ = 29, n ♂ = 15. A pair of m-chromosomes is present. At primary spermatocyte metaphase they form a bivalent of approximately the same size as the univalent X.

The *Pseudostigmatidae* are a highly specialised and in some respects "aberrant" family (FRASER, 1957). Unfortunately, only two of its members, both of the genus *Mecistogaster*, have been studied cytologically. In the other species, also unidentified, the complement is strongly reduced, the chromosome number being 2n ♂ = 12, n ♂ = 6 (CUMMING, 1964).

Hetaerina rosea Sel.

The chromosome number, n = 14, has been recorded in Bolivian material by CUMMING (1964) (including an m-bivalent), but no figures or any other data were ever published. The same author reported on chromosome numbers of four other species of the genus viz. *americana* (Fabr.) (U.S.A.; from the same country examined and illustrated also by CRUDEN, 1968), *charca* Calv. (Bolivia), *sanguinea* Sel. (Bolivia) and *titia* (Drury) (U.S.A.). The haploid chromosome number of all of these is 13. The m-bivalent is lacking only in *H. sanguinea*.

The specialised genus has a Nearctic and Neotropical distribution.

Libellula (*Platetrum*) *depressa* L. (Figs. 7–14)

This is one of the two dragonflies which were studied cytologically already by CARNOY (1885), but his paper has a historic value only. A detailed and reliable account of the cytological conditions in this species was given by HOGBEN (1921), who reported the chromosome numbers 2n ♂ = 23, 2n ♀ = 24 and n ♂ = 12 for the English material. In many metaphase figures of spermatogonial, oogonial and follicle cells he noted one or two small "granules". Three of these were often present also in prophase nuclei of spermatogonia, while one, two or three such elements often occurred in the primary spermatocyte metaphase. HOGBEN (1921: p. 63) discussed the matter in some detail and arrived at the conclusion that these elements do not represent the m-

chromosomes, though he was unable to give any other explanation as to their nature.

KIAUTA (1968) studied the material of a single mature male, taken at the torrent "Rauscherbach" on the northern slope of the Karawanken Mts., nr. St. Kanzianiberg (Škocjan), Finkenstein (Bekštajn), Villach (Beljak), Austria, on July 10th, 1967. A more detailed account of the observations is given here.

In our material numerous figures of both spermatocyte divisions were present. Mitotic stages, however, are completely lacking.

In approximately 50% of the figures of primary spermatocyte metaphase 12 elements can be counted, including the sex chromosome. The m-chromosomes are lacking (Figs. 7–8). In other figures of the same stage an additional m-bivalent occurs (Figs. 9–12). The length of the "normal" bivalents varies between 2.4–2.1 μ approximately. Where present, the m-bivalent is 0.8 μ long. The length of the sex element amounts to 1.8 μ. The latter is usually slightly more darkly stained than the autosomes. The m-bivalent, on the other hand, is slightly negatively heterochromatic in some figures, while in others there is no difference in stain absorption between the m and the other autosomes. No unpaired m-chromosomes were seen at any stage. In secondary spermatocytes n = 12 and n = 13 complements are found with approximately equal frequencies (cf. Figs. 13–14).

Including the transitional stages, there are actually four kinds of karyotypes in this species. They were described and illustrated in a previous paper (KIAUTA, 1968), therefore they will only be briefly listed here: (1) complements where no m is present, (2) cells where one bivalent has a subterminal constriction, (3) figures where the m-chromosome is clearly separated from the original bivalent, but is found lying in the prolonged longitudinal axis close to the latter, and (4) complements in which the m occupies a random position among the other elements.

The genus *Libellula* L. is mainly Holarctic in its distribution, being found throughout the Temperate Zone of the Northern Hemisphere, but it also developed some species in the Transitional and Subtropical Zones. Most species are found at present in North America, particularly in the United States.

KENNEDY (1922a, 1922b) produced a phylogenetic tree based upon penile characters. He expressed the opinion, that the genus originated

in the Eastern Hemisphere, because there the large genus *Orthetrum* Newm. is found, which by many authors is placed close to *Libellula* and in which the penis has the straight lateral lobes of the less specialised libellulas. The dominance of *Libellula* is past in Eurasia and at present is at its height in North America. The chorogeography is rather complicated and implies several migrations from Eurasia to America and vice versa, with various independent centres of speciation in both the New and the Old World.

KENNEDY (1922b) also proposed a taxonomic revision on the basis of penile characters and erected a few new subgenera. Present cytotaxonomic evidence is insufficient for any taxonomic considerations, though so far 15 species belonging to seven subgenera were studied cytologically (cf. Table 2).

Nearly in all species so far studied cytologically, the haploid chromosome number (in the male) is 13 (with or without an m-bivalent), including the primitive *Eolibellula semifasciata* and *Syntetrum angelina*, but with the exception of *Platetrum depressa* (n = 12 and 13) and *Holotania axillena* (n = 12).

Because of its phylogenetic position the fragmentation of an autosome in *Libellula* (*Platetrum*) *depressa* is of particular interest. KENNEDY (1922b) considers this species, together with *L.* (*Eurothemis*) *fulva* Müll., as the most specialised of any of the libellulas. The species probably represents an European offshoot of the American *Plathemis*-stock. It must have migrated to Eurasia before *L. quadrimaculata* came to America, but the difference between it and *Plathemis* is great enough to suggest that it branched off even much earlier. In *P. lydia* (Drury) – the only species of the genus so far studied cytologically – there are 13 elements in spermatocytes (including the m-bivalent) (CRUDEN, 1968).

Orthetrum coerulescens (Febr.) (Figs. 15–17)

The species has not been previously studied cytologically. Five mature males were collected in Warmbad Villach (Beljaške Toplice), Carinthia, Austria, on July 8th, 1967.

In our material a few figures of spermatogonial prometaphase and most spermatocyte stages were present. The diploid chromosome number is without exception 25, but in figures of the primary spermatocyte metaphase both 12 and 13 elements were counted.

TABLE 2

SUMMARY OF CYTOLOGICAL DATA ON SPECIES OF THE GENUS LIBELLULA Linn. WITHOUT AUTOSOMAL FRAGMENTATIONS

Subgenus	Species	Locality	n	m	references
Eolibellula Kenn.	semifasciata Burm.	U.S.A.	13	yes	CRUDEN, 1968
Belonia Kirby	croceipennis Sel.	U.S.A.	13	yes	CRUDEN, 1968
	saturata Uhler	U.S.A.	13	yes	CRUDEN, 1968
Syntetrum Kenn.	angelina Sel.	Japan	13	yes	OGUMA, 1915, 1930; KICHIJO, 1942b
Libellula L.	quadrimaculata quadrimaculata L.	Europe	13	yes	FUCHSÓWNA & SAWCZYŃSKA, 1928; MAKALOWSKAJA, 1940; OKSALA, 1939, 1945
	quadrimaculata asahinai Schmidt	Japan	13	yes	OGUMA, 1915, 1930; KICHIJO, 1942b; OMURA, 1955
Neotetrum Kenn.	forensis Hag.	U.S.A.	13	yes	CRUDEN, 1968
	pulchella Drury	N. America	13	yes	CRUDEN, 1968; KIAUTA, 1969
Holotania Kirby	axillena Westw.	U.S.A.	12	no	CUMMING, 1964
	composita (Hag.)	U.S.A.	13	yes	CRUDEN, 1968
	cyanea Fabr.	U.S.A.	13	no	CRUDEN, 1968
	flavida Ramb.	U.S.A.	13	yes	CRUDEN, 1968
	incesta Hag.	U.S.A.	13	no	CUMMING, 1964; CRUDEN, 1968
	luctuosa Burm.	U.S.A.	13	yes	SMITH, 1916
	vibrans Fabr.	U.S.A.	13	yes	CRUDEN, 1968

In primary spermatocyte metaphase of the n = 12 complement the bivalents are of gradually decreasing magnitude, the sex chromosome being the smallest element of the set (Fig. 15). In the n = 13 cells a slightly negatively heterochromatic m-bivalent occurs at this stage. It is inferior in size to the sex element (Figs. 16–17). No unpaired m-chromosomes were ever seen at metaphase I.

The genus is related to the (cytologically studied) genera *Libellula* L. and *Orthemis* Hag. (etc.), though in some structural characters it also shows some more primitive features (cf. LONGFIELD, 1955). It has

a wide distribution throughout the Old World. Many of its species show a pronounced tendency to infraspeciation. This is particularly true of *O. coerulescens* in the Central and Eastern Mediterranean area.

So far ten species were examined (cf. Table 3). The haploid chromosome number (in the male) in eight of these is 13 (including, in most cases, a small or very minute m-bivalent). In *O. brachiale* there are 11 elements (apparently due to a fusion; cf. below), but *O. coerulescens* is the only member of the genus in which fragmentation of an autosome can be demonstrated.

TABLE 3

SUMMARY OF CYTOLOGICAL DATA ON SPECIES OF THE GENUS ORTHETRUM Newm. WITHOUT AUTOSOMAL FRAGMENTATIONS

Species	Locality	n	m	References
albistylum speciosum Uhler	Japan	13	yes	OGUMA, 1917, 1930, 1942; KICHIJO, 1942b; OMURA, 1955
brachiale (Beau.)	Kenya	11	no	this paper
cancellatum (L.)	India, Netherlands	13	yes	DASGUPTA, 1957 and KIAUTA, 1969 respectively
glaucum (Br.)	India	13	yes	DASGUPTA, 1957
japonicum (Uhler)	Japan	13	yes	OGUMA, 1917, 1930; KICHIJO, 1942b; OMURA, 1957
pruinosum neglectum (Ramb.)	India, Taiwan	13	yes	DASGUPTA, 1957 and this paper respectively (cf. KIAUTA, 1969)
sabina (Drury)	India	13	yes	ASANA & MAKINO, 1935; MAKINO, 1935; KICHIJO, 1942b; RAY CHAUDHURI & DAS GUPTA, 1949
triangulare Sel.	Taiwan	13	yes	this paper (cf. KIAUTA, 1969)
triangulare melania Sel.	Japan	13		OMURA, 1955
azureum (Ramb.)	Madagascar	13	yes	KIAUTA, unpublished

In view of the phylogenetic and structural affinities between the genera *Orthemis* and *Orthetrum* it is interesting to note, that the type number of the former is 12. It is supposed that this condition is due to secondary fusion (KIAUTA, 1969).

Diplacodes bipunctata (Br.) (Figs. 18–22)

The species has not been previously studied cytologically. Two mature males were taken at Mona Vale Road, North of French's Forest Turnoff, N.S.W., Australia and in the sand dunes South of Sydney, between Kurnell and Cronulla, N.S.W., Australia, on April 22nd, 1968.

No mitotic figures are available in our material. The haploid chromosome number, n = 13, was found in approximately 60% of figures of primary spermatocyte metaphase and in all secondary spermatocytes. An m-bivalent is present. It is negatively heterochromatic at primary and secondary metaphase and has approximately the same size as the unpaired sex element (Figs. 18–19, 22).

In the remaining figures of primary spermatocyte metaphase (of both specimens) 15 chromosomes were counted. The additional two elements are negatively heterochromatic and of unequal size, though they are similar in length to the m-bivalent and the X. It is not clear whether or not they have a bivalent structure (Figs. 20–21).

Diplacodes haematodes (Burm.) (Figs. 23–25)

This is another species of the genus which has not been previously studied cytologically. Our observations are based on preparations of a single mature male taken at Perth, Australia, on March 3rd, 1968.

No analysable mitotic figures are available. The original haploid chromosome number, n = 12, is found in approximately 50% of figures of primary spermatocyte metaphase. The bivalents are of gradually decreasing magnitude (3.3–2.4 μ), the sex element (1.3 μ) is clearly distinguishable. There are no m-chromosomes in the n = 12 set (Fig. 23).

In the n = 13 cells an extremely minute (0.9 μ) m-bivalent occurs. It is negatively heterochromatic. No delayed pairing has been observed (Figs. 24–25). Due to the minute size of the m, its origin can not be ascertained.

This modern genus is distributed throughout tropical and subtropical regions of the Old World. So far five species were studied cytologically. In three of these no pecularities were observed, their haploid number being 13 (*lefebvrei* /Ramb./, Madagascar, KIAUTA, unpublished; *nebulosa* /Fraser/, India, DASGUPTA, 1957; *trivialis* /Ramb./, India, ASANA & MAKINO, 1935; MAKINO, 1935; DASGUPTA, 1957;

PLATE 1

Figs. 1–6. Chromosomes of *Enallagma cyathigerum* (Charp.) (Wasmeer, Hilversum, Netherlands). (1500 ×): (1–2) Spermatogonial metaphase. (Note a pair of constricted autosomes indicated by the arrows). – (3–5) Primary spermatocyte metaphase of the normal, n = 14, complement. – (6) Primary spermatocyte metaphase with an additional m-bivalent. (n = 15).

PLATE 2

Figs. 7–14. Chromosomes of *Libellula depressa* L. (St. Kanzianiberg /Škocjan/ nr. Villach /Beljak/, Karawanken Mts., Austria). (1500 ×): (7–8) Primary spermatocyte metaphase of n = 12 complement. – (9–12) Primary spermatocyte metaphase with an additional, negatively heterochromatic m-bivalent. (n = 13). – (13–14) Secondary spermatocyte metaphase with negatively heterochromatic m-element. (n = 13).

PLATE 3

Figs. 15–25. Chromosomes of some *Libellulidae*. (1500 ×): Figs. 15–17: *Orthetrum coerulescens* (Fabr.) (Warmbad Villach /Beljaške Toplice/, Carinthia, Austria): (15) Late spermatocyte diakinesis of n = 12 complement. – (16–17) Late spermatocyte diakinesis (16) and primary spermatocyte metaphase (17) of n = 13 cells. – Figs. 18–22: *Diplacodes bipunctata* (Br.) (surroundings of Sydney, Australia): (18–19) Late spermatocyte diakinesis (18) and primary spermatocyte metaphase (19) of the n = 13 cells. – (20–21) Primary spermatocyte metaphase with two additional m-elements of unequal size; $\underline{m}$ is the original m-bivalent. (n = 15). – (22) Secondary spermatocyte metaphase with 13 elements. – Figs. 23–25: *Diplacodes haematodes* (Burm.) (Perth, Australia): (23) Primary spermatocyte metaphase of n = 12 complement. – (24–25) Primary spermatocyte metaphase of cells with an additional, negatively heterochromatic m-element. (n = 13).

PLATE 4

Figs. 26–29. Chromosomes of a low-n and a "normal"-n *Sympetrum* species. (1500 ×): Figs. 26 and 28: *Sympetrum eroticum eroticum* Sel. (Ikuta nr. Tokyo, Japan). (2n ♂ = 21, n = 11): (26) Spermatogonial metaphase and karyogram. (Note a pair of large autosomes characteristic of the secondary low-n complement). – (28) Primary spermatocyte metaphase. (Note a large bivalent characteristic of the secondary low-n complement). – Figs. 27 and 29: *Sympetrum corruptum* (Hag.) (Ladona nr. Tucson, Arizona, U.S.A.). (2n ♂ = 25, n = 13): (27) Spermatogonial metaphase and karyogram. – (29) Primary spermatocyte metaphase.

PLATE 5

Figs. 30–37. Chiasma frequency in the low-n and some of the "normal"-n species of the genera *Orthetrum* and *Sympetrum*. (1500 ×): Figs. 30–31: *Orthetrum brachiale* (Beauv.) (Shimba Hills, Kenya) (n = 11): (30) Spermatocyte diakine-

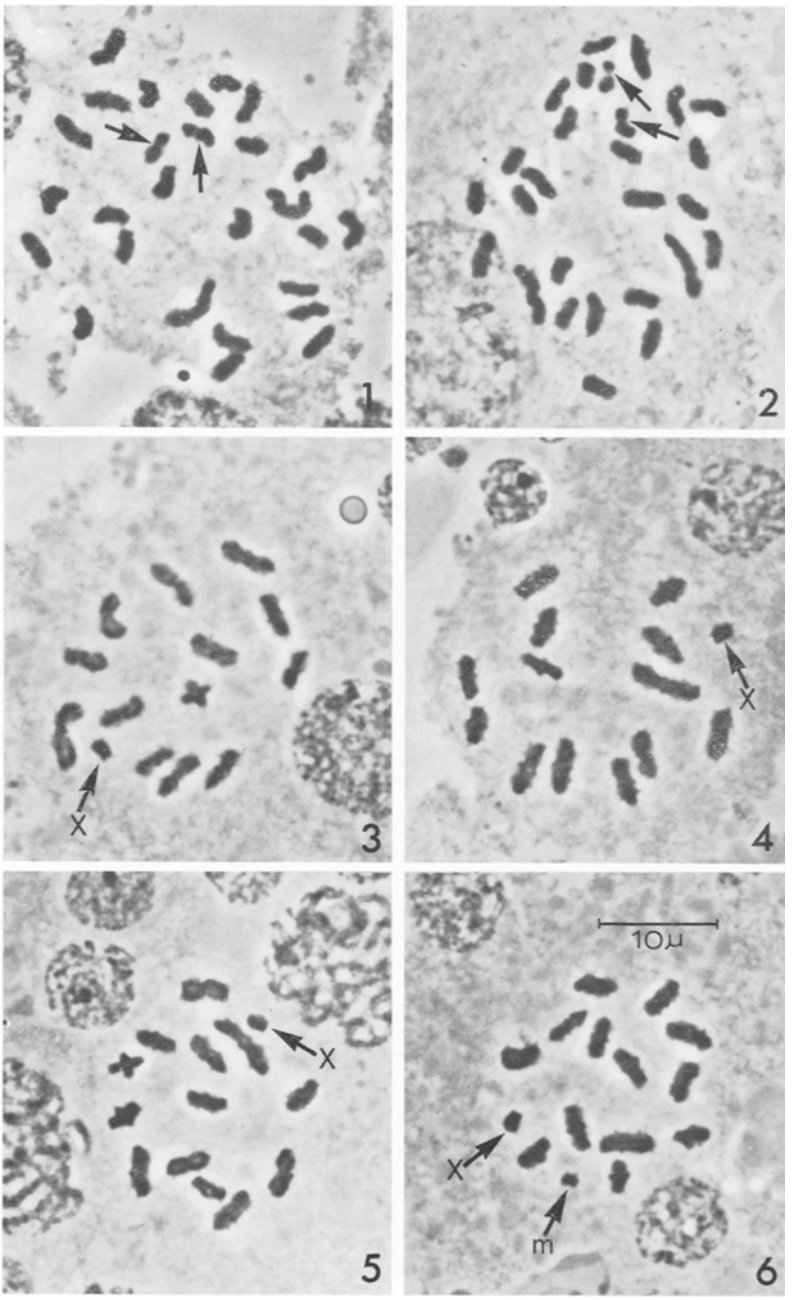

Plate 1.

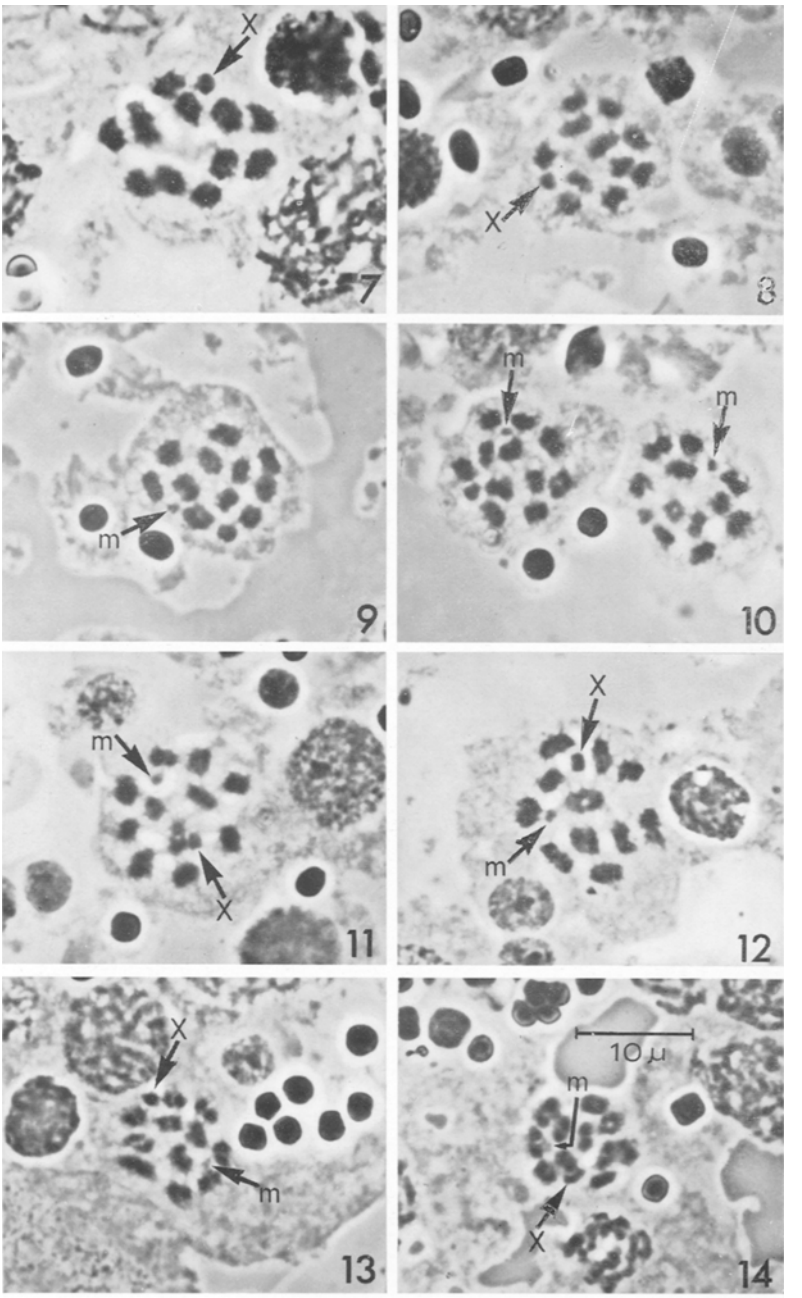

Plate 2.

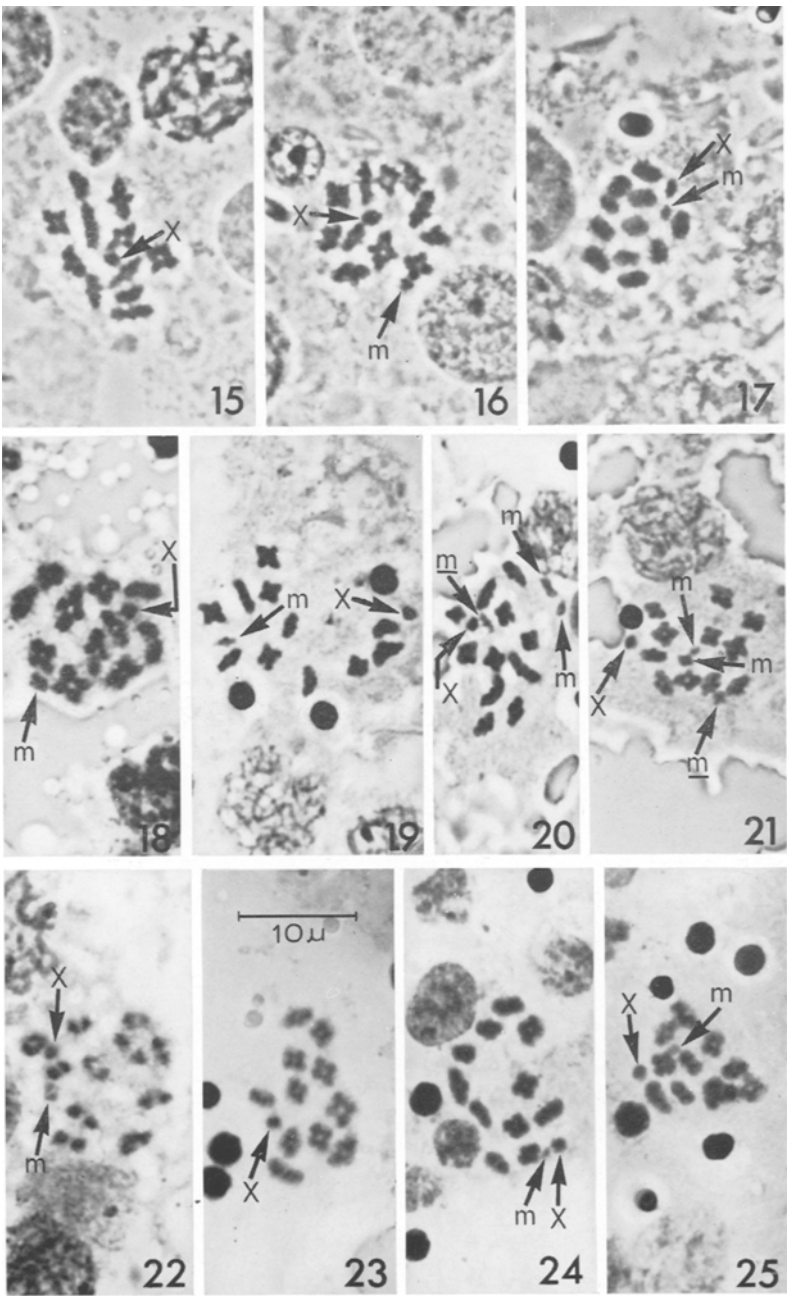

Plate 3.

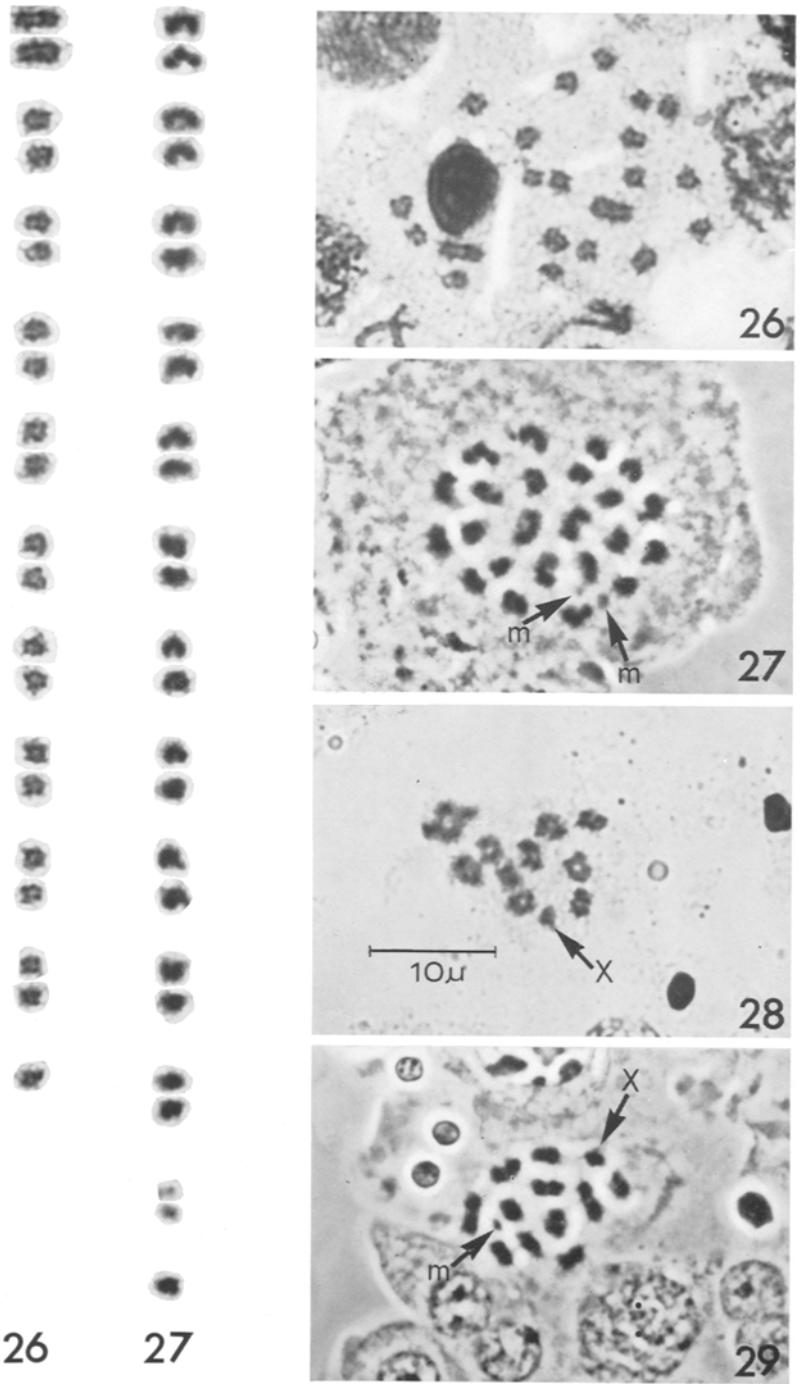

Plate 4.

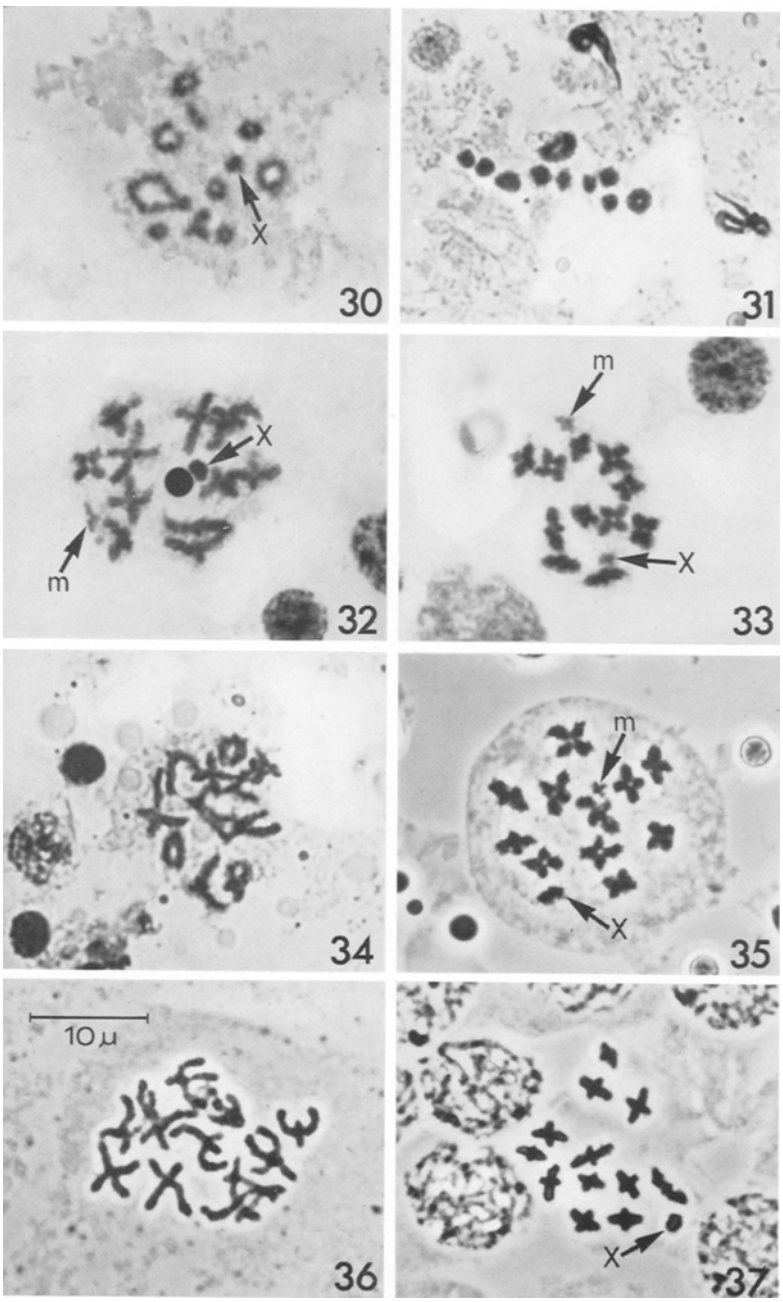

Plate 5.

sis. Most bivalents appear as rings and have at least two chiasmata per bivalent. – (31) Primary spermatocyte metaphase. (Note the ring-like structure of the big three bivalents). – Figs. 32–33: *Orthetrum pruinosum neglectum* (Ramb.) (Yung Nin San Park, Taipei, Taiwan) (n = 13) (Note the occurrence of a single chiasma per bivalent): (32) Early spermatocyte diakinesis. – (33) Late spermatocyte diakinesis. – Fig. 34: *Sympetrum eroticum eroticum* Sel. (Ikuta nr. Tokyo, Japan) (n = 11): Early spermatocyte diakinesis. At least three bivalents have two chiasmata per bivalent (rings). – Fig. 35: *Sympetrum corruptum* (Hag.) (Ladona nr. Tucson, Arizona, U.S.A.) (n = 13): Late spermatocyte diakinesis. A single chiasma occurs per bivalent. – Figs. 36–37: *Sympetrum striolatum* (Charp.) (Bridel, Luxembourg) (n = 13) (Note the occurrence of a single chiasma per bivalent): (36) Early spermatocyte diakinesis. – (37) Late spermatocyte diakinesis.

Australia, KIAUTA, unpublished). A pair of m-chromosomes is present in all n = 13 pecies.

CONSIDERATIONS AND CONCLUSIONS

The above observations can be summarized in the following points:

(1) Autosomal fragmentations were observed in only 1.5% of the species thus far studied cytologically. Taking into account the fact, that fragmentations and fusions of chromosomes represent the only way of changing the recombination index in dragonflies, the low number of cases where these occur clearly indicates the uniformity of requirements for the optimal adaptive level of the recombination index within a family. This level may have been reached in a relatively short space of time and nearly simultaneously by all members of a family.

As will be shown later in this paper, fragmentations are in fact the only way by which the recombination index is *obligatorily* changed. In fused complements the original situation can be retained by the increase in chiasma frequency. Therefore the low number of species in which chromosome fragmentation occurs is all the more significant.

(2) In at least five out of the seven cases considered the chromosome number is not stabilized: there is a numerical variation among the cells of the same individual. This indicates that the "search" for a

new adaptive level of the recombination index by an organism is an "experimental" evolutionary process and not a sudden event. If this were not so, only the species with successful karyotypic rearrangements of this kind would survive for record and no transitional stages would ever be found in nature.

(3) The karyotypic morphology in the course of mitosis is known only in one of the six species in which fragmentation occurs (*Enallagma cyathigerum*). It is evident, from metaphase figures in this species, that at least a "predisposition" for fragmentation (constrictions) already exists in mitosis, although no actual fragments were found at this stage. Furthermore, constrictions of various intensity always occur in the same mitotic elements. It is not certain whether or not the additional m-elements are formed exclusively in mitosis, or at a later stage, during meiosis. Some primary spermatocyte metaphase figures found in *Libellula depressa* seem to suggest that actual fragmentation, at least in some cases, takes place not until the first maturation division (cf. complements No. 2 and 3 above and KIAUTA, 1968, figs. 3–4).

(4) Fragmentations were observed only in some specialized forms of both major living suborders. This would indicate that structurally specialized dragonflies are capable of retaining their flexibility by increasing their recombination index. If they did not possess this possibility, they would be unable to adapt themselves to changing conditions and – being specialized – to survive in an altered situation. Probably, less specialized forms have other means to achieve the necessary adaptation before they are urged to resort to the modification of the recombination index.

(5) In four out of the seven cases considered the fragmentation led to an increase of the chromosome number above the family type number. In three of these the resulting chromosome number is the highest ever reported in dragonflies. On the other hand, in three species only, fragmentation led to an increase of the family (and order) type number level. The observation is thus in agreement with the general parallel between the increase of specialization and that of the chromosome number (vide the material of *Enallagma, Libellula, Orthetrum, Diplacodes*).

(6) The element(s) formed by fragmentation always has(ve) the size and usually also the heterochromatic features of the m-chromo-

somes irrespective of their presence or absence in the original complement.

Autosomal Fusion

Chromosome fusions are responsible for the decrease of chromosome numbers in the secondary complements and are not related to the phylogenetic status of the taxa considered (KIAUTA, 1967). They occur in species assigned to most families. Those in which the sex element is involved were reported in the preceding paper (KIAUTA, 1969).

There is an essential difference between the occurrence of fusions of an autosome with the sex element, and fusions of two or more autosomes. The former fusions are characterized by the appearance of both fused and unfused complements in one individual, whereas the latter fusions are specifically characteristic. If an autosomal fusion occurs in a species, it is found in all cells, in all individuals and in all populations. Now, the question arises, whether such complements may be considered as a secondary phenomenon, or represent but a primary set in which the fragmentation of one (or more) pair(s) has not yet been effected. There is no doubt that many dragonfly complements, lower than the family type number, are primary rather than secondary by origin.

To the author's opinion, there are two features which are characteristic for the complements reduced secondarily by an autosomal fusion and which are not found in the primary complements. Within a genus in which one or some species have slightly lower chromosome numbers than the type number of the group, a karyotype can be assumed to be of secondary rather than of primary origin (1) if one chromosome pair (bivalent) of the low-n species is essentially longer than the others (in primary complements the chromosomes are always more or less gradually decreasing in size, save for the m-chromosome, if present), and (2) if the number of chiasmata per bivalent is increased in the male. The complements with a strong numerical reduction are in all cases easily recognizable as such.

Autosomal fusions were reported by CUMMING (1964) in *Aeshna diffinis diffinis* Ramb. (Bolivia; $2n\ \delta = 21$, $n = 11$), *A. intricata* Martin (Bolivia; $2n\ \delta = 19$, $n = 10$) and *Perithemis lais* (Perty) (Bolivia; $2n\ \delta = 17$, $n = 9$). An autosomal fusion probably also

caused numerical reduction in *Lestes forcipatus* Ramb. (U.S.A.; n = 11) where one very large bivalent is present in addition to the large bivalent that characterizes the genus (CRUDEN, 1968, fig. 7). The fusion, however, cannot be ascertained on the basis of the single figure of a primary spermatocyte metaphase published.

The present author has had the opportunity to study the original material of two additional species in which autosomal fusion can be assumed without doubt. There are dealt with here in some detail.

DESCRIPTION OF MATERIAL

Orthetrum brachiale (Beauv.) (Figs. 30–31)

The species has not been studied cytologically until now. A single male was collected in Shimba Hills, Kenya, East Africa, on January 20th, 1968.

No mitotic figures suited for analyses are available in our material. The haploid chromosome number, n = 11, was observed in all metaphase figures of primary and secondary spermatocytes. This number is unique among the species of the genus studied so far (cf. Table 3). One bivalent is exceptionally big, the others are of decreasing magnitude. There are no m-chromosomes in this species (Figs. 30–31).

In eight bivalents, or perhaps in nine, there are at least two chiasmata per bivalent. The biggest bivalent probably has three chiasmata and appears as a ring even when maximally contracted at metaphase I. A single chiasma can be assumed with certainty in one of the smaller elements only (Fig. 30). Since, on the average, no more than one chiasma appears per bivalent in the other species of the genus (n = 13 or 12), the recombination index of *O. brachiale* is even higher than in any of the high-n *Orthetrum*-species.

Sympetrum eroticum eroticum Sel. (Figs. 26, 28, 34)

The spermatocyte conditions in this Japanese species were studied originally by KICHIJO (1942a, 1942b) and were reexamined by HIRAI (1956), the latter two publications being illustrated. Our observations are in agreement with earlier descriptions and are based on preparations of a single male taken at Ikuta near Tokyo, Japan, on August 12th, 1968.

At spermatogonial metaphase there are 21 elements. The longest pair is approximately twice as long (3.7 μ) as the second longest (2.0 μ). Save for the longest pair, the mitotic chromosomes are nearly equal in size, the shortest pair still being nearly 1.6 μ long. There are no m-chromosomes in this species (Fig. 26).

In at least three bivalents two chiasmata per bivalent occur at diakinesis, but the longest bivalent cannot be singled out in our material (Fig. 34). In this way the recombination index in this species is approximately the same as in the n = 13 species of the genus (cf. Table 5).

At primary (Fig. 28) and secondary spermatocyte metaphase the big bivalent is easily recognizable.

The large genus of more than fifty species belongs predominantly to the North Temperate Region, within which a Euro-Siberian, a Sino-Japanese and an American group can be distinguished. On the basis of structural characters it was divided by BARTENEV (1915, 1919) into eleven groups, but their phylogenetic relations are obscure.

Chromosome conditions in 15 species belonging to nine groups of BARTENEV are now known, including the group VIII (*corruptum*) for which a separate generic status (*Tarnetrum*) has been proposed by NEEDHAM & FISHER (1936). There are very slight structural characteristics in support of this opinion (cf. also NEEDHAM & WESTFALL, 1955: pp. 426–427, 545), whereas any cytological differences are completely absent. A summary of the main cytological data on the species of *Sympetrum* is given in Table 4.

Aside from *S. eroticum*, *S. frequens* is the only species in which fewer than 13 elements occur at primary spermatocyte metaphase. Judging from the figure published by OGUMA (1930, fig. 2d) no bivalent is exceptionally long in the karyotype of this dragonfly. It seems likely, therefore, that the complement of *S. frequens* is of primary rather than of secondary origin.

COMPARISON OF KARYOTYPES WITH AND WITHOUT AUTOSOMAL FUSIONS WITHIN THE GENERA ORTHETRUM AND SYMPETRUM

The chromosome number, 2n ♂ = 25, n = 13, is the usual number in the genera *Orthetrum* and *Sympetrum* (cf. text above and Tables 3 and 4). For most species studied reliable figures are available, although

TABLE 4

TAXONOMIC CLASSIFICATION AND MAIN CYTOLOGICAL DATA ON SPECIES OF THE GENUS SYMPETRUM Newm.

BARTENEV's classification (number and name of the group)	Species	Locality	n	m	References
I. eroticum	eroticum Sel.	Japan	11	no	KICHIJO, 1942a, 1942b; HIRAI, 1956; this paper
II. flaveolum	flaveolum (L.)	U.S.S.R.	13	yes	MAKALOWSKAJA, 1940
	madidum (Hag.)	U.S.A.	13	yes	CRUDEN, 1968
	pedemontanum elatum Sel.	Japan	13	yes	OGUMA, 1917, 1930; KICHIJO, 1942b
III. obtrusum	obtrusum (Hag.)	U.S.A.	13	yes	CRUDEN, 1968
	rubicundulum (Say)	U.S.A.	13	yes	CRUDEN, 1968
IV. cordulegaster	parvulum Bart.	Japan	13	yes	KIAUTA, unpublished
VI. frequens	frequens Sel.	Japan	12	no	OGUMA, 1917, 1930; KICHIJO, 1942b
VII. danae	danae (Sulz.)	U.S.S.R., Finland, U.S.A.	13	yes	MAKALOWSKAJA, 1940, OKSALA, 1945 and CRUDEN, 1968, respectively
VIII. corruptum [1])	corruptum (Hag.)	U.S.A.	13	yes	CRUDEN, 1968; this paper
	illotum (Hag.)	Jamaica, U.S.A.	13	yes	CUMMING, 1964 and CRUDEN, 1968 respectively
IX. uniforme	costiferum (Hag.)	U.S.A.	13	yes	CRUDEN, 1968
	semicinctum (Say)	U.S.A.	13	yes	SMITH, 1916; CRUDEN, 1968
	vicinum (Hag.)	U.S.A.	13	yes	CRUDEN, 1968
X. striolatum	striolatum (Charp.)	Luxembourg	13	no	KIAUTA, 1966

[1]) The group is considered by some workers as a separate genus, *Tarnetrum* Needham & Fisher (cf. text)

mitotic figures exist only for *O. albistylum speciosum* (OGUMA, 1930, fig. 1a; OMURA, 1955, fig. 2, unclear), *O. coerulescens* (KIAUTA, unpublished), *O. japonicum* (OMURA, 1930, fig. 3, unclear), *O. sabina* (ASANA & MAKINO, 1935, fig. 8; KICHIJO, 1942b: p. 1078, fig. 1; RAY CHAUDHURI & DAS GUPTA, 1949, fig. 2a), *O. triangulare melania* (OMURA, 1955, fig. 4), *S. corruptum* (this paper, Fig. 27), *S. eroticum* (this paper, Fig. 26) and *S. semicinctum* (SMITH, 1916, figs. 6ff.).

In Plates 4 and 5 microphotographs of low-n and some "normal"-n species of the two genera are given. When the karyotypes of the "normal"-n species (n = 13) are compared to those with low-n complements, the following observations should be stressed:

(1) In none of the "normal"-n species does an unusually large autosomal pair (bivalent) occur (Figs. 27, 29, 33, 35). This holds true also for the n = 12 complements of *O. coerulescens* (Fig. 15). Such a pair (bivalent), on the other hand, is always present in *O. brachiale* (Figs. 30–31) and *S. eroticum* (Fig. 28), but it does not occur in *S. frequens* (n = 12; cf. OGUMA, 1930, fig. 2d). The complement of the latter species may, therefore, be considered as a primary complement and analogous to the n = 12 set in *O. coerulescens*.

(2) With the exception of *S. striolatum* (Fig. 37) a pair of m-chromosomes is present in all (twenty two) "normal"-n species of the two genera, but is lacking in the n = 12 sets of *O. coerulens* (Fig. 15) and *S. frequens* (OGUMA, 1930, fig. 2d). This is another proof of the analogous (and primary) nature of the karyotypes of these two species (cf. also, p. 172, point 6, mentioned above).

(3) As a rule, a single chiasma occurs per bivalent in males of "normal"-n species (primary complements) (Figs. 32–33, 35–37), whereas the chiasma frequency is higher in the secondary low-n species (Figs. 30–31, 34). In this way, the recombination index in primary and secondary complements remains the same, or may even slightly increase in secondary complements.

As often before, this time again the cytological preparations made in the field by Prof. J. W. BOYES (Montreal) of some of the species used in this research (*Orthetrum brachiale* /Beauv./, *Diplacodes bipunctata* /Br./, *D. haematodes* /Burm./, *Sympetrum eroticum* Sel.) were of decisive importance for the studies reported on in the present paper. The material of *Orthetrum pruinosum neglectum* (Ramb.) and *Sympetrum corruptum* (Hag.) was also provided by him (cf. Plates 4 and 5). The author feels deeply obliged for his generous help.

Thanks are due to Dr. J. M. VAN BRINK (Utrecht) for valuable suggestions and critical reading of the manuscript.

Dr. M. A. LIEFTINCK (Leiden) identified and/or verified the identifications of the overseas material, whereas Miss Dr. C. LONGFIELD (Cloyne, Eire) kindly gave advice on the taxonomy of the genus *Orthetrum*, and identified the specimen of *O. azureum* (Ramb.). Dr. K. HARA (Utrecht) was helpful with the translation of the Japanese papers by KICHIJO & HIRAI.

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