


(MCH, PDS, IFG) DEPARTMENT OF BIOLOGY, TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS 77843, AND (JWS) DEPARTMENT OF ZOOLOGY, BRIGHAM YOUNG UNIVERSITY, PROVO, UTAH 84602. PRESENT ADDRESSES: (MCH) DEPARTMENT OF BIOLOGY, WASHINGTON UNIVERSITY, ST. LOUIS, MISSOURI 63130, AND (PDS) BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE 37830. Accepted 19 Jan. 1990.

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Synaptonemal Complex Analysis of Sex Chromosomes in Two Species of Sceloporus

KENT M. REED, PHILIP D. SUDMAN,

JACK W. SITES, JR. AND

IRA F. GREENBAUM

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 heteromorphisms 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 Roze, 1976), and complex sex chromosomes occur in some groups (Gorman and Gress, 1970; Wright, 1973; Lamborot and Navarro-Suárez, 1984; Moritz, 1984).

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Within the genus *Sce1oporus*, sex-chromosomal heteromorphisms have been detected in the microchromosomes of some species (Lowe et al., 1967; Axtell and Axtell, 1971; Hall, 1973; Cole, 1978, and references therein). In the majority of species where sex chromosomes have been described, males are the heterogametic sex and possess either XY or X1X2Y sex chromosomes. In species characterized by X1X2Y/X1X1X2X2 sex chromosome systems, the males exhibit an odd number of microchromosomes in mitotic preparations and an appropriate trivalent at diakinesis (Cole et al., 1967). Some XY/XX species (*Sce1oporus chrysostictus*, *S.couchii*, *S.lundelli*, *S.merriami*, *S.pyrocephalus*, *S.uitiformis*, and *S.variabilis*) show distinct size heteromorphism between the X and Y chromosomes (Cole, 1970, 1971a, 1971b, 1978). Whereas many species lack distinctly heteromorphic chromosomal pairs, several are strongly suspected of possessing a minute Y chromosome in an XY system (Lowe et al., 1967; Jackson and Hunsaker, 1970; Hall, 1973; Cole, 1978, and references therein). Because of the minor size differences between the microchromosomes, and the limited resolution of the morphology and pairing behavior obtained from conventional mitotic and meiotic data, it is likely that sex-chromosomal heteromorphisms have gone undetected in several species.

Both *S.gracioso1* (Jackson and Hunsaker, 1970) and *S.undulatus* (Cole, 1972) lack conspicuous sex-chromosomal heteromorphisms. *Sce1oporus gracioso1*, comprising the monotypic gracioso1 species group, possesses an invariant karyotype (2N = 30, 12 macrochromosomes and 18 microchromosomes) not found in other species of *Sce1oporus* (Jackson and Hunsaker, 1970; Cole, 1971a). *Sce1oporus undulatus* (2N = 22, 12 macrochromosomes and 10 microchromosomes) is a member of the undulatus group, which includes six species (*S.cautus*, *S.exsul*, *S.occidentalis*, *S.undulatus*, *S.virgatus*, and *S.woodi*) all possessing similar karyotypes (Cole, 1972; Dixon et al., 1972; Sites and Haiduk, 1979).

Interspecific and geographic variation has been reported in the largest pair of microchromosomes within the undulatus species group (Cole et al., 1967; Cole, 1971a, 1972; Hall, 1973). This chromosome ranges from telocentric in *S.occidentalis* to metacentric in *S.cautus* and *S.virgatus*. Similar variation is present both within and among the subspecies of *S.undulatus* (Cole, 1972). Because of an apparent geographical pattern and the lack of discernable differences between males and females, this variation was not attributed to sex-chromosomal heteromorphism.

As shown recently for lizards (Hedin et al., 1990) and birds (Hale et al., 1988), application of the surface-spread technique (Counce and Meyer, 1973) for visualization of whole-cell complements of synaptonemal complex (SC) provides increased resolution for examining both the meiotic pairing behavior and morphology of microchromosomes. Although virtually unutilized in reptilian systems, the SC technique has proven valuable in the study of chromosomal polymorphisms including inversions (Kaelbling and Fechheimer, 1983, 1985; Hale, 1986, and references therein), deletion/additions (Moses and Poorman, 1981; Sharp, 1986; Sudman et al., 1989), and translocations (de Boer et al., 1986; Switonski et al., 1987) in other vertebrates. Additionally, the technique has been particularly useful in the analysis of the sex chromosomes of several vertebrate species (Solari, 1974, 1977; Moses, 1977b; Sharp, 1982; Hale and Greenbaum, 1986). In this study, SC preparations of testicular material from *S.gracioso1* and *S.undulatus* were examined to ascertain if differences in behavior or morphology of the SCs could be useful in testing for the presence of cryptic sex chromosomes in these species.

**Methods and Materials**

Specimens of reproductively mature *S.gracioso1* and *S.undulatus* were collected by one of us (JWS) during April from three localities in Utah. Somatic karyotypes were prepared using a modification of the technique of Cole and Leavens (1971). The testes were removed and part of this material was processed for meiotic karyotypes (Evans et al., 1964). The remaining testicular material was either immediately prepared for visualization of SCs or preserved cryogenically (Sudman, 1989) for later preparation.

Synaptonemal complexes were prepared following the surface-spreading technique of Counce and Meyer (1973) as modified by Moses (1977a). Surface-spread SCs were stained with silver nitrate (Howell and Black, 1980), mounted on copper grids (100 mesh), and examined using a Zeiss EM10C transmission electron microscope operated at 60 kV. A minimum of 150 nuclei were examined and 58 were fully analyzed from electron photomicrographs. Nuclei were assigned to meiotic substages based on the
morphology of the chromosomal axes (lateral elements) of the SCs, and nucleoli (Greenbaum et al., 1986). Quantitative analyses of SC lengths were performed using a Zeiss SEMIPS image analysis system. All specimens used in this study are deposited in the Texas Cooperative Wildlife Collections (TCWC), Texas A&M University, College Station, Texas.

Results

Sceloporus graciosus.—The somatic karyotype (Fig. 1) of the S. graciosus examined was identical to that initially reported for the species (Jackson and Hunsaker, 1970). The six macrochromosomal pairs were metacentric excepting pair 2 which was submetacentric and possessed a telomeric secondary constriction (NOR) on the long arm. The microchromosomal morphologies could not be characterized from the somatic karyotype. Meiotic preparations (Fig. 1) were consistent with mitotic preparations and displayed six macrochromosomal and nine microchromosomal bivalents at diakinesis.

The SCs of S. graciosus appeared generally similar to those reported from other vertebrates (Solari, 1974, 1977, 1980; Moses, 1977a; Hale et al., 1988). The lateral elements, centromeric regions, and attachment plaques (Moses, 1977a) were all easily identifiable. The morphologies of the macrochromosomal SCs were consistent with both the mitotic and meiotic chromosomal data. However, the SC data provided improved resolution of the microchromosomes (Fig. 1). Based on SC data, the morphologies of the microchromosomes were interpreted as follows; pairs 7–9 subacrocentric, pairs 10–14 metacentric, and pair 15 acrocentric (identified from left to right in Fig. 1).

The second largest microchromosomal SC (corresponding to chromosomal pair 8) appeared heteromorphic in early to mid-pachytene nuclei and was morphologically distinct from the remaining SCs (arrow in Fig. 1). Whereas at early pachynema the remaining bivalents were fully synapsed, a portion of this bivalent remained asynapsed (Fig. 2a–b). Although synapsis of the bivalent appeared to proceed interstitially from two terminal initiation sites, the rate of SC formation on either side of the centromere appeared variable. At mid pachynema, the heteromorphic SC exhibited a buckle configuration due to length inequalities between the lateral elements. The position of the interstitial buckle was variable with respect to the position of the centromeric region (Fig. 2c–g). The lengths of the lateral elements of this SC differed by a maximum of 22.35%. At late pachynema (Fig. 2h), this bivalent appeared fully synapsed with the longer lateral element asymmetrically twisted around the shorter element.

Sceloporus undulatus.—The somatic and meiotic karyotypes of S. undulatus (Fig. 3) were also consistent with previous reports (Cole, 1972). The macrochromosomal morphology resembled that of S. graciosus (pairs 1, 3–6 metacentric; pair 2 submetacentric). The morphology of the microchromosomes was not clearly discernable, but all appeared to be metacentric. Surface-spread cells indicated normal synapsis for 10 chromosomal pairs and the SCs verified the metacentric condition of the microchromosomes (Fig. 3). As in S. graciosus, one SC (pair 7) appeared heteromorphic and displayed a buckle con-
Fig. 2. Electron micrographs of the eighth largest SC (pair 8) from Sceloporus graciosus. a–b) Early-pachytene pair 8 SCs displaying regional asynapsis; c–g) mid-pachytene pair 8 SCs showing variation in the position of the buckle; and h) fully synapsed late-pachytene pair 8 bivalent exhibiting asymmetrical twisting of the SC. Centromeric regions are indicated by arrowheads. All magnifications approximately equal, bar represents 3 μ.

Fig. 3. Mitotic (top), meiotic (middle) and microchromosomal SC (bottom) karyotypes from a male Sceloporus undulatus (2N = 22, TCWC 66201). The karyotype includes six pairs of macrochromosomes and five pairs of microchromosomes. The SCs of the microchromosomes are arranged by overall size and are placed with centromeric regions on the horizontal line.

uration in early-pachytene nuclei (Fig. 4a). Based on measurement of this SC in one cell (Fig. 4a), lateral elements differed in length by 13.5%.

As pachynema progressed, the buckle was eliminated and the SC exhibited marked asymmetrical twisting (Fig. 4b). By late pachynema, the lateral elements of this SC became fully straight paired (Fig. 4c) and electron dense (silver-stain positive) structures formed along the axis as the SC became more contracted. These argentophilic structures, apparently the result of a protein association, were only associated with this SC and were most prevalent in the fully adjusted (late-pachytene) configuration.

**DISCUSSION**

We hypothesize that the presence of heteromorphic SCs (representing chromosomal pairs 8 and 7 in *S. graciosus* and *S. undulatus*, respectively), is indicative of XY/XX sex chromosome systems in these species. Sex-correlated variation involving the microchromosomes is common within the genus *Sceloporus*. With the exception of those species reported to possess a minute Y (*S. chrysostictus*, *S. couchii*, *S. merriami*, *S. pyrocephalus*, *S. utiformis*, and *S. variabilis*), most XY/XX and X,XYY/X,XXY,XX systems involve the largest microchromosomal pairs (Lowe et al., 1967; Jackson and Hunsaker, 1970; Hall, 1973; Cole, 1978, and references therein). Thus, the designation of these microchromosomal bivalents in *S. graciosus* and *S. undulatus* as the sex bivalents is consistent with the previously observed sex-correlated variation reported within the genus.

In vertebrates, the SCs of heteromorphic sex bivalents are readily distinguished from those of the autosomes (Solari, 1974, 1977; Moses, 1977b; Hale and Greenbaum, 1986, and references therein; Hale et al., 1988). Differences between the sex-chromosomal and autosomal bivalents include asynchronous synapsis and degree of association and resulting SC morphology. Sex chromosomes typically pair later than
the autosomes and may not initiate synapsis until the autosomal bivalents become fully paired (Moses, 1977b, 1980; Hale and Greenbaum, 1986, and references therein). Detailed analysis of the temporal relationship of synapsis between the heteromorphic bivalents and remaining bivalents in *S. graciosus* and *S. undulatus* was not within the scope of this study. However, the apparent delayed synapsis of portions of these bivalents is consistent with the hypothesis that they represent the sex chromosomes.

Heteromorphic sex bivalents typically exhibit morphological configurations unlike those of the autosomes. In most mammals and birds, the sex chromosomes synapse in an apparently homologous fashion to form a length of SC (although see Sharp, 1982). Among those species in which the sex chromosomes differ substantially in size, the degree of synapsis of the XY or ZW is variable. For example, in mammals, the Y chromosome has become highly heterochromatic and much reduced relative to the X. Synapsis of the Y chromosome with the typically longer X chromosome varies from involving the entire length of the Y to including as little as one-half or less of the Y. This pattern results in a substantial portion of both the X and Y chromosomes being unpaired (Solari, 1974; Moses, 1977b; Joseph and Chandley, 1984; Gillies and Cowan, 1985).

In the birds examined to date, uniterminal synaptic initiation and linear pairing of the ZW bivalent results in complete association of the W with the longer Z (Solari, 1977; Ryder, 1989). Similar to the mammalian X, synapsis results in a portion of the Z being unpaired. Length differences between the Z and W may persist as in mammals, or become equalized through synaptic adjustment (Solari, 1977; Ryder, 1989).

In those species in which the sex chromosomes do not fully synapse, the unpaired regions typically exhibit heteropycnosis with conspicuous condensation and thickening. Additionally, fusiform bulbous excrescences are often associated with these and other unpaired segments (Moses, 1977b; Gillies and Cowan, 1985; Shi et al., 1988). Whereas heteropycnosis was not observed in the unpaired regions of the heteromorphic SCs of *S. graciosus* or *S. undulatus*, the presence of electron-dense excrescences in *S. undulatus* supports the designation of pair 7 as the sex pair.

The degree of association between the X and Y in both *S. graciosus* and *S. undulatus* may be indicative of the degree of homology which exists between these chromosomes (Moses and Poorman, 1981). The buckle configurations, representative of the relative differences in the amount of chromatin, could be explained by either the addition or deletion of genetic ma-
terial. Similar buckle configurations have been observed in individuals heterozygous for interstitial euchromatic insertions/deletions (Moses and Poorman, 1981; Mahadevaiah et al., 1984; de Boer et al., 1986) and interstitial heterochromatic additions (Sharp, 1986; Sudman et al., 1989). Although our data do not provide information as to which chromosome (X or Y) is larger in S. gracilis or S. undulatus, the nature of the length differences between the X and Y could be determined through a mensural comparison of the SCs of males and females of each species.

Previous studies of the SCs of insertion/deletion carrying Peromyscus (Sudman et al., 1989) and Mus (Moses and Poorman, 1981) have documented variation in the position of the associated buckle. The variation in the position of the buckle seen in S. gracilis may be the result of either asynchronous synaptic initiation or unequal rates of synopsis. If synopsis proceeds from both telomeres at an equal rate, the variation in position of the buckle could be the result of differences in the timing of initiation at these two sites. However, if biterminal initiation is synchronous, differences in rate of synopsis (homo- or heterosynapsis) could result in the observed variation in buckle position. Finally, the position of the buckle could be indicative of the differences in sequence arrangement between the sex chromosomes (Moses and Poorman, 1981).

Synaptic adjustment, a mechanism which acts to reduce the amount of unpaired chromat in through heterosynapsis, has been reported for both euchromatic and heterochromatic rearrangements in several vertebrate species (Hale and Greenbaum, 1986, and references therein; Sudman et al., 1989). This process, which converts heteromorphic SCs to straight-paired configurations, appears to represent the loss of required homology for pairing during the latter phase of synopsis (Moses and Poorman, 1981; Moses et al., 1982; von Wettstein et al., 1984). This saturation of DNA pairing sites, through non-homologous pairing, has been hypothesized to result in a more "stable" configuration. The asymmetrical twisting observed in the heteromorphic SCs in both S. gracilis and S. undulatus, and the lack of the buckle configuration in some late-pachytenic cells, is consistent with the phenomenon of synaptic adjustment (Moses and Poorman, 1981).

The evolutionary differentiation of heteromorphic sex chromosomes from a presumably homomorphic pair is thought to result from reduction in recombination due to structural rearrangements (Ohno, 1967, 1969) or through the amplification of unique satellite DNA sequences (Singh et al., 1976, 1980; Olmo et al., 1984, 1987). Lack of crossing over (recombination) could isolate the sex determining regions and augment the differentiation of the X and Y chromosomes. Heterosynapsis in S. gracilis and S. undulatus, as the result of synaptic adjustment of the buckle configuration, should effectively prevent crossing over in the heterosynapsed region (Hale, 1986).

The relationship of the observed heteromorphism in pair 7 to the geographic variation reported for this chromosome in S. undulatus (Cole, 1972) remains problematic. The distance between our collection sites (approx. 360 km), dismisses the possibility that the heteromorphism observed in S. undulatus is the result of a locally isolated phenomenon. Cole (1972) examined 1039 cells from 81 individuals (47 and 349) collected from throughout the range of S. undulatus and found no individuals heteromorphic at chromosomal pair 7. In light of the present observations, the previously reported pattern is indicative of geographic variation within the sex chromosomes of this species.

**Material Examined**

Scoloporus gracilis.—Utah: Sanpete County (Co.): 0.9 km E Hwy 89 on paved road to Milburn (TCWC 66205); San Juan Co.: Window Rock, 32 km S Moab (TCWC 66198); Washington Co.: Oak Grove Campground, 13.5 km W Leeds (TCWC 66199, 66200).

Scoloporus undulatus.—Utah: San Juan Co.: Window Rock, 32 km S Moab (TCWC 66197); Washington Co.: Oak Grove Campground, 13.5 km W Leeds (TCWC 66201).

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(KMR, PDS, IFG) DEPARTMENT OF BIOLOGY, TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS 77843, AND (JWS) DEPARTMENT OF ZOOLOGY, BRIGHAM YOUNG UNIVERSITY, PROVO, UTAH 84602. PRESENT ADDRESS: (PDS) BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE 37831. ACCEPTED 12 JAN. 1990.