Synaptonemal Complex Analysis of Sex Chromosome Pairing in the Common Ground Skink, *Scincella lateralis* (Sauria, Scincidae)

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Techniques for the electron microscopic visualization of synaptonemal complexes were used to investigate the XY and X,X,Y sex chromosomal systems in *Scincella lateralis*. Synaptonemal complex analyses revealed differences in the pattern of synapsis between the sex chromosomal and autosomal bivalents within each cytotype, and differences in sex chromosomal pairing between the cytotypes. Sex chromosomes of the XY cytotype synapsed in homology to form a bivalent. Sex chromosomes of the X,X,Y cytotype synapsed in a trivalent configuration, with localized regions of nonhomologous synapsis. These data support previous hypotheses that the X,X,Y condition is derived from the XY system by translocation of the Y chromosome to one homolog of the smallest macrochromosomal pair, and implicate centromeric inactivation in the fusion event.

The common ground skink, *Scincella lateralis* (the specific name used here follows Brooks, 1975), displays well documented male heterogamety, with conspicuous size dimorphism between the sex chromosomes (Wright, 1973). With one exception, populations of *S. lateralis* surveyed from localities throughout the southeastern United States are characterized as having a diploid number (2N) of 30, with an absence of autosomal heteromorphisms, and a heterogametic XY sex chromosome system (Wright, 1973). A distinctive population from the Edwards Plateau in Texas, however, has a stable X,X,Y sex chromosome system. Males in this population have a diploid number of 29, while females retain the 2N = 30 condition. This X,X,Y sex chromosomal system is hypothesized to have been derived from the more widespread XY condition by the fusion of the ancestral Y chromosome to a metacentric macrochromosome (Wright, 1973).

The majority of studies concerning patterns of meiotic chromosomal pairing behavior in lizard species have utilized hypotonic-treated, nondifferentially stained testicular preparations (Bickham, 1983). The accuracy and breadth of such studies are restricted typically by the limited resolution achieved in these conventional pachytene and diakinet preparations. In contrast, the surface-spreading technique (Counce and Meyer, 1973) for visualization of whole cell complements of synaptonemal complexes (SC) provides excellent resolution of chromosomal pairing. The SC is a ribbonlike proteinaceous structure, required for chromosomal pairing during zygonema and pachynema of meiotic prophase I. The synapsed axes of meiotic autosomes and sex chromosomes are represented by the paired lateral elements of the SC. Silver-stained lateral elements are easily resolved by electron microscopy, and proteinaceous material associated with centromeric regions can often be distinguished as darkly stained structures on the SC lateral elements (Moses, 1977; Hale
et al., 1988). As such, SC analyses provide for a simple linear representation of chromosomal pairing behavior and orientation.

Although SC data have been applied widely to cytogenetic analyses of both mammalian and avian species, this technique has yet to be utilized to address questions relating to reptilian cytogenetics. Herein, we report an SC analysis of the pairing behavior of both autosomal and sex chromosomal elements in male individuals representing the XY and X1X2Y cytotypes of S. lateralis. The data demonstrate clearly the applicability of the SC technique to cytogenetic study of reptiles, and the results allow for an initial evaluation of sex chromosome pairing in reptiles.

**Methods and Materials**

Specimens of S. lateralis were collected during April and May 1989, from natural populations at two localities in Texas. Data from two individuals from an XY (hereafter referred to as X1Y1) population in Sabine County were compared with those from three animals from the X1X2Y (hereafter referred to as X1X2Y2) population on the Edwards Plateau. To assure the availability of spermatogenically active individuals, collection of specimens coincided with the reported periods of peak reproductive activity and testicular development for S. lateralis (Johnson, 1953; Mather, 1970).

Microscope slides were prepared by dipping them into a Coplin jar containing a 0.6% (w/v) solution of plastic (Falcon Optilux petri dish) dissolved in chloroform. Nail polish was used to seal the edges of plastic coating on the desired side of the slides. The coated and sealed slides were then allowed to dry.

Preparation of testicular material for visualization of SC was accomplished using the surface-spread technique of Counce and Meyer (1975) as modified by Moses (1977). Seminiferous tubules from a single testis of each individual were removed from the tunica albuginea and placed in a spot plate depression containing Eagle's Minimum Essential Media (MEM). The testicular material was minced using a scalpel blade and suspended in 10 ml of MEM. The cell suspension was centrifuged at 150 × g for 5 min, the supernatant discarded, the cell button resuspended in 1.5 volumes of fresh MEM, and the testicular solution placed on ice.

A micropipette was used to touch a minute volume of the testicular solution onto the convex surface of a 0.5% (w/v) NaCl solution. Meiotic cells, now spread on the surface of the saline solution (surface-spread), were transferred onto plastic coated slides by slowly lowering the slide onto the convex NaCl surface. The adhered cells were fixed for 5 min in a Coplin jar containing 50 ml of a 4% paraformaldehyde solution (adjusted to pH = 8.2 with 10% formic acid and 1.0 N NaOH) with 0.03% Sodium Dodecyl Sulfate (SDS). Slides were then fixed for an additional 5 min in 4% parafor-
maldehyde without SDS (pH = 8.2), washed for 8–10 sec in a 0.4% Photoflo (Kodak) solution, and allowed to dry. The method of Howell and Black (1980) was used to silver stain the surface-
spread preparations. In preparation for electron microscopic (EM) analysis, the plastic sur-
rounding the desired cell area was etched with a diamond-tipped scribe, and floated off the slide into a dissecting bowl filled with distilled water. Fontax forceps were used to mount copper grids (100 mesh) onto the floating plastic, which was removed from the water surface with a strip of wax film. Electron microscopic analysis of SC was performed using a Zeiss EM10C transmis-
sion electron microscope at an accelerating voltage of 60 kV.

In addition to the methods described above, the cryogenic technique developed by Sudman (1989) was used to preserve testicular material for processing at a later date. Testicular ma-
terial (minced in MEM as above) was buffered in a 1:9 suspension of glycerol: Ham’s F-10 cell culture media, placed in cryogenic tubes, and frozen in either liquid nitrogen or an ultra-low freezeer (–80 C). For surface-spreading, the frozen material was thawed, resuspended in MEM, and processed as described above.

Chromosomal preparations of testicular ma-
terial for light microscopic (LM) observation of meiotic stages (cells at diakinesis/metaphase I and metaphase II) were accomplished using a modification of the technique of Evans et al. (1964). Seminiferous tubules from the other testis were macerated in MEM, suspended in 10 ml of aqueous 1% sodium citrate, and incubated for 20 min at 37 C. Following incubation, the preparation was centrifuged at 250 × g for 2 min, the supernatant decanted, and the pellet resuspended in 5 ml of freshly prepared Carn-

oy’s fixative (3:1 absolute methanol: glacial acetic acid). The testicular suspension was cen-
trifuged and washed in Carnoy’s fixative three times, dropped onto wet slides, air-dried, and stained with Giesma (2% in 0.01 M phosphate buffer, pH 7.0).

Results

Meiotic preparations of diakinesis/metaphase I cells from X1Y1 specimens contained six macrochromosomal bivalents, eight microchromosomal bivalents, and a heteromorphic biva-

lent assumed to represent the sex (X1Y1) chro-

mosomes (Fig. 1a). Examination of metaphase II cells revealed the expected 6+1+8 and 6+0+9 secondary spermatocytes (6+1+8 no-
mencature represents six macrochromosomes, the intermediate-sized X1 chromosome, and eight microchromosomes; Y1 is a microcho-

mosome [Wright, 1979]). For the X1X2Y2 spec-

imens, cells in diakinesis/metaphase I revealed five macrochromosomal bivalents, eight micro-

chromosomal bivalents, and a large trivalent, assumed to represent the sex (X1X2Y2) chro-

mosomal elements (Fig. 1b). Analysis of meta-

phase II cells revealed no evidence for an un-

balanced assortment of sex chromosomes, with both 6+1+8 (5 macrochromosomes and large X2 element; intermediate X1) and 6+0+8 (5 macrochromosomes and large Y2 element) sec-

ondary spermatocytes observed.

The morphology of skink SC was comparable with that previously described as “convention-
al” for both mammals and birds (Moses, 1977; Hale et al., 1988) (Fig. 2). Chromosomal axes represented by lateral elements of the SC were well resolved, and argentophilic (i.e., silver-

stained) centromeric regions appeared to overlap and span the lateral elements of synapsed bivalents. The location of these densely-stained structures corresponded with expected centro-

meric positions, as based on the description of the mitotic chromosomes (Wright, 1973). Twisting of the SC along their axes was often observed, as was stretching due to surface ten-

sion resulting from the spreading procedure.

Results of the SC analysis of surface-spread cells from the X1Y1 specimens were consistent with those of meiotic chromosomal prepara-
tions. Fifteen SC bivalents were discernible in 21 whole-cell complements examined. Six mac-

rochromosomal bivalents and the largest micro-

chromosome were metacentric, whereas seven microchromosomal bivalents were clearly ac-

rocentric (Fig. 2). Surface-spread cells also had an heteromorphic submetacentric bivalent, for which the morphology differed from that of the other elements (Fig. 3a–c). The consistent syn-

aptic deviations observed for this SC bivalent lead us to identify it as the sex chromosomal (X1Y1) pair. In the X1Y1 SC configurations (Fig. 3a–c) the Y1 chromosome was submetacentric, rather than acrocentric as proposed by Wright (1973). Synapsis between the X1 and Y1 ele-

ments occurred along approximately one-half of the length of the X1 chromosome. The distal region of the X1 long arm remained unpaired. In several cells, the unpaired axis of the X1 chro-

mosome appeared to “loop-out,” and there was evidence of fold-back pairing along the length
of this sex chromosome (Fig. 3a–c). Our data do not allow a robust evaluation of differences in the temporal sequence of X,Y, sex bivalent pairing relative to autosomal elements.

Analysis of 24 surface-spread cells from X,X,Y,Y specimens indicated synopsis of 13 autosomal bivalents (five macrochromosomal and eight microchromosomal) and a single trivalent. The trivalent was observed in all cells which contained a full diploid complement, and is considered to represent pairing of the sex chromosomal (X1, X2, Y2) elements. Some trivalent configurations revealed asynapsed regions identified as the unpaired segments of the X1 and X2 chromosomes (Fig. 3d). As trivalent synopsis progressed, the unpaired regions became fully paired along most of their lengths (Fig. 3e). This late pairing is assumed to represent synopsis between nonhomologous regions of the ancestral X1 chromosome and the autosomal (X2) element. In all observed trivalent configurations, the X2–Y2 portion of the synapsed trivalent had a centromeric region which spanned both lateral elements. Alternatively, the centromeric region within the X1–Y2 portion of the trivalent was less intensely stained, and apparently associated only with the lateral element of the X1 chromosome (Fig. 3d–e).

**DISCUSSION**

Two hypotheses have been forwarded to explain the initial differentiation of sex chromosomes from originally homomorphic, but sex determining, chromosomes. One model cited extensively implicates a marked reduction in recombination between homologous chromosomes due to a primary structural rearrangement (Ohno, 1967, 1979; Bull, 1980). Subsequent to this structural rearrangement there is a loss of functionality in regions not involved in sex determination, due to secondary heterochromatinization or loss of genetic material. Alternative hypotheses implicate a process of primary sex chromosome differentiation via incorporation and amplification of unique satellite DNA sequences (Singh et al., 1976, 1980; Olmo et al., 1984), with structural rearrangements of secondary importance in the development of sex chromosome heteromorphism.
Fig. 3. Electron micrographs and schematic representations of sex chromosome pairing configurations for both sex chromosome cytotypes in *Scinella lateralis*. a). X\(_1\)Y\(_1\) sex chromosome pair during early synopsis. The unpaired portion of the X\(_1\) is "looped-out" and a region of fold-back pairing is apparent. The non-centromeric end of X\(_1\) is in association with the distal end of Y\(_1\). Aligned centromeres are indicated by the arrow. b). X\(_1\)Y\(_1\) bivalent at a later stage of synopsis. A short region of X\(_1\) fold-back pairing is apparent. Aligned centromeres are indicated by the arrow. c). X\(_1\)Y\(_1\) bivalent with a configuration in which the unpaired axis of the X\(_1\) chromosome appears "clumped" at the distal end. Aligned centromeres indicated by arrow. d). Incompletely paired X\(_1\)X\(_2\)Y\(_2\) sex chromosome trivalent. X\(_1\)-Y\(_2\) synopsis is interpreted to exist on the upper fork of the sex trivalent. Centromere of X\(_1\) chromosome is at large arrow. X\(_1\)-Y\(_2\) synopsis occurs on the opposite side of the configuration, with aligned centromeres indicated by the smaller arrow. The Y\(_2\) chromosome is stretched between regions of synopsis. Both the X\(_1\) and X\(_2\) chromosomes have unpaired axes to the left of the trivalent fork. e). Fully-synapsed X\(_1\)X\(_2\)Y\(_2\) trivalent. Y\(_2\) extends in a vertical plane, with proposed X\(_1\)-Y\(_2\) pairing at the upper fork of the trivalent. Centromere of X\(_1\) chromosome is at large arrow. X\(_1\)-Y\(_2\) pairing occurs below the trivalent fork, with aligned centromeres indicated by the smaller arrow. Hypothesized region of non-homologous X\(_1\)-X\(_2\) pairing occurs left of the trivalent fork. Y\(_2\) chromosome is stretched in the middle, between regions of synopsis (bar = 1\(\mu\)m).

Under both models, the processes of X-Y crossover suppression and Y chromosome degeneration lead to characteristic sex chromosome differences, including differences in chromosome size (and/or shape), gene content, and chromatinn content (Bull, 1983).

The morphological heteromorphism of the X\(_1\)Y\(_1\) chromosome pair in *S. lateralis* suggests a possible high level of genetic differentiation (Bull, 1980). However, this morphological difference is confounded by complete synopsis of the Y\(_1\) to the X\(_1\) (Fig. 3a–c). Also, the synapsed region of this sex bivalent does not differ in general appearance from fully-synapsed, homologous regions of autosomal SC elements. Although there is no well-defined microscopically detectable difference between homologous and nonhomologous regions of SC (Solari et al., 1988), we observed no asymmetrical twisting or misaligned centromeres suggestive of nonhomologous pairing for the X\(_1\)Y\(_1\) bivalent. The retention of full synopsis between segments of the X\(_1\) and Y\(_1\) chromosomes suggests considerable homologous pairing between these elements, although data on sequence composition are needed to adequately test this supposition (Jones and Singh, 1982; Lloyd et al., 1989).

Assuming that the X\(_1\)Y\(_1\) condition is derived from an initially homomorphic pair of autosomes, the observation of "differential" regions
suggest that either the Y1 chromosome has lost genetic material, or that there has been an addition of chromatin (possibly heterochromatin) to the X1 chromosome (Fig. 4a). While distinguishing between these hypotheses remains problematic, the complementary use of SC data, in addition to chromosomal banding and molecular genetic data, may prove useful in an unequivocal evaluation of the mode of X1Y1 sex chromosomal divergence in \textit{S. lateralis}.

The SC data from X1X2Y2 males support Wright's (1973) hypotheses on the evolutionary relationship between the sex chromosomal cytotypes of \textit{S. lateralis}. The X1X2Y2 condition appears to be the consequence of a translocation of the submetacentric microchromosomal Y1 to an autosome, resulting in a derived Y2 chromosome with partial pairing homology to both the X1 and the autosomal (X2) chromosomes (Fig. 4b). Based on size comparisons between the primitive (X1Y1) and derived (X1X2Y2) sex chromosomes, the hypothesized Y-autosome translocation involves the smallest macrochromosomal pair (designated here as autosome \#6 following Wright, 1973).

Cytogenetic studies of trivalent pairing have indicated that nonhomologous synapsis is often characteristic of SC formation in late pairing trivalent axes (Moses et al., 1979; Bogdanov et al., 1986; Solari et al., 1988). The observed pairing delay has been attributed to mechanical characteristics of trivalent synapsis, and may represent a process termed synaptic adjustment (Moses and Poorman, 1981; Carpenter, 1987). We hypothesize that the delayed and apparently non-homologous association of portions of the X1 and X2 chromosomes within the X1X2Y2 sex trivalent represents a form of synaptic adjustment. Synaptic adjustment is a process characterized by the loss of the requirement for homology in late pairing chromosomal regions. This process results in fully synapsed SC segments with aligned axial telomeres, and thus reduces the amount of unpaired chromatin in cases where length inequalities or nonhomologous chromosomal segments exist. Such a mechanism has been hypothesized to result in saturation of DNA pairing sites, thus providing for a more stable meiotic configuration (Miklos, 1974). As such, synaptic adjustment of unpaired chromosomal axises may be essential for normal meiotic or postmeiotic development.

The structural rearrangement proposed for the derivation of the Y2 chromosome implicates the fusion of two chromosomes at their telomeric ends. When two biarmed (in this case submetacentric) chromosomes fuse in this manner, the resulting element is expected to be dicentric (Hsu et al., 1975), and its proper functioning would require inactivation of one of the two centromeres. Differential staining intensity of the centromeric regions in the X1X2Y2 trivalent provides some indirect evidence for a centromeric inactivation event in the evolution of the Y2 of \textit{S. lateralis}. This lack of centromere differentiation on one lateral element may implicate an important structural difference between the active and inactive centromeric regions of the Y2 chromosome (i.e., presence or absence of a kinetochore plate).

The application of the SC technique, primarily for avian and mammalian species, has contributed considerably to current understanding of heteromorphic sex chromosome systems. Studies of the mammalian XY (Hale and Greenbaum, 1986 and references therein) and of the avian ZW chromosomes (Solari, 1977; Solari et al., 1988) have revealed that SC configurations of heteromorphic sex chromosomes are distinguished from those of autosomal elements by differences in pairing behavior, axial length equality, staining intensity (i.e., heteropycnosis), and relative timing of synaptic initiation. Heteromorphic sex chromosomes are
typical of these vertebrate classes and can be hypothesized to have evolved once in each class (Bull, 1983).

Although not rare, heteromorphic sex chromosomes are the exceptional condition in the Reptilia and may have evolved independently in several lineages (Peccinini-Seale, 1981; Becak, 1983; Olmo, 1986). The heteromorphic sex chromosomes of reptiles are not, therefore, necessarily expected to have general similarities to those of either birds or mammals. Phylogenetic studies of reptile lineages with several stages of X-Y heteromorphism, ranging from cases of morphologically indistinguishable or slightly heteromorphic sex chromosomes (Bull, 1978), to cases of extreme XY heteromorphism (Gorman et al., 1967; Cole et al., 1967; Pennock et al., 1969), may provide data relevant to an improved understanding of the initial differentiation and evolution of sex chromosomes in lizards.

There are numerous examples of reptilian taxa with intra- and/or interspecific chromosomal heteromorphisms (King, 1981; Bickham, 1983). These cases of widespread chromosomal variation suggest the occurrence of meiotic mechanisms which may serve to prevent chromosomal underdominance (King, 1981). An adequate evaluation of the biological significance (i.e., potential as an isolating mechanism) of structural heterozygosity should include, among other things, in-depth analyses on the meiotic consequences of chromosomal heteromorphism (Sites and Moritz, 1987). The cytogenetic manifestations of chromosomal rearrangements such as pericentric and paracentric inversions (inversion loop formation) and centric fusion-fission events (trivalent formation) are most effectively studied during the pairing stages of meiosis. If the patterns and processes of chromosomal synopsis (i.e., meiotic system) have been described for a structural rearrangement in a given lineage, the potential for production of meiotic abnormalities such as aneuploidy or duplications/deficiencies can be assessed (Greenbaum et al., 1986).

The application of the SC technique has been particularly informative for the study of chromosomal mutations such as pericentric inversions, centric fusions, translocations, and heterochromatic heteromorphisms. The study of these structural rearrangements in lizard taxa should be as equally informative (King, 1979; Sites, 1983; Moritz, 1984). Analyses of the synaptic behavior of additional X, X,Y systems (Gorman and Atkins, 1966; Gorman et al., 1967; Lamborot and Navarro-Suarez, 1984), supernumerary chromosomes (Bezy et al., 1977) and accurate determinations of reported microchromosomal variation (Blake, 1986; Lamborot and Alvarez-Sarret, 1989) would also prove beneficial. This study exemplifies the applicability of synaptonemal complex methodology to laboratory studies of reptilian cytogenetics. The cryogenic method (Sudman, 1989) provides the field herpetologist with an additional research tool, as it allows for the collection and preservation of testicular material under field conditions.

**Material Examined**

The specimens used in this study are deposited in the Texas Cooperative Wildlife Collection (TCWC), College Station, Texas. (Texas: Angelina County (Co.), 7.5 km E of Zavalla (TCWC 0066190, 0066191); Hays Co., 8 km E of Wimberly, Fern Bank Springs (TCWC 0066192, 0066193, 0066194).

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Synaptonemal Complex Analysis of Sex Chromosomes in Two Species of Sceloporus

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Silver-stained synaptonemal complexes were analyzed to examine chromosomal pairing in two species of Sceloporus (S. graciosus and S. undulatus) that have indistinct sex chromosomes. Electron microscopic analyses revealed distinct length heteromorphism between the lateral elements of one of the largest microchromosomal synaptonemal complexes in each species. The morphology and behavior of the heteromorphic synaptonemal complex in S. graciosus and S. undulatus were congruous with those described for heteromorphic sex bivalents in other vertebrates and are hypothesized to represent synapsis of the sex chromosomes. Synaptic behavior of the heteromorphic bivalents was similar between species and differed from that of the homomorphic (autosomal) bivalents within each species. In both species, synapsis of the heteromorphic bivalent was characterized by the formation of a buckle in the synaptonemal complex at early to mid-pachynema. Synaptic adjustment was observed to result in equalization in length of the lateral elements.

SEX determination in reptiles follows one of two general modes. Sex is determined either by temperature or by the genotypic constitution of the individual (Bull, 1980; Bickham, 1983). Although the presence of heteromorphic sex chromosomes has been used traditionally as an indicator of genotypic sex determination in reptiles (Bull, 1980), verification of genotypic sex determination may be problematic when heteromorphic chromosomes are not discernable cytogenetically. Sex chromosome systems in reptiles are represented by both male (XY/XX) and female (ZZ/ZW) heterogamety (Ohno, 1967; Pennock et al., 1969; Gorman, 1973; King and Rofe, 1976), and complex sex chromosomes occur in some groups (Gorman and Gress, 1970; Wright, 1973; Lamborot and Navarro-Suárez, 1984; Moritz, 1984).