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Chromosome Number in the Lizard Genus *Uta* (Family *Iguanidae*)*

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Abstract. A chromosome number of 34 (12 macro- and 22 microchromosomes) was found to be characteristic of the bone marrow in 47 animals including males from the species *Uta antiquus*, and both males and females from the following species and subspecies: *Uta stansburiana stansburiana, Uta stansburiana stejnegeri, Uta stansburiana elegans, Uta stansburiana klauberi, Uta stansburiana mannophorus, Uta nolascensis, Uta palmeri,* and *Uta squamata.* — Diploid chromosome numbers of 34 and haploid numbers of 17 were found in the nine testis smears examined. — The presence of a large number of hypodiploid figures in the bone marrow smears is attributed to cell fragmentation and the problem of distinguishing the small microchromosomes. — Series of polyploid figures whose chromosome numbers increased in arithmetic rather than geometric progressions were observed in the testis dry smears. Possible alternatives for the origin of these figures are presented. — Problems encountered in the use of chromosome number as a taxonomic character are discussed.

Introduction

Cytological techniques have in the past been so time consuming and the karyotype record so fragmentary that reptilian taxonomists have seldom utilized chromosome variation as a source of information. Karyotype comparison, when used, has been largely at intergeneric and interfamilial levels, and variation at specific and subspecific levels has remained virtually unexplored.

With the advent of more effective cytological techniques, comparative chromosome study has become a more practical taxonomic tool, and it is being employed with increasing frequency by herpetologists. The present report deals with chromosome number in the *Uta* species and subspecies which have thus far been examined.

The genus *Uta* is composed of 6 species (BALLINGER and TINKLE, 1968) of small iguanid lizards, and it occupies a range limited to the arid portions of western North America. The genus exhibits its greatest diversity on the islands of the Gulf of California, where several species occur which are quite different from those on the mainland.

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Materials and Methods

Ten to 14 hours prior to sacrifice, the lizards were injected with 0.1 ml of a 0.004% Velban solution for each 4 g of body weight. During exposure to this drug the animals were kept in a small cage heated by two 60-watt light bulbs. Following sacrifice, leg bones, and in some cases vertebral column (minus spinal cord) and gonads were removed and placed separately in small containers of DULBECCO and VOGT'S (1954) saline solution in which calcium chloride and magnesium chloride had been omitted, and 0.01 μg/ml Velban added. All glassware used in subsequent steps was siliconized. Tissues were macerated with scissors and forceps, the residual pulp removed, and the suspension repeatedly aspirated through an 18 gauge syringe needle. Bone marrow suspensions were filtered through a 50-micron mesh nylon monofilament screen. All suspensions were centrifuged for 5 minutes at 800 RPM (105 RCF), the supernatant removed, the cells suspended in a 1% sodium citrate solution containing 0.1μg/ml Velban, and placed in a 37° C water bath for 18 minutes. The suspensions were recentrifuged and 2 ml of fixative (1 part glacial acetic acid to 3 parts absolute ethanol) added near the pellet-citrate interface, taking care not to disrupt the pellet. All of the fluid was removed and fresh fixative added, keeping the pellet intact. Fixative was replaced after 10 minutes and the tube chilled for one hour in the refrigerator. Fixative was removed, chilled 45% acetic acid added, and the tube returned to the refrigerator for a period of 2 to 20 hours. The pellet was finally resuspended in 0.2 to 0.4 ml of fresh 45% acetic acid, two drops of the suspension placed on a dry slide, the drops allowed to spread, and excess fluid blown from the slide. The resulting dry smears were stained in aceto-orcein (4 g synthetic orcein/100 ml 45% acetic acid) or ammoniacal Giemsa, and mounted in balsam. The Giemsa-stained slides faded rapidly and were of little value after 6 or 8 months.

Reference specimens are on deposit at the University of Michigan Museum of Natural History. The following collecting areas are represented in the sample: *Uta stansburiana stansburiana*, New Mexico (San Juan Co.), Colorado (Delta Co.); *Uta stansburiana stejnegeri*, Texas (Winkler Co.), New Mexico (Bernalillo Co.); *Uta stansburiana elegans*, California (San Diego Co., Riverside Co.), Mexico (Baja California); *Uta stansburiana klauberi*, San Esteban Island, Mexico; *Uta stansburiana mannophorus*, Carmen Island, Mexico; *Uta antiquus*, San Lorenzo Island, Mexico; *Uta nolascensis*, San Pedro Nolasco Island, Mexico; *Uta palmeri*, San Pedro Martyr Island, Mexico, and *Uta squamata*, San Lorenzo Island, Mexico. All of the above islands are in the Gulf of California.

Chromosome counts were made in a total of 48 animals from 9 species and subspecies. The species and sex of the animals, as well as the tissues utilized, are summarized in the Table. The nomenclature is that followed by BALLINGER and TINKLE (1968) in their revision of the genus.

Observations

As a preliminary step in assessing the degree and pattern of variability in chromosome number, a sample of 50 or more bone marrow figures was obtained from each of 10 individuals. This group contained one male and one female from the species *Uta palmeri*, and one individual from each of the other species or subspecies included in the study. Four of the single representatives were male, and 4 were female.

These samples, subsequently referred to as unselected samples, were composed of all of the analyzable figures observed during systematic

scanning of one or more slide sectors under a 16 × objective. Isolated macrochromosomes and small clusters of chromosomes which contained at least one macrochromosome were considered to be figures. Figures were excluded only if spreading, staining, or fixation were so poor as to make even approximate counts unfeasible.

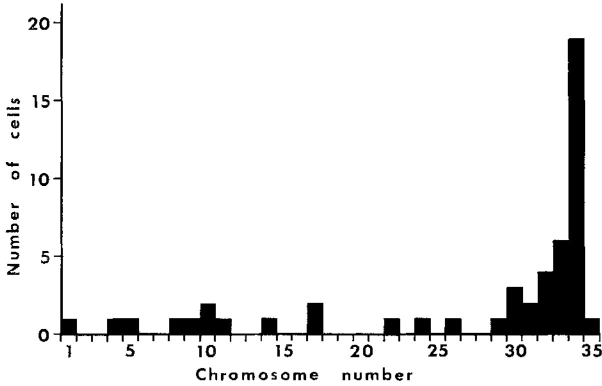


Fig. 1. Distribution of chromosome number in a sample of 50 unselected mitotic figures from the bone marrow of a female *Uta palmeri*

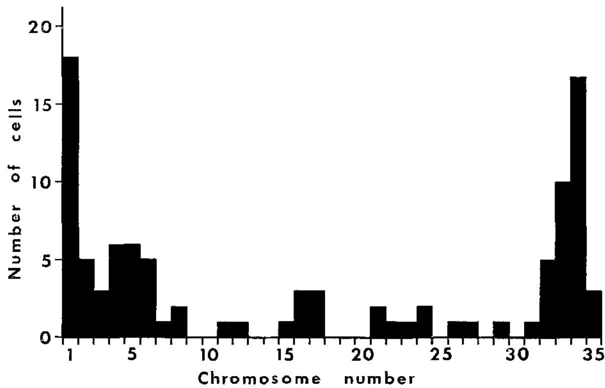
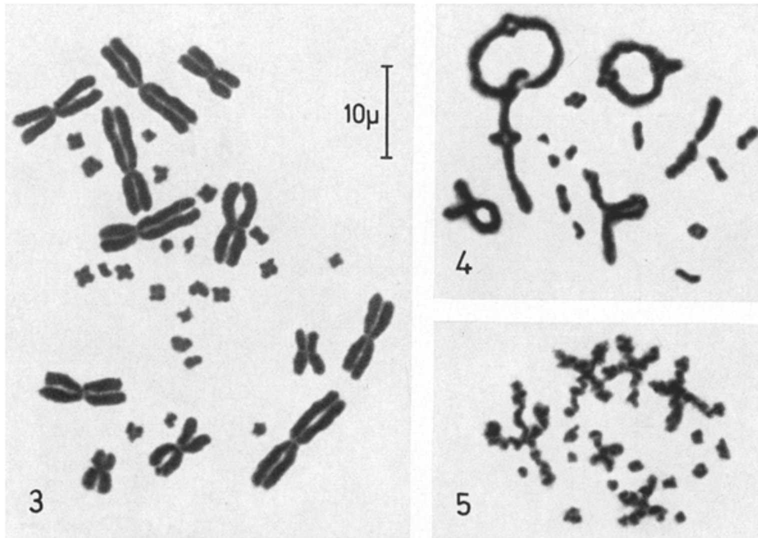


Fig. 2. Distribution of chromosome number in a sample of 100 unselected mitotic figures from the bone marrow of a male *Uta antiquus*

The most striking feature revealed by counts made from the unselected samples was the extent of variability in chromosome number present within each preparation. A modal chromosome number of 34, and a distribution similar to that shown in Fig. 1 was observed in 8 of the 10 unselected samples. The *Uta antiquus* and *Uta stansburiana stansburiana* samples exhibited a bimodal or U-shaped distribution curve (Fig. 2) in which chromosome numbers of 1 and 34 were most frequently represented. In all samples, the 34 chromosome complement

was composed of 12 macrochromosomes and 22 microchromosomes (Fig. 3). This chromosome number and distribution of macro- and microchromosomes is in agreement with that reported by Hall (1965) for *Uta stansburiana*. Polyploid figures did not happen to fall within the sample sectors, although one or two were generally observed in each preparation.

Chromosome number in the bone marrow smears from the remaining 37 individuals (Table) was assessed using biased or selected samples com-



Figs. 3—5. Mitotic metaphase (Fig. 3) chromosomes from the bone marrow of a *Uta stansburiana stansburiana* female, and meiotic metaphase I (Fig. 4) and metaphase II (Fig. 5) chromosomes from the testis of a *Uta palmeri* male

posed of 10 of the better figures from each individual. Mitotic figures with deficiencies in chromosome number sufficient to be detected before counting, as well as those in which counting would be difficult, were excluded from the samples.

A modal chromosome number of 34 was observed in all but one of these selected samples. The exception was a male *Uta stansburiana elegans* sample in which figures with 33 chromosomes were as abundant as those with 34, however in 3 other males from the same subspecies, the modal number was clearly 34.

Sixty-two percent of the figures in the 37 biased samples exhibited a chromosome number of 34, 20% had 33 chromosomes, 14% had fewer than 33 chromosomes, and only 4% had more than the modal number of 34.

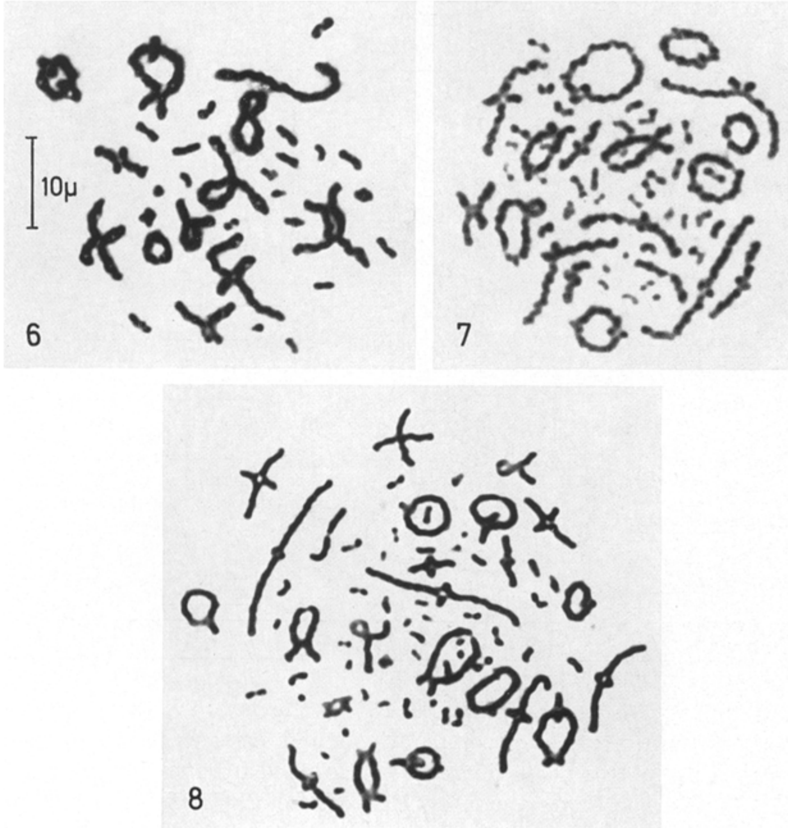
Table. *Summary of samples from 27 male and 21 female Uta*

Species	Sex	Number of individuals sampled							
		bone marrow		spermatogonia		prim. spermatocytes		sec. spermatocytes	
		50+ cells	10 cells	30 cells	10 cells	30 cells	10 cells	30 cells	10 cells
Uta stansburiana	♀	—	2	—	—	—	—	—	—
stansburiana	♂	1	1	—	—	—	—	—	—
Uta stansburiana	♀	—	2	—	—	—	—	—	—
stejnegeri	♂	1	2	—	—	—	1	—	1
Uta stansburiana	♀	1	2	—	—	—	—	—	—
elegans	♂	—	4	—	1	—	1	—	1
Uta stansburiana	♀	—	1	—	—	—	—	—	—
klauberi	♂	1	4	—	—	—	—	—	—
Uta stansburiana	♀	1	2	—	—	—	—	—	—
mannophorus	♂	—	2	—	—	—	—	—	—
Uta antiquus	♀	—	—	—	—	—	—	—	—
	♂	1	2	—	—	—	4	—	2
Uta nolascensis	♀	1	2	—	—	—	—	—	—
	♂	—	1	—	—	—	—	—	—
Uta palmeri	♀	1	3	—	—	—	—	—	—
	♂	1	2	—	1	—	1	1	1
Uta squamata	♀	1	2	—	—	—	—	—	—
	♂	—	3	—	—	—	—	—	—
Total number of cells		550	370	30	20	30	70	30	50

In contrast to the preparations from bone marrow, the few testis smears examined contained comparatively high percentages of polyploid figures (Figs. 6—8). These figures were excluded from the samples used to determine the chromosome number characteristic of different divisional stages in the germ line.

Chromosome counts were made in unselected samples of 30 mitotic metaphase (spermatogonial) figures, 30 meiotic metaphase I (primary spermatocyte) figures, and 30 meiotic metaphase II (secondary spermatocyte) figures from a single *Uta palmeri*, and in selected (10 cells/divisional stage) samples from the testes of 8 other individuals (Table). In all of the samples, the modal number of metaphase I bivalents and metaphase II chromosomes was 17 (Figs. 4, 5). A modal number of 34 chromosomes was observed in each of the three mitotic metaphase samples. The percentages of cells in the selected samples which exhibited the reported modal numbers was 60% in metaphase I, 77% in metaphase II, and 64% in mitotic metaphase.

The relative abundance of figures in different divisional stages and at different polyploid levels varied from preparation to preparation. In a sample of 200 metaphase cells from the one individual (*Uta palmeri*) examined in detail, 12% of the cells were in mitotic metaphase, 72%



Figs. 6—8. Polyploid metaphase I figures from testis of a male *Uta palmeri*. The figures are 4n (Fig. 6), 6n (Fig. 7), and 8n (Fig. 8), and contain 12, 18, and 24 macrochromosome bivalents respectively

were in meiotic metaphase I, and 16% were in metaphase II. Seventy-six percent of the cells in this sample had the expected chromosome number (based on counts of macrochromosomes only), 9% had twice the number of chromosomes normally found in their respective divisional stages, 6% had three times the normal number, 1% had 4 times the normal number, and 8% were aneuploid. Polyploid levels higher than four times the normal chromosome number characteristic of a divisional stage were not found within the 200 cell sample, but in other sections of the same

preparation, mitotic metaphase figures were observed which had 2, 3, 4, 5, 6, or 7 times the normal diploid number of chromosomes, metaphase I figures with 2, 3, 4, 6, 8, or 10 times the normal number of bivalents, and metaphase II figures with 2, 3, 4, 5, and 8 times the normal haploid number of chromosomes.

Discussion

Interest in the question of aneuploid and polyploid cells is stimulated by the increasing number of reports dealing with intraindividual polymorphism in chromosome number and morphology.

Among the possible explanations for the high variability in chromosome number observed in the *Uta* bone marrow samples are: (1) the variability is an illusion and stems from the observer's inability to distinguish all of the small chromosomes due to their clumping and overlapping, (2) the variability is characteristic of the tissue *in situ*, and (3) the variability is induced by the technique.

The first explanation probably accounts for the majority of cells scored as having chromosome numbers slightly less than 34. Microchromosomes are most frequently the missing elements in these cells. The ratio of lost microchromosomes to lost macrochromosomes is 26:1 in the 104 cells (found among the 550 cells in the unselected samples) which lack one or two chromosomes. In cells with chromosome numbers well below 34, this selective loss of microchromosomes is not apparent. The loss ratio is only 2:1 in the 93 cells (from the same samples) which lack 10 to 20 chromosomes. This is the approximate ratio of micro- to macrochromosomes in the complete cell. Fragmentation and disruption of the cells by maceration and by the acetic acid schedule (Genest and Auger, 1963) are probably the major factors in chromosome loss at this level.

The cytoplasmic boundary observable in wet squash preparations provides a criterion for recognizing broken cells, but since the material studied consisted entirely of air-dried smears, the possible contribution of naturally occurring aneuploidy can not be evaluated.

Multiple factors must also be considered in attempting to explain the origin of the polyploid figures which are so frequently encountered in the testis smears.

The superimposition of two or more cells offers a possible explanation for these figures, but in contradiction to this explanation are the facts that the majority of polyploid figures have round or oval perimeters, the chromosomes are evenly distributed with few overlaps, and they exhibit the same divisional stage, degree of contraction, and intensity of staining.

Endoreduplication, in its normal form, is an unlikely cause of the polyploidy, as the polyploid cell series follow an arithmetic progression (2n, 4n, 6n, 8n, 10n in spermatogonia) rather than the geometric progression (2n, 4n, 8n, 16n, 32n) one would expect to be produced by this mechanism.

A third alternative is suggested by PAINTER's report (1921) that in sectioned lizard testis one can observe rounded masses of cytoplasm which contain from two to eight nuclei, but in which distinguishable cell walls are absent. Within single syncytial masses he found different spindles which were closely associated, but which retained their individual integrity. The formation of sperm heads appeared to progress normally in the syncytia, although giant spermatozoa were occasionally observed. Painter mentions similar reports in turtles, birds, and mammals.

Support for PAINTER's interpretation is provided by the presence of clustered nuclei in the *Uta* dry smears. Nuclei within the same cluster frequently appear to be synchronous with respect to cell cycle. In many of the prophase clusters, the member nuclei show the same staining intensity, and their chromosomes exhibit a similar degree of contraction. In clusters with ruptured nuclear membranes, the chromosomes are often dispersed as though the membranes had ruptured at approximately the same time.

It is possible that the combination of mitotic inhibition, maceration of the tissue to form a cell suspension, hypotonic shock, fixation, and flattening may convert a metaphase syncytium filled with multiple independent spindles into a single giant "polyploid" cell in which the chromosomes, once grouped on separate spindles, are evenly dispersed throughout the cytoplasm. Within the preparations one can select different stages of clusters and metaphase figures which together form an evolutionary sequence which is compatible with this explanation.

Such a sequence may be induced by mechanisms other than cytological technique. The presence of giant sperm in the testis sections prepared by PAINTER, and of large variably sized interphase nuclei in the *Uta* dry smears, suggests that at least some of the polyploid figures are the products of naturally occurring events.

Polyploid series similar to those described here have been reported in human testes by SASAKI and MAKINO (1965). Their conclusion regarding the origin of these cells is equally applicable to those found in *Uta*. SASAKI and MAKINO comment: "At the present state of our knowledge any conclusive statement should be avoided as regards the cause and significance of such a frequent occurrence of polyploid cells ..."

It is difficult for the cytologically oriented taxonomist to find an acceptable middle ground between the practice of reporting chromosome number without supporting data (a tradition in the literature on rep-

tiles), and the standards of large sample size imposed on taxonomists utilizing more readily accessible characters. While the former approach is no longer acceptable, the analysis of karyotypes is of limited value as a taxonomic tool if the burden imposed by sample size becomes prohibitive. Furthermore an accurate accounting of the observed chromosome numbers in a truly unbiased sample is not always the best indicator of the investigator's degree of certainty regarding the chromosome number characteristic of an animal or tissue.

The present study points up ways in which two generally accepted procedures, the use of samples composed of the "best" figures, and the use of "modal chromosome number", may be misleading.

During the initial examination of a chromosome preparation, one develops a mental image of what he believes to be the complete chromosome complement, and without counting chromosomes, he can recognize figures whose chromosome number significantly departs from the expected. The question is raised as to how frequently the "better" figures are the ones which conform to the observer's image of the complete cell. A portion of the samples used in this study ("selected" samples) are composed of figures which so conform. Inherent in such a practice is the danger that variability which is of biological significance may be overlooked by the investigator, as well as the danger that the person reading his report may be given a false sense of security with respect to the frequency with which the reported chromosome number actually occurred in the preparations.

The assumption that the chromosome number most frequently observed in a sample is the number characteristic of the living cell may also be hazardous. In organisms which possess high numbers of minute, easily obscured microchromosomes, such as lizards or birds, it is quite possible that one may observe a higher number of cells in which one microchromosome is hidden, than cells in which all of the microchromosomes are visible. In several of the *Uta* samples, the number of cells with 33 chromosomes closely approached the number with 34, and in one sample the two chromosome numbers were equally represented. One may also be misled by the modal number in preparations which contain a large number of cells with diminished chromosome numbers (whether due to natural or artificial causes). Using the modal criterion in its strictest definition, one of the lizards (Fig. 2) would be characterized as having a single chromosome. This is obviously absurd. For the purpose of the present study, the "cliff" at the right side of the histograms (Figs. 1, 2) which occurs between the 34 and 35 chromosome classes appears to be a more reliable criterion than the mode in establishing the chromosome number. A sharp drop in cell frequency at this point was present in all of the samples.

It is concluded that the diploid chromosome number characteristic of the bone marrow in each of the 47 animals examined is 34. The presence of the same chromosome number in 9 different forms of *Uta* is not surprising since *Uta* is considered (BALLINGER and TINKLE, 1968) to be of comparatively recent origin, and chromosome number is a rather conservative character at intra- and interspecific levels in the majority of vertebrate groups.

The taxonomic significance of single factor analysis in general, and chromosome number in particular is of limited value. The literature dealing with reptile chromosomes is so sparse, however, that much remains to be learned from the karyotype, and a knowledge of chromosome number serves as a necessary prerequisite to the study of other karyotype parameters.

Observations on sex chromosome heteromorphism, and a detailed morphologic decription of the *Uta* karyotype derived from chromosome measurements will be presented in separate communications.

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