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2 DBCP was the active ingredient in Fumazone and Nemagon, the trademarks of the Dow Chemical Company and the Shell Chemical Corporation, respectively.
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Karyology of the primitive salamanders, family Hynobiidae[1]

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Summary. Karyotypes have been studied in 3 species of *Hynobius* and in 1 species each of the remaining genera of Hynobiids *(Ranodon, Batrachuperus, Salamandrella* and *Onychodactylus).* All species have large diploid numbers, between 56 and 66, and asymmetrical and bimodal karyotypes. DNA contents (2C) were found to vary between 33 and 51 pg. Determination was not possible in *Onychodactylus* where higher values may be suspected. Some of the karyotypes investigated are similar to those of Cryptobranchids. Phylogenetic implications are discussed.

The members of the family of the Hynobiidae, found throughout palaearctic Asia, are the most primitive of the living Urodeles (Amphibia, Caudata). It is probable that all, or nearly all, the present-day families of the order were derived from ancient hynobiid forms[2, 3]. The relationships among the different genera of the family are not well known at present. Most of the species live in areas difficult to reach. According to Noble[2], *Hynobius* is the central genus from which the other Hynobiids were derived by independent specializations. Within the genus, the Japanese species are usually more evolved than the continental ones. However, other authors consider that *Ranodon* retains the greatest number of primitive characters[4], even though it is the only hynobiid able to fabricate a rudimentary spermatophore[5].

Salamandrella, which is monotypical, is often included in the genus *Hynobius.* It is the most widespread of the family and the only one which has reached Europe. Other genera, such as *Batrachuperus* and *Onychodactylus,* consist of a few species adapted to life in mountain waters. The species *boulengeri,* sometimes assigned to the genus *Pachypalaminus,* is more probably a species of *Hynobius*[6]. The karyology of various species of *Hynobius* and of *Salamandrella keyserlingii* was studied by Makino[7] and Sato[8], who showed the chromosomal affinities between the Hynobiids and the Cryptobranchids, and the differences between these 2 families (often combined in the suborder Cryptobranchoids) and the other families of the Urodeles. Other investigators have since confirmed these conclusions in general[9-11].

In this note we report on the chromosomes of *S. keyserlingii* and of 3 species of *Hynobius* (described also by the Japanese investigators) and describe those of 1 species each of the genera *Ranodon, Batrachuperus* and *Onychodactylus.* We have also evaluated the amounts of nuclear DNA (2 C) in nearly all of these species. The karyology of these primitive Urodeles is also compared to that of the other families of the order[11].

Materials and methods. The species investigated were *Ranodon sibiricus, Batrachuperus mustersi, Salamandrella keyserlingii, Hynobius dunni, H. tsuensis, H. nebulosus* and *Onychodactylus japonicus*[12]. In the case of *Ranodon,* we were able to find only 1 juvenile specimen of undifferentiated sex. In the other cases, except for *Onychodactylus,* we had available live animals of both sexes, from which we obtained fresh material suitable for investigation on the karyotypes and also histophotometrical assay of nuclear DNA. For *Onychodactylus* only fixed material was available, and therefore the data on the karyotype must be considered as approximate and provisional, while DNA measurements were not possible. The methods used for the investigation of the chromosomes and for the determination of the genome size were those described in a previous paper[13].

Results. Ranodon sibiricus ($2n=66$) has 5 pairs of large bi-armed chromosomes (metacentric, or M), 9 pairs of chromosomes with only 1 arm or with the 2nd arm extremely short (acrocentric, or A), and finally 19 pairs of very small, dot-like chromosomes (microchromosomes) (figure 1). The nuclei of the erythrocytes of this species contain 50.7 pg of DNA.

Batrachuperus mustersi (2n = 62) has 7 pairs of large M and 5 of large A, plus 19 pairs of microchromosomes (figure 2). The nuclear DNA content is 43.3 pg.

Salamandrella keyserlingii, with a diploid number (2n = 62) and nuclear DNA content (42.3 pg) similar to those of *B. mustersi*, nevertheless has chromosomes of somewhat different form: 7 pairs of M and 12 of A, plus 12 pairs of microchromosomes (figure 3). In this species, as in *Hynobius*, the distinction between the smallest of the A chromosomes and the microchromosomes is not as marked as it is in *R. sibiricus* and in *B. mustersi*.

Hynobius dunni, *H. nebulosus* and *H. tsuensis* all have 2n = 56 and similar karyotypes, with 9 pairs of large M, 6 pairs of medium-to-small M, 3-5 pairs of small A and 8-10 pairs of microchromosomes, where the distinction between the last 2 classes of chromosomes is not very clear. In figure 4 the karyotype of *H. dunni* is shown. The nuclear DNA contents of the 3 species are, respectively, 33.8, 38.4 and 33 pg.

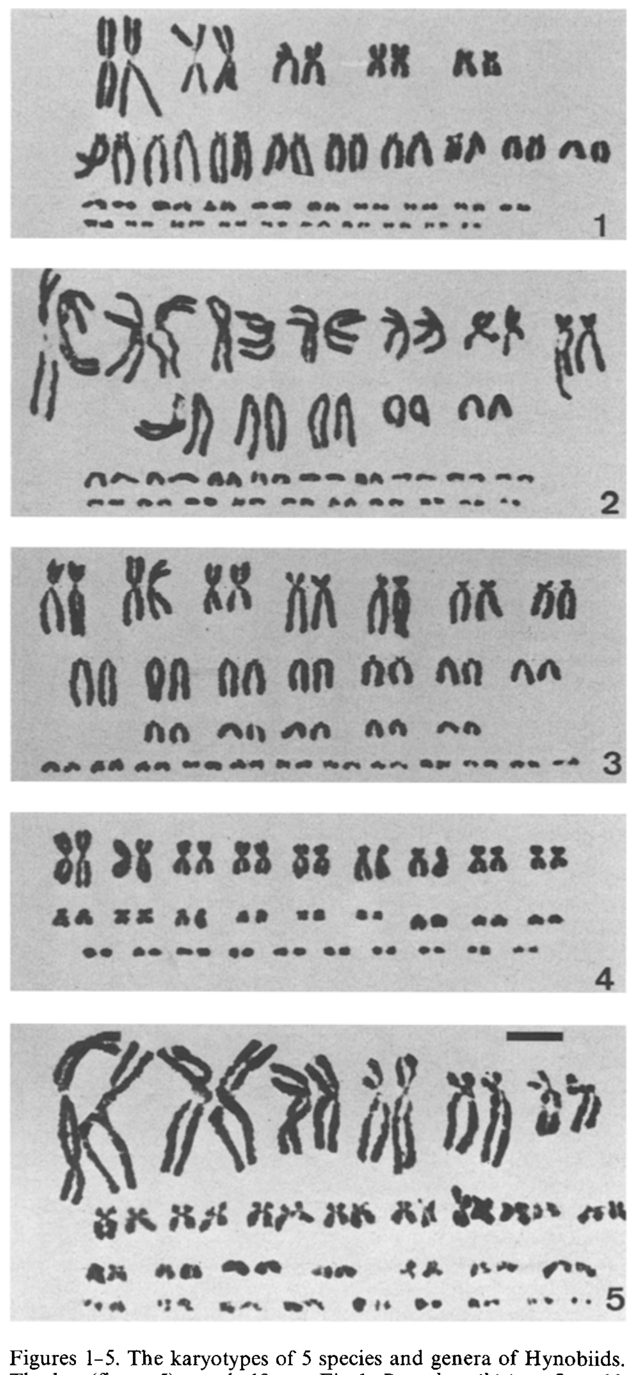
Onychodactylus japonicus, on the uncertain basis of fixed material, has at least 56 chromosomes, perhaps more. In the best metaphase obtained, that from which the karyotype shown in figure 5 was constructed, it is possible to count 60 chromosomes: 6 pairs of large M, 8 pairs of medium-sized M, 7 pairs of small M and A chromosomes, and 9 pairs of microchromosomes. There is considerable doubt about the morphology of the small chromosomes and the total number of microchromosomes: the diploid number of this species is therefore said provisionally to be 58 ± 2. The chromosomes seem to be considerably larger than those of the other Hynobiids studied. While this may be an effect of the storage solution, it is also possible that this species has a large amount of nuclear DNA, perhaps near to that of the living Cryptobranchids (93-112 pg[13]).

Discussion. All the Hynobiids in this study show a large chromosome number of 2n = 56-66 and karyotypes which can be defined as asymmetric (with both M and A chromosomes of various size) and bimodal (with both 'normal' and dot-like chromosomes)[14]. Among the Urodeles only the Cryptobranchids have similar karyological characters, with asymmetric and bimodal karyotypes of 2n = 60. Except for 2 transitional families, the Proteids and Sirenids, showing asymmetric but unimodal karyotypes, i.e., without microchromosomes, the other Urodeles have small numbers of 2n = 22-28, and symmetrical karyotypes, i.e., M chromosomes only[11].

There are at least 3 reasons to consider that the karyological formulas of the Cryptobranchoids are of the primitive type: they are typical of a fundamentally primitive and bradytelic group of Urodeles; similar formulas are found in other primitive families of the Anurans and Apodans; asymmetrical and bimodal karyotypes are also frequent in many groups of lower tetrapods[10, 11, 15].

Earlier data obtained for various species of *Hynobius*, for the most part Japanese, showed that the characteristic karyotype for this genus is bimodal with 56 chromosomes: this is true for at least 11 species, including *boulengeri*, belonging, to all the major groups in which the genus is currently subdivided[7, 8]. 1 island species, *retardatus*, has only 40 chromosomes and no microchromosomes[7, 9]: the unimodal karyotype of *retardatus* was probably gained in a secondary way, since other species of the same group *(sadoensis, nigrescens)* show the 'basic' formula of the genus, 2n = 56.

Within the family, the karyotype of *Ranodon, Batrachuperus* and *Salamandrella* differ from those of most *Hynobius* and of *Onychodactylus* in that there are various large A chromosomes, not seen in the last two genera, and in having larger diploid numbers, depending especially on the number of the microchromosomes. The karyotypes rich in large A chromosomes are similar to those shown by the Cryptobranchids[13], a family which is usually considered an early offshoot from an ancient hynobiid stock: thus, they may be more primitive than the 56-chromosome karyotypes. However, among the *Hynobius* there are probably transitional karyotypes between those presumed to be more primitive and those basic in the genus: *H. lichenatus* has 2n = 58, and a population of *H. kimurai* (or perhaps a new



Figures 1-5. The karyotypes of 5 species and genera of Hynobiids. The bar (figure 5) equals 10 μm. Fig. 1. *Ranodon sibiricus*, 2n = 66. Fig. 2. *Batrachuperus mustersi*♂, 2n = 62. Fig. 3. *Salamandrella keyserlingii*♀, 2n = 62. Fig. 4. *Hynobius dunni*♂, 2n = 56. Fig. 5. *Onychodactylus japonicus*♂, 2n = 58 ± 2. In no case have heterochromosomes been detected.

species) has $2n = 60$: in both there is a larger number of A chromosomes than in the other species of *Hynobius*[7,8].

These last data indicate the existence, among the Hynobiids, of many of the initial stages that occurred along the karyological differentiation of the Urodeles from bimodal karyotypes rich in chromosomes and microchromosomes, to the symmetrical and numerically reduced karyotypes of the 'higher' families.

As to the diversification of genome size, the Hynobiids comprise species with a relatively low amount of DNA, compaired to other Urodeles, with the possible exception of *Onychodactylus*[11].

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Attractive behavior toward human constructions helps to explain the domestic and cosmopolitan status of some Drosophilids

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Summary. Big differences in the Drosophilid adults collected by traps placed within a short distance were observed during a study in tropical Africa. These can be explained mainly by divergent behavior towards man-made constructions. In most cases, adults strictly avoided entering buildings. On the other hand, some species, mainly *D. melanogaster,* appeared to concentrate within man-made constructions. This preference can be considered as a preadaptation which allowed exploitation of new, poorly competed food sources and also favored transportations with fruit shipments all over the world.

Among Drosophilids, more than 15 species have reached a subcosmopolitan and, in most cases, a domestic status (David and Tsacas, in preparation). Although these species are generally considered as opportunist, generalist and r strategist, a careful analysis failed to show any consistent general genetic properties[2,3] leading to the conclusion that a special analysis should be made in each case. Recently, attention has been drawn[4] to a high stress tolerance which could characterize cosmopolitan species. Behavioral characteristics, much studied in the laboratory[5] seem, however, to have received only little attention in the wild. During a survey of the drosophilid fauna of the popular republic of Bénin, in tropical Africa, it was noticed that species collected with banana baits differed according to the position of traps. The problem was further studied in special experiments. Results, presented here, gave consistent conclusions which certainly help to explain the ecological status of some *Drosophila* species and particularly of the most widespread *D. melanogaster.*

Experiments were made on the campus of the University of Bénin, about 10 km north to Cotonou. The University is built in the country and is surrounded by cultivated fields, orchards and small woods, with no village in the vicinity. 5 different trapping sites were used and flies were collected every day, usually in the morning. The location of the traps, with respect to university buildings, is given on the map figure 1. These places were chosen as function of the local possibilities and, mainly, of the availability of shadow.

Altogether more than 1800 flies, belonging to 14 different species, were collected and results are given in the table. No indication of any modification of the fauna for the duration of the experiment was obtained. Results are, however, highly different between traps. Traps 1 and 2, outside the buildings and placed below trees, collected an abundant fauna with the highest diversity. Trap 1 is remarkable for the rarity of *D. melanogaster* and the predominance of *D. malerkotliana* and of various species of the *Zaprionus* genus. Trap 3, although close to trap 2 and to the outside of the building, yielded a very different fauna. Flies were clearly less abundant, only 6 species were found and *D. melanogaster* represented about 90% of the total catch. Trap 4 confirmed the above difference. A great number of flies were collected there but they all belonged to only 5 species and *D. melanogaster* constituted more than 95% of the catch. Trap 5 was set a few days after the beginning of the experiment in order to check if the avoidance behavior shown by most species toward tall concrete buildings could also be observed versus a low, primitive shelter, and also to determine if *D. melanogaster* could be found in abundance at a distance of 100 m from the main buildings. Results were clearly conclusive: almost no *Zaprionus* were collected in trap 5, which however produced 117 *D. melanogaster.*

All these results are coherent. *D. melanogaster* was more abundant in the vicinity of the university buildings (traps 2, 3 and 4) than in traps 1 and 5 placed at a distance of 100 meters. Apparently such a short distances was sufficient to provide a fairly strong isolation of 2 populations. Perhaps, in this special case, the isolation was favored by the occurrence of bare, non-shadowed area between the build-