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Cytogenetic analysis of the pleurodine turtle *Phrynops hogei* and its taxonomic implications

Kent M. Reed[1], Brian G. Hanks[2], John W. Bickham[2], Anders G. J. Rhodin[3], Ira F. Greenbaum[1], Russell A. Mittermeier[4], Luiz P. Fedullo[5]

[1] Department of Biology, Texas A&M University, College Station, TX 77843-3258, USA
[2] Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-3258, USA
[3] Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138 and Orthopaedic Associates, P.C., Nichols Road, Fitchburg, MD, 01420, USA
[4] Conservation International, 1015 18th Street NW, Washington, DC, 20036, USA
[5] Fundação Rio Zoo, Quinta da Boa Vista, São Cristóvão — Rio de Janeiro, Brasil

Abstract. Conventional chromosomal preparations of the Brazilian sidenecked turtle *Phrynops hogei* indicate the karyotype of this species (diploid number 2n = 58; fundamental number FN = 64) is identical to that reported for other members of the genus. Electron microscopic analysis of whole-cell complements of synaptonemal complexes corroborated the karyotypic description and provided definitive resolution of the acrocentric condition of the microchromosomes. Additionally, this approach provided a continuous sequence of prophase I nuclei which enabled detailed description of chromosomal behavior from leptonema through diakinesis and the sequence of centriolar replication.

Introduction

Chromosomal evolution in the Testudines is exemplified by extreme conservatism at higher catagorical levels (Bickham, 1983; Bickham and Carr, 1983). For example, each of the families and subfamilies of hidden-necked turtles (suborder Cryptodira) is typically characterized by a unique karyotype and there is almost no karyotypic variation within genera and species (Bickham and Baker, 1979). Karyotypic variation in the side-necked turtles (suborder Pleurodira) is typified by a dichotomy of diploid numbers between the families Pelomedusidae (2n = 26-36, few acrocentric chromosomes; Bull and Legler, 1980; Rhodin et al., 1978) and Chelidae (2n = 50-64, 3n = 96, many acrocentric chromosomes; Bull and Legler, 1980; McBee et al., 1985). Based on the current understanding of the supraspecific taxonomy of the Chelidae, karyotypic variation within the family appears to be primarily at the generic level, with few recognized genera sharing the same karyotype. However, karyotypic comparisons

among turtle genera are often restricted by the limited resolution of conventional preparations, especially with regards to the microchromosomes. As shown recently for birds (Hale et al., 1988) and lizards (Hedin et al., 1990; Reed et al., 1990), analyses of whole-cell complements of synaptonemal complexes (SCs) provide increased resolution of both the morphology and pairing behavior of microchromosomes.

The chelid genus *Phrynops* comprises 12 currently recognized species, including *P. dahli, P. geoffroanus, P. gibbus, P. hilarii, P. hogei, P. nasuta, P. raniceps, P. rufipes, P. tuberculatus, P. vanderhaegei, P. williamsi*, and *P. zuliae* (Bour and Pauler, 1987; King and Burke, 1989). Karyotypic data previously have been reported for 5 of these species (table 1), four of which possess a karyotype with 2n = 58 and FN = 64. The one exception, *P. dahli*, has a greater fundamental number (FN = 66) due to the metacentric condition of chromosomal pair 8 (Bull and Legler, 1980). *Phrynops hogei* is the most osteologically divergent member of the genus, differing from all other South American chelids in the extent of the plastral bridge (Rhodin, unpubl. obs.). As in the Australian short-necked chelids, the axillary buttress reaches the second marginal bone.

Table 1. Reported karyotypic data on turtles of the genus *Phrynops*.

Species	*2n*	*FN*	*Source*
dahli	58	66	Bull and Legler, 1980
gibbus	58	64	McBee et al., 1985
geoffroanus	58	64	Bull and Legler, 1980
hogei	58	64	Present study
nasuta	58	64	Gorman, 1973
rufipes	58	64	McBee et al., 1985

Phrynops hogei, however, is a very rare and elusive animal (Mittermeier et al., 1980; Rhodin et al., 1982), and previous efforts at obtaining animals for study have been unsuccessful. Recently, a live specimen of *P. hogei* was obtained for cytogenetic analysis. In this study, mitotic and meiotic preparations of this specimen of *P. hogei* were examined as a representative description of the chromosomal phenotype in order to assess the taxonomic and karyological relationship of this species.

Material and methods

The adult male specimen of *P. hogei* was obtained from the Rio Paraiba drainage, Rio de Janeiro, Brazil. To increase meiotic and mitotic activity, the turtle was injected daily with 10 units of FSH and Concanavalin A for eight and three days respectively, prior to sacrifice. The spleen and part of the testicular material were used to prepare somatic and meiotic chromosomes (Bickham, 1975; Evans et al., 1964). The remaining testicular material was either immediately prepared for visualization of SCs or cryogenically preserved (Sudman, 1989) for later preparation. The chromosomal preparations were C-banded using a modification of Sumner's (1972) technique.

Synaptonemal complexes were prepared following the surface-spreading procedure of Counce and Meyer (1973) as modified by Moses (1977). Surface-spread SCs were stained with silver nitrate (Howell and Black, 1980), mounted on copper grids (100 mesh), and examined using a Zeiss EM 10C transmission electron microscope operated at 60 kV. Cells were assigned to meiotic stage based on the appearance of the nuclei and structures contained therein (Gillies, 1975; von Wettstein et al., 1984).

Results

The somatic karyotype (2n = 58, FN = 64; fig. 1) is comprised of three pairs of large biarmed chromosomes (pair 1, submetacentric; pair 2, metacentric; and pair 3, subtelocentric) and a graded series of 26 pairs of presumed acrocentric chromosomes. The eight largest chromosomal pairs can be considered as macrochromosomes. How-

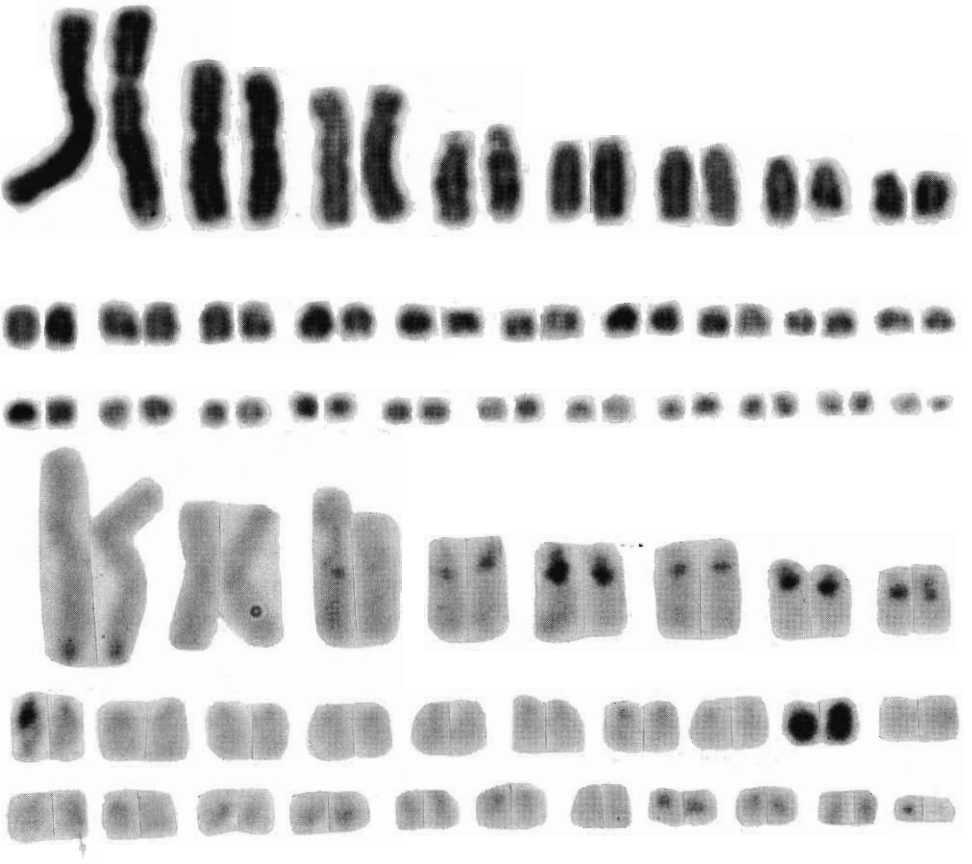

Fig. 1. Standard (top) and C-band (bottom) karyotypes of *P. hogei*. Chromosomes are arranged in descending order of size

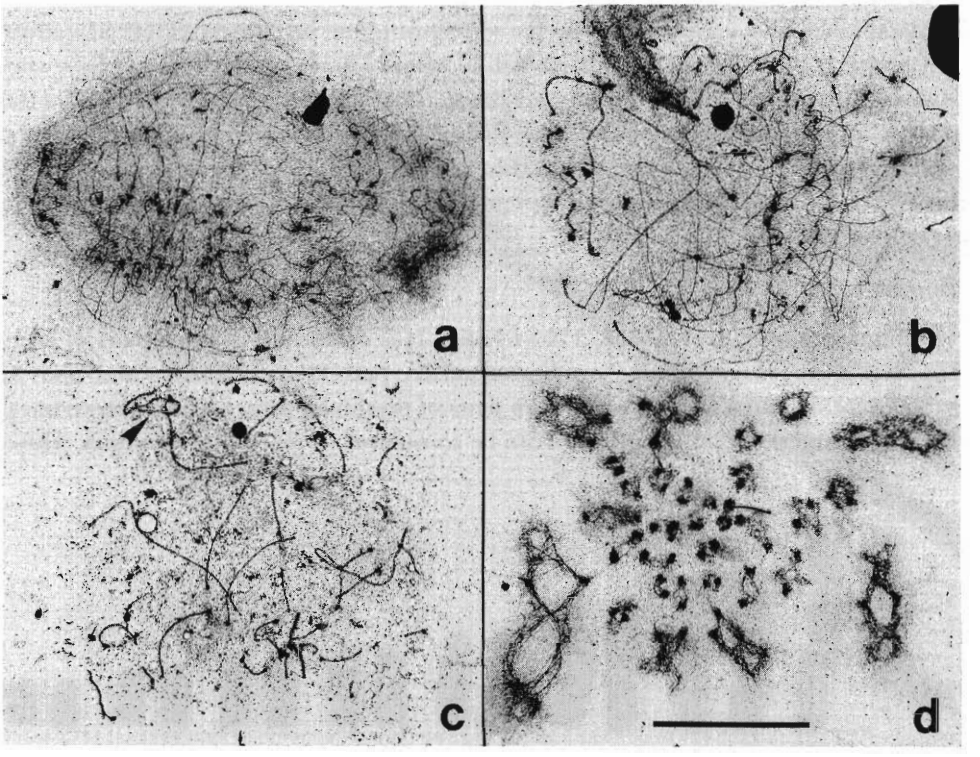

Fig. 2. Electron micrographs of silver-stained primary spermatocytes from surface-spread preparations. a) Late-leptotene nucleus just prior to synaptic initiation Axial elements appear fully formed with distinctly stained centromeric regions. b) Mid-zygotene nucleus with bivalents at various stages of synapsis c) Pachytene nucleus exhibiting complete synapsis of all bivalents except pair 1 (arrowhead). d) Late-diplotene/early-diakinesis nucleus Although the SCs have disassembled, centromeric regions and chromosomal infrastructures are visibly distinct Magnifications are equal, bar represents 10 μm

ever, because of the graded nature of the remaining chromosomes, the distinction between macrochromosomes and microchromosomes is somewhat arbitrary. C-band hereochromatin was primarily limited to the centromeric regions of most chromosomes and the telomeric region of the long arm of chromosome 1. Additionally, two microchromosomes, which formed a bivalent at diakinesis, stained entirely C-band positive. Sex-chromosomal heteromorphism was not observed.

Primary spermatocytes from each stage of prophase I (leptonema through diakinesis) were recovered in the surface-spread testicular preparations. Leptotene nuclei (fig. 2a) were characterized by the formation of electron dense axial elements each with a thickened (dark staining) region corresponding to the position of the centromere. Attachment plaques (the sites of attachment of the axial elements to the nuclear membrane) were visible at the axial termini. Complete axial elements were formed prior to the initiation of synapsis.

During zygonema, synaptic initiation of the biarmed chromosomes occurred at both telomeric regions and SC formation proceeded medially. Synapsis of the acrocentric chromosomes was variable with pairing being initiated at either one or both telomeric regions. Within individual cells, the bivalents exhibited various degrees of synapsis with no apparent relationship to size (fig. 2b).

At pachynema, the fully formed SCs of *P. hogei* (fig. 2c) appeared generally similar to those reported for other vertebrates (Hale et al., 1988; Moses, 1977; Reed et al., 1990; Solari, 1977, 1980). The SCs were somewhat contracted and could easily be traced over their entire lengths. Although lateral elements were prominent, central elements were not distinctly stained. The appearance of the centromeric regions and attachment plaques was similar to that observed in leptotene nuclei. The morphologies of the SCs (fig. 3) were consistent with the mitotic chromosomal data and confirmed the acrocentric condition of chromosomal pairs 4 - 29. Obvious heteromorphism indicative of a sex-chromosomal pair was not observed in any of the bivalents examined.

Post-pachytene primary spermatocytes were also recovered in the surface spread preparations. In diplotene nuclei, the SCs disassembled and the centromeric regions became increasingly dense. At the diplotene/diakinesis transition (fig. 2d), the chromosomal infrastructure was visible, the centromeric regions remained densely stained and chiasmata were frequently observed.

As reported in the South American chelid *Platemys platycephala* (Hale et al., 1989), centrioles were evident in the surface spread preparations of *P. hogei* (fig. 4). The centrioles of the primary spermatocytes were large (3-5 μm in length) and were visible in nuclei throughout prophase I (figs. 2 and 3). In cells not obviously disrupted by the surface spreading procedure, the centrioles usually associated in a linear end-to-end fashion or at angles of roughly 90°. Replicating centrioles were observed in several late-pachytene and diplotene nuclei. The inferred sequence of the replication of the centrioles is shown in fig. 4. During replication, the new "daughter" centrioles formed at one end of each procentriole (fig. 4b) and elongated as the parental centrioles dissociated (fig. 4c) during the progression of prophase I.

Discussion

The karyotype of *P. hogei* is indistinguishable from that reported for other members of the genus, except *P. dahli*. The occurrence of C-band heterochromatin primarily at the centromeric regions is consistent with the banding patterns reported for other pleurodirans (Bull and Legler, 1980; McBee et al., 1985). The presence of a heterochromatic pair of microchromosomes is a feature common to both suborders of turtles (Bickham and Baker, 1976; Bickham et al., 1983; Bull and Legler, 1980; Sites et al., 1979*b*).

Bickham (1976) discussed the utility of meiotic analyses in turtle karyology. Whereas examination of meiotic chromosomal configurations has proven useful

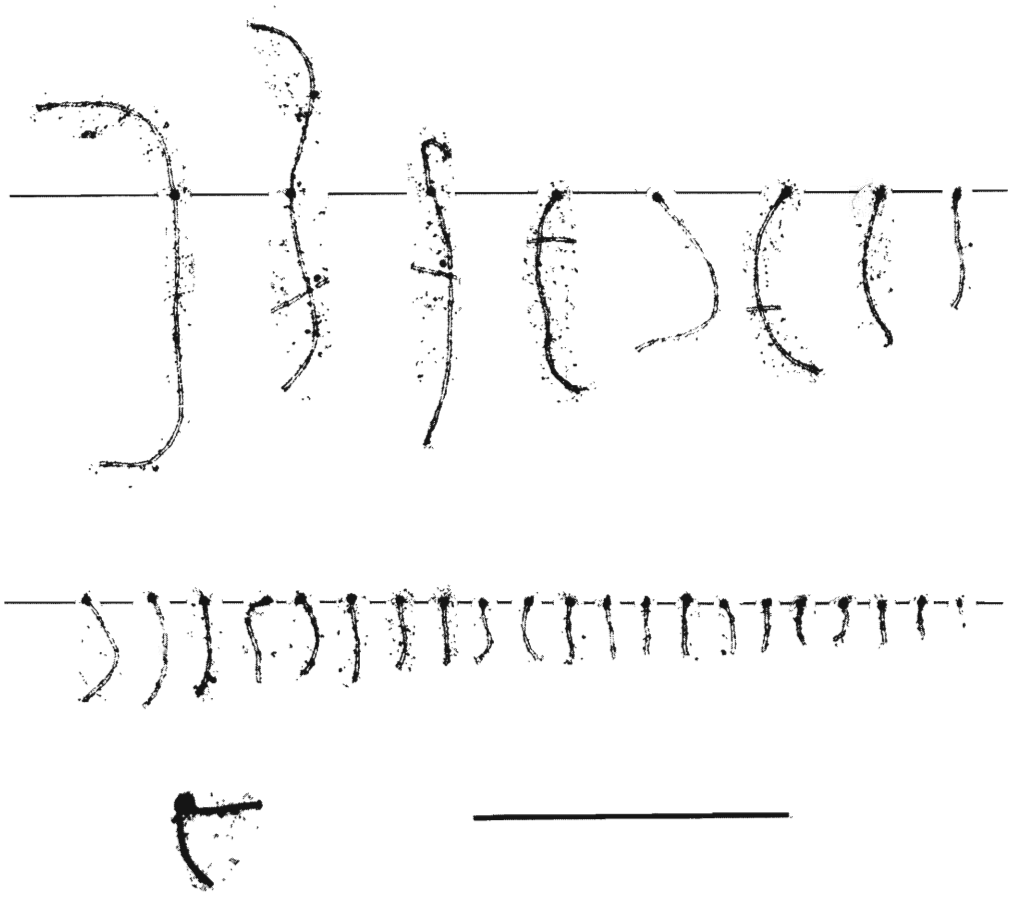

Fig. 3. Synaptonemal complex karyotype constructed from an electron micrograph of a pachytene nucleus. The SCs are arranged in descending order of size and placed with centromeric regions on the horizontal lines. The centrioles of this cell are included for size comparison. Bar represents 10 μm.

(Bickham, 1983), such studies were often restricted by the limited resolution of conventional preparations. The present observations on *P. hogei*, demonstrate the applicability of SC techniques to turtle cytogenetics. Surface spreading provides large numbers of nuclei amenable to electron microscopic analysis. In silver-stained nuclei, the axial elements, SCs with associated kinetochores and attachment plaques, and the centrioles are easily visualized. Individual bivalents can be identified based on size and centromere position allowing for examination of the orientation and synaptic behavior of specific chromosomal pairs.

For the most part, analyses of surface-spread SCs have not included the examination of leptonema. The typically reduced number of leptotene nuclei in these prepara-

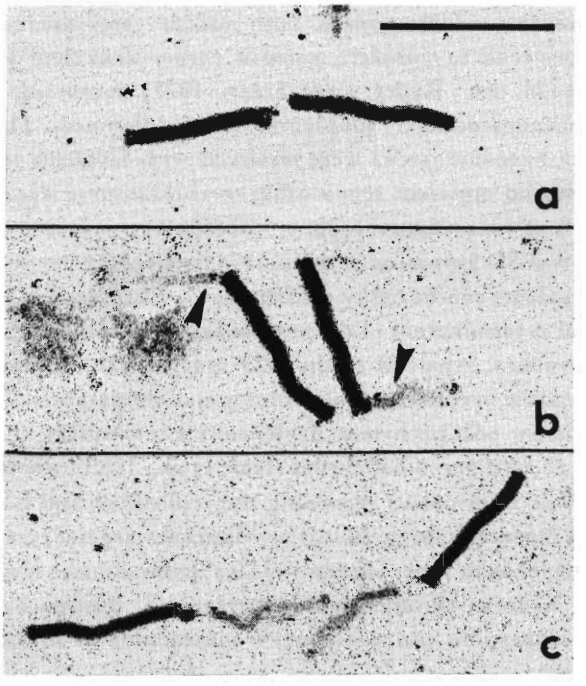

Fig. 4. Inferred sequence of centriolar replication during prophase I a) Centriolar pair in linear association. b) Centriolar pair initiating replication. The lighter staining "daughter" centrioles (arrowheads) appear at the ends of the procentrioles. c) Centriolar pair near the completion of replication. Daughter centrioles lie medially and are nearly equal in length to the parentals. Magnifiations are equal, bar represents 5 μm.

tions may be an indication of the relative duration of this meiotic stage (Moses, 1977). Additionally, the extent of development of the axial elements during leptonema differs substantially among diverse organisms. The synthesis of complete axial elements in leptonema of *P. hogei* represents one extreme of this variation. In many organisms, the axial elements are absent or incomplete until after synaptic initiation (Moses, 1977; von Wettstein et al., 1984). The presence of complete axial elements in presynaptic nuclei facilitates the examination of the nuclear organization.

Zygonema is potentially the most informative stage of prophase I. Examination of nuclei at this stage provides data on the temporal sequence of bivalent formation, including the number and position of synaptic initiation sites, and the directionality and relative rate of synapsis. The analysis of zygotene nuclei from *P. hogei* indicates that the pattern of synpasis differs between the biarmed and acrocentric chromosomes. Differences in the initiation and directionality of SC formation have been implicated in the maintenance of certain types of chromosomal rearrangements (Greenbaum et al., 1986; Hale, 1986).

In those organisms which possess both macro- and microchromosomes, the microchromosomes tend to complete synapsis earlier than their larger counterparts (K. M. Reed unpubl. obs.; Ryder, 1989; Solari, 1977). Assuming similar rates of SC formation, this phenomenon may simply reflect size differences. This was not the case in *P. hogei* in that zygotene nuclei were observed with bivalents in various stages of SC formation with no apparent relationship to chromosome size. These differences may be the result of either asynchronous initiation or synapsis.

The completion of SC formation at the start of pachynema results in nuclei in which the individual bivalents can be easily distinguished. Examination of these nuclei provides unequivocal determination of the morphology and centromeric position of even the microchromosomes. Analysis of the SCs may reveal chromosomal heteromorphisms not discernable in conventional chromosomal preparations (Reed et al., 1990).

As with leptonema, post-pachytene nuclei were less frequent in the surface-spread preparations. In *P. hogei* and other turtles (Hale et al., 1989), the latter stages of prophase I can provide information regarding the replication and behavior of the centrioles. Although distinctly visible in turtles, centrioles are not routinely visualized in surface-spread nuclei from other vertebrates (for discussion see Hale et al., 1989). As in conventional meiotic chromosomal preparations, post-pachytene nuclei from surface-spread preparations can also provide information as to the position and frequency of chiasmata.

Chromosomal banding techniques have been effective in determining homologies for use in systematic comparisons. However, because of the reduced resolution and difficulty in determining centromeric position, the microchromosomes have either been eliminated from such comparisons or included only in determination of diploid numbers (Bickham, 1975; Bickham and Baker, 1976; Bickham et al., 1983; Bickham and Carr, 1983; Sites et al., 1979*b*). The increased resolution of the SCs provides a superior means for determination of the number and morphology of the microchromosomes. The surface-spreading technique is simple to use and could be applied to investigate chromosomal heteromorphisms, such as the sex-chromosome systems reported in *Acanthochelys, Platemys, Siebenrockiella,* and *Staurotypus* (Bull et al., 1974; Carr and Bickham, 1981; McBee et al., 1985; Sites et al., 1979*a*), and the chromosomal mosaicism in *Platemys* (Bickham et al., 1985).

Despite its osteological divergence, *P. hogei* shares karyological affinities with the other currently recognized species of *Phrynops*. Lacking an appropriate outgroup comparison, the question remains whether this karyotype is synapomorphic or symplesiomorphic for the genus. Clearly, additional characters need to be examined to determine if *P. hogei* is sufficiently divergent to warrant generic status.

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