Among the Salamandrids, both the species of *Taricha* 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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to a fresh culture vial and 3 males hemizygous for the *S* allele to the *To* locus were added. The female and the 3 males were transferred to a fresh culture vial every 4 days. Males that died were replaced with males of a similar genotype. All cultures were kept at 25 °C. When progeny eclosed from the vials, 8 females were assayed for their genotype at the *To* locus.

The second series of experiments utilized flies of the Centroamerican and Interior semispecies. 2 strains of flies each homozygous for the sex-linked, white-eyed allele were derived from strains collected in Lancetilla, Honduras (Centroamerican) and Llanos, Colombia (Interior). White-eyed virgin females were confined with wild type males for 4 days. The females were then individually isolated and left to oviposit for 4 days. All inseminated females produced white-eyed male and red-eyed female progeny as expected. The original females were now placed in fresh culture vials, 1 female per vial, with white-eyed males. Progeny hatching from these vials were scored. Two sets of replicas for each semispecies were completed.

A third series of experiments consisted of testing females of the Andean semispecies which were taken from 2 population cages polymorphic for the *F* and *S* alleles at the *To* locus. These females were individually isolated and when larvae appeared, they were electrophoresed to determine their *To* genotype. When the progeny appeared, 6–10 females were analyzed for their *To* genotype.

Results and discussion. Table I shows the results of the first series of experiments. Of the 19 females which were homozygous for the *F* allele and initially mated to *F* males, 8 had also mated with the *S* males after being confined with them for 4 days. These 8 females produced eggs which were inseminated by sperm from both males. 4 females produced no progeny either because they died or presumably had not mated. After a total of 4 transfers (16 days), all the remaining females had mated a second time. Sperm from the initial mating with homogametic males lasted a total of 3 transfers or 12 days in 1 female. By the fifth transfer (20 days) all remaining females[7] were producing eggs fertilized by sperm from a second mating. This table demonstrates that *D. paulistorum* females will remate well before sperm from a previous mating is exhausted.

Table I. The origins of paternal gametes among progeny of *Drosophila paulistorum* females (Andean semispecies) given the opportunity to mate twice

Origin of sperm	Transfer number					
	1	2	3	4	5	6
F male	7	4	1	0	0	0
S male	—	5	13	14	7	6
S + F males	8	6	1	—	—	—
No progeny	4	4	4	5	12	13
Total	19	19	19	19	19	19

The data refer to the number of females producing progeny whose paternal genomes have the origin shown.

Table II. Incidence of double mating among white-eyed females of the Centroamerican and Interior semispecies of *D. paulistorum*

	Semispecies Interior	Centroamerican
No. females	123	40
Total mated	45	31
No. double matings	29	6
No. single matings	16	25
Percent double matings	64.4	19.4

Table II gives the results of tests using the sex-linked white-eyed stocks of the Interior and Centroamerican semispecies. These results again support the contention that repeated mating occurs in *D. paulistorum*. The large difference in the proportion of double matings in these two semispecies were unexpected. This difference may be due in part to differing degrees of ethological isolation between the mutant stocks and the wild type cultures from which the mutants were originally isolated. It is also possible that the difference observed represents a genetic difference in the propensity for the double mating in these stocks.

In order to determine whether repeated matings occur under the conditions of mass culture in population cages, females of the Andean semispecies were sampled from previously established cages. Each female was individually isolated and her progeny examined. A total of 82 females from 2 separate cages gave progeny. Of these females 18 or 22% had mated at least twice. These figures are an underestimate of the true proportion of double matings since a female which choses a second mate whose genotype is identical to that of the first mate will not be scored as having mated twice.

The observations show that *D. paulistorum* will mate repeatedly under experimental conditions. Whether or not multiple matings occur in nature is unknown, but tests of wild collected females under appropriate conditions would answer this question. The further observation that females will remate before exhausting stored sperm from a previous mating and that sperm mixing may occur suggests an adaptive value for repeated mating in nature. Competition among larvae is more likely to be reduced if they differ in genotype[5,6] and such a reduction is more probable if a female is producing eggs which may carry any 2 of 6 instead of 4 genomes. As these observations and those of other workers dealing with different species of *Drosophila* show[1–4], repeated mating in Drosophila may be a much more common phenomenon than has previously been postulated.

Résumé. Trois séries d'expériences ont été fait pour vérifier la possibilité d'accouplements répétés chez le *Drosophila paulistorum*. A l'aide de marqueurs morphologiques et de marqueur à enzyme, nous avons démontré que l'accouplement double se pratique fréquemment parmi cette espèce, même pour une population vivant en cage. Les femelles qui se sont accouplées deux fois utilisent le sperme des deux mâles pour fertiliser une seule couvée d'œufs.

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