Chromosomal Studies of Two Populations of *Peromyscus difficilis felipensis* (Rodentia: Muridae)

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Summary This study evaluated and compared the chromosomes of 2 populations of *Peromyscus difficilis felipensis* which has a broad but discontinuous distribution. One population occurs throughout the central highlands of Mexico, 2000 m above sea level, where the population range extends through the States of Mexico and Morelos; the second population is found in a locality called Cerro San Felipe, State of Oaxaca. The chromosomes were obtained from bone marrow cells, and their classification is described here. The diploid chromosome number ($2n$) found is 48 and the fundamental number (fn) is 76 for both populations. The principal differences reside in one pair of the morphology of bichromosomes. The chromosomal formula for the population in the States of Mexico and Morelos is $1M+1SM+13ST+8T$, and for the population in the southeast of Oaxaca is $2M+13ST+8T$. In both populations, the X chromosome is submetacentric and the Y chromosome is metacentric. Several comparisons with other subspecies are presented here, as well as the description of a karyotype of *Peromyscus difficilis felipensis* which is different from other reported subspecies.

Key words Karyotype, *Peromyscus*, Rodentia.

Peromyscus difficilis extends from northern Colorado southward into Oaxaca, and comprises 8 subspecies, but only 6 occur in Mexico: *P. penicillatus*, *petricola*, *difficilis*, *amplus*, *felipensis* and *saxicola* (Hoffmeister and de la Torre 1961, Carleton 1989, Hall 1981).

P. d. felipensis is characterized by the following: a large body and tail, large hind foot, large skull (the largest of all subspecies), long nasals, fairly long tooth-rows, a broad braincase and a blackish color suffused with silver. It differs from *P. d. amplus* in that it has a much darker color and a large skull size; however, *P. d. amplus* has a contrasting karyotype and its habitat is rocky (Arellano-Meneses *et al.* 2000). *P. d. felipensis* is the darkest of all subspecies and inhabits the highest altitudes.

The extent of *P. d. felipensis* is discontinuous. This is interesting to note when comparing the karyotype of the population that occurs throughout the central highlands of Mexico, 2000 m above sea level, where the population extends through the States of Mexico and Morelos, with the karyotype of the second population which is distributed in a locality called Cerro San Felipe, State of Oaxaca.

Comparison reveals that specimens of *P. d. felipensis* from Oaxaca and those from central Mexico are quite similar in color, skull size, proportions, and shape. If both population groups (Mexico-Morelos and Oaxaca) are truly of the same subspecies, geographical continuity must have been achieved at one time by way of the narrow corridor of highlands connecting central Mexico and Oaxaca. We felt, as did Hoffmeister and de la Torre 1961, that these 2 populations should be studied for several reasons: the southern population actually belongs to a different stock, but reacts in a similar manner to comparable environmental conditions as does the central population; also, both populations are derived from the same stock (possibly *P. amplus*-like) and thus represent parallel adaptations by different segments of a single stock which evolved in response to new environmental conditions that resembled the earlier ones.

P. difficilis is one of the species of the genus which shows cytological polymorphism. Of the 6 specimens analyzed, there is no standard karyotype for this species (Hsu and Arrighi 1968). Only *P. d. amplus* has been described and the others have been reported to some degree. The purpose of this paper is to present the karyotype of *P. d. felipensis* and to indicate differences that exist between the central and southern populations, besides making a comparison between *P. d. felipensis* and *P. d. amplus*.

Materials and methods

Eight adults (4 female and 4 male) *P. d. felipensis* were collected in a wet oak-pine forest using Sherman traps from the following localities in central Mexico: Santiago Cuautenco, 4 km north-east of Amecameca, Estado de México; and Lagunas de Zempoala, Huitzilac, Morelos. The southeastern locality is in Sierra Norte, Santa Catarina Ixtepeji 17°11'N and 96°38'W, 2901 m a.s.l. (a National Protected Area known as La Cumbre) in an oak-pine forest in Oaxaca, Mexico. Voucher specimens were deposited as museum specimens in the mammal collection of the Universidad Autónoma Metropolitana Iztapalapa.

Metaphase chromosome spreads were obtained from bone marrow with the colchicine-hypotonic method of Baker and Qumsiyeh (1988). Fifty fields on 15 chromosome slides were examined to determine the diploid number ($2n$) of each specimen examined. Conventional karyotypes were prepared from photographs of selected metaphase spreads. Chromosomes were classified on the basis of centromere position following Levan *et al.* (1964).

Classification	PB	IC	D
M	1.00–1.67	37.50–50.00	0.00–2.50
SM	1.67–3.00	25.00–37.50	2.50–5.00
ST	3.00–7.00	12.50–25.00	5.00–7.50
T	>7.00	0.00–12.50	7.50–10.00

The fundamental number was established on the basis of the number of autosomal arms (excluding the sex pair) as defined by Patton (1967). Karyograms of the mitotic chromosomes were prepared from photomicrographs. Individual chromosomal elements were cut from prints and paired with their presumed homologue.

Results

The chromosomal complement of *P. d. felipensis* from the States of Morelos and Estado de México comprises the following: 1 pair of (small) metacentric autosomes, 1 pair of (small) submetacentric autosomes, 13 pairs of (large to small) subtelocentric autosomes, 8 pairs (ranging from mid-size to small) of telocentric autosomes, a large subtelocentric X, and a small metacentric Y chromosome. The population of Cerro San Felipe, Oaxaca has the same chromosome set except for the submetacentric autosome which is missing, but it has two pairs of (small) metacentric autosomes. Both populations share the same $2n=48$ and the same $fn=76$. Sex chromosomes correspond to the typical XX/XY mammalian system and their karyotypes are structurally homologous.

A statistical analysis of each population was made based on 10 karyotypes in order to determine the individual lengths of the chromosomes and of chromosome arms p and q. The position of the centromere was established according to Levan *et al.* (1964). Tables 1 and 2 show results of the analyses made in the two populations. Number 1 is from central Mexico (States of Mexico and

Table 1. Relative length and classification of the chromosomes of *Peromyscus difficilis felipensis* from central Mexico (States of Mexico and Morelos)

Chromosome pair	Relative length of p	Relative length of q	PB=q/p	I.C=p/(p+q)(100)	D=(PB-1)(10)/(PB+1)	Classification
1	1.8625	2.41296875	1.29555369	43.57013016	1.285973967	M
2	1.4459375	3.36140625	2.32472444	30.08496867	3.983006265	SM
3	2.77140625	11.26390625	4.0643288	19.74506452	6.050987096	ST
4	2.3896875	10.13125	4.23957107	19.0999472	6.180010561	ST
5	2.03171875	11.19015625	5.50772899	15.37130098	6.925739805	ST
6	1.82828125	9.58484375	5.24254337	16.02310221	6.795379557	ST
7	1.77203125	8.84359375	4.99065338	16.69174917	6.661650166	ST
8	1.775	8.2253125	4.63397887	17.75854216	6.448291568	ST
9	1.6790625	7.34578125	4.37493021	18.60607608	6.278784784	ST
10	1.5565625	6.530625	4.19554306	19.2505997	6.14988006	ST
11	1.4296875	5.9396875	4.15453552	19.386521	6.122695801	ST
12	1.4215625	5.5790625	3.92459881	20.29223859	5.941552282	ST
13	1.32203125	5.295625	4.00567309	19.98331661	6.003336678	ST
14	1.3275	5.36984375	4.04508004	19.82030667	6.035938666	ST
15	1.15984375	4.75421875	4.09901657	19.61797803	6.076404395	ST
16	0.65625	9.84265625	14.9983333	6.250202978	8.749959404	T
17	0.625	9.3171875	14.9075	6.286802439	8.742639512	T
18	0.625	7.8328125	12.5325	7.389796039	8.522040792	T
19	0.6125	6.77546875	11.0619898	8.300455326	8.339908935	T
20	0.55	6.189375	11.2534091	8.163819666	8.367236067	T
21	0.53125	5.895	11.0964706	8.266175253	8.346764949	T
22	0.50625	5.47215625	10.8091975	8.472919389	8.305416122	T
23	0.49375	4.87109375	9.86550633	9.221342699	8.15573146	T
X	2.45	15.67	6.39591837	13.5209713	7.29580574	ST
Y	1.57	2.3025	1.46656051	40.54228535	1.891542931	M

Table 2. Relative length and classification of chromosomes of *Peromyscus difficilis felipensis* from Oaxaca

Chromosome pair	Relative length of p	Relative length of q	PB=q/p	I.C=p/(p+q)(100)	D=(PB-1)(10)/(PB+1)	Classification
1	2.3	2.999375	1.304076087	46.12800952	0.774398096	M
2	2.5534375	3.818125	1.495288214	39.30616074	2.138767851	M
3	3.6109375	12.815	3.548939853	21.59258704	5.681482593	ST
4	3.2325	12.065625	3.732598608	21.70139185	5.65972163	ST
5	2.5053125	12.2749375	4.899563428	17.43389356	6.513221288	ST
6	2.279375	11.6246875	5.09994516	18.55033867	6.289932266	ST
7	2.22625	9.970625	4.478663672	19.52722056	6.094555887	ST
8	2.153125	9.2409375	4.291872279	20.06304098	5.987391805	ST
9	2.116875	9.09625	4.29701801	19.86060314	6.027879372	ST
10	2.0425	7.9665625	3.900397797	21.74319078	5.651361843	ST
11	1.935	7.1225	3.680878553	20.42950558	5.914098884	ST
12	1.625	6.08125	3.742307692	20.46220353	5.907559295	ST
13	1.5375	6.115625	3.977642276	21.953603	5.6092794	ST
14	1.491875	6.1325	4.110599078	21.22136152	5.755727696	ST
15	1.3584375	5.3784375	3.959282264	21.28260345	5.74347931	ST
16	0.6625	6.1053125	9.215566038	2.838751677	9.432249665	T
17	0.65	9.9171875	15.25721154	6.113914979	8.777217004	T
18	0.625	9.058125	14.493	6.087219699	8.78255606	T
19	0.625	7.57375	12.118	7.30998937	8.538002126	T
20	0.6125	6.7528125	11.025	8.014416522	8.397116696	T
21	0.6125	6.4671875	10.55867347	8.258033733	8.348393253	T
22	0.6	5.7496875	9.5828125	8.662099378	8.267580124	T
23	0.559375	5.4765625	9.790502793	9.572198744	8.085560251	T
X	3.738125	16.73	4.475505768	19.34640523	6.130718954	ST
Y	1.64	2.17	1.323170732	43.0904933	1.381901339	M

Fig. 1. Karyotype (A) and Metaphase (B) of *Peromyscus difficilis felipensis* from central Mexico.

Fig. 2. Karyotype (A) and Metaphase (B) of *Peromyscus difficilis felipensis* from Oaxaca.

Fig. 3. Idiograms of *Peromyscus difficilis felipensis* from central Mexico (a) and from Oaxaca (b).

Morelos) and number 2 is from Oaxaca. Figures 1 and 2 show the karyotypes and some of the mitosis used to describe each karyotype taken from the central population and the southeastern population from Oaxaca, respectively. Figure 3 shows the idiograms for both populations.

Discussion

Many of the most extreme examples of karyotypic variation are those encountered in the order Rodentia. With few exceptions, cytogeneticists have found a different karyotype for every rodent species investigated and, in many cases, have even found several different karyotypes within a species (Mascarello *et al.* 1974).

Our findings on the $2n$ and fn of *Peromyscus difficilis felipensis* are similar to results reported for this species by Hsu and Arrighi in 1968. Our data indicate that this species show some intrasub-specific chromosomal polymorphism. We detected karyotypic polymorphism occurring between these two populations from central and southeastern Mexico. *Peromyscus difficilis* is one of the species in this genus which shows cytological polymorphism. Of the specimens previously reported (Diersing 1976, Lee *et al.* 1972, Hsu and Arrighi 1968, Arellano-Meneses *et al.* 2000), there is no "standard" karyotype for this species. If we compare it with *P. d. amplus* (another subspecies from central Mexico), we can see the differences (see next table).

<i>P. d. amplus</i>	2M	0 SM	8 ST	13 T	X=ST	Y= Small T	$2n=48$	$nf=66$	Arellano <i>et al.</i> 2000
<i>P. d. felipensis</i> central Mexico	1M	1 SM	13 ST	8 T	X=ST	Y= Small M	$2n=48$	$nf=76$	Present study
Oaxaca	2M	0 SM	13 ST	8 T	X=ST	Small M	$2n=48$	$nf=76$	Present study

The subspecies *amplus* has more telocentric chromosomes than *difficilis*. Chromosomal rearrangements such as fusions, pericentric inversions, and heterochromatin addition have been identified as the factors responsible for turning uni-armed chromosomes into biarmed chromosomes (Patton and Rogers 1993). Both the southern and northern populations could be derived from the same stock (possibly *amplus*-like), yet at present they denote similar adaptations by different segments of a single stock evolving in response to similar but new environmental conditions (Hoffmeister and de la Torre 1961).

Perhaps cytological characteristics are no more reliable than other morphological characters in this regard. Breeding experiments and morphometric studies must be performed in the future to provide answers to this problem and to determine the degree of isolation.

This heteromorphism could be interpreted as constituting a polymorphism within a continuum. The karyotypic differences and the geographic distribution of the various groups point to the beginning of a changing gene pool.

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