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Application of Synaptonemal Complex Techniques for Determination of Diploid Number and Chromosomal Morphology in Birds

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Avian karyological studies are often complicated by the large numbers of microchromosomes which characterize the chromosomal complements of most bird species (Shields 1982, 1983). The exact number of microchromosomes can be difficult to ascertain, as they are often covered by the macrochromosomes or lost during preparation of mitotic material for cytological analysis. In addition, the small size of the microchromosomes makes it difficult or impossible to determine the position of their centromeres. Consequently, many reports of bird karyotypes present modal or approximate diploid numbers (e.g. Biederman et al. 1980), or emphasize the morphology of the macrochromosomes (e.g. Bartlett and Threlfall 1987). We discuss a technique for accurate determination of the diploid number and chromosomal morphology of birds by electron microscopic analysis of silver-stained synaptonemal complexes.

Fig. 1. Giemsa stained somatic karyotype prepared from hypotonically-treated bone marrow of a female *Colinus virginianus*. The diploid number is clearly 82; however, the morphology of the smaller chromosomes cannot be determined from this type of preparation.

The synaptonemal complex (SC) is a ribbonlike proteinaceous structure which forms the longitudinal axis of pachytene bivalents during meiotic prophase I. Each meiotic chromosome forms an axis which, upon pairing with its homolog, becomes one of the lateral elements of the SC. Staining with silver differentiates preferentially the two lateral elements of the SC from their associated chromatin and surrounding nucleoplasm, thereby providing simple linear representations of chromosomal behavior and orientation during pachynema. In addition, the positions of the centromeres of the meiotic chromosomes are often discernible as dark or thickened regions on the lateral elements of the SCs in early pachytene nuclei.

The technique for obtaining pachytene nuclei from the testes of adult male birds (in breeding condition) was modified from one described for mammalian testicular material (Moses 1977). We used plastic-coated microscope slides to pick up the spermatocyte nuclei after spreading on the convex surface of a 0.5% (w/v) NaCl solution. The slides were prepared by dipping them into a Coplin jar that contained a 0.6% (w/v) solution of plastic (Falcon Optilux petri dish) in chloroform. After air-drying, the edges of the plastic coat

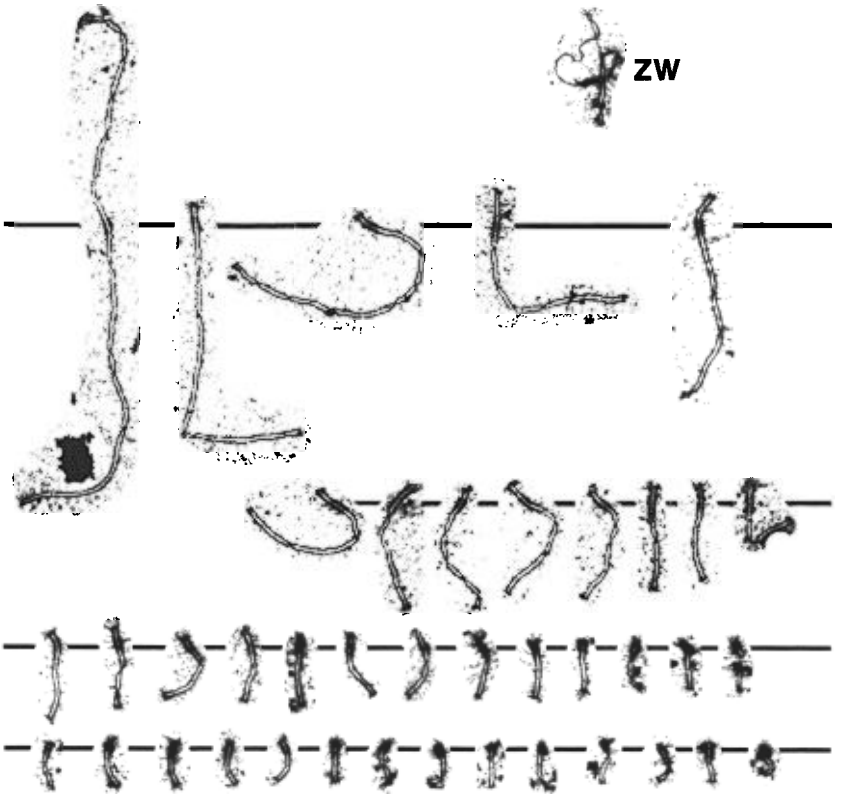

Fig. 2. Synaptonemal complex karyotype constructed from an electron micrograph of a silver-stained pachytene oocyte from *Colinus virginianus*. The autosomal SCs are arranged by length and positioned with their darkly stained centromeric regions on the horizontal lines. The partially paired ZW bivalent is shown at the upper right. The first row comprises the 5 macrochromosome pairs, excluding the sex bivalent. The second row includes the intermediate-sized autosomes, and the third and fourth rows contain the 27 pairs of microchromosomes. Magnification ×3,435.

were sealed with clear nail polish on the preferred side of the slide. The 4% paraformaldehyde fixative was prepared without sucrose or DMSO, and adjusted to pH 8.2–8.5 with 10% formic acid (diluted from 88% stock) and 1.0 N NaOH. Fixation of the surface-spread spermatocytes was accomplished in two steps. First, the slides were immersed for 5 min in a Coplin jar containing 4% paraformaldehyde with 0.03% SDS (48.5 ml paraformaldehyde solution with 1.5 ml 1.0% SDS). The slides were then immersed for an additional 5 min in paraformaldehyde without SDS in a second Coplin jar. After the second fix, the slides were drained briefly on end and then swished in 0.4% Photoflo (Kodak) for 10–15 s. When dry, the testicular material was stained 6–8 min with silver nitrate (Howell and Black 1980).

For electron microscopy (EM), the plastic around the desired cell area was etched with a diamond-tipped scribe, and floated off by slowly slipping the slide into a large dissecting bowl filled with distilled water. Fontax EM forceps were used to place copper grids (100 mesh) onto the floating plastic, which was then lifted off the water surface with a strip of Parafilm. Once dry, the grids can be lifted off the Parafilm with forceps and stored in a dessicator until EM analysis. The best electron micrographs were obtained with a Zeiss EM10C transmission electron microscope at 60 kV with low magnification setting. Nuclei were photographed at magnifications between 1,000× and 2,000×.

Zygotene and pachytene oocytes were obtained from female chicks soon after hatching. For Northern Bobwhite (*Colinus virginianus*), the largest numbers of pachytene oocytes were recovered from the ovaries of 0–3 day-old chicks using a "settle" technique (Mahadevaiah et al. 1984). The techniques for preparing the slides, fixative, and EM samples were as described above for male birds.

The somatic karyotype of the Northern Bobwhite (Fig. 1) has been reported to consist of 82 chromosomes (Benirschke and Hsu 1971). Six autosomal pairs are submetacentric or subtelocentric; the Z chromosome is submetacentric, and the W is acrocentric or subtelocentric. The remaining 68 chromosomes ap-

pear to be acrocentric. Well spread, silver-stained pachytene nuclei from male and female Northern Bobwhite clearly display a full complement of 41 SCs (Fig. 2). This confirmed the diploid number previously determined from feather pulp preparations (Benirschke and Hsu 1971). Our SC data generally corroborate the description of the chromosomal morphology from somatic metaphases. The SC data revealed 6 pairs of macrochromosomes, including the sex chromosomes. The largest autosomal bivalent is submetacentric, 2 macrochromosome pairs are acrocentric, and the remaining 2 pairs are subtelocentric. The Z chromosome is acrocentric, and approximately twice as long as the subtelocentric W chromosome. Of the 8 "intermediate-sized" chromosome pairs, 4 appear subtelocentric and 4 are acrocentric. Two microchromosome pairs are subtelocentric; the remaining 25 pairs of microchromosomes are clearly acrocentric. The higher number of subtelocentric autosomes indicated by the SC data reflects the less contracted condition of the prophase I chromosomes and the increased precision with which the morphology of the microchromosomes can be resolved.

The surface spreading and settle techniques for obtaining pachytene meiocytes and visualizing SCs are readily applicable for studies of avian cytotaxonomy and cytosystematics. These preparations provide large numbers of well-spread pachytene nuclei amenable to analysis by transmission electron microscopy. The silver-stained SCs are easily counted, and provide a useful and superior means of accurately determining the diploid number of male and female birds. Because the meiotic chromosomes are completely paired at pachynema, there is the added advantage of analyzing a haploid number of configurations. Moreover, the centromeric regions are visible on the SCs of both the macrochromosomes and microchromosomes, which resolves the chromosomal morphology to a degree generally not attainable by conventional karyological methods. The prophase I configurations are more elongate than the corresponding somatic metaphase chromosomes; hence small chromosomal arms are more easily detected in SC preparations. Although the SC techniques are somewhat involved, they are substantially less time-consuming and expensive than some of the conventional procedures for obtaining avian chromosomal material (i.e. embryo incubation, blood and tissue culturing).

The SC techniques also have considerable potential in cytogenetic analyses of known or suspected chromosomal rearrangements in birds. As pachytene SC configurations faithfully reflect the orientation of the paired meiotic chromosomes, these techniques can be employed to investigate the nature and behavior of chromosomal rearrangements in the heterozygous state. Although these procedures have been widely applied in studying chromosomal heteromorphisms in mammals (Mahadevaiah et al. 1984, Hale 1986), similar SC analyses in birds have thus far been limited to examinations of experimentally induced reciprocal translocations (Kaelbling and Fechheimer 1983) and pericientric inversions (Kaelbling and Fechheimer 1985) in heterozygous domestic chickens (*Gallus domesticus*). Synaptonemal complex data on chromosomal rearrangements which occur in natural populations of birds are not presently available; however, numerous examples of intraspecific chromosomal variation have been documented (reviewed by Shields 1982). These include polymorphism for pericentric inversions (Shields 1973, 1976; Hammar and Herlin 1975; Thorneycroft 1975; Ansari and Kaul 1979a; Bass 1979) and translocations (Ansari and Kaul 1978, 1979b; Kaul and Ansari 1979). It is unclear how these types of polymorphisms persist in avian populations, as heterozygous individuals are generally expected to exhibit reduced reproductive fitness (Shields 1982). Analysis of SC configurations could provide insight into the mechanisms involved in the maintenance of chromosomal polymorphisms in avian populations, and yield information relative to the evolutionary implications of chromosomal rearrangements in birds.

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Influence of Desert Nesting and Foraging Distance on Growth Rates in Gray Gulls (*Larus modestus*)

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By nesting 35–100 km from the coast in the waterless pampas of the interior Atacama Desert of northern Chile, Gray Gull adults limit themselves to a single daily foraging trip (Howell et al. 1974; Guerra et al. in press [a, b]; Fitzpatrick et al. in press [a]). Limitations on feeding frequency and the amount of food that adults can carry (ca. 18 g dry weight; Fitzpatrick et al. in press [a]; unpubl. data), presumably affects growth rates in young Gray Gulls. General conditions of the Atacama and specific descriptions of the Gray Gull nesting sites were reported by Howell et al. (1974), Guerra and Cikutovic (1983) and Guerra et al. (in press [a]). Information on Gray Gulls and their problems associated with nesting far from food and water in the Atacama, where daily surface and air temperatures range from 2–61°C and 2.5–38°C, respectively, are well documented (Howell et al. 1974; Guerra and Fitzpatrick 1987; Guerra et al. 1988, in press [a, b]; Cikutovic et al. in press; Fitzpatrick et al. in press [a, b]).

We studied growth rates in free-ranging and captive Gray Gull young, and compared them to other semiprecocial species within the Laridae. We hypothesized that, because of food and water limitations imposed by nesting far from the coast, Gray Gulls would have a lower mass growth-rate than other Laridae.

During early January 1986 we hand-captured 23 Gray Gull chicks (33–250 g; 1–ca. 45 days old) at a nesting site located at Cerro Negro, 100 km east of Antofagasta (23°41'S), in the Atacama Desert. Cerro Negro nesting sites were described by Cikutovic and Guerra (1983) and Guerra et al. (in press [a]). Each gull was tagged with a numbered metal band (Model 1242-M, size 10, Nat. Band and Tag Co., Kentucky, USA). Chicks were weighed with Pesola scales, and wing, culmen, tarsus and total lengths measured as described in Fitzpatrick et al. (in press [b]). Twelve gulls were released and 11 were taken to the outdoor aviary on the Universidad de Antofagasta campus, located several hundred meters from the beaches where free-ranging gulls forage. We attempted to recapture and remeasure the released gulls on six occasions between 17 January and 10 February 1986. Eight were recaptured four times; three, three times; and one, twice. The free-ranging gulls were also used to study water-turnover and feeding energetics using labeled water (MS in prep.).

Gulls maintained in the aviary were fed once daily an *ad libitum* diet of fresh or previously frozen sardines (*Sardinops sagax*) and anchovies (*Engraulis ringens*), the principal fish component of Gray Gulls' diet (Guerra et al. in press [b]). We weighed and measured captive gulls every 2–10 days, depending on size, until the age of 100 days. Free-ranging gulls beyond 50 days were not recaptured easily and growth data on them are limited.

We estimated age of chicks from an empirically determined wing length-age curve (Ricklefs et al. 1980). The curve was based on wing length-age relationships for free-ranging and captive chicks which