Karyological relationships between the Cryptobranchid salamanders[1]

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Summary. The 3 living Cryptobranchids (*Andrias japonicus*, *A. davidianus* and *Cryptobranchus alleganiensis*) show 2n = 60 and nuclear DNA amounts of respectively 92.9, 100.1 and 112.5 pg. Karyologically, the 2 genera differ in the morphology of 2 chromosome pairs. The hypotheses are advanced that either the *Cryptobranchus* karyotype is derived from that of *Andrias* through an unequal translocation, or the karyotypes of both genera are derived from that of a common (perhaps hynobiid) ancestor with at least 62 chromosomes.

The amphibia Caudata belonging to the family Cryptobranchidae make up a group of semilarval giant forms, possibly of hynobiid descent. Phylogenetically, they are of great interest, since together with Hynobiidae they represent the most generalized extant Caudata[2-4], grouped into the suborder Cryptobranchoidea. Despite the paucity of present knowledge of fossil remains of these urodeles, the Cryptobranchoidea are believed to be very ancient. As concerns the Cryptobranchids, the less recent remains date back to the Lower Eocene, but the majority of them are miocenic[5-8]. Undoubtedly, the most famous is *Andrias scheuchzeri* from Central Europe, described as *Homo diluvii testis* in 1732, and identified as a giant urodele by Cuvier in 1824. Some workers sponsor the view that many of these fossils may belong to extant species (*A. japonicus*); this would be evidence of a bradytelic evolution of Cryptobranchids (a phenomenon also shared by other Caudata).

At any rate, between the 2 living genera (*Andrias* or *Megalobatrachus*, Asiatic at present; and *Cryptobranchus*, from North America); *Andrias* might represent the most ancient form, since the fossil Cryptobranchids from Asia and America seem to bear greater affinity with *Andrias* than with *Cryptobranchus*[5-8].

In the present work, the chromosome morphology and other karyological characteristics of the 3 cryptobranchid species of present day (*A. davidianus*, *A. japonicus* and *C. alleganiensis*) are described, and the possible impact of our findings on the knowledge of the phyletic relationships of these Caudates is considered.

Materials and techniques. Live specimens from the 3 above-mentioned species, supplied by specialized dealers, were used. The squash technique was applied to spleen, gonad and intestine fragments after colchicine and hypotonic pretreatments. In order to draw a more detailed comparison between the karyotypes of the 3 species, the following parameters, listed in the table, were estimated on 3 complete plates for each species:

- The centromeric index (IC), i.e. the ratio ($\times 100$) of the short arms to the total length of each homologue pair;
- percent length (L%), i.e. the length of each pair of the complement as percent of the length of the largest chromosomes of the karyotype.

This second parameter was held more suitable than the absolute length of each pair, which varies according to the chromosome degree of spiralization. Anyhow, the quantitative relationships between the genomes of the 3 species under study are inferred by comparing the DNA nuclear contents (in pg per nucleus, pg/N) of the 3 cryptobranchid species with one another.

The DNA nuclear amount was evaluated histophotometrically by means of a Leitz MPV microphotofluorimeter at 546 nm wavelength, on Feulgen-stained blood smears. The high DNA content of these paedogenetic urodeles is known to be made up of largely similar nucleotidic sequences, repeated millions of times[9,10]. In order to find out the chromosomal localization of the more highly repetitive sequences (C-bands) and of ribosomal cistrons (NO-bands), we used the chromosome banding techniques described by several workers[11], slightly modified. The last investigations are essentially directed to *A. japonicus*, owing to the low number of specimens of the other species available to us.

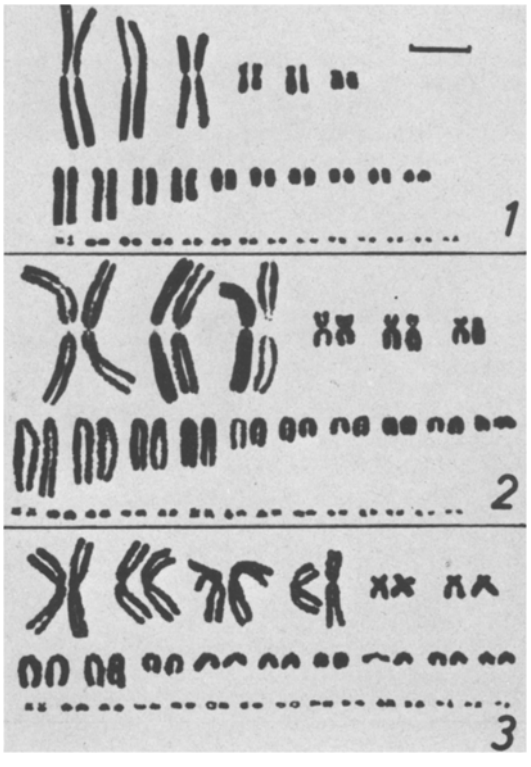

Fig. 1. The karyotype of *Andrias davidianus*. The bar is 10 µm long.
Fig. 2. The karyotype of *A. japonicus*.
Fig. 3. The karyotype of *Cryptobranchus alleganiensis*.

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Chromosome		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	...	30
Andrias davidianus	IC	49.1	47.4	41.7	45.8	35.4	33.0	–	–	–	–	–	–	–	–	–	–	43.7	–	...	–
	L%	100	84.4	66.5	19.5	19.0	13.0	43.6	38.7	27.3	25.3	16.5	12.9	11.1	10.2	9.3	8.4	6.0	4.8	...	1.5
Andrias japonicus	IC	46.7	49.9	48.9	47.8	40.6	33.3	–	–	–	–	–	–	–	–	–	–	48.7	–	...	–
	L%	100	89.5	69.2	18.3	17.6	15.8	41.5	37.8	30.2	27.6	17.2	13.9	10.9	10.3	9.5	8.3	4.7	3.8	...	1.6
Cryptobranchus alleganiensis	IC	47.3	49.4	48.9	44.3	47.1	29.2	–	–	–	–	–	–	–	–	–	46.9	–	–	...	–
	L%	100	91.4	80.4	63.7	20.1	22.8	33.5	31.9	28.2	16.1	15.8	14.9	12.6	12.0	11.5	5.1	4.2	3.9	...	2.6

Results and discussion. The 3 species under inspection have 2 n = 60 (figures 1–3). This differs from formerly reported findings, namely 64 chromosomes in *A. japonicus*[12] and 62 chromosomes in *C. alleganiensis*[13].

The karyotypes of *A. davidianus* and *A. japonicus* are almost equal, in that they consist of 3 pairs of large 2-armed chromosomes (metacentric, MC), 3 middle-sized MC pairs, 10 one-armed (acrocentric, AC) pairs and, lastly, 14 pairs of microchromosomes all AC but one MC (the 17th pair) (figures 1 and 2). In these 2 species, the nucleolus organizers (NO) are localized next to the centromere on the shortest arms of the 3rd chromosome pair consisting of large MCs (figure 5).

C. alleganiensis karyotype consists of 4 pairs of large MCs, 2 pairs of middle-sized MCs, 9 AC pairs and lastly of 15 pairs of microchromosomes, all AC, but the 16th pair, made up of MC (figure 3). In this species, too, the nucleolus organizers lie on the 3rd pair, in a procentromeric position, corresponding to that of *Andrias* NOs (figure 6).

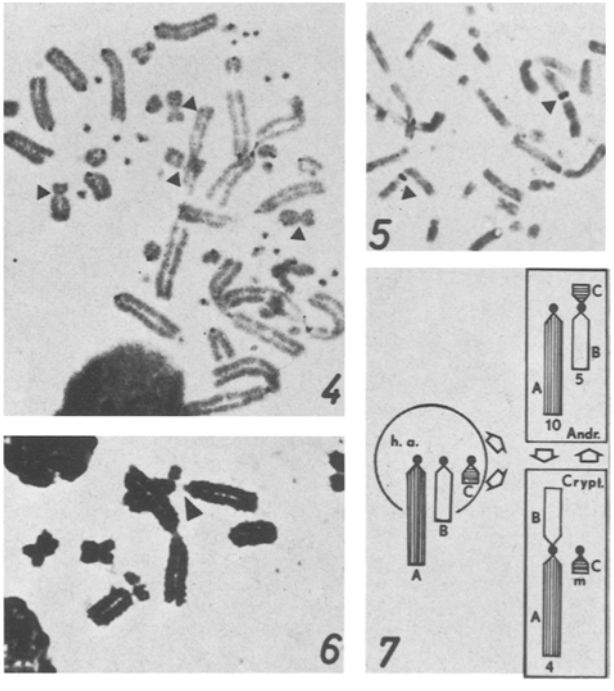

Fig. 4. C-bands in *A. japonicus*. All the centromeres are deeply stained, except in 4 middle-sized metacentrics (arrows) and some microchromosomes. Fig. 5. Procentromeric NO-bands on the 3rd pair of *A. japonicus*. Fig. 6. Nucleolar heterochromatin on 1 of the chromosomes of the 3rd pair of *C. alleganiensis*. Fig. 7. The presumed karyological relations between *Andrias*, *Cryptobranchus* and a hypothetical common ancestor (h.a.), according to the hypotheses mentioned in the text.

The nuclear DNA content is 92.9 pg/N in *A. japonicus;* 100.1 pg/N in *A. davidianus;* 112.5 pg/N in *C. alleganiensis*. In *A. japonicus*, the most highly repetitive sequences appear localized at the centromeric level in all the chromosomes, except 2 pairs of medium-sized MCs, i.e. the 4th and 6th pairs, and except 4 pairs of microchromosomes (figure 4).

Inspection of figures 1–3 and the data reported in the table reveals that the majority of *Andrias* and *Cryptobranchus* chromosomes are comparable in size and shape, this being true in particular of the NO-containing pair (i.e. the 3rd one in both genera) and of another pair of readily identifiable 'markers', namely, the MC microchromosomes (17th pair in *Andrias*, 16th in *Cerypto-branchus*). The karyological differences between the 2 genera seem to concern essentially 2 pairs of homologues for each of them, namely, 1 pair of large ACs (10th pair) and 1 of middle-sized MCs (possibly the 5th pair) would be characteristic of *Andrias*, whereas a pair of large MCs (4th pair) and 1 microchromosome pair would be typical of *Cryptobranchus*. In each of these pairs, the chromosomes consist of 3 arms of different lengths, i.e. a long arm (A), a middle one (B) and a rather short arm (C). Theoretically, considering for simplicity sake a haploid karyotype, the 2 typical chromosomes of 1 of the 2 genera might turn into the typical ones of the other by means of a simple unequal translocation (B on A, when shifting from *Andrias* to *Cryptobranchus;* B on C for the reverse shift) (see scheme in figure 7).

In paedogenetic Caudates, possessing huge amounts of DNA compared to the other Urodeles and to amphibians of other orders, the quantitative differentiation of the genome was plausibly achieved through progressive increases in nuclear DNA[10]. Hence, *Cryptobranchus* possessing a greater DNA amount than *Andrias* might be the more differentiated karyologically among the extant ones of the family. For the foregoing reasons, of the 2 above-mentioned hypotheses on the derivation of the karyotype of the 2 cryptobranchids, the first one (i.e. the descent of the *Cryptobranchus* set from that of *Andrias*) seems to be the more probable. As stated earlier, this would also be supported by paleontological findings.

However, yet another hypothesis can be formulated on the origin of the karyotype of these Urodeles, having the same degree of 'parsimony' (i.e. in karyological terms, likewise requiring a single chromosome mutation) as the origin just postulated. According to this 3rd hypothesis, there would have been a common ancestor of the 2 extant Cryptobranchids, which possessed a similar chromosome complement, except for the presence of 3 AC pairs of different length instead of the 2 typical pairs displayed by

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each of genera living today (2 n = 62). From these 3 AC pairs (A, B, C) might have arisen – through a simple centromeric (Robertsonian) fusion – the chromosomes typical of *Andrias* (B + C, A) or those of *Cryptobranchus* (A + B, C) (figure 7).

During the evolution of Caudates, the karyotype seems to have differentiated by a progressive reduction in the number of the chromosomes, notably ACs and microchromosomes, due perhaps to centric fusions or other mutational events[10]. From this standpoint, the Cryptobranchids do not appear the most primitive forms of the order, karyologically, since within Hynobiids some species have more than 60 chromosomes[10]. Among these, *Ranodon sibiricus* (2 n = 66) is likely to be the species karyologically closest to the 2 living Cryptobranchids[14]. It is of interest to note that *Ranodon*, owing to various characteristics of its development, is regarded as the most primitive genus within the Hynobiids[15]. In view of the fact that the Cryptobranchids probably represent a paedogenetic derivation from ancient Hynobiids[2–4], a hypothetical ancestral form common to *Andrias* and *Cryptobranchus* may have been equipped with a chromosome set of more than 60 elements, though nearly akin enough to the set of the 2 extant genera, so as to be able to turn into it with a minimum number of chromosome mutations. (These urodeles are in fact likely to be bradytelic also from a karyological point of view.)

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Interchange trisomics in pearl millet

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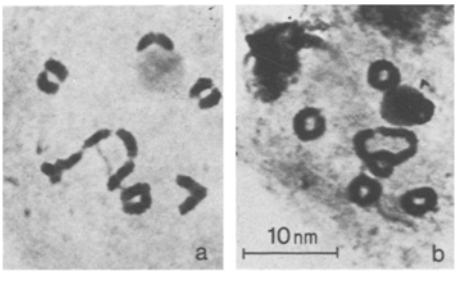
Summary. In the progeny of interchange heterozygotes of *Pennisetum typhoides*, 2 interchange trisomic plants were obtained. These were selfed over 2 generations and the cytology of the parents, s_1 and s_2 plants was studied. The types of multiple associations in the s_1 and s_2 generations differed from those of the parent. A significant decrease in mean chiasma frequency was noted in s_1 and s_2.

The term interchange trisomic applies to a situation where an extra chromosome occurs in an interchange background. If the extra chromosome consists of 2 arms of 2 nonhomologous chromosomes, it is known as a tertiary trisomic. Both interchange and tertiary trisomics can be obtained in the progeny of a translocation heterozygote. These 2 types of trisomics can be distinguished from one another by their characteristic chromosome associations. In the tertiary trisomics, when the extra chromosome is present as a univalent, the other chromosomes will be in pairs and the critical configuration is the dumbbell-shaped association of 5 chromosomes, viz. 2 ring bivalents attached to a single middle chromosome, whereas in an interchange trisomic besides associations of 5 chromosomes, a univalent and a ring of 4 are expected to occur. Interchange trisomics were previously reported in a number of plants, e.g. maize[2], Pisum[3], barley[4]. In these cases, they were obtained in the progeny of translocation heterozygotes.



Types of associations of chromosomes in the interchange trisomics at diakinesis. [a] PMC showing 1 chain of 4 chromosomes, 5 bivalents and a univalent. [b] PMC showing frying pan type (1 trivalent attached to a ring bivalent) association of 5 chromosomes and 5 bivalents.

Results and conclusions. In *Pennisetum typhoides* S. & H. (Gramineae) in the progeny of interchange heterozygotes, 2 plants with the chromosome number 2n = 15 instead of the normal 2n = 14 were obtained. A study of PMC meiosis in these plants was carried out and a dumbbell-shaped association of 5 chromosomes was never observed. Further, when the extra chromosome was present as a univalent, a ring of 4 chromosomes was frequently observed indicating translocation background of this aneuploidy.

In an interchange trisomic of this type, there are 2 completely homologous chromosomes and 2 interchange chromosomes. The 5th chromosome is a normal one and is homologous to one of the chromosomes involved in the translocation. Under these conditions, a total of 9 types of pachytene configurations can be formed which result in 9 groups of configurations at diakinesis and metaphase I. These are possible only under 2 conditions: a) chromosome pairing is initiated exclusively at the chromosome ends; b) the 3 ends have equal probability of being involved in pairing. When there are no interstitial chiasmata, these 9 types will consist of 6 as chains of 5 chromosomes, 1 as chain of 3 chromosomes plus ring bivalent, and 2 each as a ring of 4 chromosomes and a univalent[7]. The data relating to the types of associations observed at diakinesis are presented in the table. Among the PMC's examined, 54% showed associations of 5

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