layer. The peroxidase-labelled GAR was applied for 60–90 min to the sections pretreated with the above antibodies. After three 20 min washes with PBS, the sections were reacted with diaminobenzidine (DAB[7]) for 20–30 min. The reaction and the last wash with PBS occurred at room temperature. The sections were then rinsed and washed in 3 changes of distilled water for 3–5 min each and mounted in Elvanol[8]. Controls consisted of sections treated with non-immune rabbit γ-globulins as an intermediate layer.

Results and conclusions. The chromatographic pattern shown in Figure 1 has been reproduced 4 times. The procedure results in a satisfactory separation of conjugated globulins from the rest of the reaction mixture. This was demonstrated by the following tests: 1. Agar immunodiffusion revealed cross reaction with rabbit γ-globulins for fractions 31–52 of Figure 1; 2. When the same fractions were applied to sections of *Rana pipiens* embryonic lenses, pretreated with specific antibodies against γ-crystallins, specific staining in the lens fiber area was found only for fractions 31–43, being most pronounced for fractions 35–41. Therefore fractions 31–41 were pooled and absorbed 3 times with mouse tissue powder (20 mg, each time). After centrifugation at 12,100 *g* for 1 h the resulting 25 ml of supernatant were dialyzed against 4 changes of 50 volumes of distilled water for 8 h and then lyophilized. The yellow powder obtained can be kept indefinitely under vacuum in the cold. For use, it is dissolved in PBS (6–8 mg/ml). This solution shows strong cross reaction with rabbit non-immune 7 S γ-globulins (0.5 mg/ml) in OUCHTERLONY tests[9].

Sections through lenses of *R. pipiens* or *N. viridescens* regenerates stained with peroxidase-labelled antibodies show that γ-crystallins are present in the fiber cells, but absent in the lens epithelial cells. This finding confirms previously published results[2,10]. Thus, peroxidase as a label for antibodies gives the same results as does an FITC label (Figures 2 and 3)[11].

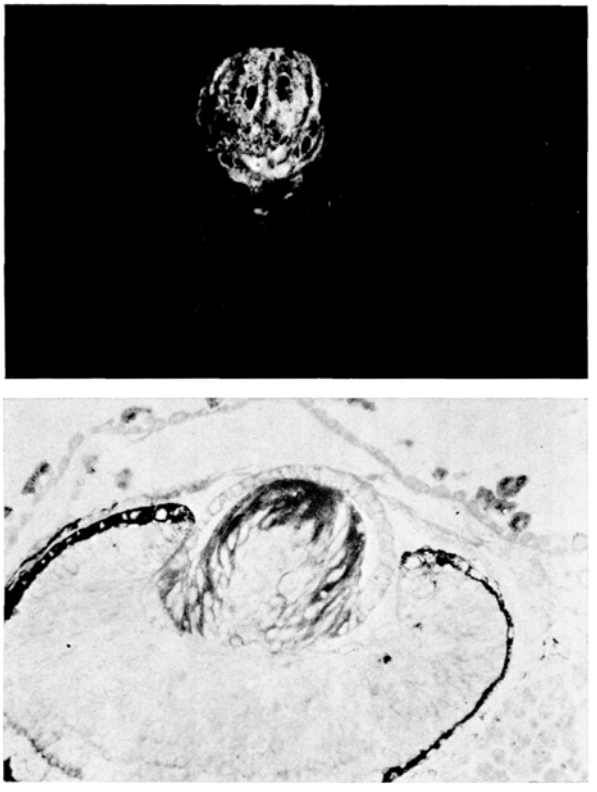

Fig. 3. Embryonic lens of *R. pipiens* (stage IX/X) treated with *R. pipiens* anti-γ crystallin, and stained with FITC-GAR (a), or peroxidase-GAR (b). Labelling in both cases was restricted to the lens fiber area. In (a) the label is bright, the background dark; in (b) the label is dark, the background bright. The black area in (b) is the pigmented layer of the optic cup.

Zusammenfassung. Antikörper, die entweder mit Fluoresceinisothiocyanat oder mit Peroxidase markiert waren, wurden auf ihre Spezifität im Erkennen von Linsen-Antigenen untersucht. Beide Markierer ergeben gleiche Resultate: die γ-Kristalline können in den Linsenfaserzellen lokalisiert werden, nicht aber in den Linsenepithelzellen.

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Biology Division, Oak Ridge National Laboratory, Oak Ridge (Tennessee 37830, USA), 16 October 1970.

[7] 3,3'-diaminobenzidine (Sigma Chemical Company): 50 mg of DAB in 100 ml of 0.05 *M Tris*-HCl buffer, pH 7.6, 0.001% H_2O_2 were used as reagent for peroxidase.

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[11] Acknowledgment. We gratefully thank Drs. P. K. NAKANE and J. N. DUMONT for their valuable suggestions and advice.

[12] Supported by the Damon Runyon Memorial Fund for Cancer Research, New York; and by the 'Kredit zur Förderung des akademischen Nachwuchses an der Universität Zürich'.

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Mitotic Karyotype and Nucleoli of the Marbled Newt *Triturus marmoratus* (Latreille)[1]

Triturus marmoratus (Latreille, 1800) is an European newt species confined to the Iberian Peninsula and southwestern France. It can be grouped with *T. cristatus* as a superspecies or Artenkreis[2]: in fact they are 2 related species[3] that give rise to the natural hybrid *T. blasii* de l'Isle (1862) in the region in France in which their ranges overlap. Such area is rather small compared with the ranges of both species: *T. marmoratus* and *T. cristatus* can therefore be regarded as allopatric in the sense of MAYR[4], apart from the zone of overlap and hybridization.

The phenotypical and anatomical characters of *T. marmoratus*, *T. cristatus* and *T. blasii* have been widely dealt

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[2] L. A. LANTZ, Proc. zool. Soc., London *117*, 247 (1947).

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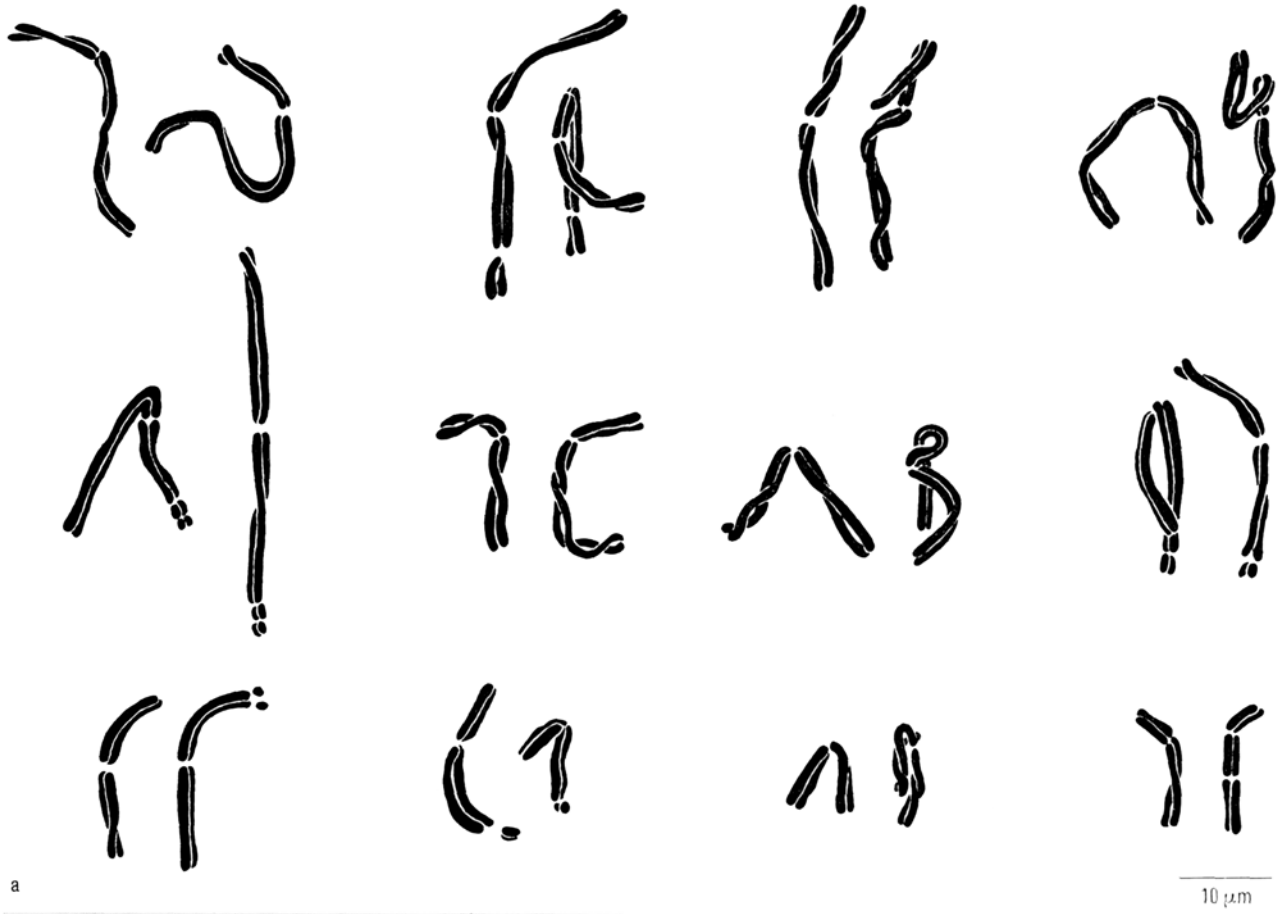
with in the literature[5]. Also the spermatogenesis in the 2 parental species and their hybrids have been analyzed[6–8]; the morphology of the mitotic chromosomes has been described only in *T. cristatus*[9–11].

The present study is therefore a further contribution to the knowledge of the karyology of *T. marmoratus*, to be subsequently completed with a detailed description of the maps of lampbrush chromosomes. This will permit greater precision in establishing the degree and nature of the karyological differentiation within the superspecies *T. cristatus* in the course of speciation.

Karyotype of mitotic chromosomes. The elements composing the karyotype of *T. marmoratus* can be divided into 3 groups. The first consists of the 4 longest chromosomes, which according to the position of centromeres, are respectively[12]: I subtelocentric; II, III and IV metacentric. The second group comprises the 4 middle-length chromosomes, of which V, VII and VIII are metacentric, while chromosome VI is submetacentric. Lastly, the third group consists of the 4 shortest chromosomes, 2 metacentric (IX and XI) and two submetacentric (X and XII) (Figure 1). The mean values of the centromere indexes are in both embryos and adult specimens shown in the Table.

The recognition of the homologues, based primarily on the centromere indexes, has been facilitated by the



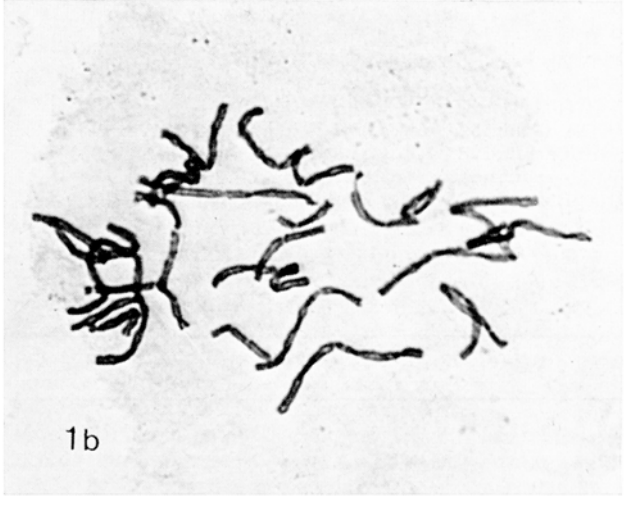


Fig. 1. The 24 mitotic chromosomes of the diploid complement of *T. marmoratus* from an embryonic cell. The serial alignment of the chromosomes does not strictly follow the criterion of the decreasing length order to permit an easier comparison with the karyotype of *T. cristatus*, which will be discussed in a next paper. a) Camera lucida drawings; b) microphotograph. Squashed in aceto-orcein. ×660.

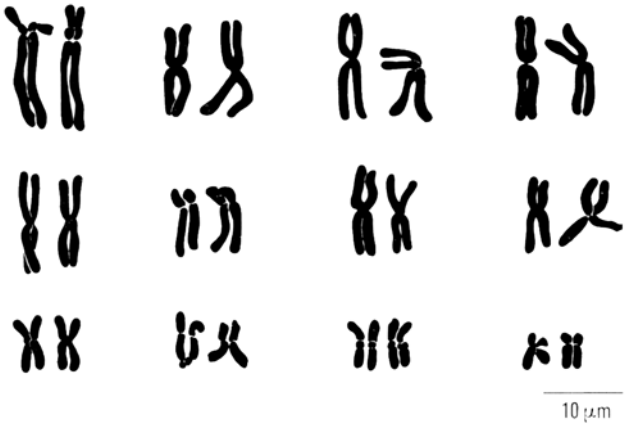


Fig. 2. The mitotic chromosomes from the regenerating tailtip of an adult specimen. Camera lucida drawings. Further explanation in text.

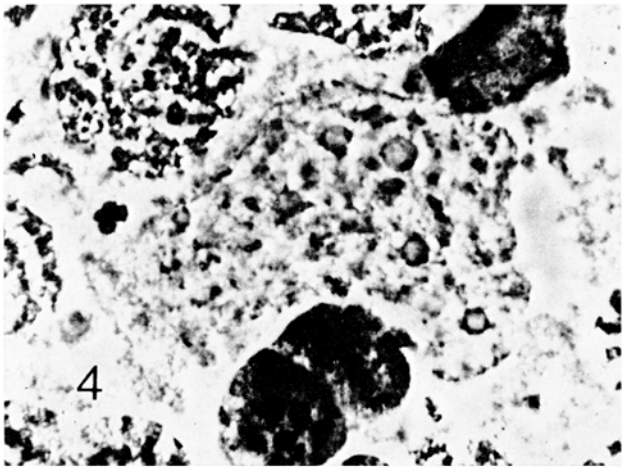


Fig. 4. Large polymorphous spermatogonium nucleus showing multiple nucleoli. Phase contrast. ×680.

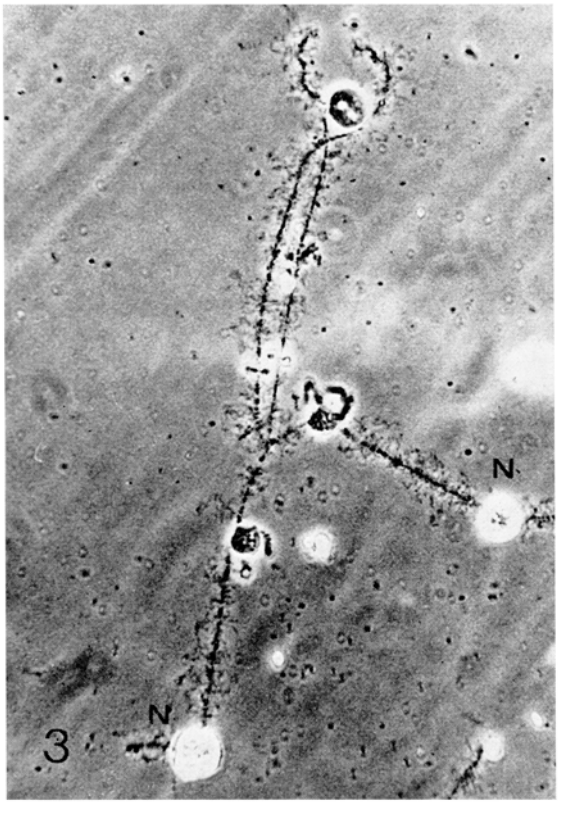


Fig. 3. Lampbrush chromosome X, isolated from the germinal vesicle of an oocyte (diameter: 1.33 mm), showing a nucleolus (N) inserted at the level of the nucleolus organizer on both homologues. Phase contrast. ×345.

presence of secondary constrictions. In the embryos, in fact, chromosomes II, VIII and X have consistently a subterminal secondary constriction and chromosome V displays 2 of them close to each other in subterminal region. Another heterochromatic tract is frequently encountered in the mid-region of the longer arm of chromosome XII, often restricted to a single homologue. In one instance, a subterminal constriction has been found also on 1 homologue of chromosome IX (Figure 1). In mitotic metaphases from adult tissues only the subterminal constriction of chromosome X is constant, while the others previously noticed in the embryos can no longer be seen. Different constrictions, e.g. one about midway along the short arm of chromosome I and another next to the centromere of chromosome XI seem to be peculiar to the adult, although they are found only occasionally (Figure 2). This particular morphological change in the karyotype may be in relation to the process of embryonic differentiation as previously observed in the axolotl[13] and in *Pleurodeles*[14].

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Mean values of centromere indexes of the twelve mitotic chromosomes

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Embryos	0.235	0.472	0.392	0.436	0.454	0.288	0.453	0.439	0.462	0.364	0.447	0.315
Adults	0.263	0.468	0.401	0.428	0.486	0.302	0.480	0.448	0.446	0.369	0.428	0.311

Nucleoli in somatic cells. The results obtained by means of specific stains (methyl-green and pyronin method, followed by RNase tests) indicate that 1 or sometimes 2 nucleoli are present in each interphase nucleus of adult tissues. Hence, *T. marmoratus* karyotype is characterized by a single pair of nucleolus organizers. From the outset of the present study it appeared that chromosome X was the element endowed with the nucleolus-organizing region, since it is the only one displaying consistently the secondary constriction from the early stages of embryonic development.

This assumption turned out to be true when, upon inspection of the lampbrush chromosomes (unpubl.), only chromosome X was found to carry a nucleolus inserted in subterminal region of the long arm (Figure 3).

Nucleoli in spermatogonia. This investigation has been carried out on both first and second spermatogonia[15] mainly of *T. marmoratus* as well as of other newt species. First spermatogonia are generally enclosed by a few follicle cells and recognizable by their large polymorphous nucleus with poorly stainable diffuse chromatin. They may be identified with the cells already known as 'large polymorphous spermatogonia' in *Batrachoseps*[16] and *'cellules-mères primitives'* in several *Triturus* species[17]. Their most remarkable character is the presence of endonuclear bodies of varying sizes, ranging from 2 to 20 per cell in number. Such bodies are clearly identifiable under phase-contrast microscope (Figure 4); they display an outer Feulgen-positive ring, are stained by Unna-Pappenheim methyl-green and pyronin, and are disrupted by RNase. Owing to these characteristics they may be considered as nucleoli. On the contrary, second spermatogonia display only 1 or, occasionally, 2 nucleoli per cell.

The presence of multiple nucleoli recalls the phenomenon of gene amplification of the cistrons for ribosomal RNA which, already known in oocytes I, has been recently suggested also in amphibian oogonia[18]. This may be the case of the first spermatogonia as well: however, at present, their degree of ploidy has yet to be ascertained. If gene amplification is proved to occur in primitive spermatogonia beside oogonia, it will be of great interest to examine more closely the cytological process of the germ-line cell differentiation.

Riassunto. I cromosomi mitotici di *Triturus marmoratus* sono stati ordinati in cariotipo. Gli organizzatori nucleolari sono due per cellula, ma negli spermatogoni primitivi sono presenti numerosi nucleoli liberi.

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Istituto di Zoologia e Anatomia comparata dell'Università, Via Volta 4, Pisa (Italy), 26 October 1970.

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Étude comparée de la multiplication des cellules germinales à la fin de la première semaine de la vie embryonnaire chez trois espèces de Gallinacés (*Gallus domesticus, Meleagris gallopavo, Coturnix coturnix japonica*)

Chez le Poulet (*Gallus domesticus*) et le Dindon (*Meleagris gallopavo*), les populations de cellules germinales subissent un accroissement de type exponentiel entre le moment qui suit leur installation dans les ébauches gonadiques et l'époque de la multiplication rapide qui va les transformer en gonies. Toutefois, chez ces deux espèces, les populations de départ et les taux d'accroissement ne sont pas identiques: le Poulet possède une population de gonocytes primaires plus dense au départ mais dont la prolifération est moins active que chez le Dindon[1]. L'étude de la progression du nombre des gonocytes a été réalisée chez une troisième espèce (*Coturnix coturnix japonica*) et pour la même période de la vie embryonnaire (entre 4 et 7 jours d'incubation) afin d'en comparer les résultats aux précédents.

Techniques. Des embryons de Caille ont été sacrifiés toutes les 24 h entre 4 et 7 jours d'incubation. 2 specimens sont fixés simultanément pour chacun des 4 stades étudiés. Le premier est fixé au Gendre à 4 °C pour être ultérieurement traité par la technique du PAS[2] qui s'est avérée efficace chez la Caille comme chez les deux autres espèces; le second est fixé au Bouin en vue de la coloration des coupes à l'hématoxyline-éosine. Les embryons ont été inclus dans la paraffine et on a débité en coupes transversales de 7,5 µm d'épaisseur une région du corps incluant largement les ébauches gonadiques. Les gonocytes primaires ont été identifiés grâce à leurs enclaves glycogéniques chez les sujets traités par la technique du PAS., et d'après des critères morphologiques classiques sur les autres préparations. Les cellules germinales ont été dénombrées coupe par coupe, pour chacun des stades étudiés, mais on s'est efforcé de ne pas compter plusieurs fois une même cellule.

Résultats. Les résultats des dénombrements de gonocytes effectués chez la Caille sont les suivants: 366 et 412 gonocytes au 4e jour d'incubation, 795 et 816 au 5e, 1457 et 1623 (2 individus ♀) au 6e jour, 2884 (♀) et 3199 (♂) au 7e. Ils montrent que les nombres totaux de gonocytes augmentent assez régulièrement avec le stade de développement, bien que l'on puisse noter un écart plus ou moins grand entre les valeurs trouvées à chaque stade, ce qui est aussi le cas chez les deux autres espèces[1]. La différenciation morphologique du sexe qui se manifeste dans les ébauches gonadiques à la fin de la période étudiée (un peu plus précocement chez la Caille et le Poulet que chez le Dindon) n'entraîne pas aussitôt une disparité d'effectif global des cellules germinales entre le mâle et la femelle, la répartition bilatérale des gonocytes pouvant être néanmoins très asymétrique (indice G/D des gonocytes variant de 1,20 à 6,9 chez les sujets examinés). En portant les valeurs obtenues pour les populations de gonocytes sur un axe logarithmique, l'échelle

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