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CYTOTAXONOMIC STUDIES OF THE HARVEST MICE OF THE SAN FRANCISCO BAY REGION

HOWARD S. SHELLHAMMER

ABSTRACT.—The karyotypes of *Reithrodontomys megalotis longicaudus*, *R. raviventris halicoetes* and *R. r. raviventris* in the San Francisco Bay region were studied to understand better the taxonomic relationships of the three taxa. *Reithrodontomys megalotis* has 44 chromosomes whereas the two endemic suspecies of *raviventris* have 38. The karyotypes of *R. r. halicoetes and R. r. raviventris* are different enough to suggest that the two are in the terminal stages of speciation.

During the past decade there has been increased use of cytotaxonomic techniques as aids in solving difficult taxonomic problems (Nadler and Block, 1962; Nadler and Hughes, 1966; Nadler and Sutton, 1962; Matthey, 1965; Singh and McMillan, 1966; Wahrman and Zahavi, 1953, 1955, 1958). Investigators in this field have been careful to combine cytological results with the more standard systematic criteria, i.e., morphological, physiological, and behavioral variation, for as Matthey (1949) has stated "chromosome formulae provide only indications but in themselves are not criterion [*sic*] for exact conclusions." The goal of the present study has been to ascertain whether cytotaxonomic studies can clarify further the taxonomic relationships between the three kinds of harvest mice in the San Francisco Bay region.

Reithrodontomys raviventris is a species endemic to the salt marshes bordering San Francisco Bay. It is divided into two subspecies—*R. r. raviventris* in the southern part of the bay area and *R. r. halicoetes* in the northern part (San Pablo and Suisun bays). A second species of harvest mouse, *Reithrodontomys megalotis*, is common to the grasslands surrounding the bay. Its geographic distribution is extensive, including most of western United States and much of Mexico. Throughout the rest of this paper, *R. r. raviventris*, *R. r. halicoetes*, and *R. megalotis* will be referred to simply as *raviventris*, *halicoetes*, and *megalotis*, respectively.

It is presumed that *megalotis* was the progenitor of the marsh species and while *megalotis* is known to inhabit areas near marshes, there is no evidence of interbreeding between the two species (Hooper, 1944). Fisler (1965) studied the three taxa utilizing morphological, behavioral and ecological characters. He suggested that the endemic species evolved from *megalotis* during recent times, i.e., in the last 25,000 years, accompanying the formation of marshes in the bay. He further suggested that the xerophilic parent species was somewhat preadapted to the saline environment of the newly developing bay and that populations were isolated in marshes somewhat separated from the edge of the bay.

As the level of the bay rose these isolated populations became geographically contiguous with those on the edge of the bay, but enough time had elapsed

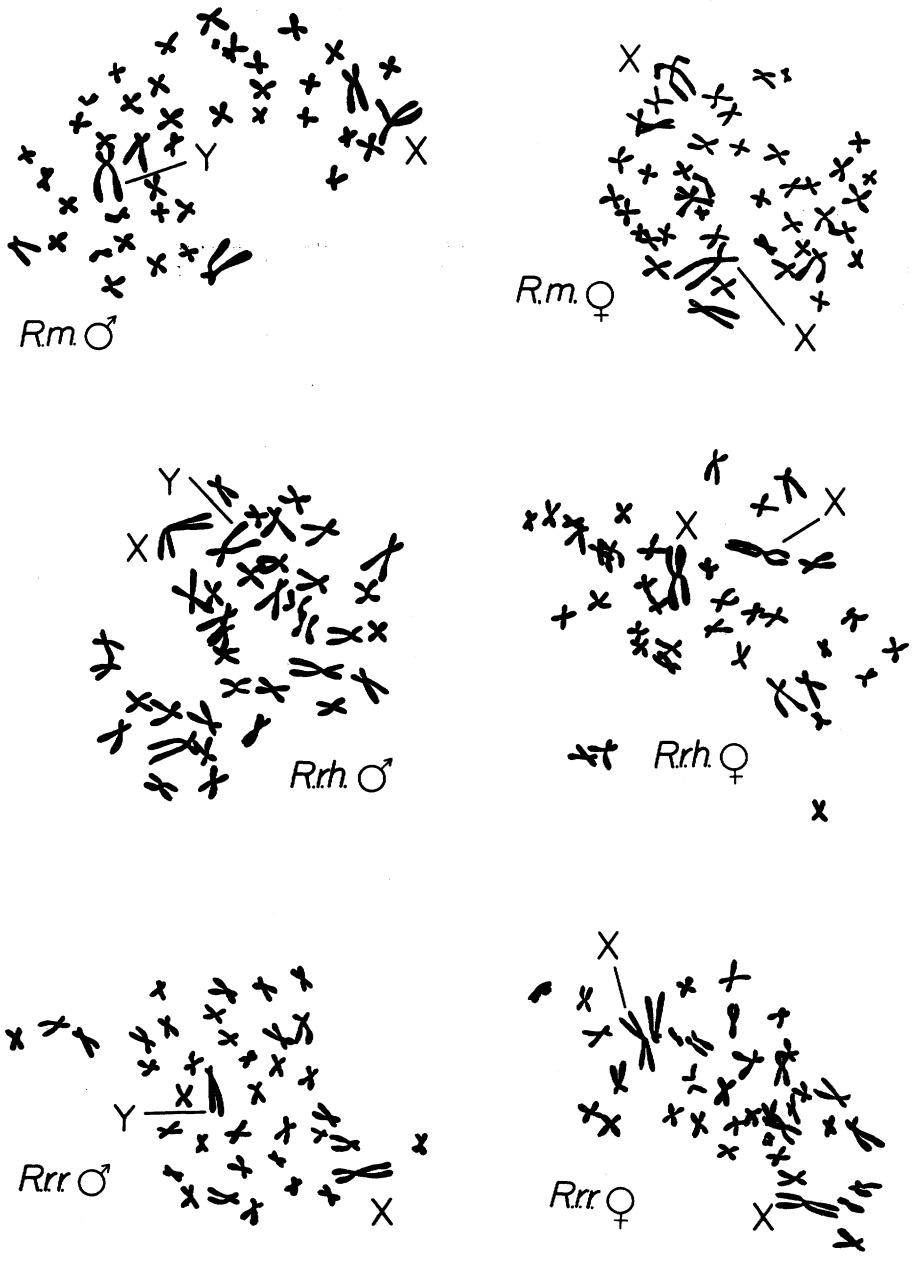

FIG. 1.—Camera lucida drawings of chromosomes prepared by Matthey's technique (1953). All are metaphase plates from spleenic material except for *R. r. raviventris* ♂, which is from testis.

TABLE 1.—*Cytotaxonomic data on the three kinds of harvest mice found in the San Francisco Bay region.*

Subspecies	Sex ♂	Sex ♀	2n	Autosomes* M	Autosomes* SM	Autosomes* ST	Autosomes* A	X	Y	FN*
R. megalotis longicaudus	18	9	44	20	16	6	–	SM	ST	84
R. raviventris halicoetes	11	9	38	14	16	6	–	SM	ST	72
R. raviventris raviventris	7	7	38	10	18	8	–	SM	ST	72

* M = metacentric, SM = submetacentric, ST = subtelocentric, A = acrocentric, FN = fundamental number.

so that allopatric speciation had occurred, effectively separating the two species. Fisler found *megalotis* to differ from the two subspecies of *raviventris* in many ways—size, pelage coloration, cranial length, genitalia, salt tolerance, activity patterns, temperament, and social system. There is little variation in *megalotis* itself throughout the bay region. The subspecies *raviventris* and *halicoetes* differed less from one another than they did from *megalotis* but did differ in cranial depth, per cent distribution of pelage types, tail length, nest building activities, and in control of body temperature. Fisler considered mate preference to be the only reproductive mechanism isolating *raviventris* and *halicoetes* but considered it, at least under laboratory conditions, to act only incompletely. He concluded that "*raviventris* and *halicoetes* have thus nearly reached the status of species by arising out of a condition of normal geographic races."

MATERIALS AND METHODS

Mice were live-trapped between 1962 and 1967 in at least three separate localities in the range of each of the three taxa studied (see list of specimens examined). Each animal was weighed, measured, and injected intraperitoneally with a buffered solution of "Colcemide" (CIBA) at 20 mg/kg of body weight. Two hours later the animal was killed and bits of testis, spleen, or bone marrow were removed and prepared on permanent slides using one of the following methods.

The first method used was that of Makino and Nishimura (1952) as modified by Matthey (1953). Figures for analysis were drawn either with the aid of a camera lucida and magnified 2900× or photographed using a Polaroid MP-3 camera and enlarged to various magnifications for study. During the last year of the study bone marrow cells were prepared for analysis by the method of the Ford and Hamerton (1956) colchicine-hypotonic citrate technique as modified by Blanks (1967). Patton (1967) discussed the general method used.

All chromosome figures prepared by this technique were photographed rather than drawn. The chromosomes were described using a four class system based on relative chromosome arm ratios: (1) metacentric (arm ratio of less than 1 : 1.1); (2) submetacentric (between 1 : 1.1 and 1 : 1.9); (3) subtelocentric (1 : 2 or greater); and (4) acrocentric (or telocentric) with no visible second arm. Fundamental number (or "Nombre Fundamental" after Matthey, 1951) is defined as the number of chromosome arms in the autosomal complement of a given species.

Fifty or more metaphase cells were counted to determine the diploid number of animals investigated. Approximately eight figures were studied per individual.

Patau (1960) has pointed out numerous sources of errors in measuring chromosomes. In an attempt to minimize mensural errors the following procedures were used in this

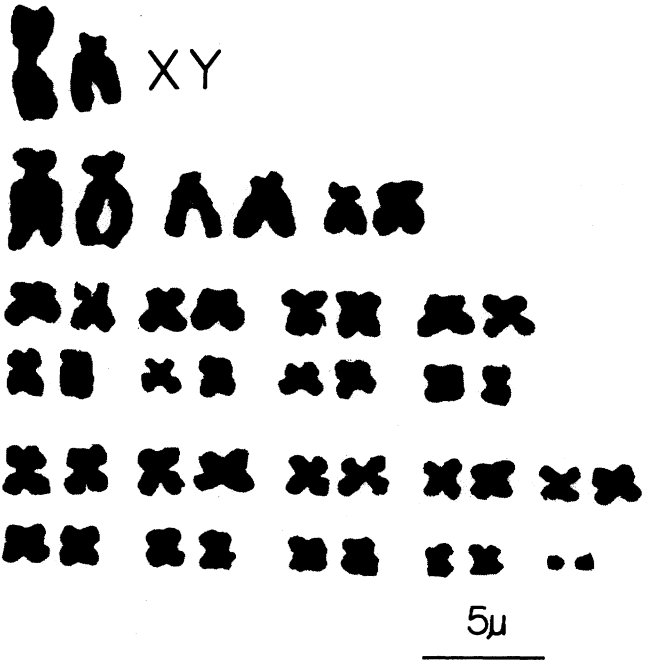

FIG. 2.—Karyotype of *Reithrodontomys megalotis longicaudus* (♂, SJ no. 79, bone marrow).

study. The length of time the animals were injected with colcemide was varied, resulting in differing states of contraction of the chromosomes. Some of the error induced by the disproportionate shrinkage of the longer chromosomes was hence reduced by comparing figures in differing states of contraction. Camera lucida drawings of the squash technique and photographs of the same technique were compared to assess variation induced by the technique on the representational medium. Camera lucida drawings were made with each chromosome in the center of the field of view to reduce optical errors in the microscope. The chromosomes drawn with the camera lucida method were measured and then redrawn on graph paper for purposes of analysis and comparison.

RESULTS

The results are given in Table 1. Representative karyotypes for each species are presented in Figs. 1–4. The karyotype descriptions are as follows:

Reithrodontomys megalotis longicaudus (Baird).—The diploid number of 44 chromosomes (see Figs. 1 and 2) confirms the report of Matthey (1961). The sex chromosomes are the longest pair with the subtelocentric Y-chromosome approximately 90% the length of the submetacentric X-chromosome. There are 20 metacentrics, 16 submetacentrics, and six subtelocentrics. No observable variation was found in the various populations sampled. The smallest metacentrics are usually contracted enough by the colcemide-hypotonic citrate treatment as to appear as microchromosomes or dotlike chromosomes.

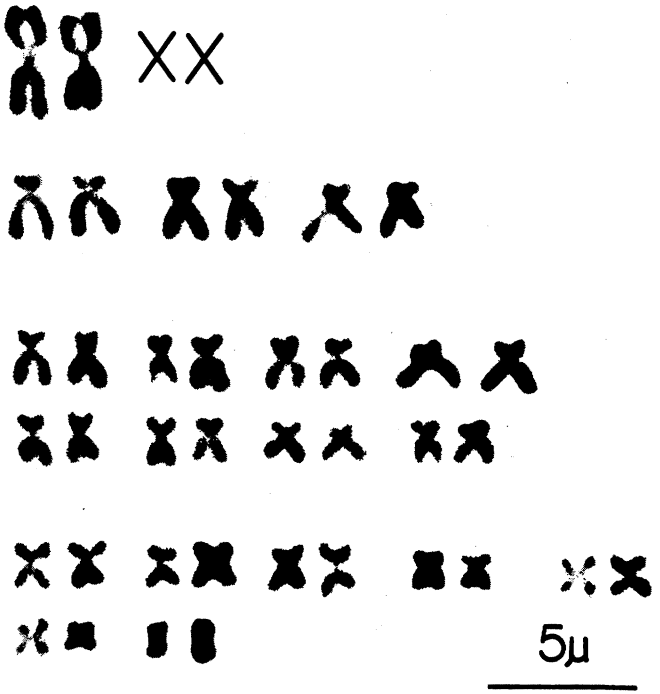

FIG. 3.—Karyotype of *Reithrodontomys raviventris halicoetes* (♀, SJ no. 76, bone marrow).

Reithrodontomys raviventris halicoetes Dixon.—The diploid number of 38 includes 14 metacentrics, 16 submetacentrics, and six subtelocentrics (see Fig. 1 and 3). The sex chromosomes are the longest pair and in this subspecies occurs the shortest Y-chromosome in relation to the X-chromosomes (approximately 72% of the length of the X).

Reithrodontomys raviventris raviventris Dixon.—The diploid number, 38, equals that of the other subspecies, but in the subspecies *raviventris* there are only 10 metacentrics, 18 submetacentrics, and eight subtelocentrics (see Fig. 1 and 4). The sex chromosomes are intermediate between the condition found in *R. megalotis* and *R. r. raviventris*, i.e., the Y chromosome is approximately 86% of the length of the X chromosome.

DISCUSSION

This cytotaxonomic study confirms in part the relationships between the three taxa (Fisler, 1965). They seem, however, more distantly related than had been suggested by previous studies. The two endemic subspecies of *raviventris* differ from *megalotis* by six chromosomes. The fundamental number possessed by *megalotis* is 84; *raviventris* and *halicoetes* both have 72. These differences are great enough to cast doubt on the assumption that *megalotis* is the ancestral form, but other evidence suupports this assumption.

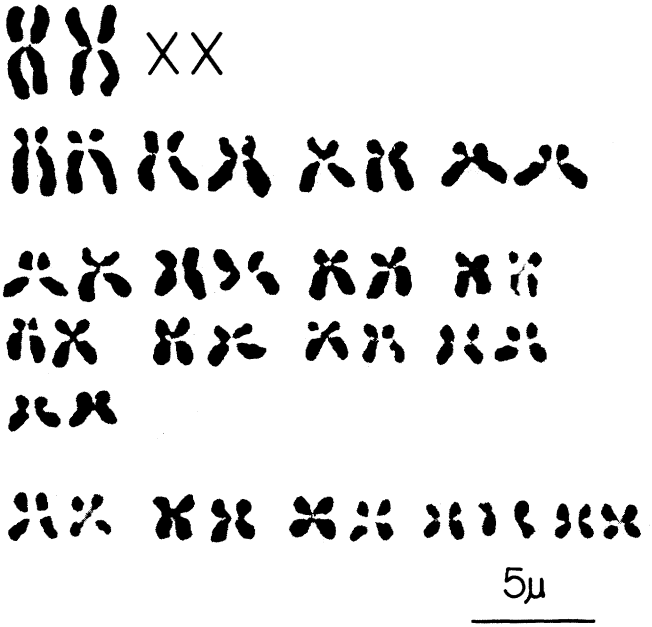

FIG. 4.—Karyotype of *Reithrodontomys raviventris raviventris* (♀, SJ no. 74, bone marrow).

Fisler (1965) demonstrated numerous similarities between *raviventris* and *megalotis*. Cytologically, the X chromosomes are similar, and there are no other harvest mice other than *megalotis*, Recent or fossil, that seem to be ancestral to *raviventris*.

A few cytological studies of groups of rodents, in which the taxa were morphologically similar but differed behaviorally and ecologically, have revealed a series in which chromosome numbers are reduced by the fusion of acrocentrics to produce a metacentric (Matthey, 1953; Wharman and Zahavi, 1958). Recently Patton (1967) observed a similar case of this type of variation in several species of *Perognathus*. In his study, one group in particular (the *P. intermedius* group) had a wide range in diploid number but a narrow range in fundamental number. Such a phenomenon was not found in the present study. Indeed there are no acrocentric chromosomes in *megalotis* and nothing can be said concerning a possible ancester that could have given rise to two or more of the mice used in this study.

The most likely explanation for the cytological differences is that the selection pressures which gave rise to the salt-marsh populations were so intense as to cause rapid speciation in which the cytology was greatly changed. The resultant evolution of the two salt-marsh subspecies of *raviventris* has been along divergent lines. It is obvious that the number of inter- and intrachromosomal rearrangements as well as deletions have been numerous.

On the basis of their chromosomal differences and the differing characters discussed by Fisler (1965), I suggest that *raviventris* and *halicoetes* are in the final stages of speciation. Matthey (1959, 1960) suggested that large chromosomal mutations, such as centric fusions, are not chronologically related to the process of speciation, but appear tardily as mechanisms of isolation. The final proof that Matthey's contention is valid in this case may never be obtained. Little evidence can be expected from mating experiments. Fisler found *raviventris* and *halicoetes* to have slightly different social systems and mate preferences. He was able, under carefully programmed laboratory studies, to get 15 partially successful *raviventris-halicoetes* pairings (in three of these, one animal was killed by its mate after 21 or more days). Out of these 12 pairings one litter was produced. The fact that it was produced precludes complete sexual prezygotic isolation, but hybrid inviability seems likely. Another answer to the question might come from experiments involving artificial insemination. Any studies must be completed soon as the two endemic subspecies of *raviventris* are being exterpated by the filling and contamination of San Francisco Bay.

Somewhat similar karyotypic variation in the chromosome number of other endemic kinds of harvest mice related to *R. megalotis* have been found by Blanks (1967). It appears that changes in chromosome number frequently occur in this group, hence it offers a fertile field for cytotaxonomic study. Such variation also raises the question of the basic chromosome number of *R. megalotis*. This species is widespread, ranging from southern Mexico to southwestern Canada, and from California to southern Wisconsin and eastern Texas, but to date all determinations of its diploid number have come from animals from the central coastal region of California.

Specimens Examined

Reithrodontomys megalotis longicaudus (Baird).—Total, 27 (18 males, 9 females). *Contra Costa Co.*: Avon (2). *San Benito Co.*: 1 mi. NE Hollister (4). *Santa Clara Co.*: Alum Rock Park, San Jose (6); 1.7 mi. NNE junction of Watsonville Road and Hecker Pass Road, State Route no. 152 (13); 2 mi. SW Coyote (2).

Reithrodontomys raviventris halicoetes.—Total, 20 (11 males, 9 females). *Solano Co.*: Grizzly Island (8); 4.1 and 4.3 mi. W Napa River on State Route no. 48 (12).

Reithrodontomys raviventris raviventris.—Total, 14 (7 males, 7 females). *Santa Clara Co.*: west end of railroad bridge, Dumbarton (2); 1.7 mi. NNE Alviso (4); 2.0 mi. N Alviso (7); Palo Alto (1).

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