were in 10^{-9} M and control solution respectively. However, the background or control migration was 41 ± 12.7% of cells in the absence of FMLP. Furthermore, the migration of PMN in the non-gradient presence of the highest concentration (10^{-7} M) of FMLP was 66 ± 8.0%. The difference between the migrations of cells in the non-gradient presence of FMLP and the complete absence of the factor is an indication of the chemokinetic effect of the factor and was therefore substantial in these experiments using the standard membrane.

Discussion. The present studies have shown that chemotactic migration of PMN towards FMLP can be demonstrated in a Boyden chamber without significant influence of either control (background) migration or chemokinetic effect of the agent by the use of a sparse-pore (0.1% of surface area) polycarbonate (Nuclepore) membrane, confirming earlier observations using *E. coli* as chemoattractant[15]. While the percentage of cells migrating to the lower surface is greater when a membrane having pores (5% of surface area; 'standard' Nuclepore membrane) is used, both the control migration and chemokinetic migration contribute to the total percentage of cells on the lower surface at the end of incubation. The present results show in addition, that migration of PMN is maximal in the highest gradient of concentration between the two compartments of the Boyden chamber, supporting earlier observations made using visual assay techniques[24]. However, the precise concentration of FMLP to which the cells respond is better defined using the new membrane in these experiments, since the migration of cells towards 10^{-7} and 10^{-8} M could not be distinguished with standard polycarbonate membrane, while with the use of the new membrane, maximal responses of PMN were demonstrated with 10^{-7} M FMLP.

This concentration (10^{-7} M) of FMLP providing a maximal chemotactic response across the new membrane is slightly higher than that reported by other authors[6, 25–27], but the discrepancy could be accounted for by the fact that many of the cells are initially separated at a distance from the widely separated pores, and the chemotactic factor forms a gradient on the upper surface of the membrane by diffusion or convection from the upper mouths of the pores. Thus many of the cells would be responding to a lesser concentration of FMLP. Such an explanation of the mechanism by which polycarbonate membranes are suitable for measuring chemotactic responses of PMN is different to the suggestion by Horwitz and Garrett[14] that the cells respond to a gradient formed only by diffusion along the intramembranous length of the pores.

Nevertheless, the new polycarbonate membrane is demonstrated to be superior to standard membrane for distinguishing chemotaxis from chemokinesis using 10^{-6} M and 10^{-8} M as well as 10^{-7} M FMLP, and thus in many circumstances, the former may be the membrane of choice for use in the Boyden chamber. The studies show further that comparisons of motility of PMN using the two membranes can be used to study the relative chemokinetic and chemotactic effects of factors affecting the locomotion of these cells.

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0014-4754/88/060518-04$1.50 + 0.20/0

A new chromosome number for *Bombina* (Anura, Discoglossidae)

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Received 11 December 1987; accepted 10 February 1988

Summary. The karyotype of a primitive discoglossid anuran, *Bombina maxima*, native to southwestern China, has $2n = 28$ chromosomes with 6 large and 8 small bi-armed homologous pairs. This is a higher chromosome number than described for other *Bombina* species, all of which have $2n = 24$.
Key words. Bombina maxima; Amphibia; Discoglossidae; karyotype.

The family Discoglossidae is an old[1] and primitive[2] group of Anura distributed in Europe and Eastern Asia. This group comprises about 15 species divided into four genera which are highly diversified with respect to morphology and ecology. *Alytes* are terrestrial, *Discoglossus* and *Bombina* are semi-aquatic, whilst *Barbourula* are permanently aquatic[3].

Chromosome numbers and chromosome morphology recorded in this family vary greatly[4]: $2n = 38$ in *Alytes*, $2n = 28$ in *Discoglossus* and $2n = 24$ in *Bombina*. (None of the two known *Barbourula* species have been karyotyped). High chromosome numbers, the presence of microchromosomes *(Alytes)* and telocentrics *(Alytes, Discoglossus)* as well as interstitial chiasmata in male meiotic configurations in all three genera are considered primitive characters in the karyotypes[5]. This observation is consistent with the primitiveness of the group.

In contrast to other discoglossids, the genus *Bombina* has bi-armed chromosomes typical of many advanced frogs. All of the three investigated species, namely *B. bombina*, *B. variegata* and *B. orientalis*, have $2n = 24$ chromosomes and their karyotypes are virtually identical[6–10].

In this paper we report a chromosome number $2n = 28$ for *B. maxima* and describe its karyotype. The present study supplements our studies of the relationships within the genus[11] and within the family[12].

Material and methods. Chinese fire-bellied toads, *B. maxima*, were obtained from a dealer, Mr van Mourik from Rotterdam, the Netherlands. The precise location of their collection is not known. They were collected in southern China, probably in Yunnan Province, and were part of a 1980 shipment that arrived in Europe through Hong Kong. A male and a female were induced to spawn by injection of 100 units of human chorionic gonadotropin into the dorsal lymph sac. The female laid 114 eggs all of which were fertilized. Heartbeat embryos (Gosner stage 19)[13] were kept in 0.05% colchicine solution for 12–24 h at room temperature. After removal of the ventral part filled with yolk, the embryos were treated with 0.075 M KCl solution for 20 min and fixed in Carnoy's fluid (methanol:glacial acetic acid, 3:1). Air-dried preparations were made from cell suspension prepared from minced embryos in 45% acetic acid and chromosome spreads were stained in 8% Giemsa stain for 2–5 min. In addition, testes of a single male were excised and subjected to the same treatment. However, colchicine treatment was omitted when preparing testis.

Results. In *B. maxima*, $2n = 28$ (table, figs 1–3). There are two groups of chromosomes differing greatly in size. Like in the other three species of *Bombina* investigated so far, there are 6 pairs of large metacentric or submetacentric chromosomes. However there are 8 pairs of smaller chromosomes (figs. 1–3, table) whilst other *Bombina* species have 6. We observed no telocentric chromosomes, microchromosomes

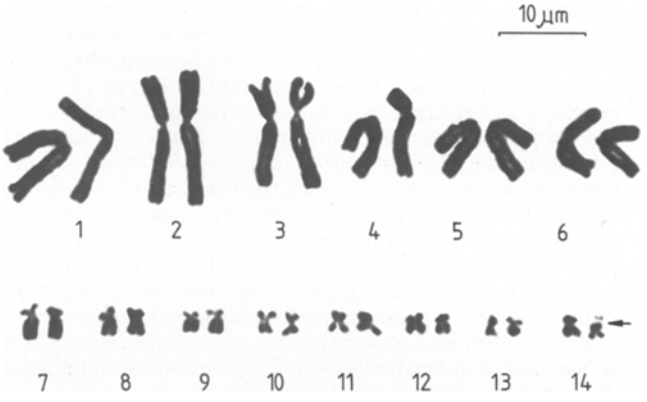

Figure 1. Karyotype of *Bombina maxima*. The arrow indicates the location of the nucleolus organizer region.

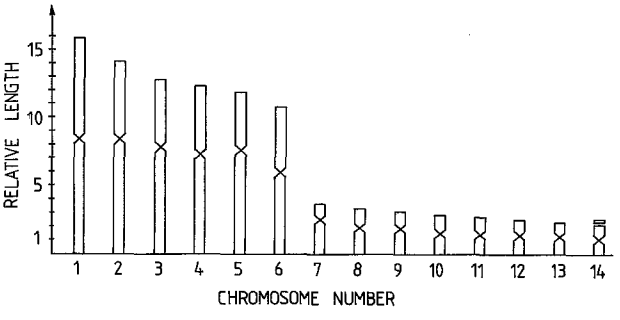

Figure 2. Idiogram of *B. maxima* based on the quantitative data presented in the table.

Quantitative characteristics of *Bombina maxima* chromosomes based on average of 10 well-spread mitotic cells. Arm ratio = length of long arm/length of short arm. Centromere index = length of short arm × 100/length of entire chromosome. Centromere position according to the quantitative definition of Levan et al.[18]; m = metacentric, sm = submetacentric

Chromosome pair No.	Relative length	Arm ratio	Centromere index	Centromere position
1	15.8	1.1	46.7	m
2	14.1	1.5	40.0	m
3	12.8	1.6	38.4	m
4	12.3	1.5	40.2	m
5	11.9	1.8	35.7	sm
6	10.8	1.3	43.5	m
7	3.7	2.0	33.2	sm
8	3.3	1.3	44.8	m
9	3.1	1.6	37.2	m
10	2.7	1.4	42.2	m
11	2.7	1.2	46.3	m
12	2.5	1.3	43.2	m
13	2.3	1.2	44.3	m
14	2.1	1.2	45.2	m

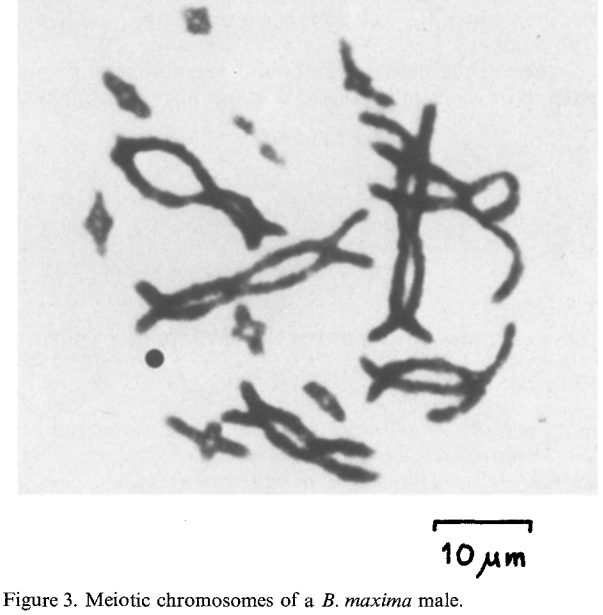

Figure 3. Meiotic chromosomes of a *B. maxima* male.

or supernumeraries in *B. maxima*. Chromosome No. 14 of *B. maxima* frequently had a secondary constriction (fig. 1), a marker for chromsome No. 7 in the other fire-bellied toad species[6–8, 10].

Heteromorphic chromosomes were not observed. We studied 8 embryos so it is unlikely that all of them were of the same sex. Male meiotic chromosomes (fig. 2) revealed interstitial chiasmata: one per chromosome arm. This is typical of the discoglossids.

Discussion. Apart from the chromosome number and the localization of the secondary constrictions, the chromosome set of *B. maxima* does not significantly differ in overall chromosome morphology from other species of *Bombina*. According to Morescalchi[5], karyological evolution in the discoglossids has led from a primitive, 'ascaphid' karyotype (i.e. *Alytes*) rich in acrocentrics with few microchromosomes, to a 'higher' type with few chromosomes all of which are bi-armed (i.e., *Bombina*). Karyotype evolution was accompanied by a loss of microchromosomes and acrocentrics resulting in a reduction in the total number of chromosomes. *B. maxima* ($2n = 28$) has a higher chromosome number than *B. bombina, B. variegata* or *B. orientalis*. These three species have $2n = 24$. However, as there are no acrocentrics in *B. maxima,* karyotypes of the latter species cannot be derived from the former by simple chromosome fusions. Therefore it is more reasonable to assume that *B. maxima* on one side and *B. bombina, B. variegata* and *B. orientalis* on the other represent two separate lineages within the genus, in which chromosome evolution proceeded independently. A common ancestor of all the *Bombina* species probably had a primitive karyotype with many acrocentrics.

To Mertens[14] and Bolkay[15] *B. maxima* was a close relative of *B. variegata,* whereas *B. bombina* was akin to *B. orientalis*. Present karyological data do not support such a relationship. Results of immunological[12] and electrophoretic[11] studies as well as artificial hybridization experiments[16] demonstrate that the European species, *B. bombina* and *B. variegata,* are the closest pair within the group. The East-Asian species are distantly related to them yet *B. orientalis* is closer to the European pair than it is to *B. maxima*. This relationship is also supported by the present chromosome analysis. *B. bombina, B. variegata* and *B. orientalis* have karyotypes practically identical to one another[7]. The split between *B. orientalis* and the European *Bombina* is estimated to have taken place in the Miocene, about 8–12 Myr ago[12]. Chromosome evolution in this lineage of small-bodied fire-bellied toads has been extremely slow and proceeded at a slow pace typical of the anurans in general[17]. If the same is true for the other lineage of large-bodied species from the *Bombina maxima* group, we predict that the other two Chinese species: *B. microdeladigitora* and *B. fortinuptialis* should have karyotypes very similar to, or indistinguishable from that of *B. maxima*.

Acknowledgments. We thank Dr J. W. Arntzen for help in obtaining the animals. This work was supported by a grant from the Polish Academy of Sciences (project CPBP 04.03) to J. M. S.

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0014-4754/88/060521-03$1.50 + 0.20/0

Insect photoperiodism: various ways of regulating univoltinism in lacewings (Planipennia: Chrysopidae)

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Received 2 November 1987; accepted 23 February 1988

Summary. Reproduction in *Tjederina gracilis* adults depends on short-day followed by long-day conditions. In *Nineta pallida*, development of overwintering first-instar larvae is retarded by short days; later on, that of second- and third-instar by long days. *Nineta flava* and *Chrysopa perla* prepupae automatically enter diapause, but in *N. flava*, photoperiod regulates diapause termination, and long days may retard egg laying.
Key words. Photoperiodism; dormancy; diapause; voltinism; overwintering; seasonal adaptation; Neuroptera; Chrysopidae.

The developmental strategies of insects in the temperate zone are variable, ranging from one generation every two years[1] up to 7 or 8 generations per year along the Mediterranean border of the tropical zone[2]. Whichever strategy of voltinism is chosen, the cycle has to be well synchronized with seasonal changes. This synchronization is most often established by perceiving variations in daylength, a perfectly repetitive geophysical signal. This cue, traditionally called 'photoperiod', is shown here to influence development in some Palearctic lacewings[3]. Four univoltine European species exhibiting different strategies were compared: *Chrysopa perla* (L.), *Nineta flava* (Scopoli), *Nineta pallida* (Schneider)