Chromosomes of *Elaphe subocularis* (Reptilia: Serpentes), with the Description of an in vivo Technique for Preparation of Snake Chromosomes

Thirty-four of the thirty-five species of snakes examined by us have a diploid number of 36 with the karyotypes of most species divisible into 16 macrochromosomes and 20 microchromosomes. One species, *Elaphe subocularis*, has a diploid number of 40 with 18 macro-sized and 22 micro-sized chromosomes. Described below is the karyotype of *E. subocularis*, with suggested changes involved in its evolution.

Methods and materials. The specifics of our technique are as follows: 1. Inject the live snake i.p. with a solution of phytohemagglutinin-P at the following rate: 0.02 ml/g of body weight for the first 35 g and 0.01 ml per each additional gram. The phytohemagglutinin-P solution is prepared by diluting a 5 ml ampule (of Difco) to a volume of 75 ml. 2. 24 h later repeat step 1. 3. At 42 h after the first injection, the snake is injected i.p. with a 0.025% solution of Velban at a rate of 0.005 ml/g of body weight (1 cm^3 maximum). 4. 6 h after the Velban injection the animal is sacrificed and blood is removed with a heparinized syringe. A quantity of at least 1 cm^3 is desirable. After allowing the blood to set for 15 min, it is centrifuged for 3 min at 500 *g*. 5. The supernatant and the top layer of cells are pipetted and centrifuged for 3 min at 1,500 *g*. 6. The supernatant is discarded and two cm^3 of a 1.0% sodium citrate solution are added, and the cell button is resuspended and allowed to set for 10 min. 7. Recentrifuge at 1,500 *g* for 3 min and discard the supernatant. Add 2 ml of freshly prepared fixative (1 part acetic acid: 3 parts methanol), resuspend cells and allow to set for 10 min. 8. Centrifuge at 1,500 *g* for 3 min and discard supernatant. Add 2 ml of fixative and resuspend cells. Repeat this step 4 times. 9. Dilute cell button with 1–2 cm^3 of fixative and resuspend cells. Add 3–4 drops of suspension to a microscope slide. Ignite and allow the fire to extinguish itself. Sling residue from slides. 10. After slides dry, stain with an appropriate stain.

Results. The chromosomes of *Elaphe subocularis*, *E. guttata*, *E. obsoleta*, and *Crotalus molossus* are described below. *E. subocularis* is the only species that has the unique $2N = 40$ karyotype. *Elaphe guttata* and *E. obsoleta* are the closest relatives of *E. subocularis* that we have karyotyped.

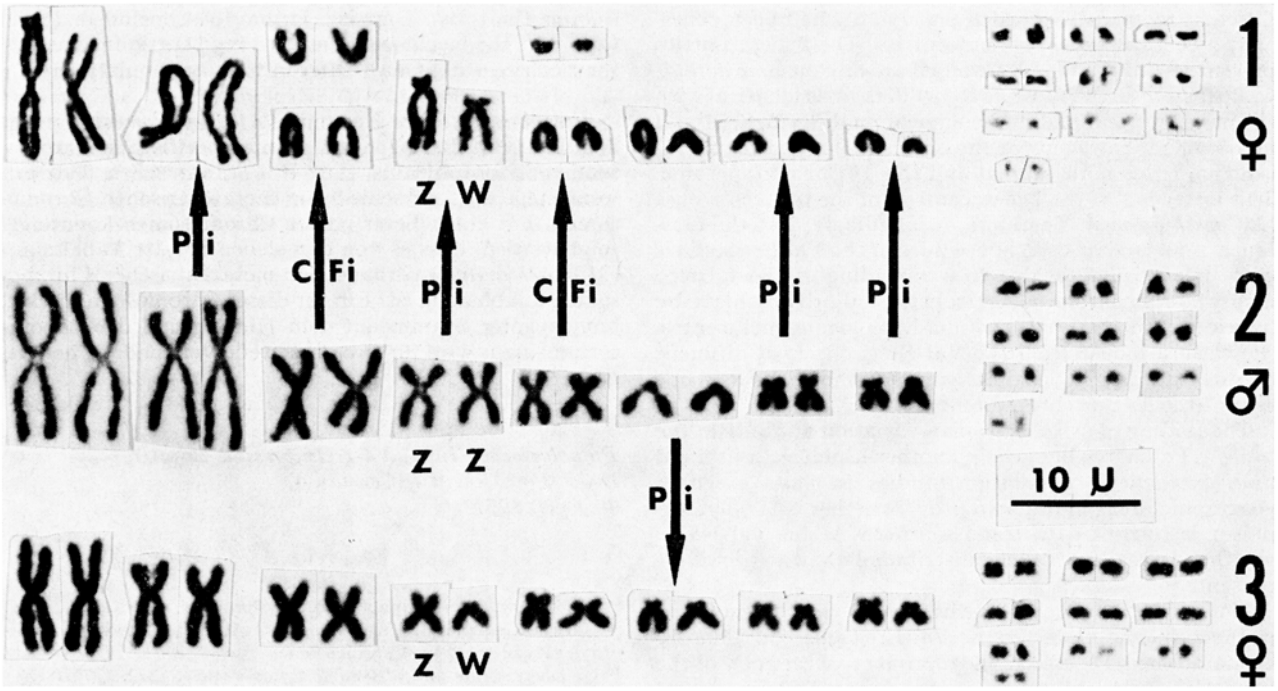
The karyotype of *Crotalus molossus* is presented because it is representative of the most common Crotalid karyotype which is essentially like that of *E. guttata* and *E. obsoleta* as well as several other Colubrids[1]. A brief description of the 4 species follows.

Elaphe subocularis. $2N = 40$. (1♂; 1♀) Figure 1. The largest pair of chromosomes are submetacentric, with the only other distinctly biarmed elements being the Z and W, which are the third largest pair in size. Although both the Z and W are subtelocentric in nature, the Z is distinguished by its larger size. The remainder of the chromosomes are acrocentric or near acrocentric. There are 11 pairs in the micro-size range.

Elaphe guttata. $2N = 36$. (3♂♂; 2♀♀) Figure 2. The 5 largest pairs of chromosomes are submetacentric. The 6th largest pair is acrocentric, and the remaining 2 pairs of macros are submetacentric and subtelocentric, respectively. There are 10 pairs of micro elements. The submetacentric *Z* is smaller than the 3 largest pairs of autosomes. The *W* is smaller than the *Z* and nearly metacentric. The autosomes and *Z* chromosome of *E. obsoleta* (3♂♂; 3♀♀) are indistinguishable from those of *E. guttata*. The *W* equals the *Z* in size but is subtelocentric.

Crotalus molossus. $2N = 36$. (1♂; 1♀). Figure 3. The 8 pairs of macrochromosomes are biarmed with pair 6 ap-

[1] W. BECAK and M. L. BECAK, Cytogenetics *8*, 247 (1969).
[2] W. BECAK, M. L. BECAK, H. R. S. NAZARETH and S. OHNO, Chromosoma *15*, 606 (1964).



Karyotypes of 1. *Elaphe subocularis*, 2. *Elaphe guttata*, and 3. *Crotalus molossus*. Arrows indicate changes from the central karyotype. Pi = Pericentric Inversion; CFi = Centric Fission.

proaching a subtelocentric centromere placement. The submetacentric Z is smaller than the 3rd largest pair of autosomes, and the W is smaller than the Z and subtelocentric. 10 pairs are in the microchromosome size range.

Discussion. The available data on snake chromosomes were reviewed by BECAK and BECAK[1], and they found a karyotype with a $2N = 36$ (with 8 pairs macro and 10 pairs of micro elements) in the families Boidae, Colubridae, and Crotalidae. Such a karyotype was probably characteristic of the primitive line of snakes that gave rise to the 3 families.

Although most Colubrids have a diploid number of 36[1], variation within the family in diploid number ranges from 50 in *Clelia*[2] to 24 in *Hydrodynastes*[3]. Most of this variation is accounted for by reduction of the number of microchromosomes[1].

In Figures 1–3 the karyotypes of *Elaphe subocularis*, *E. guttata*, and *Crotalus molossus* are shown. Arrows indicate changes necessary to derive one karyotype from another. Although the direction of the change between the karyotypes of *E. guttata* and *Crotalus molossus* is open to question, it is probable that the $2N = 36$ karyotype is primitive and the increase in diploid number to 40 was due to 2 centric fissions in macrochromosomes. Further changes between the two *Elaphe* karyotypes can be explained by 3 pericentric inversions. From a gross morphological basis only 1 pericentric inversion is required to explain the differences between the autosomes of *E. guttata*, *E. obsoleta*, and *Crotalus molossus*. A similar karyotype is characteristic of many other Colubrids and Crotalids[1,4].

Although centric fissions are not frequently reported as a mechanism of chromosomal evolution in vertebrates, such seems to be the most plausible mechanism in this case. In most species of snakes studied by BECAK et al.[1-3] and by us, the 4th largest pair is the sex chromosomes. In *E. subocularis* the ZW pair is the 3rd largest in size. A centric fission in the 3rd largest pair of autosomes resulting in 2 smaller acrocentric pairs would explain this change in relative size. In view of the generally conservative nature of chromosomal variation in snakes, the degree of chromosomal divergence between *Elaphe subocularis* and the other two species of *Elaphe* is extraordinary.

The genus *Elaphe* is complex, and it is possible that chromosome morphology may be a useful phylogenetic indicator within the genus. However, of the other species of the genus that have been studied by other workers (*E. carinata*[7], *E. climacophora*[6], *E. longissima*[5], *E. obsoleta*[7], *E. quadrivirgata*[6]), all have had diploid numbers of 36.

All voucher specimens are deposited in the Collection of Amphibians and Reptiles, Department of Biology, Texas Tech University.

Zusammenfassung. 34 von 35 Schlangenarten, die bei uns geprüft wurden, haben eine doppelte Nummer 36. Nur eine Art, *Elaphe subocularis*, hat eine doppelte Nummer 40. Die Chromosomen von *E. subocularis*, *E. obsoleta*, *E. guttata*, und *Crotalus molossus* sind beschrieben, und mögliche Evolutionsveränderungen sind angenommen. Alle vier Arten haben ein *ZZ/ZW*-Geschlechtschromosomensystem. Karyotypische Glasplättchen wurden durch eine in vivo Technik hergestellt, die in allen Einzelheiten beschrieben ist.

R. J. BAKER, J. J. BULL and G. A. MENGDEN

Department of Biology, Texas Tech University, Lubbock (Texas 79409, USA), 20 April 1971.

[3] W. BECAK, M. L. BECAK and H. R. S. NAZARETH, Mem. Inst. Butantan, Simp. int. *33*, 151 (1966).
[4] R. J. BAKER, G. A. MENGDEN and J. J. BULL, unpublished data.
[5] H. R. KOBEL, Genetica *38*, 1 (1967).
[6] W. BECAK and M. L. BECAK, Cytogenetics *8*, 247 (1969).
[7] H. K. FISCHMAN, J. MITRA and H. DOWLING, Proc. Meet. Genet. Soc. Amer. (Abstr.) *60*, 177 (1968).

Shock Effects on Some Cryptogamic Plants

Shock stimuli are known to inhibit growth in higher plants[1-3]. Microchemical changes were also observed in lichens[4], and induced tropic responses after shock treatment were hastened in euglenas[5]. The present paper reports on further cytological studies on other cryptogams.

Materials and methods. Light microscopy was used for all observations on living materials. An air loader, previously described[2], was used for developing shock pressures. Unialgal cultures were maintined in nutrient solution indoors near a northwest window. Several *Spirogyra* species, *Chlamydomonas reinhardii*, *Euglena gracilis*, *Closterium sp.* and *Cosmarium sp.* were subjected to shock pressures from 10 to 75 ψ depending on the alga. The algae were suspended in nutrient solution during shock treatment. Spore caps from a common moss, *Polytrichum sp.* were sterilized in 25% ethyl alcohol, then punctured to release the spores. The spores were cultured on an enriched agar medium[6] under a gro-lux fluorescent tube for a 12-h light period at ambient room temperature. 2 cultures of different ages (12 day and 40 day protonemata) were shocked at 60 ψ for about 6 sec duration. Both were shocked within 10 min of each other to eliminate any complications from diurnal rhythms[7]. Protonemata were teased off the agar onto slides 5–60 min after shock. The microscopic image was projected onto a drawing board to facilitate accurate drawings of the cell wall angles. The angles were measured by a protractor. Post-shock studies were not attempted because the cultures were exposed to the atmoshere during shock treatments and the cultures were too old to subculture. Fungus contamination ensues shortly after such exposure.

Fern fronds bearing sporangia from *Polypodium polycarpa* and a mature strobilus from *Equisetum arvensis* were cut in half. Half of the sporebearing structures served as controls, whereas the other half was shocked at 60 ψ with a pressure duration of 4–6 sec. Spores were collected overnight on paper then sprinkled on an enriched agar medium[6] and cultured similarly as the moss spores. Prior to shock treatment the strobilus was placed in a 35 °C oven

[1] S. A. MURRAY and C. L. NEWCOMBE, Radiat. Bot. *10*, 563 (1970).
[2] S. A. MURRAY, Experientia *26*, 319 (1970).
[3] S. A. MURRAY, Am. J. Bot. *58*, 119 (1971).
[4] S. A. MURRAY, Experientia *27*, 11 (1971).
[5] S. A. MURRAY, Experientia *27*, 757 (1971).
[6] Turtox Service Leaflet No. 44.
[7] R. BIEBL and K. HOFER, Radiat. Bot. *6*, 225 (1966).