Analysis of haploid macrochromosome complements

Species of		Macrochromosome No. 1	2	3	4	5	6
Liolaemus pictus	length	117	112	86	79	64	42
	ratio	1.11	1.70	1.21	1.15	1.15	1.49
	type	*m*	*m*	*m*	*m*	*m*	*m*
L. cyanogaster	length	115	109	87	80	64	43
	ratio	1.13	1.86	1.12	1.12	1.15	1.30
	type	*m*	*sm*	*m*	*m*	*m*	*m*

The value for the arm ratio is determined by dividing the long arm by the short arm; type designates the chromosome as determined by the position of centromere (*m*, metacentric; *sm*, submetacentric); length refers to the normalized length of the macrochromosome complement.

664, 790) and 3 females (IZUA-R 464, 637, 638): near Valdivia city (Valdivia Province). *Liolaemus pictus*. This species has a diploid number of 34 chromosomes; these can be arranged in pairs and numbered in order of decreasing length (Figure 1 a). There are 6 pairs of metacentric (*m*) macrochromosomes and 11 pairs of microchromosomes, pair 7 being considerably larger than the others. In the testicular cells (diakinesis) we found 6 large bivalents and 11 microbivalents.

Liolaemus cyanogaster. The karyotype (Figure 1 b) is essentially the same as *L. pictus*, except that in *cyanogaster* chromosome pair number 2 (macrochromosome) is submetacentric (*sm*). In diakinesis there are 6 macrobivalents and 11 microbivalents. Thus, besides known morphological differences in both species, karyologically they are distinct at the level of No. 2 chromosome (metacentric in *L. pictus* and submetacentric in *L. cyanogaster*).

The karyological pattern of the lizards of the genus *Liolaemus* examined (*L. lutzae*[1], *L. pictus* and *L. cyanogaster*) is very similar. The three species present the formula $2n = 34$ and the karyotypes show 6 pairs of macrochromosomes and 11 pairs of microchromosomes, of which the first pair is very 'large'. Within the family Iguanidae, a similar formula ($2n = 34$) and karyotype (6 macrochromosomes and 11 microchromosomes) is found also in some species of *Anolis* (*r. roquet, r. extremus, aeneus*[7], *vermiculatus*[8]) and the lizards of the genera *Uma* (*notata*[1]) and *Scelophorus* (*orcutti*[9], *chrysostictus, maculatus, utiformis, gadavidae*[10,11], *nelsoni, pyrocephalus*[11]). According to GORMAN et al.[1], a karyotype consisting of 6 pairs of macrochromosomes and 12 pairs of microchromosomes ($2n = 36$) is considered primitive for the family Iguanidae. The species of *Liolaemus* (*lutzae*[1], *pictus, cyanogaster*) differ from this karyological patterns by having only 11 pairs of microchromosomes. Reduction in chromosome number appears to be the evolutionary trend in iguanids[7]. If this is true, *Liolaemus* species would have derived karyological forms of the primitive formula ($2n = 36$). The presence of a pair of 'large' microchromosomes (pair 7) in *L. lutzae* has made GORMAN[1] believe in the existence of centric fusion among two of these pairs. We are in agreement with this hypothesis in order to explain the occurrence of formula $2n = 34$ in *L. pictus* and *L. cyanogaster*, since in these two species also the chromosomic pairs No. 7 are very 'large'.

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A Possibility to Achieve Genetic Transformation in the Platyfish-Swordtail System *(Platypoecilus maculatus – Xiphophorus helleri)*[1]

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Summary. In order to determine how informative homologous donor DNA might be made available to propigment cells of the recipient *Xiphophorus helleri* for transformation, labelled heterologous DNA from *E. coli* was injected into the neural crest region or the yolk sac of embryos of the recipient. On the basis of the degradation rate of the donor DNA and the incorporation rate of radioactivity into the recipient DNA, it is concluded that injection into the neural crest region may be a suitable method to make available informative homologous donor DNA for transformation.

Since AVERY, MACLEOD and MCCARTY[2] originally observed genetic transformation after treatment of pneumococcal recipient strains with DNA from donor strains, many attempts have been made to achieve genetic transformation also in higher organisms. A variety of genetic alterations has been obtained[3]; it has been argued, however, that they might not be due to transformation.

One objection against interpreting these alterations as the result of a transformational event has been that DNA mediated mutations or conversions[4] of the already present allele[5] might have pretended genetic transformation. This objection cannot be brought against the transformation system we have chosen, in which the platyfish (*Platypoecilus maculatus*) is the *donor*, carrying up to 12 different luxurious loci for distinct pigment cell patterns, and the swordtail (*Xiphophorus helleri*) is the

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[3] J. M. OLENOV, Int. Rev. Cytol. *23*, 1 (1968). – L. LEDOUX, *Informative Molecules in Biological Systems* (North-Holland Publ. Co., Amsterdam/London 1971); *Uptake of Informative Molecules by Living Cells* (North-Holland Publ. Co., Amsterdam/London 1972). – D. HESS, Naturwissenschaften *59*, 348 (1972); Umschau *75*, 501 (1975).
[4] Mutations could be demonstrated in *Drosophila* after treatment with DNA (O. G. FAHMY and M. J. FAHMY, Nature, Lond. *196*, 873 1962), and are known to be induced by degradation products of DNA (J. J. BIESELE, R. F. BERGER, M. CLARKE and L. WEISS, Expl Cell Res., Suppl. *2*, 279 (1952). – B. A. KIHLMAN, Expl Cell Res. *25*, 694 (1961); J. cell. comp. Physiol. *62*, 267 (1963).
[5] All transformation experiments were done so far on the basis of the replacement of one allele by another.

Rate of degradation of the $^2H^3H$-donor DNA in the recipient embryo, and rate of incorporation of material from donor DNA into the recipient DNA

Time after injection (h)	Degradation	Incorporation	
	(mean mol.wt. of donor DNA)	(spec. radioactivity of recipient DNA dpm per μg)	
	DNA injected into	DNA injected into	
	Neural crest region or yolk sac	Neural crest region	Yolk sac
0	50×10^6	0	0
2	1×10^6	250	0
5	$< 3 \times 10^5$	600	0
10	$< 1 \times 10^4$	1500	0

recipient, lacking these loci[6,7]. A certain donor character occuring after treatment on the recipient could only be due to a transformational event.

Another objection was that it has not been made sure that informative donor DNA actually had been available to competent recipient cells; it is known that donor DNA undergoes rapid degradation in the recipient[8–10].

The present investigation was undertaken to find out how the donor DNA has to be applied to the recipient *X. helleri* so that it might be available in informative size for transformation.

Materials and methods. $^2H^3H$-labelled donor DNA from *E. coli* (specific radioactivity 5×10^5 dpm per μg), taken for technical reasons instead of fish DNA[7,9,10], was injected into the neural crest region, where the pigment cells originate, or alternatively into the yolk sac of 3- to 5-day-old embryos of *X. helleri*. 50 embryos were homogenized immediately, and 2, 5 and 10 h after injection. Using centrifugation, a yolk cytoplasm fraction, containing the injected donor DNA, and a nuclear fraction, containing the DNA of the recipient, were prepared. The fractions were lyzed in 1% sodiumdodecylsulphate, and the DNA present in the fractions, was re-isolated by CsCl density gradient centrifugation. The degradation rate of the donor DNA was measured by determining the molecular weight of the re-isolated dialyzed donor DNA by sucrose gradient centrifugation, and the incorporation rate of material from the donor DNA into the recipient DNA was measured by determining the specific radioactivity of the recipient DNA.

Results and discussion. a) *Degradation rate.* The re-isolated, dialyzed donor DNA has, regardless of the site of injection, immediately after injection a molecular weight of about 50×10^6 (see Table). After 2 h it is 1×10^6, after 5 h less than 3×10^5, and after 10 h less than 1×10^4. Because the average size of a gene appears to be in the molecular weight range between about 5×10^5 and 10^6[11], this result shows that informative donor DNA may be available for transformation during the first 2 to 3 h after injection.

b) *Incorporation rate.* The incorporation rate depends critically on the injection site. If the donor DNA is injected into the *neural crest region*, 2 h later the specific radioactivity per μg recipient DNA is 250 dpm, after 5 h it is 600 dpm, and after 10 h it is 1500 dpm. These data show that injected material had been incorporated into the DNA of the embryo. It should be emphasized, however, that it cannot be distinguished whether this material represents degradation products or long sequences of donor DNA.

If, in contrast,the donor DNA is injected into the *yolk sac*, no radioactivity is detected within 2 to 3 h, during which time informative donor DNA is available. In corresponding experiments, made by other authors in other systems, the average amount of donor DNA incorporated into the recipient genome was about 0.02% of the recipient DNA[12]. If a similar amount of donor DNA had been incorporated into the DNA of the embryos, we would have detected it, because the specific radioactivity of the donor DNA is relatively high. The fact that no radioactivity is detectable, would imply that no appreciable amounts of informative donor DNA could have been incorporated and that therefore this method of application is not suitable for obtaining genetic transformation.

This investigation shows that, by injection into the *neural crest region*, relatively long sequences of donor DNA can be offered over a period of 2 to 3 h for the incorporation into the recipient genome. In order to assess whether in fact donor genes may become incorporated, it is worth noting the results of LAVAL, MALAISE and LAVAL[13], who found high rates of uptake of donor DNA by recipient cells in vitro within the first 2 h of DNA treatment; CHARLES, REMY and LEDOUX[14], furthermore, working with ascites tumor cells, observed incorporation of donor DNA into the recipient DNA within less than 2 h after application. It may be concluded, therefore, that by injection into the neural crest region donor genes may be made available to propigment cells of *X. helleri* for transformation.

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