daily intake of fluid was recorded. At the end of the treatment period each test and control male was then individually caged for 1 week with 3 untreated virgin 8-week-old females. Females were replaced weekly and consecutively with fresh animals for a total of 6 weeks. During the mating period, mice were fed laboratory chow and had access to tap water ad libitum. All females were autopsied on day 13–15 of their exposure to males. They were scored for pregnancy and implants comprising of normal living implants, late fetal deaths, and early fetal deaths; the latter appeared as black deciduomata. Details of the assay system and technique[8,9] and satistical anylsis have been reported[10].

The mean fluid intake of lead sub-acetate solution for the entire treatment period was 107.4 ml/mouse ranging from 207 to 220 ml/mouse; total mean intake of lead amounted to 1.64 g. Body weights of the treated and control groups were similar at the end of the treatment period. In the treated group there was no mortality during the treatment period, but 1 male died during the 4th week and another died in the 6th week of mating; 1 control male died in the 2nd week; the cause of death of these males was not attributed to treatment.

The overall incidence of pregnancy, indicative of fertility, was 52.7% in control group, as compared to 27.6% in the treated group (Table). The data of the individual weeks also showed that fertility of the treated males was consistently lower, except in the 1st week which was perhaps due to sexual inexperience of the males. Fertility was lowest in the 4th week. Thus, the treatment with lead reduced the fertility of the males by 50%. Testicular degeneration and oligospermia based on histological exmination in the rat has been reported following repeated administration of lead acetate[11].

Number of total implants per pregnancy in the treated group when compared with the corresponding value in the controls did not indicate any systematic variation; the overall mean values were 8.7 and 8.8 respectively. Thus, there were no preimplantation losses either due to paternal or maternal causes.

Statistical analysis of the differences in mutagenicity index between the test and controls were highly significant ($p \geq 0.1$) in the 3rd week and were significantly different ($p \geq 0.05$) in the 6th week; this index was not statistically significant in the 1st week. Chromosomal aberrations in leukocytes cultured in vitro have been reported in mice fed with led acetate in their diet[12].

The study indicates that lead caused infertility in mice as evidenced by reduced pregnancy rates among the females. In addition to this, perhaps of greater importance is the genetically-related mutagenicity that was also detected. Undoubtedly more extensive dose-response study is required to establish the threshold limits for human exposures.

Zusammenfassung. Nach 28tägiger Verabreichung von 2% Blei-Acetat im Trinkwasser wurden männliche Mäuse mit Gruppen unbehandelter Weibchen wöchentlich über 6 aufeinanderfolgende Wochen gepaart und die Weibchen in der mittleren Gestationsperiode seziert und untersucht. Das Gesamtvorkommen von Schwangerschaften war 27,6% und 52,7% in behandelten, bzw. unbehandelten Tieren. Die Fruchtbarkeit war in der 4. Woche am geringsten und der Mutagenitäsindex zeigte in der 3. und 6. Woche statistisch signifikante Differenzen.

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DNA Renaturation Kinetics in Some Paedogenetic Urodeles

Paedogenesis, namely the persistence of larval characters throughout life, has played an important role in the evolution of the Amphibia *Caudata*[1,2]. 4 out of the 8 living families of this order include only paedogenetic forms, either permanently larval (Proteids and Sirenids) or semi-larval (Cryptobranchids and Amphiumids)[3]. These last families show the same karyotype morphology (though with larger chromosomes) of the families from which they presumably originated: in fact the Cryptobranchids ($2n = 60$–64) have karyotypes similar to those of the primitive Hynobiids, and the Amphiumids ($2n = 28$) have the same karyotypes as the ambystomatoid stock (Ambystomatids and Plethodontids). The Proteids ($2n = 38$) have karyotypes intermediate between those of the Hynobiids and the ones of the more advanced ambystomatoid stock while the still problematic Sirenids show peculiar characters in their karyotypes which are possibly originated by polyploidization[4,5].

Considering the DNA content per nucleus, all paedogenetic species are characterized by very large amounts in the nuclear DNA, the largest among the other families of the same order and, together with the Dipnoi among all vertebrates; this amount is very likely to be of secondary origin since none of these species is basic in the phylogeny of the Urodeles[4,6].

It would have been of interest to find out possible correlations between the large genomes of paedogenetic species and their content in highly repetitive DNA, and to have some indications on the genome complexity (the amount of diverse DNA sequences)[7] of species from different evolutionary stages within the order. To these

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Species	2n	Nuclear DNA content (pg/N)	Component with repetition frequency 10^2		Component with repetition frequency 10^6		Theoretical Cot 1/2 for unique sequences
Cryptobranchids *M. japonicus*	60	93	11%	(10.2 pg)	29%	(26.9 pg)	83.000
Proteids *N. maculosus*	38	165	10%	(16.5 pg)	28%	(46.2 pg)	147.000
Amphiumids *A. means*	28	150	30%	(45.0 pg)	17%	(25.5 pg)	133.800
Ambystomatids *A. tigrinum*	28	55	40%	(22.0 pg)	10%	(5.5 pg)	43.000
Plethodontids *D. fuscus*	28	30	21%	(6.3 pg)	35%	(10.5 pg)	26.000
Salamandrids *T. cristatus*	24	44	45%	(19.8 pg)	12.5%	(5.5 pg)	39.000
Salamandrids *T. torosa*	22	56	27%	(15.1 pg)	22%	(12.3 pg)	49.900
Salamandrids *T. rivularis*	22	60	22%	(13.2 pg)	19%	(11.4 pg)	53.300

purposes DNA renaturation kinetics have been measured for 3 species of paedogenetic families and 5 species belonging to 'advanced' families of Urodeles.

The DNA has been extracted from erythrocytes following MARMUR's methods[8] slightly modified. The reassociation reactions have been carried out at 60 °C in 0.12 *M* neutral phosphate buffer (PB) and the reassociated DNA has been separated from the single stranded by absorption on hydroxiapatite from Calbiochem (HAP)[9].

It is well known that when a DNA molecule is denatured by heat or alkali, the 2 separated strands tend to reassociate with each other at a speed which is proportional to the concentration of the DNA sequences in the solution[10]: thus, keeping constant other parameters (temperature, length of DNA fragments and salt concentration), reiterated DNA reanneals faster than single copy. Using a *Cot* plot where on the abscissa the product of DNA concentration (*Co* = Moles of nucleotides/l) and time of incubation (*t*) is reported, and on the ordinate the percentage of reassociated DNA, a curve is obtained whose shape depends on the complexity of the material examined[11].

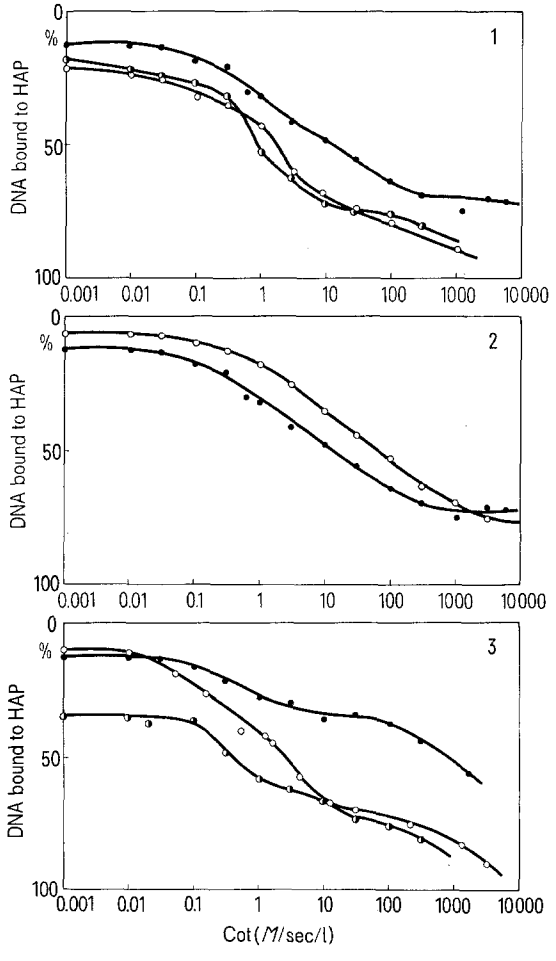

Fig. 1–3. DNA reassociation curves. 1. ● 1 *Amphiuma means*; ○ 2 *Megalobatrachus japonicus*; ◑ 3 *Necturus maculosus*. 2. ● 4 *A. means*; ○ 5. *Ambystoma tigrinum*. 3. ● 6 *Triturus cristatus*; ○ 7 *Taricha torosa*; ◑ 8 *Desmognathus fuscus*. The curve of *Taricha rivularis* is practically coincident with that of *T. torosa*.

The following species have been examined: *Megalobatrachus (Andrias) japonicus* (Cryptobranchids), *Necturus maculosus* (Proteids), *Amphiuma means* (Amphiumids), *Ambystoma tigrinum* (Ambystomatids), *Desmognathus fuscus* (Plethodontids), *Triturus cristatus (carnifex)*, *Taricha torosa* and *Taricha rivularis* (Salamandrids).

Plots of DNA renaturation of the species studied are presented in Figures 1–3. Two arbitrary components have been chosen, with a repetition frequency of 10^2 and 10^6 respectively, and their percentage in the DNA of the different species is reported in the Table. Theoretical $Cot_{1/2}$ (the *Cot* value at which 50% of a DNA component is reassociated) for unique sequences is shown in the last column. The values for nuclear DNA (in picogram per nucleus = pg/N) reported in the Table have been obtained in this laboratory by E. OLMO[12] using histophotometrical methods: his values are in rather good agreement with those from other sources[13,14].

The DNA reassociation curves of *Megalobatrachus* and *Necturus* (Figure 1) are very similar, both indicating very fast reactions. Moreover, these reactions being almost completed at *Cot* values much lower than those expected for unique sequences, both these genomes seem to be constituted essentially by repetitive DNA. These results agree with those obtained by STRAUS[15] who did not show a unique component in the 2 Urodele species he examined.

As for *Amphiuma*, though its DNA content is nearer to that of *Necturus*, its renaturation curve shows a reaction slower than those of the other 2 paedogenetic species (Figure 1) being almost parallel to the reassociation curve of *Ambystoma* (Figure 2). This could indicate that *Amphiuma* has a genome complexity more similar to the one of a genus belonging to the advanced family of Ambystomatids; this is in good agreement with recent hypotheses on the phyletic affinities between Amphiumids and the ambystomatoid stock (cf.[4]).

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Among the Salamandrids, both the species of *Tarïcha* examined seem to have a genome complexity smaller than that of *Triturus cristatus* (Figure 3), the latter belonging to a genus from which *Taricha* might be derivated.

The Table shows that the highly repeated component reachs its greatest absolute values (pg/N) in the paedogenetic species. While the DNA of *Desmognathus fuscus*, a metamorphosing species of the advanced family Plethodontids, shows a higher percentage of the same component.

It has been shown in many organisms that highly repeated DNA is characteristically localized in the centromeric heterochromatin[16], and in some Urodeles a relationship between chromosome size and the amount of a highly repeated DNA fraction (satellite) present in the centromeric region has been demonstrated[17].

The fact that also in other Amphibia different amounts of highly repeated DNA are present at the centromere of different chromosomes could be supported by our finding that no clear relationships exist either between genome size and the amount of highly repeated DNA (probably mostly centromeric) or between the latter and the chromosome number. Our results also suggest that in general species belonging to more advanced families show greater genome complexity than the primitive groups, even if the latter may have a larger DNA content per nucleus.

We know that the paedogenetic Urodeles studied show the same chromosome number and shape but higher nuclear DNA content than the families from which they are presumably derived: this could imply that the increase in DNA might have been achieved by tandem gene duplications along the chromosomes[4,18].

The role played in evolution by this duplication mechanism is still difficult to interprete[19], however tandem gene duplication seems to have found its utmost expression among living Tetrapods in the paedogenetic families of Urodeles, with the possible exception of the Sirenids, whose entire genome might have been duplicated by polyploidization[5].

Riassunto. Gli Urodeli delle famiglie pedogenetiche posseggono enormi quantità di DNA nucleare; qui è stata studiata la cinetica di rinaturazione del DNA di specie di tre famiglie pedogenetiche (due delle quali primitive) e di specie appartenenti a famiglie «superiori», generalmente a metamorfosi completa, sempre di questo Ordine di Anfibi. I risultati sembrano indicare che le specie di famiglie superiori, pedogenetiche o non, hanno una maggiore complessità cinetica nel loro DNA rispetto alle specie di gruppi primitivi, anche se hanno meno DNA totale. L'incremento nel DNA tipico delle famiglie pedogenetiche, che hanno cariotipi simili a quelli delle famiglie da cui si sono forse originate, può essere avvenuto per duplicazioni *tandem* sui singoli cromosomi; possibile eccezione sono forse i Sirenidi, che mostrano tracce di poliploidia.

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The Incidence of Repeated Mating in the Superspecies, *Drosophila paulistorum*

Observations of repeated mating have been made in several species of *Drosophila*[1–3]. Recent work on *D. melanogaster* has shown that this phenomenon may be more frequent than had been previously suspected[4]. The sperm of the two or more males involved in a multiple mating event may be used to inseminate eggs within a single batch laid by one female[1]. This observation may be of more than incidental interest if competition among larvae developing from a single egg batch occurs in nature. It has been shown that larvae developing in media previously inhabited by larvae of a different genotype produce more adults than in media previously utilized by larvae of the same genotype[5,6]. Whether or not these phenomena occur in nature is unknown, but natural selection would be expected to produce increased receptivity to multiple mating in females if larval competition among similar genotypes constitutes an important competitive interaction.

Drosophila paulistorum is a widely distributed, neotropical species. A series of observations by Dobzhansky et al.[7] have demonstrated that this species is a superspecies in the process of forming 5 sibling species. The semispecies are often locally extremely abundant and have been extensively used by Ehrman et al.[8] to elucidate the mechanisms involved in ethological isolation between and within semispecies. The experiments described below were designed to test for the presence of double mating in 3 semispecies of *D. paulistorum*: Centroamerican, Andean, and Interior.

Methods and materials. Three kinds of experiments were conducted to test for the presence of double mating in *D. paulistorum*. In the first series of experiments, virgin females of the Andean semispecies known to be homozygous for the *F* allozyme allele at the *To* locus[9] were placed singly in culture vials with 3 males of the same strain hemizygous for the *F* allele. *To* is sex-linked in *D. paulistorum*. After 2 days the female was transferred

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