Technical note

1. Princée, F. P. G. & de Boer, L. E. M. : **A new technique for obtaining chromosome preparations of small reptiles**

To date, the only way to obtain material for chromosome research on small reptiles, *i.e.* blood for *in vitro* leucocyte cultures (Gorman *et al.* 1967) or tissues with high mitotic indices, was to sacrifice the animal. Even techniques for *in vivo* stimulation of leucocytes introduced by Baker *et al.* (1971) and Taylor and Bolaños (1975) required the animals to be sacrificed because of the relatively large volume of blood necessary to yield enough metaphases.

The availability of a technique for chromosome research on small reptiles which does not require the sacrifice of the animals involved would be desirable for several reasons. Firstly, it would enable further studies to be undertaken on specimens whose karyotypes have been established. This is of particular interest in cases in which chromosomal differences constitute the main distinction between taxa. Secondly, in studies of species isolation mechanisms hybridization experiments using animals of known karyotypes would be possible. Thirdly, such a technique, by virtue of requiring less material to yield sufficient mitoses, would allow the application of several banding techniques on the chromosomes of one specimen.

During a project on specific and subspecific distinctions in *Anolis*, a technique was developed that meets the above requirement. This technique involves the *in vivo* stimulation of leucocytes based on the principle of boostering, a method commonly used in immunology. Using this technique very small volumes of blood (less than 0.05 ml) proved to be sufficient to yield large numbers of metaphases. Even in the smallest reptiles, those weighing only a few grams, such volumes of blood can be obtained without sacrificing the animals.

Small lizards such as *Anolis carolinensis* and *A. gingivinus* (Sauria : Iguanidae ; with body-weights varying between 2.5 and 5.5 g) and *Eublepharis macularius* (Sauria : Gekkonidae ; body-weights 20-30 g) were injected intra-peritoneally with a solution of phytohaemagglutinin-M (Difco, dissolved according to the instructions on the label) using a dosage of 0.018 ml per g body-weight, or with pokeweed mitogen (Gibco, dissolved according to the instruction on the label) using a dosage of 0.028 ml per g body-weight. In accordance with the method of Taylor and Bolaños (1975) this injection was repeated after 24 hours. However, whereas Taylor and Bolaños collected blood for chromosome preparation 72 hours after the first injection, in our experiments the animals were given a third injection between the 12 th and the 25 th day (Table 1 ; during the full experiment the animals were kept at room temperature, 20-22℃). Then, 72 hours after this booster-injection the tip of the lizard's tail was cut off and blood was collected using heparinized hematocrit tubes (diameter 1 mm ; the tail was cut at a position where its diameter was roughly the same as that of the tube). The blood was transferred into a 10 ml centrifuge tube and incubated at 37℃ for 1 hour with colchicine (2 ml of a 0.00015% solution of colchicine in RPMI 1640 tissue culture medium ; Difco). Hypotonic treatment and fixation in Carnoy's fixative were carried out according to the usual procedures employed for human and mammalian leucocyte cultures.

Three drops of the final cell suspension (with a total volume of 0.5 ml) were used per slide. Slides were air-dried in the usual way. For each animal the numbers of metaphases were counted in 5 slides, the average numbers per slide are shown in Table 1.

Given, that at least 15 slides could be prepared per animal, the numbers of metaphases available varied roughly from 100 to 1500. This is considerably more than could be obtained

Table 1

	mitogen used	day of 3_{rd} injection	ml blood collected	metaphases per slide
Anolis carolinensis ♀	PHA-M	22	0.050	90
A. carolinensis ♂	PKW	25	0.024	10
A. carolinensis ♂	PKW	19	0.037	5
A. gingivinus ♀	PHA-M	12	0.020	30
Eublepharis macularius ♂	PHA-M	12	0.016	20
E. macularius ♀	PHA-M	12	0.034	25

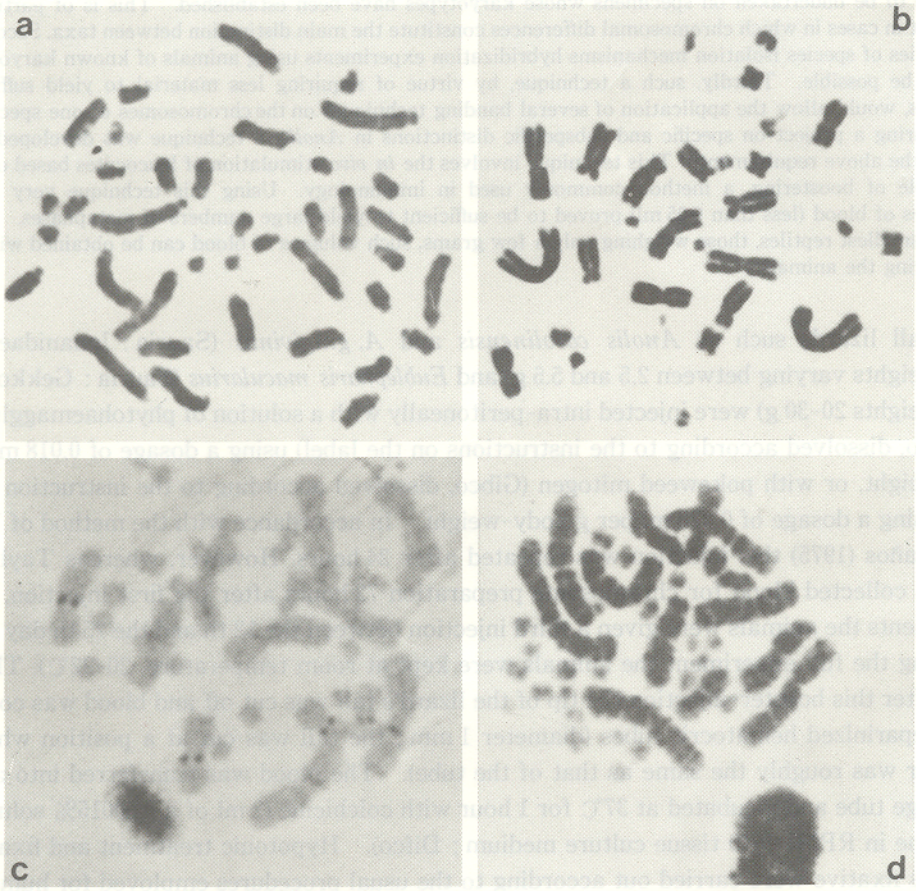

Figure 1. Metaphase plates of small reptiles obtained with the booster-technique described in the present communication: a. *Eublepharis macularius*, male (orcein stain); b. *Anolis gingivinus*, female (orcein stain); c. *Anolis carolinensis*, male (C-banding); d. *Anolis carolinensis*, female (G-banding).

using the technique of Taylor and Bolaños (1975) omitting the booster injection. In our experience this technique has seldom yielded more than 10 metaphases per animal (tested on several *Anolis* species, *Eublepharis macularius* and *Natrix natrix*). The availability of high numbers of mitoses of good quality allowed the application of various staining techniques, some examples of which are shown in Fig. 1. In no case did the animals show any apparent ill effects from the repeated injections of phytohaemagglutinin or pokeweed mitogen; this means that the animals could be used for further studies.

It should be stressed that the above experiments are fairly limited and that the results are preliminary. However, since we were struck by the richness of the preparations obtained it is our opinion that this technique deserves further study. In particular the influence of different mitogenic stimulators, the optimal day of giving the booster injection, and the temperature at which the animals are maintained during the experiment need to be studied in detail.

References

Baker, R.J., Bull, J.J. & Mengden, G.A. 1971. Experientia **27**: 1228-1229. Gorman, G.C., Atkins, L. & Holzinger, T. 1967. Cytogenetics **6**: 286-299. Taylor, R. & Bolaños, R. 1975. Revt. trop. **23**: 177-183.

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Original research notes

2. Yeh, Mau-shing*, Maekawa, Fumio** & Yuasa, Hiroshi** : **Chromosome numbers of *Dunbaria villosa* (Thunb.) Makino and *Dumasia truncata* Sieb. *et* Zucc.**

The chromosomes of two leguminous species of *Dunbaria* and *Dumasia* are newly described in this study. The somatic chromosome numbers are 22 in *Dunbaria villosa* and 20 in *Dumasia truncata*. This result suggests that the basic number of *Dunbaria* is X=11, and that of *Dumasia* is X=10.

Wild seeds of *Dunbaria villosa* (Thunb.) Makino were collected from Min-shan, province of Yunnan, China (Yuasa, (No. 193), 1979); and *Dumasia truncata* Sieb. *et* Zucc. were collected in Tokyo (Yuasa, (No. 1600), 1982). These two species belong to subtribes Cajaninae and Glycininae respectively of the tribe Phaseoleae (Leguminosae). Both species grow in temperate regions in Japan, and *Dunbaria villosa* is distributed also in Korea and China.

The root tips were pre-treated with water for 7 hrs. at 5℃ in a refrigerator. Acetic alcohol (1 : 3) were used for fixation, then aceto carmine squash and smear techniques were followed.

In the present study, 22 chromosomes, max.=1.6μ, min.=0.8μ, were observed in *Dunbaria villosa*, and 20 chromosomes, max.=1.3μ, min.=0.7μ, in *Dumasia truncata* (Figs. 1, 2).

Bir and Kumari (in Löve 1975) reported that *Dumasia cordifolia* Benth. has a haploid number of n=11 in the pollen mother cells. The result suggests that the basic number (X) is 10 or 11 in *Dumasia*, while it is 11 in *Dunbaria*, though no other species has even been studied in the latter genus.