does not appear to have any obvious deleterious effect on sheep, although experiments in progress indicate that GSH^l type sheep are more prone to kale anaemia than are GSH^H type individuals.

Resumen. Se medió glutation reducido (GSH) en glóbulos rojos de 427 ovejas pertenecientes a 6 razas diferentes. 25% de las ovejas de raza «Finnish Landrace» dieron un contenido de GSH de un tercio del valor obtenido en el resto. Datos familiares indican que esta deficiencia es heredada como un factor autosómico recesivo. En las ovejas que tienen esta deficiencia, la concentración media de potasio en glóbulos rojos fué significativamente menor que la observada en las ovejas con niveles normales de GSH.

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Interspecific Variability of DNA Content in Amphibia[1]

Gene and chromosome duplications are important mechanisms in the evolution of pluricellular organisms. The duplications can be accomplished by several means, such as unequal crossing-over between homologous chromosomes during meiosis, unequal exchange of chromatids during mitosis, redundant duplication of DNA in certain regions of the chromosomes, and polyploidy or polyteny.

Higher vertebrates seem to be remarkably uniform in DNA content. Indeed, the class Mammalia as a whole, the class Aves, and the order Squamata exhibit almost unvarying DNA content values[2–5].

However, among plants and lower animals, several cases of DNA increasing series have been demonstrated. Indeed, the anemone plants of the family Ranunculaceae[6], the grasshoppers of the family Acrididae[7], the teleost fish of the orders Percomorphi and Heterosomata[8], and the frogs of the family Ranidae[9] have stable karyotypes but show increased DNA content values. Proportional increase of DNA was also demonstrated in the polyploid amphibians of the family Ceratophrydidae[10–12]. Attempts to correlate DNA content of vertebrates, especially amphibians, with evolution patterns and ecology have recently been made[13,14].

The present paper deals with DNA content values of 30 species of Salientia, 2 species of Caudata, and 1 species of Gymnophiona.

Material and methods. Smears of air-dried blood were fixed in neutral 50% formol for 1 min, washed in tap and distilled water, hydrolysed in *N* HCl at 60 °C, and stained in Feulgen. 20 blood smears of a *Bufo paracnemis* LUTZ were prepared, air dried and stored. These slides were used as controls for every batch of specimens to be stained. The DNA value of each species was always determined in relation to that of *B. paracnemis*.

The DNA content was determined[15,16] with the histophotometer of VIALLI and PERUGINI[17] (Fratelli Koristka, Italy). The nuclei were enlarged to 1500 diameters, the area of the plug being more or less 20% the optical area of the nucleus. Generally, the mean of 3 plugs through the nuclear area was used but in some cases 4 to 6 measurements were made of material with irregular shaped chromophore masses. For each nucleus, a blank measurement was made and the galvanometer adjusted to the value of 100. Each absorption reading of the plus was given in % of the standard light of the blank. A stabilized lamp with an interference filter of 5400 Å was used.

Through the average determination of absorption for each nucleus, the DNA content was calculated according to the formula $DNA = D^2 (2 - \log In)$. D^2 = optical area of the nucleus; In = value of the absorption, and $2 = \log 100$. For each specimen 25 measurements were obtained and the mean (X), the standard deviation (S), and the standard error of the mean (SE) calculated. The overlapping of segments to 2 SE indicates roughly the significance of the differences between the two mean values at the level of $P \leqq 0.05$[18] (Figure).

In the Table and in the Figure the DNA values were adjusted for the Feulgen baths, and also expressed as % in proportion to mammal DNA measured on lymphocytes. The blood was collected in heparinized capillary tubes and centrifuged. The tubes were sectioned at the leucocytes strate level, smeared on slides, dried, fixed and Feulgen stained. Bird lymphocytes were also measured and compared with erythrocytes in the same smear.

In order to compare amphibian DNA with mammal DNA content, we adjusted mammal lymphocyte DNA values using a ratio between lymphocyte DNA and erythrocyte DNA, determined in the quail.

In the species where more than one specimen was analyzed, the arithmetic mean, the S, and the SE are calculated from the data of all the specimens together.

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The following species have been studied:

(A) Salientia:
  (1) Family Brachycephalidae: *Melanophryniscus moreirae* MIRANDA-RIBEIRO.
  (2) Family Bufonidae: *Bufo crucifer* WIED; *Bufo ictericus* SPIX and *Bufo paracnemis* A. LUTZ.
  (3) Family Ceratophrydidae: *Ceratophrys calcarata*; *Ceratophrys dorsata* WIED; *Odontophrynus americanus* DUMÉRRIL and BIBRON; *Odontophrynus carvalhoi* SAVAGE and CEI; *Odontophrynus cultripes* REINHARDT and LUTKEN; *Odontophrynus occidentalis* BERG; *Oocormus microps* BOULENGER; *Stombus boiei* (WIED) and the hybrid *Odontophrynus cultripes* × *Odontophrynus americanus*.
  (4) Family Hylidae: *Hyla albomarginata* SPIX; *Hyla faber* WIED; *Hyla fuscomarginata* A. LUTZ; *Hyla fuscovaria* A. LUTZ; *Hyla multilineata* A. LUTZ and B. LUTZ; *Hyla nana* BOULENGER; *Hyla parkeri* GAIGE; *Hyla polytaenia* COPE and *Hyla pulchella prasina* BURMEISTER.
  (5) Family Leptodactylidae: *Cycloramphus asper* WERNER; *Cycloramphus dubius* (MIRANDA-RIBEIRO); *Eupemphix nattereri* STEINDACHNER; *Leptodactylus fuscus* (WIED); *Leptodactylus ocellatus* (LINNAEUS); *Physalaemus fuscomaculatus* (STEINDACHNER) and *Pseudopaludicola ameghini* (COPE).
  (6) Family Microhylidae: *Dermatonotus mülleri* (BOETTGER).

(B) Caudata:
  Pleurodeles waltlii MICHAELIS and *Triturus cristatus* LAURENTI.

(C) Gymnophiona:
  Siphonops annulatus (MIKAN).

Results and discussion. In general, our results indicate DNA variations within each family. The DNA contents are significantly different, even among species with the same chromosome number. The smallest DNA values were found in *L. occelatus* and *C. asper* (Leptodactylidae) respectively with 43% and 44% of DNA as compared to mammals, in *D. mülleri* with 44%, the only species analyzed in the family Microhylidae, and in *O. carvalhoi* and *O. occidentalis* (Ceratophrydidae) both with 45%. The anurans with the highest DNA values are *H. pulchella prasina* (Hylidae) with 147% and the octoploid species *C. dorsata* (Ceratophrydidae) with 181% (Table, Figure).

Caudata and Gymnophiona show very high DNA content values of about 400% in relation to mammals (Table). Different DNA values have been found for species of Caudata by other authors[19]. While those data were obtained through DNA measurements of oocytes, our measurements were made in erythrocytes and they are consistent, e.g. in *T. cristatus* (Milan, Italy), with the values found in the liver and in the spermatogenetic series.

We should also consider the existence of a certain amount of giant erythrocytes (macrocytes) in the circulating blood with a double amount of DNA. The deviations from the expected mean DNA content values may perhaps be explained by this fact. The spermatid would be the more appropriate cell for DNA determination since it is not liable to metabolic or interphasic variations.

Considering the species in each salientian family, in accordance with a phylogenetical criterion of evolution, we may conclude that, in general, the primitive species have smaller DNA content values compared with the values found for the most highly evolved ones. Evidently there are several exceptions to this conclusion. Furthermore, we should consider that a phylogenetical approach frequently implies a subjective criterion.

Apparently, the DNA increase was independent in several families. We found low content species and high content species in the different families analyzed (Figure). Among the Hylidae, *H. nana* ($2n = 30$) has 54% of DNA. This value rises to 147% in *H. pulchella prasina* ($2n = 24$)[20]. Among the Leptodactylidae the values range from 43% in *L. ocellatus* ($2n = 22$) to 70% in *L. fuscus*, also with ($2n = 22$)[20]. In the family Ceratophrydidae, in which polyploidy has been demonstrated for some

DNA content measurements and somatic number of chromosomes of 33 amphibians including 30 species of Salientia, 1 species of Gymnophiona and 2 species of Caudata

Species	$2n$	DNA	S	SX	% Mammalia
Salientia					
Brachycephalidae					
Melanophryniscus moreirae	22	93	12.8	1.8	84%
Bufonidae					
Bufo ictericus	22	91	10.8	1.7	83%
Bufo crucifer	22	99	7.5	1.5	90%
Bufo paracnemis	22	103	8.3	1.7	94%
Ceratophrydidae					
Odontophrynus carvalhoi	22	50	5.5	1.1	45%
Odontophrynus occidentalis	22	50	5.7	0.8	45%
Ceratophrys calcarata	26	59	5.6	1.1	54%
Oocormus microps	26	61	6.9	1.4	55%
Odontophrynus cultripes	22	65	16.0	1.2	59%
Stombus appendiculatus	22	68	7.7	1.5	62%
Hybrids (*O. cultripes* × *O. americanus*)	33	72	9.2	1.3	65%
Stombus boiei	22	76	5.8	1.2	69%
Odontophrynus americanus	44	107	13.0	1.0	97%
Ceratophrys dorsata	104	199	29.0	2.6	181%
Hylidae					
Hyla nana	30	60	7.7	1.5	54%
Hyla fuscomarginata	24	72	8.5	1.7	65%
Hyla parkeri	24	73	8.1	1.6	66%
Hyla polytaenia	24	74	9.5	1.9	67%
Hyla albomarginata	24	83	18.2	2.1	75%
Hyla faber	24	86	19.1	2.7	78%
Hyla multilineata	24	117	9.4	1.9	106%
Hyla pulchella prasina	24	162	32.8	3.7	147%
Leptodactylidae					
Leptodactylus ocellatus	22	47	7.5	0.6	43%
Cycloramphus asper	26	49	8.4	1.7	44%
Cycloramphus dubius	26	55	7.3	1.5	50%
Eupemphyx nattereri	22	55	7.6	1.5	50%
Physalaemus fuscomaculatus	22	58	5.1	1.0	53%
Pseudopaludicola ameghini	20	76	9.3	1.9	69%
Leptodactylus fuscus	22	77	9.6	1.8	70%
Microhylidae					
Dermatonotus mülleri	22	49	8.8	1.8	44%
Gymnophiona					
Siphonops annulatus	–	438	45.7	9.1	398%
Caudata					
Triturus cristatus	24	448	50.4	11.0	407%
Pleurodeles waltlii	24	464	53.8	10.8	422%

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species, the values range from 45% in *O. carvalhoi* and *O. occidentalis* ($2n = 22$) to 181% in *C. dorsata* ($2n = 104$)[21].

The polyploid species of Ceratophrydidae show successive values of DNA content corresponding to the duplicated genomes. The triploid hybrid ($3n = 33$) produced by interspecific mating of the diploid *O. cultripes* ($2n = 22$) and the tetraploid *O. americanus* ($4n = 44$)[22] has a DNA content almost intermediary to the diploid and the tetraploid species.

The high values of DNA found in Gymnophiona and Caudata may be related to their phylogenetic origin from a common ancestral genome. On the other hand, the Salientia, whose DNA content is smaller, probably derived from another Stegocephalian ancestral genome that evolved in a different direction, from which the primitive reptiles originated too.

Genic duplications, detected by successive increases in DNA, played an important role in the evolution of the species. While the original genes are, for instance, in charge of basic functions for the maintenance of the species, the duplicated genes supply raw material for new mutations, whose adaptative values are possibly higher. This mechanism may produce a faster diversification and a higher genetic polymorphism. Eventually, during the evolutive process, new proteins and enzymes may be coded by the duplicated DNA.

Resumen. Fueron determinados por citofotometría los valores de DNA en 33 especies de Amphibia, en las que estaban incluidas 30 especies de Salientia, 1 de Gymnophiona y 2 de Caudata, y fueron comparadas con sus constituciones cromosomicas. Las variaciones encontradas sugieren que hubo aumento de DNA, independientemente, en las diferentes familias estudiadas y que en general, las especies más primitivas filogeneticamente, poseen menor cantidad de DNA, cuando comparadas a los valores encontrados para las más evoluidas.

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Sex Chromosomes of a Pygopodid Lizard, *Lialis burtonis*

The lizard family Pygopodidae of the Australia–New Guinea region contains about 15 species, all limbless. Despite a superficial resemblance to snakes (Figure 1), anatomical studies imply a close relationship to the large family Gekkonidae[1]. The karyotypes of pygopodids are thus of interest to serve as a basis of comparison with other morphological features. To our knowledge, there are no other published reports on the chromosomes of any pygopodid lizard.

Through the courtesy of Dr. RICHARD ROSS, and the cooperation of the Museum of Comparative Zoology, we received one male and one female *Lialis burtonis* from Mareeba, Atherton Tableland, Queensland (Australia). Knowing nothing about the reproductive states of the lizards (they had been in captivity for some months before we received them in mid-June) we injected them 3 times on alternate days with 100 IU per injection of Equinex Serum Gonadotropin (Ayerst Laboratories) during the week prior to sacrifice. Our aim was to stimulate meiotic and mitotic divisions. 12 h preceding sacrifice the lizards were injected with 0.1 ml Colcemide (CIBA), in a solution of 50 mg/l, to accumulate mitotic metaphase cells. Following sacrifice, gonads were minced in a hypotonic citrate solution and permanent slides were made as previously described[2].

Male: All stages of meiotic and mitotic divisions were seen in abundance. At diakinesis there are 16 bodies. Close examination reveals that one is a trivalent, the other 15 bivalents (Figure 2). In the first meiotic metaphase, cells with 16 and 17 chromosomes appeared equally abundant. Mitotic metaphase cells confirm that the diploid number is 33 (Figure 3). There is one large pair of submetacentric chromosomes; a smaller pair of even more submedian chromosomes; approximately 3 pairs of subtelocentric chromosomes in which a short arm may be seen; approximately 5 pairs of telocentrics decreasing gradually in size, and not differing markedly from the subtelocentrics; and approximately 5 pairs of microchromosomes, 1 of which is clearly bi-armed. There are no sharp breaks between macro- and microchromosomes, nor between the telocentrics and subtelocentrics – hence the use of the word 'approximately'. Thus there are 15 autosomal pairs and 3 unpaired sex chromosomes.

Female: Only 3 cells were clear enough for analysis in the female. The diploid number appears to be 34, with 17 pairs of chromosomes, the 2 largest pairs are two-armed (Figure 4). One of the smallest pairs of chromosomes appears to be metacentric.

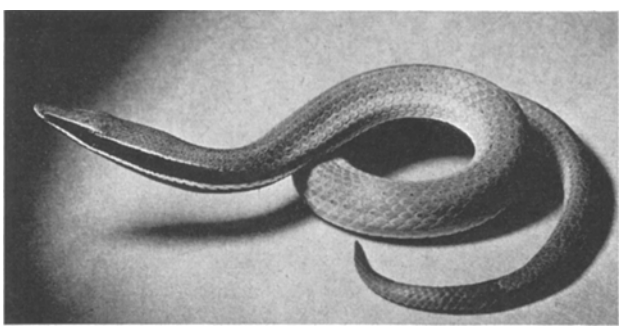

Fig. 1. *Lialis burtonis*, female.

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