Ploidy, pigment patterns
and species specific antigenicity in interspecific
nuclear transplantations in newts

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It was shown by Simnett (1964) that in Xenopus laevis skin grafts in adult frogs between members of the same nuclear clone were tolerated in the same way as autografts, but in skin grafts made between individuals belonging to different nuclear clones a homograft rejection occurred. The nucleus is therefore responsible for the synthesis of specific transplantation antigens. It seemed to us useful to investigate the species-specific antigenicity of animals derived from eggs transplanted with foreign nuclei in correlation with their ploidy and with the development of their species-specific pigment patterns, as a proof of functional activity of transplanted nuclei. For this purpose we used two species of Triturus, T. vulgaris and T. alpestris, because of earlier studies carried out in our laboratory on the pigmentation of their hybrids (Romanovský & Štefanová, 1960; Mazáková-Štefanová, 1965) and on their species-specific antigenicity (Romanovský, 1962a, b), in spite of the known difficulties and limitations of nuclear transplantation experiments in these species (Lehman, 1955; Sládeček & Mazáková-Štefanová, 1964, 1965).

MATERIALS AND METHODS

Triturus vulgaris L. and T. alpestris Laur. were used both as hosts and donors in nuclear transplantation. For implantations advanced blastula animal pole cell nuclei and early or middle gastrula chordomesoblast or entoblast nuclei were used. For some experiments tetraploid nuclei of T. vulgaris gastrulae obtained by hot-shock treatment (36.5 °C, 10 min) of eggs during the formation of the first cleavage furrow were used. Different series of eggs were prepared as hosts:

(a) Androgenetic series, obtained by UV-irradiation of the animal pole of artificially inseminated eggs. A Tesla high-pressure mercury lamp, 500 W, was used at the experimentally proved distance of 25 cm, without filtration, irradiation time 120 sec.

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(b) **Gynogenetic series**, obtained by artificial insemination of eggs with UV-irradiated sperm (the same parameters as in preceding series, irradiation time 90 sec.), or by electric activation of unfertilized eggs by a discharge of a condenser (70 V, 8 µF) (Signoret & Fagnier, 1962). This second procedure was found to be effective only for *T. vulgaris* eggs.

(c) **Totally enucleated series**, obtained by UV-irradiation of eggs artificially inseminated with UV-irradiated sperm, or by electric activation of unfertilized eggs, followed by UV-irradiation.

(d) **Fertilized series**, transplanted with tetraploid nuclei.

To disaggregate donor cells treatment for about 20–30 min in Niu-Twitty solution without Ca\(^{2+}\) or Mg\(^{2+}\) ions, supplemented with EDTA (concentration 5 × 10\(^{-4}\) M), was used. Transplantation was carried out in Niu-Twitty solution in Petri dishes with an agar bottom, using an air-filled micro-injection system in a Zeiss Gleit-Mikromanipulator holder. To prevent the formation of exovates the operated eggs were kept overnight in full-strength Niu-Twitty solution and then transferred into Holtfreter solution, diluted 1:8 with distilled water. To this culture medium 0-20% of Gantrisin (Roche) and 50 i.u./ml. of Streptomycin sulphate were added.

The development of experimental and control embryos was recorded daily and some of the animals at different developmental stages (especially those with signs of diminishing vitality) were used to make squash preparations to test their ploidy. For the same purpose tail-tip preparations from larvae were made. Squash preparations from early stages (blastula-neurula) were stained with acetic orcein after pretreatment with distilled water (1–2 h). Tail-tip preparations were stained with Harris’s haematoxylin after fixation in Bouin-urea. The ploidy was estimated on suitable metaphase figures and by comparison of nuclear size.

For the detection, of species-specific antigens antisera against *T. vulgaris* and *T. alpestris* were prepared by immunization of rabbits with homogenates of adult animals together with Freund’s adjuvant (Romanovsky, 1964). The antisera were stored at −30 °C until used. The antisera obtained were examined in
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macro-reactions using double agar diffusion according to Ouchterlony and the absorption technique of Björklund, and were found to be highly species specific. In the experimental series it was necessary to test individual larvae and therefore a micromodification of the method on micro-glasses was used to examine the small amounts of antigens available. Two tests were carried out for every animal examined: in the first the antiserum against *T. vulgaris* was used while the absorption was with heterologous antigens (*T. alpestris*) in the respective agar plate; in the second an opposite cross was used. Antigens from control animals of both species were used as controls. The standard arrangement of the wells is shown in Text-fig. 1.

RESULTS

The development of all the experimental series, and especially the completely enucleated one, was relatively poor, as referred earlier (Sládeček & Romanovský, 1966). In the androgenetic series out of 213 operated eggs only 24 reached larval stages, 2 metamorphosed and 1 died during metamorphosis. Out of 63 animals tested for ploidy at different developmental stages 9 were haploid, 21 diploid, 1 triploid, 11 mosaic (haplo-diploid) and 21 cases were not clearly diagnosed. Out of 46 treated control animals tested for ploidy 19 were found to be haploid, 14 diploid and 13 could not be determined. In the gynogenetic series, out of 50 operated eggs 5 reached larval stages, but none metamorphosed. Of 11 animals tested for ploidy, 3 were haploid, 5 diploid, 2 mosaic (haplo-diploid), and 1 could not be determined. Out of 6 relevant control animals tested 4 were haploid and 2 were not clear. In the totally enucleated series, out of 223 operated eggs only 5 reached tail-bud stage and none of them developed further. Out of 5 animals tested for ploidy, 2 were diploid, 1 was mosaic (haplo-diploid) and 2 could not be diagnosed. Of 109 treated control animals only 1 reached tail-bud stage; 2 animals tested for ploidy were both haploid. In the fertilized series, out of 14 operated eggs 6 reached larval stages and 2 metamorphosed. Out of 7 animals tested for ploidy, one was found to be tetraploid, 1 was a diplo-tetraploid mosaic and the other 5 could not be diagnosed. All 3 relevant control animals tested were found to be diploid. The failure of the ploidy estimation in some of these cases was due to cytolytic changes in the cells of dead embryos.

The development of species-specific pigment patterns cannot be followed during advanced embryonic and larval stages because of its great variability within both species and in their hybrids. The situation was further complicated by the possibility of changes in ploidy since in haploids small, numerous and dispersed melanophores occurred, while for example in triploids the melanophores were large and sparse (Plate 1, figs. A–F). The formation of the continuous dorsal pigment bands was found not to be species specific, and the same was found to be true for the later appearance of the second, lateral, pigment band, which occurs both in some *T. alpestris* and *T. vulgaris* larvae and is missing in others. Also the white pigment cell border of the tail fin occurred
irregularly in control larvae of both species, though more often in *T. alpestris*. This border was found in the *T. vulgaris* larva produced by a transplanted *alpestris* nucleus, which metamorphosed into an animal with typical hybrid pigmentation (with predominantly *alpestris* pigment pattern) and exhibited positive *alpestris* antigenic specificity (Plate 1, figs. E, H; Plate 2, fig. B; Text-fig. 4). On the other hand, this border was completely absent in another larva of the same experimental series with the same positive *alpestris* antigenicity (Plate 1, fig. I; Text-fig. 3). Generally, the expression of this trait was also very variable in *alpestris* controls and *alpestris* transplanted with *vulgaris* nuclei (Plate 1, figs. D, G; Plate 2, fig. D; Text-fig. 5) and could not be regarded as a species-specific marker. The only significant species-specific difference during larval development of both species was the size of respective stages: *vulgaris* was always much smaller than *alpestris* and the same was found in nuclear transplant larvae, which in their size were always close to the host species without regard to the success of nuclear transplantation as proved by postmetamorphic pigmentation and by the presence of antigens specific for the implanted nucleus.

Unlike larval stages, the pigmentation of postmetamorphic animals differs very distinctly between the species used. The hybrids of these species have an adult pigmentation which is closer to *alpestris* type, so that in nuclear transplant animals the positive results could be definitely proved in *vulgaris* hosts with implanted *alpestris* nuclei but were not so clear in the reverse combination (Plate 2, figs. A–D).

Species-specific antigens could be successfully detected only in larger larvae or in metamorphosed animals because of the larger amount of antigenic

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**PLATE 1**

Fig. A. Larva $X. 5$, haploid: gynogenetic series, *alpestris* host, *vulgaris* donor, no species antigens detected. Evidently failure of nuclear transplantation.

Fig. B. Larva $II. 5$, haploid: androgenetic series, *vulgaris* host, *alpestris* donor, not tested for antigens. Again, failure of nuclear transplantation.

Fig. C. Larva $II. 30$, diploid: androgenetic series, *vulgaris* host, *alpestris* donor. The presence of *alpestris* antigenicity only indicates success of nuclear transplantation. See also fig. I.

Fig. D. Larva $V. 5$, diploid: androgenetic series, *alpestris* host, *vulgaris* donor. Not tested for antigens, but tetraploidy could be taken as a proof of the success of nuclear transplantation.

Fig. E. Larva $II. 7$, triploid: androgenetic series, *vulgaris* host, *alpestris* donor. The presence of both *alpestris* and *vulgaris* antigenicity indicates the success of nuclear transplantation. See also this Plate, fig. G, and Plate 2, fig. D.

Fig. F. Larva $VIII. 10$, tetraploid: fertilized series, *alpestris* host, tetraploid *vulgaris* donor. Not tested for antigens, but tetraploidy could be taken as a proof of the success of nuclear transplantation.

Fig. G. Tail of the larva $V. 5$ (fig. D): white pigment cell border.

Fig. H. Tail of the larva $II. 7$ (fig. E): white pigment cell border.

Fig. I. Tail of the larva $II. 30$ (fig. C): absence of white pigment cell border.
material necessary for agar diffusion tests. The tests upon small larvae often failed for both species—the method used was not sensitive enough for such small amounts of material. Positive results were obtained only in the androgenetic series. In combination II (vulgaris egg receiving a diploid alpestris nucleus) 6 experimental and 1 control animals were tested, 3 experimental larvae (all diploid) were too young and in 1 of them neither host-specific nor donor-specific antigens could be found. In the second only host-specific antigenicity was detected, but in the third both vulgaris- and alpestris-specific antigenicity was found (Text-fig. 2). In the fourth, advanced larva (a haplo-diploid mosaic)

Text-fig. 2. Larva II. 57: androgenetic series, vulgaris host, alpestris donor: diploid. Missing lines between the antibody and experimental wells (compared with one or the other parent wells) due to the fact that parent wells contained antigens prepared from a number of adult individuals while experimental well contained antigens from a single larva. Using control tests with antigens prepared from a single larva of parental species the number of precipitation lines was the same as for experimental larva (not shown on the figure). The same is true for Text-fig. 3.

Text-fig. 3. Larva II. 30: androgenetic series, vulgaris host, alpestris donor: diploid.

Plate 2
Fig. A. Metamorphosed Triturus vulgaris.
Fig. B. Metamorphosed animal II. 7: triploid, androgenetic series, vulgaris host, alpestris donor, both vulgaris and alpestris species antigens detected. Hybrid pigment pattern with meander-like (alpestris) design. Warty skin and the size and shape of the head are alpestris-like.
Fig. C. Metamorphosed Triturus alpestris.
Fig. D. Metamorphosed animal V. 5: diploid, androgenetic series, alpestris host, vulgaris donor, both alpestris and vulgaris species antigens detected. Hybrid or alpestris-like pigment pattern and other characters.
no species antigenicity was found (technical failure), in the other more advanced larva (diploid) only \textit{alpestris} antigenicity was demonstrated (Text-fig. 3). In the single metamorphosed animal (7 months) both \textit{vulgaris} and \textit{alpestris} antigenicity could be detected (Text-fig. 4). This postmetamorphic animal was triploid and its pigmentation was clearly hybrid, close to \textit{alpestris} pattern (Plate 2, fig. B). The sole advanced control larva (diploid) showed only \textit{vulgaris} antigenicity. In combination V (\textit{alpestris} eggs transplanted with diploid \textit{vulgaris} nuclei) 3 experimental and 2 control animals were examined. In early larva only \textit{alpestris} antigenicity was found and the same was true for the other more advanced larva (just before metamorphosis, 3$\frac{1}{2}$ months). Both were diploid. In the third, postmetamorphic animal (6 months) both \textit{alpestris} and \textit{vulgaris} antigens could be detected (Text-fig. 5). The pigmentation of this diploid animal was, however, of \textit{alpestris} type with possible signs of hybrid phenotype (Plate 2, fig. D). In the early control larva no species antigenicity was found; in the second, more advanced control larva only \textit{alpestris} antigenicity could be proved. The results for the androgenetic series are summarized in Table 1.

In the gynogenetic series of the combination X (\textit{alpestris} egg transplanted with diploid \textit{vulgaris} nucleus) 4 experimental and 5 control animals were examined without positive results. Of 3 experimental early larvae 2 were both \textit{alpestris}- and \textit{vulgaris}-negative, the third (haploid) was only \textit{alpestris}-positive, and the same was found also in the fourth advanced diploid larva (1 month old). Of 5 young control larvae 2 were completely negative, the other 3 only \textit{alpestris}-

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{fig4}
\caption{Text-fig. 4. Metamorphosed animal II. 7: androgenetic series, \textit{vulgaris} host, \textit{alpestris} donor; triploid.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{fig5}
\caption{Text-fig. 5. Metamorphosed animal V. 5: androgenetic series. \textit{alpestris} host, \textit{vulgaris} donor; diploid.}
\end{figure}
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...positive. Two of these were haploid, the third was not examined for ploidy. Negative results were also obtained for two animals tested in the fertilized series VIII (alpestris eggs transplanted with tetraploid vulgaris nuclei). Both were alpestris-positive but vulgaris-negative; one was a small larva, the other a postmetamorphic animal with alpestris pigment pattern. Their diploidy indicates the failure of the transplantation procedure.

Table 1. Antigens, pigmentation and ploidy in the androgenetic series

<table>
<thead>
<tr>
<th>Combination</th>
<th>Host/donor</th>
<th>Animal no.</th>
<th>Antigens</th>
<th>Developmental stage</th>
<th>Pigmentation</th>
<th>Ploidy</th>
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<tr>
<td>II</td>
<td>vulg./alp.</td>
<td>6</td>
<td>— -</td>
<td>Early larva</td>
<td>?</td>
<td>2n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71</td>
<td>+ -</td>
<td>Early larva</td>
<td>?</td>
<td>2n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57</td>
<td>+ +</td>
<td>Early larva</td>
<td>?</td>
<td>2n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>- -</td>
<td>Advanced larva</td>
<td>?</td>
<td>2n/1n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>- +</td>
<td>Advanced larva</td>
<td>?</td>
<td>2n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>+ +</td>
<td>Metamorphosed</td>
<td>hybrid</td>
<td>3n</td>
</tr>
<tr>
<td>Control vulg.</td>
<td></td>
<td>+ -</td>
<td></td>
<td>Advanced larva</td>
<td>?</td>
<td>2n</td>
</tr>
<tr>
<td>V</td>
<td>alp./vulg.</td>
<td>14</td>
<td>- +</td>
<td>Early larva</td>
<td>?</td>
<td>2n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>- +</td>
<td>Advanced larva</td>
<td>?</td>
<td>2n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>+ +</td>
<td>Metamorphosed</td>
<td>alp.-hybr.</td>
<td>2n</td>
</tr>
<tr>
<td>Control alp.</td>
<td></td>
<td>- -</td>
<td></td>
<td>Early larva</td>
<td>?</td>
<td>2n?</td>
</tr>
<tr>
<td>Control alp.</td>
<td></td>
<td>- +</td>
<td></td>
<td>Middle larva</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

DISCUSSION

The ploidy in some experimental and control animals of different series showed a relatively large variety of haploid, diploid and haplo-diploid mosaic cases. This is not surprising as it is known that nuclear transplantation procedures often result in chromosomal aberrations and in changes of ploidy (Briggs & King, 1952; Lehman, 1955). But the differences in the proportion of haploid and diploid animals in experimental and control series, and especially the absence of mosaics in the controls, indicate the success of nuclear transfers in some cases. The limited material, and the haphazard selection of animals used for karyological analysis, do not allow us to arrive at any definitive conclusions on the reasons for the poor development of newts in comparison with some frogs in nuclear transplantation experiments. The same is true for the question of the participation of the host nuclei in development in the androgenetic or gynogenetic series, which is better than the development in the completely enucleated series. A more detailed cytological and karyological analysis is needed.

The pigmentation of the experimental animals provided a definitive proof of the activity of injected nucleus only in postmetamorphic individuals and this only in the cases of vulgaris host egg and alpestris donor nucleus. Here, the
pigmentation clearly differs from the *vulgaris* pattern and is of hybrid type. In the reverse combination the situation is more difficult because in the hybrid pigment pattern the *alpestris* type dominates over *vulgaris* (Romanovský & Štefanová, 1960) and thus the participation of *vulgaris* donor nucleus can be neither proved nor excluded. Evidently, one cannot compare nuclear transplant animals with normal hybrids. They differ in quantity of species-specific genomes as in cytoplasmic environment. In the tests for species antigenicity it was found in suitable cases (advanced larvae or postmetamorphic animals) that not only in triploids, but also in some diploids (probably with functional nuclei descended only from the donor nucleus) both types of species-specific antigens could be detected. It seems that host cytoplasm must be responsible for the host species-specific antigenicity in these cases. It has been shown (Romanovský, 1962b) that even after temporary embryonic parabioses of these two species of newts the foreign species-specific antigens could be detected at least 8 days after the separation of the partners. As to pigment patterns the *alpestris* (or hybrid) type of pigmentation found in one metamorphosed animal (*vulgaris* host, *alpestris* donor, androgenetic series, triploid) exhibiting both *vulgaris* and *alpestris* species antigenicity is easy to understand (fusion of implanted diploid nucleus with haploid host nucleus). Another positive case of the same series, diploid larva also with double antigenicity, should be interpreted as a case of persistence of maternal species antigenicity even in the probable absence of host nucleus. The third positive case of this series, a diploid advanced larva with only *alpestris* antigenicity, could be regarded as a case in which in the absence of the host nucleus the level of host-species antigenicity fell under the threshold of sensitivity of the method used. The only positive case with respect to the presence of donor (and host) antigenicity in the reciprocal combination of the androgenetic series (*alpestris* host, *vulgaris* donor), a diploid animal with *alpestris* (or hybrid) pigment pattern, is difficult to explain. In this case one should expect *vulgaris* pigment patterns reflecting the activity of the injected nucleus. In her experiments on triploid hybrids of these two species of *Triturus*, Mazákova-Štefanová (1965) showed that a double *vulgaris* genome dominated over a single *alpestris* one. However, in her experiments diploid *vulgaris* and haploid *alpestris* genomes were functioning in *vulgaris* cytoplasm while in the present case of nuclear transplantation it was probably a diploid *vulgaris* genome functioning in *alpestris* cytoplasm. In similar experiments of nuclear transplantations in different species of frogs it was usually found that the implanted nucleus is responsible for the type of pigmentation (McKinnell, 1960, 1962; Gurdon, 1961; Simpson & McKinnell, 1964). In other experiments some influence of maternal cytoplasm on the pigmentation was suggested (Sambuichi, 1957, 1959; Kawamura & Nishioka, 1963a, b). All these nuclear transplantations were carried out with completely enucleated eggs. In transplantation experiments with androgenetic eggs the temporary participation of the host haploid nucleus during at least the early stages of development cannot, however, be excluded and this
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could be the reason for the persistence of host antigens and the appearance of host adult pigmentation.

The size of experimental animals during embryonic and larval development was found to be host-specific, evidently related to the different egg size of the two species. This was evident even in cases of *vulgaris* host injected with *alpestris* nuclei which later proved to be the results of successful transfer. Not until metamorphosis did they reach approximately the size of the donor nucleus (*alpestris*) species. Such a dependence on egg size was also found in interspecies nuclear transplantation in *Xenopus* (Gurdon, 1961).

**SUMMARY**

1. Transplantations of blastula animal pole cell nuclei or gastrula entoblast or chordomesoblast cell nuclei into eggs of *Triturus vulgaris* and *T. alpestris* in reciprocal combinations were carried out. In several cases tetraploid nuclei from *T. vulgaris* blastulae were used for transplantations. As hosts, different series of eggs were prepared: androgenetic, gynogenetic, completely enucleated and fertilized, obtained by artificial insemination of eggs and UV irradiation of eggs and/or sperm, or by electric stimulation of eggs followed by UV irradiation.

2. The development of eggs, injected with single nuclei, was found to be different in different series. In the androgenetic and gynogenetic series about 10% of the experimental eggs developed to advanced larval stages, but very few of them metamorphosed. In the totally enucleated series development was very poor: out of 223 operated eggs only 5 developed to the tail-bud stage. In the fertilized series about 40% of eggs reached larval stages and some of them metamorphosed. Some of the experimental animals were tested for ploidy and compared with treated, but non-recipient, control animals of the appropriate series. The shift of proportions between haploids and diploids and especially the occurrence of haplo-diploid mosaic animals in the experimental series indicate the participation of injected nuclei in the development of some at least of the experimental animals. At the same time the methods used for enucleation were found to be not absolutely efficient.

3. Antigen specific for the donor species of the transplanted nucleus were found to occur in androgenetic series in the combination *T. vulgaris* host and *T. alpestris* donor in 3 out of 6 tested animals; the only relevant control larva tested was *T. alpestris* negative. In the reciprocal combination of the same series only 1 out of 3 tested animals was *T. vulgaris* (= donor) positive and both tested control larvae were *T. vulgaris* negative. No donor species-specific antigens were found in the gynogenetic series in *T. alpestris* host and *T. vulgaris* donor combination (4 animals tested) and negative results were obtained also in two of the fertilized series of the same combination.

4. Pigmentation was found to be distinctly species-specific only in post-metamorphic animals. In larvae it could not be used as a test of the success of
nuclear transplantations because of the great variability of larval pigment patterns in both species used. The only metamorphosed animal (triploid) in the combination *T. vulgaris* host and *T. alpestris* donor in the androgenetic series, with both *T. alpestris* and *T. vulgaris* antigenicity, manifested a clearly hybrid pattern of pigmentation (with *alpestris* pigmentation predominating). The single metamorphosed animal (diploid) in the reciprocal combination of the same series exhibiting both *T. alpestris* and *T. vulgaris* antigenicity, was of *alpestris* pigment pattern which is, however, difficult to distinguish from the hybrid one in extreme cases.

5. The growth and size of experimental animals was found to be always host-specific during larval stages, irrespective of the success or failure of nuclear transplantation.

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**RÉSUMÉ**

*Ploidie, caractères de la pigmentation et antigénicité spécifique d’espèce dans les transplantations nucléaires interspécifiques chez les tritons*

1. Des noyaux cellulaires provenant de cellules du pôle animal de blastula d’entoblaste ou de chondromésoblaste, ont été transplantés dans des œufs de *Triturus vulgaris* et *T. alpestris*, selon des combinaisons réciproques. Dans plusieurs cas, des noyaux tétraploïdes de blastulas de *T. vulgaris* ont été utilisés dans les transplantations. Différentes séries d’œufs réceptrices ont été préparées: androgénétiques, gynogénétiques, œufs complètement énucléés, œufs fécondés obtenus par fécondation artificielle, œufs ou sperme irradiés aux rayons U.V., stimulation électrique d’œufs suivie d’une irradiation aux rayons U.V.

2. Le développement d’œufs recevant un seul noyau s’est montré différent dans les diverses séries. Dans les séries andro- et gynogénétiques, environ 10% des œufs en expérience se sont développés jusqu’aux stades larvaires avancés, mais très peu de larves se sont métamorphosées. Dans les séries d’œufs totalement énucléés avant la greffe nucléaire, le développement a été très faible: sur 223 œufs opérés, seuls cinq ont atteint le stade du bourgeon caudal. Dans les séries à œufs fécondés, sensiblement 40% des œufs atteignent les stades larvaires et quelques larves se métamorphosent. Le degré de ploidie de certains des animaux en expérience a été étudié et comparé avec celui d’animaux témoins traités, mais non opérés, des séries appropriées. La modification des proportions entre haploïdes et diploïdes, et spécialement la présence d’animaux en mosaïque haplo-diploïde dans les séries expérimentales, indique la participation des noyaux injectés dans le développement d’au moins quelques animaux opérés. Il apparaît que les méthodes utilisées pour l’énucléation ne se révèlent pas absolument adéquates.

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(= donneur) et deux larves témoins éprouvées furent *T. vulgaris* négatif. Aucun antigène spécifique de l’espèce donneur n’a été observé dans les séries gynogénétiques où *T. alpestris* est l’hôte et *T. vulgaris* le donneur (4 animaux éprouvés). Des résultats négatifs ont été obtenus également dans deux des séries à œufs fécondés de la même combinaison.

4. La spécificité liée à l’espèce de la pigmentation a été observée distinctement seulement chez les animaux ayant dépassé la métamorphose. Chez les larves, ce caractère ne peut être utilisé comme test du succès de la transplantation nucléaire à cause de la variabilité de la distribution des pigments larvaires dans les deux espèces utilisées. Le seul animal métamorphosé (triploïde) dans la combinaison *T. vulgaris* (hôte) et *T. alpestris* (donneur) dans les séries androgénétiques avec à la fois l’antigénicité *T. alpestris et T. vulgaris*, présentait un type (pattern) de pigmentation clairement hybride (avec prédominance du type *alpestris*). Le seul animal métamorphosé (diploïde) dans la combinaison réciproque des mêmes séries, présentant aussi la double antigénicité *T. alpestris et T. vulgaris*, avait le type *alpestris* de pigmentation, lequel est cependant difficile à distinguer de celui d’un hybride dans les cas extrêmes.

5. La croissance et la taille des animaux en expérience se présentent toujours comme spécifiques de celles de l’hôte au cours des stades larvaires, quelque soit le succès ou l’échec de la transplantation nucléaire.

REFERENCES


(Manuscript received 23 September 1966)