

efflux of 2.7 $\mu\text{M/hr cm}$ was observed for calcium-45. The values for zinc-65 varied from 0.2-0.6 $\mu\text{M/hr cm}$.

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Chromosomes of the Nevada Ground Squirrel *Spermophilus richardsonii nevadensis* (Howell)* (29615)

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Taxonomic characters currently used for investigation of the 3 geographically isolated subspecies of *Spermophilus richardsonii* include cranial and body measurements, pelage and geographic distribution(1,2). Although these characters differentiate each subspecies as a whole, they are not always reliable for individual specimens and they do not clarify evolutionary relationships between the subspecies(3).

Chromosomes from 2 of the 3 isolated subspecies of *S. richardsonii* have been analyzed previously(3). The Montana (*S. richardsonii richardsonii*) and Wyoming-Colorado (*S. r. elegans*) populations differed consistently in chromosome number and morphology. Chromosomal characters accurately identified individual specimens and, in addition, suggested that the *elegans* karyotype evolved from a chromosomal polymorphism which probably once existed in the more ancestral *richardsonii* population(3). Specimens of *S. r. neva-*

densis from Nevada representing the third isolated subspecies, were not available previously for chromosomal analysis.

The purpose of the present study is to determine the chromosome number and morphology of *S. richardsonii nevadensis* and utilize this data to provide a unified concept regarding subspecies relationships within *S. richardsonii*.

Methods. Two male and two female *Spermophilus richardsonii nevadensis* from Jiggs (Elko Co.) Nevada were trapped alive and studied in the laboratory. Specimens were identified by cranial and pelage characters and geographic distribution(1,2).

Chromosomes were analyzed from femoral bone marrow using a Colcemide, hypotonic citrate, acetic orcein squash technique(4,5). Sixteen karyotypes were constructed to determine the chromosomal pattern for *S. r. nevadensis*.

Results. Chromosome number. The data are listed in Table I. The modal diploid (2n) number was 34 in all 4 specimens of *S. r. nevadensis*.

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TABLE I. Chromosome Counts of *S. richardsonii nevadensis*.

Sex	Total cells counted	No. of chromosomes		
		32	33	34
♂	32			32
♂	53	1	4	48
♀	28		1	27
♀	21		1	20

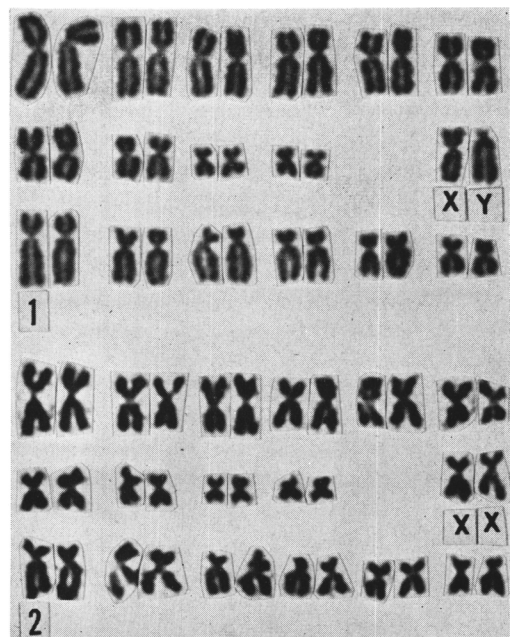
Chromosome morphology. Representative karyotypes are illustrated in Fig. 1 and 2. The karyotypes of all specimens were characterized by 10 pairs of metacentric autosomes and 6 pairs of submetacentrics (Fig. 1, 2). Male karyotypes contained an unpaired medium sized submetacentric X and a medium sized acrocentric Y (Fig. 1). In contrast, a pair of medium sized submetacentric X chromosomes occurred in all female karyotypes (Fig. 2). No karyotypic variation was found in the 4 specimens examined.

Discussion. It is now possible, using chromosomal characters described in the present and a previous investigation, accurately to differentiate individual specimens of *S. r. nevadensis* and *S. r. elegans* from specimens of *S. r. richardsonii*(3). Comparison of chromosomal characters demonstrates that *S. r. nevadensis* and *S. r. elegans* both have a diploid number of 34 and *elegans*(3) has a karyotype similar to that described for *nevadensis*, except for a possible difference in Y chromosome size. The Y of *nevadensis* is slightly larger than the Y of *elegans* but this small difference cannot be considered diagnostic until a larger number of specimens is analyzed. In contrast, the karyotype of *S. r. richardsonii* contained 36 chromosomes that included 10 pairs of metacentric, 5 pairs of submetacentric, and 2 pairs of acrocentric autosomes. The X chromosome was a submetacentric and the Y was an acrocentric that was smaller than the Y of *S. r. nevadensis* and *elegans*. An important contribution of the chromosomal characters is their reliability in differentiating *nevadensis* from *richardsonii* which, according to Howell, are quite similar in body measurements(1).

The possible explanations for two different

karyotypes within *S. richardsonii* has been discussed previously and they may be due to: 1) an intraspecific chromosomal polymorphism, 2) failure to recognize the existence of 2 different species, each characterized by a different karyotype or, 3) the existence of an intermediate stage of speciation between that of a population containing an intraspecific polymorphism and the stage characterized by 2 reproductively isolated species each possessing a different karyotype (3). The last explanation is most probable. A polymorphism may be excluded because only one karyotype was found within each population examined in this and another study(3). Unfortunately, reproductive isolation cannot be proved or disproved to evaluate satisfactorily the possible existence of two species.

Chromosomal data indicate that a chromosomal polymorphism probably once existed within the ancestral *S. richardsonii* population ($2n = 36$)(3). Centric fusion of non-homologous acrocentric autosomes resulted in formation of a metacentric autosome and produced a heterozygotic karyo-

FIG. 1. Karyotype from a male *S. richardsonii nevadensis* 1800X.FIG. 2. Karyotype from a female *S. richardsonii nevadensis* 1800X.

type containing 35 chromosomes. Interbreeding between heterozygotes would produce animals with $2n = 34$ and karyotypes roughly similar to those observed in *nevadensis* and *elegans*. Following centric fusion a pericentric inversion must have occurred to alter the metacentric produced by fusion of equal sized acrocentrics to a submetacentric. Finally, remodeling of the Y chromosomes must be postulated because the Y of *nevadensis* and *elegans* is larger than that of *richardsonii*. When peripheral populations containing the polymorphism became isolated from the parental stock, animals with $2n = 35$ and $2n = 36$ were selected against and eliminated, resulting in a population containing only the new $2n = 34$ (*nevadensis* and *elegans*) karyotypes. Because centric fusion is a more likely mechanism than centric fission or dissociation for conversion of the *richardsonii* karyotype to *elegans*, *richardsonii* is considered chromosomally more ancestral(3).

It is not possible to determine whether *nevadensis* and *elegans* evolved from one or two isolates that became separated from the ancestral *richardsonii* population. Possibly the earlier distribution of the $2n = 34$ population was much larger than it is at present and as climatic conditions became unfavorable *nevadensis* remained as a relict population in Nevada and diverged from *elegans*, at the same time retaining a similar karyo-

type. In any case chromosomal characters suggest that *nevadensis* and *elegans* are more closely related to one another than either is to *richardsonii*.

Summary. Chromosomes were analyzed from the bone marrow of 4 specimens of *Spermophilus richardsonii nevadensis*. Comparison of chromosomal characters from the 3 geographically isolated subspecies of *S. richardsonii* demonstrates that *S. r. nevadensis* and *S. r. elegans* both have a diploid number of 34 and similar karyotypes whereas *S. r. richardsonii* has a diploid number of 36 and a different karyotype. The *nevadensis* and *elegans* karyotypes probably evolved from the chromosomally more ancestral *richardsonii* karyotype by means of a chromosomal polymorphism. The data also suggest that *S. r. nevadensis* and *S. r. elegans* are more closely related to one another than either is to *S. r. richardsonii*.

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Antiparasitic Drugs. V. Anticoccidial Activity of 4-Amino-2-Ethoxybenzoic Acid and Related Compounds. (29616)

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Wyss(1), Johnson(2), Martin(3) and others have tested ring-substituted 4-aminobenzoic acids as inhibitors of the growth of 4-aminobenzoic acid (PABA)-requiring bacteria. 4-Amino-2-chlorobenzoic acid has received the widest attention, *in vitro* activity

being noted with *E. coli*, *D. pneumoniae*, *S. hemolyticus*, *A. aerogenes* and the tuberculosis organism. *In vivo* trials, however, were disappointing(1a).

The sensitivity of coccidia to PABA antagonists of the sulfanilamide and 4,4'-di-