

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 BEČAK and BEČAK<sup>1</sup>, 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<sup>1</sup>, variation within the family in diploid number ranges from 50 in *Clelia*<sup>2</sup> to 24 in *Hydrodynastes*<sup>3</sup>. Most of this variation is accounted for by reduction of the number of microchromosomes<sup>1</sup>.

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<sup>1,4</sup>.

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 BEČAK et al.<sup>1–3</sup> 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*<sup>7</sup>, *E. climacophora*<sup>8</sup>, *E. longissima*<sup>5</sup>, *E. obsoleta*<sup>7</sup>, *E. quadrivirgata*<sup>6</sup>), 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.

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<sup>3</sup> W. BEČAK, M. L. BEČAK and H. R. S. NAZARETH, Mem. Inst. Butantan, Simp. int. 33, 151 (1966).

<sup>4</sup> R. J. BAKER, G. A. MENGDEN and J. J. BULL, unpublished data.

<sup>5</sup> H. R. KOBEL, Genetica 38, 1 (1967).

<sup>6</sup> W. BEČAK and M. L. BEČAK, Cytogenetics 8, 247 (1969).

<sup>7</sup> 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<sup>1–3</sup>. Microchemical changes were also observed in lichens<sup>4</sup>, and induced tropic responses after shock treatment were hastened in euglenas<sup>5</sup>. 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<sup>2</sup>, was used for developing shock pressures. Unialgal cultures were maintained in nutrient solution indoors near a northwest window. Several *Spirogyra* species, *Chlamydomonas reinhardtii*, *Euglena gracilis*, *Closterium* sp. and *Cosmarium* sp. were subjected to shock pressures from 10 to 75  $\psi$  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<sup>6</sup> 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  $\psi$  for about 6 sec duration. Both were shocked within 10 min of each other to eliminate any complications from diurnal rhythms<sup>7</sup>. 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 atmosphere 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 arvense* were cut in half. Half of the sporebearing structures served as controls, whereas the other half was shocked at 60  $\psi$  with a pressure duration of 4–6 sec. Spores were collected overnight on paper then sprinkled on an enriched agar medium<sup>6</sup> and cultured similarly as the moss spores. Prior to shock treatment the strobilus was placed in a 35°C oven

<sup>1</sup> S. A. MURRAY and C. L. NEWCOMBE, Radiat. Bot. 10, 563 (1970).

<sup>2</sup> S. A. MURRAY, Experientia 26, 319 (1970).

<sup>3</sup> S. A. MURRAY, Am. J. Bot. 58, 119 (1971).

<sup>4</sup> S. A. MURRAY, Experientia 27, 11 (1971).

<sup>5</sup> S. A. MURRAY, Experientia 27, 757 (1971).

<sup>6</sup> Turtox Service Leaflet No. 44.

<sup>7</sup> R. BIEBL and K. HOFER, Radiat. Bot. 6, 225 (1966).

Table I. Mean values and standard errors for the acute angles between cell walls of moss protonemata. Results of the *t*-test for special comparisons are indicated

Culture age	Control	60 $\psi$	Group compared	P
12 day	80.79 $\pm$ 1.49	80.16 $\pm$ 1.54	C-60 12 day	0.8
40 day	79.05 $\pm$ 1.61	75.42 $\pm$ 1.73	C-60 40 day	0.2
Group compared	C-C	60-60		
P	0.5	0.05		

Table II. Gametophyte measurements (mm) of *Equisetum arvense* 14 days after shock treatment

	Control	60 $\psi$	<i>t</i>	P
Length	0.16	0.62	20.64	0.001
Width	0.16	0.24	6.20	0.001

Table III. Gametophyte measurements (mm) of *Polypodium polycarpa* at several post-shock periods

		Control	60 $\psi$	<i>t</i>	P
Rhizoid length		0.07	0.12	6.20	0.001
Gametophyte, 23	L	0.24	0.27	2.06	0.06
Days post-shock	W	0.09	0.10	1.34	N.S.
Gametophyte, 30	L	0.35	0.36	1	N.S.
Days post-shock	W	0.16	0.17	1	N.S.

to hasten sporangia dehiscence. Loose spores were discarded. Frequent subcultures were made to prevent crowding and to reduce fungal contamination. Gametophyte measurements were made with a filar micrometer microscope attachment.

**Results and discussion.** Algae. Species of *Spirogyra* containing 1 chloroplast were sensitive to low levels of shock (10–20  $\psi$ ), whereas species with 2 chloroplasts spiralled in opposite directions were apparently resistant to low shock pressures. In the sensitive kind the nucleus became acentric immediately after shock and the central cytoplasmic strands receded to the periphery. Cytoplasmic streaming diminished immediately after shock and was confined to the periphery of the cells. Cells appeared more normal in these respects about 3 weeks after shock. In those cultures which were conjugating, shocked filaments began about 1 week sooner, and the conjugation tubes were about  $1\frac{1}{2}$  times longer than normal, indicating softer cell walls after shock.

*Euglena* encysted immediately after a shock of 20  $\psi$ , remaining that way for at least a day, although some were still encysted 72 h after shock. This behavior is typical under unfavorable environmental conditions<sup>8</sup>. Only under conditions of intense light or heat (from the light) did the euglenas display a negative tropism, stretching out into their regular pear-shaped forms and swim away<sup>8</sup>.

*Closterium* sp. also showed signs of becoming dormant after a 20  $\psi$  shock in that the pyrenoids disappeared within 24 h (mostly within 1 h) after shock. According to LUT-

MAN<sup>9</sup> the disappearance of the pyrenoids is characteristic of the time of spore formation. Also, the cell wall was more sensitive than normal to cover slip pressure, rupture always occurring at the isthmus of the desmid. This would perhaps indicate an increased turgor in the cells.

Neither *Chlamydomonas*, shocked at 75  $\psi$ , nor *Cosmarium*, at 20  $\psi$ , responded to shock treatment. Both are somewhat spherical cells so that less area was exposed to shock.

Moss. A unique feature of moss protonemata is the frequent occurrence of oblique cell walls. Since lignin is known to be absent in mosses, the cell walls always remain plastic<sup>10</sup>. Therefore, pressures on the cells might displace the cell wall to some degree by apparent changes in cell turgor. The observations indicate that while wall obliqueness is not changed with age, shock sensitivity is a function of culture age, affecting older protonemata (Table I). The decreased wall angle indicates a decrease in cell turgor.

*Equisetum* gametophyte development. Gametophytes had undergone 2 divisions by the 6th day and were multicellular by the 14th day. At this time gametophytes from shocked spores were 4 times longer and  $1\frac{1}{2}$  times wider than the controls, a significant difference at the 0.001% level (Table II). Also, at this time photosynthetic lobes were observed in the shock group and 6 days later in the controls. The nutrient medium was not adequate to support further growth of these plants, as plants in both cultures had died by the 34th day even though the medium was not dried out.

Fern gametophyte development. Although spore germination was retarded in the shocked culture, by the 8th day the rhizoids of the shocked spores were significantly longer than the controls (Table III). Since there were no cross-walls in the rhizoids, the increased length was due to extension of the cell wall. The gametophytes in the shocked group were significantly longer than the controls for the first few weeks, whereas the width was similar for both groups (Table III). By the 35th day the gametophytes of both groups were similar in size and had developed into the familiar heart-shaped prothalli. About 6 weeks later sporogenous tissue developed in both groups.

Shock apparently has an effect on cell wall plasticity and turgor. Further, shock stimuli appear to hasten spore-forming and dormant conditions within the individuals. And, finally, sensitive organisms apparently recover from low shock stimuli. The cell shape might have some bearing on shock sensitivity, where spherical unicellular organisms are apparently resistant to shock stimuli to 75  $\psi$ .

**Résumé.** La turgescence de la cellule et la plasticité de sa paroi subissent des changements après le choc; la condition de latence et la sporogénèse sont accélérées. Les organismes blessés régénèrent. Il se peut que la forme de la cellule détermine la sensibilité à la pression du choc.

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<sup>8</sup> R. BRACHER, Ann. Bot. 33, 93 (1919).

<sup>9</sup> B. F. LUTMAN, Bot. Gaz. 49, 241 (1910).

<sup>10</sup> M. R. YOUNG, G. H. N. TOWERS and A. C. NEISH, Can. J. Bot. 44, 341 (1966).

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