

CHROMOSOMAL DIFFERENTIATION BETWEEN THE JACKRABBITS *Lepus insularis* AND *Lepus californicus* FROM BAJA CALIFORNIA SUR, MEXICO

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Resumen. Se estudiaron y compararon los cromosomas de dos especies de liebres de México. Los números diploides y fundamentales de *L. insularis* fueron 48 y 80, respectivamente, mientras que los de *L. californicus* fueron 48 and 82. Los autosomas de *L. insularis* fueron cuatro pares de metacéntricos, cuatro pares de submetacéntricos, nueve pares de subtelocéntricos y seis pares de telocéntricos. En contraste, *L. californicus* tuvo siete pares de metacéntricos, cuatro pares de submetacéntricos, siete pares de subtelocéntricos y cinco pares de telocéntricos. El cromosoma sexual X de *L. insularis* fue submetacéntrico de tamaño medio y el cromosoma sexual Y fue telocéntrico y pequeño. Los dos cromosomas sexuales de *L. californicus* fueron submetacéntricos y medianos. Se identificaron una inversión pericéntrica y dos deleciones en los cromosomas de *L. californicus*, las cuales explican las diferencias entre los patrones de bandas G de ambas especies. Sus diferencias en heterocromatina constitutiva fueron pocas. Estas diferencias cromosómicas pudieron haber aparecido en una población ancestral aislada de *L. californicus* durante el Pleistoceno y derivaron en el cariotipo actual de *L. insularis*. Los resultados complementan conclusiones de estudios morfológicos y morfométricos.

Abstract. We evaluated and compared the chromosomes of two species of Mexican jackrabbits. The 2n and FN of *L. insularis* were 48 and 80, respectively, whereas those of *L. californicus* were 48 and 82. The autosome morphology of *L. insularis* is four pairs of metacentric chromosomes, four pairs of submetacentric chromosomes, nine pairs of subtelocentric chromosomes and six pairs of telocentric chromosomes. In contrast, *L. californicus* had seven pairs of metacentric chromosomes, four pairs of submetacentric chromosomes, seven pairs of subtelocentric chromosomes, and five pairs of telocentric chromosomes. The X chromosome of *L. insularis* was medium-sized and submetacentric; the Y chromosome was small and telocentric, whereas both sex chromosomes of *L. californicus*

were medium-sized and submetacentric. A pericentric inversion and two deletions in chromosomes of *L. californicus* were identified which explain the differences between the G-banding patterns of the two species of jackrabbits. There were few interspecific differences within the amount of constitutive heterochromatin. The chromosome variation may have arisen in the isolated ancestor of *L. californicus*, and produced the karyotype of the extant *L. insularis* during the Pleistocene. These results complement conclusions from morphological and morphometric comparisons.

Key words: Chromosomes, G- bands, C- bands, jackrabbits, *Lepus insularis*, *Lepus californicus*, Baja California, México.

INTRODUCTION

The black jackrabbit (*Lepus insularis*) is endemic to Espíritu Santo Island, which lies East of La Paz Bay, in the Southern portion of the Peninsula of Baja California, Mexico (Thomas and Best, 1994). The island is 99 km² and is located 6 km offshore the peninsular mainland (Gastil *et al.*, 1983). The peninsular counterpart of the black jackrabbit is the black-tailed jackrabbit (*L. californicus*), a common and widespread leporid in Mexico (Hall, 1981). The black jackrabbit is a monotypic species and is considered rare (SEDESOL, 1994) and near-threatened (IUCN, 1996). Unfortunately, its biology is poorly known (Cervantes *et al.*, 1996; Thomas and Best, 1994). One of the urgent research activities identified by Chapman *et al.* (1990) to ensure the survival of the black jackrabbit was to carry out genetic and morphological studies to determine the relationship of this species with the black-tailed jackrabbit from the mainland.

Both species are closely related (Hall, 1981). An ancient population of the black-tailed jackrabbit of the Peninsula of Baja California may have become isolated when the Espíritu Santo Island separated from the peninsular territory (Gastil *et al.*, 1983). Later, the founding population could have diverged and speciated to the present black jackrabbit. However, no research has proved this hypothesis.

Except for the coat color, these species of jackrabbits are similar. However, some cranial characters (Hall, 1981) and a numerical taxonomic analysis of several species of *Lepus* (Dixon *et al.*, 1983) revealed enough differences with regard to the two forms as distinct species. Particularly, the jugal bones of *L. insularis* are heavier than those of *L. californicus*, and there is definite clustering separating *L. insularis* from *L. californicus* when both species are compared. Unfortunately there are not genetic data available to support or refute this taxonomic conclusion.

The conventional karyotype of the black jackrabbit has never been reported so far. Detailed karyotypic information may contribute to ascertain whether there is

genetic divergence between the black jackrabbit and the black-tailed jackrabbit. The karyotype of the genus *Lepus* is conservative. Every member of this genus - including American samples of *L. californicus* (Worthington and Sutton, 1966) - has a diploid chromosome number ($2n$) of 48 and a fundamental number (FN) of 88 (Azzaroli Puccetti *et al.*, 1996; Best, 1996; Robinson *et al.*, 1983; Stock, 1976; Uribe-Alcocer *et al.*, 1989). The only known exception is *L. callotis* with a FN = 90 (González and Cervantes, 1996). Accordingly, we would expect that the black jackrabbit display the same common $2n$ and FN.

In addition, data from differentially stained karyotypes of several species of *Lepus* have shown that there is no evidence of non-Robertsonian chromosomal rearrangements in the species examined (Azzaroli Puccetti *et al.*, 1996; Robinson *et al.* 1983). Chromosomal variation, therefore, is not a widespread phenomenon among jackrabbits. Furthermore, only a few differences in heterochromatin distribution have been reported, opposite to the case of rabbits of the genus *Sylvilagus* (Robinson *et al.*, 1983, 1984).

Conversely, the divergence among mammals of the Baja California Peninsula has occurred in oceanic islands presumably because of the relatively greater effects of isolation since the Pleistocene; distinctive chromosomal traits may be found in those well-isolated populations (Lawlor, 1983). However, genetic distinctiveness of insular mammals has resulted in a modest degree of differentiation from the related mainland species (Lawlor, 1983). On these basis, we predicted that if there is a difference between differentially stained karyotypes of *L. insularis* and *L. californicus*, it should be small. The aim of our study, therefore, is to examine the chromosomal attributes of these jackrabbit species, in order to test our hypothesis.

MATERIALS AND METHODS

Lepus insularis samples were collected in the southern portion of Espiritu Santo Island, Municipio La Paz, Baja California Sur, México (24° 24.5' N, 110° 20.9' W). *Lepus californicus xanti* was caught in the Peninsula at 60 km NW La Paz, Municipio La Paz, Baja California Sur, México (24° 36.4' N, 110° 20.9' W). The jackrabbits were prepared as museum specimens (skin plus skeleton) and deposited in the National Collection of Mammals (CNMA, formerly IBUNAM) from the Universidad Nacional Autónoma de México (Cervantes *et al.*, 1996).

Four females and 10 males of *L. insularis*, and five females and four males of *L. californicus* were examined for cytogenetic analyses. Cell suspension preparations were obtained on site from bone marrow as described by Baker *et al.* (1982), to a point where cells were suspended in fixative solution. Slide preparation for non differentially stained karyotypes and for G- and C-bands were delayed until returning to the laboratory.

Metaphase chromosomes from dividing bone marrow cells were prepared following the colchicine-hypotonic technique (Patton, 1967). Twenty-five mitotic figures per slide and ten slides per individual were prepared and the mitotic fields examined. The chromosomes were measured and classified following Levan *et al.* (1964) and Naranjo *et al.* (1983). The fundamental number was established on the basis of the number of autosomal arms (excluding the sex pair) as defined by Patton (1967). Each chromosome pair of the unbanded karyotypes was numbered according to chromosome morphology (Figs. 1 and 2). G-bands were prepared using the trypsin technique (Seabright, 1971). Comparison of homologies and chromosomal rearrangements as detected by G-banding were assessed. Chromosomes were numbered following the *L. c. xanti* format (Fig. 3). C-bands were prepared using the method of the barium hydroxyde (Sumner *et al.*, 1971). Differences in amounts and distribution of constitutive heterochromatin were recorded. Chromosomes were numbered as for conventional staining (Figs. 4 and 5).

RESULTS

Lepus insularis had a $2n = 48$ and a $FN = 80$ (Fig. 1). The autosomes consisted of four pairs of medium-sized metacentric chromosomes, four pairs of small-to-large submetacentrics, nine pairs of small-to-large subtelocentrics, and six pairs of small-to-medium telocentric chromosomes. The X chromosome was medium-sized and submetacentric while the Y was small and telocentric.

The karyotype of *L. californicus xanti* is $2n = 48$ and $FN = 82$ (Fig. 2). The autosome complement consisted of seven pairs of small-to-medium metacentric chromosomes, four pairs of small-to-large submetacentric chromosomes, seven pairs of small-to-large subtelocentric ones, and five pairs of small-to-medium telocentrics. The X chromosome was medium-sized and submetacentric; the Y chromosome was small and submetacentric.

The comparative G-banding patterns of *Lepus insularis* and *L. californicus* are shown in Fig. 3. The G-banding pattern of *L. insularis* showed distinctive bands of euchromatin along the arms of the autosomes and along the X chromosome (Fig. 3). The Y chromosome displayed euchromatic material at the centromeric region. The autosomes and the X chromosome of *L. californicus* also showed conspicuous bands of euchromatin; both arms of the Y chromosome were also G-band positive (Fig. 3). Homologous autosomes and X and Y chromosomes were identified through the matching of G-bands. The concordance between the G-banding patterns of both species showed that their karyotypes are structurally homologous, however, some differences between their banding patterns were evident (Fig. 3).

After analyzing the differential staining patterns of each pair of chromosomes from both species, two chromosome rearrangements were identified, namely a

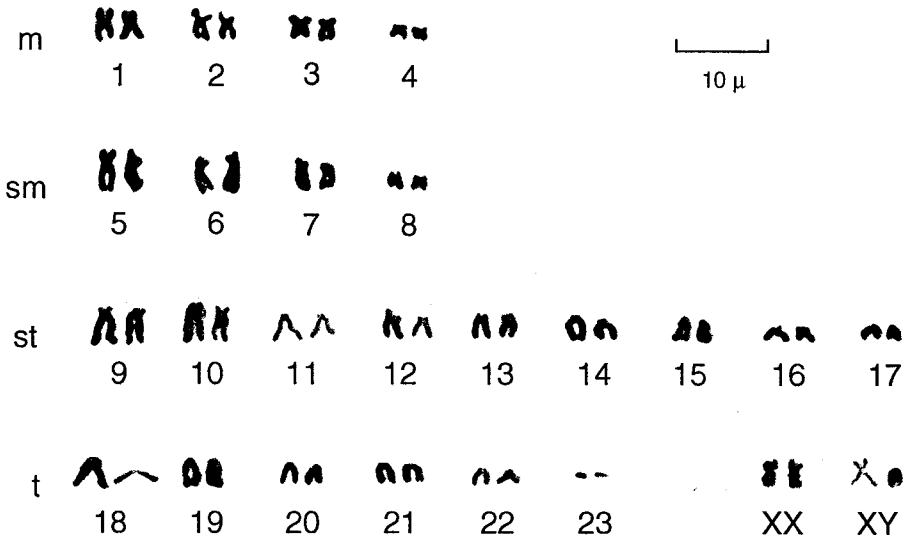


Figure 1. The karyotype of the black jackrabbit *Lepus insularis* (36544 female and 36547 male, National Mammal Collection, Instituto de Biología, Universidad Nacional Autónoma de México, from Espiritu Santo Island, Municipio La Paz, Baja California Sur, México, 24° 24.5' N, 110° 20.9' W). m = metacentric chromosome, sm = submetacentric chromosome, st = subtelocentric chromosome, t = telocentric chromosome. XX and XY = female and male sex chromosomes, respectively.

pericentric inversion and two losses of a chromosome segment by deletion (Fig. 3). The inversion took place in the metacentric chromosome pair 4 of *L. californicus* and produced the telocentric chromosome pair 6 of *L. insularis*. The G-banded karyotype comparison showed that losses of euchromatin took place in the metacentric chromosome pairs 5 and 6 of *L. californicus*, and produced the subtelocentric chromosome pairs 8 and 9, respectively, of *L. insularis*.

The C-banding pattern of *L. insularis* showed small amounts of constitutive heterochromatin (Fig. 4) in the pericentric area of the metacentric and submetacentric autosomes, and on the two largest subtelocentric autosomes. The X chromosome also appeared slightly stained while the Y chromosome was fairly heterochromatic. The genome of *L. californicus* displayed more autosome pairs with constitutive heterochromatin than *L. insularis*, and the distribution of this C-band material was distinctive (Fig. 5). Heterochromatin in the pericentric position was present on chromosome pairs 1, 2, 3, 4, 6, 7, 8, 10, 11, 15, 16, 18, and 19. In addition, interstitial constitutive

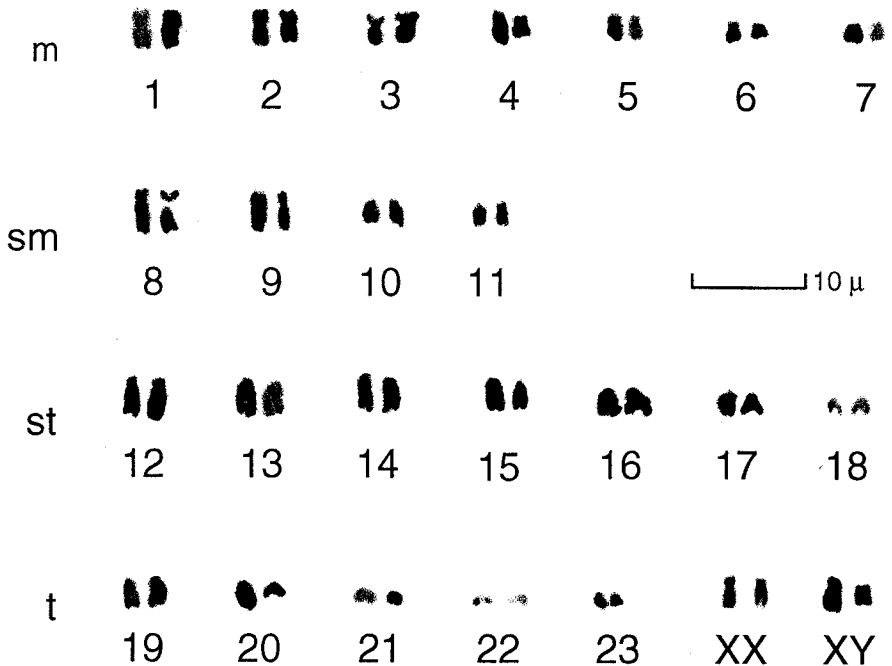


Figure. 2. The karyotype of the black-tailed jackrabbit *Lepus californicus* (38409 female and 37142 male, National Mammal Collection, Instituto de Biología, Universidad Nacional Autónoma de México, from 60 km NW La Paz, Municipio La Paz, Baja California Sur, México, 24° 36.4' N, 110° 20.9' W). m = metacentric chromosome, sm = submetacentric chromosome, st = subtelocentric chromosome, t = telocentric chromosome. XX and XY = female and male sex chromosomes, respectively.

heterochromatin was in chromosome pairs 4, 5, 6, 9, 12, 13, 14, 15, 17, and 19; telomeric constitutive heterochromatin was observed in chromosome pairs 3, 4, 10, 11, 18, and 19. The X chromosome was barely stained, while the long arm of the Y chromosome was C-band positive. Thus, the differences in the amount and distribution of constitutive heterochromatin between the two species were evident.

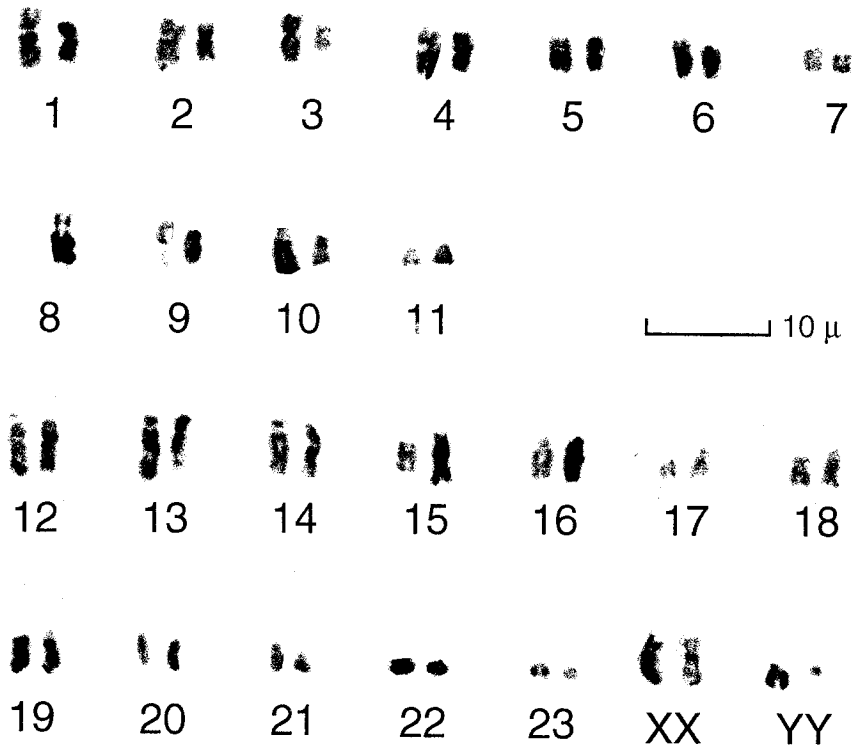


Figure 3. Comparison of G-banded chromosomes between the black-tailed jackrabbit *Lepus californicus* (38409 female and 37142 male, National Mammal Collection, Instituto de Biología, Universidad Nacional Autónoma de México, from 60 km NW La Paz, Municipio La Paz, Baja California Sur, México, 24° 36.4' N, 110° 20.9' W) and the black jackrabbit *L. insularis* (36544 female and 36545 male, Instituto de Biología, Universidad Nacional Autónoma de México, from Espíritu Santo Island, Municipio La Paz, Baja California Sur, México, 24° 24.5' N, 110° 20.9' W). Numbers refer to the chromosome-pair number. The chromosome at the left of each pair belongs to *L. californicus* and the one at the right side belongs to *L. insularis*. Chromosome-pair 4 of *L. insularis* shows a pericentric inversion, whereas pairs 5 and 6 of the same species show losses of euchromatic segments.

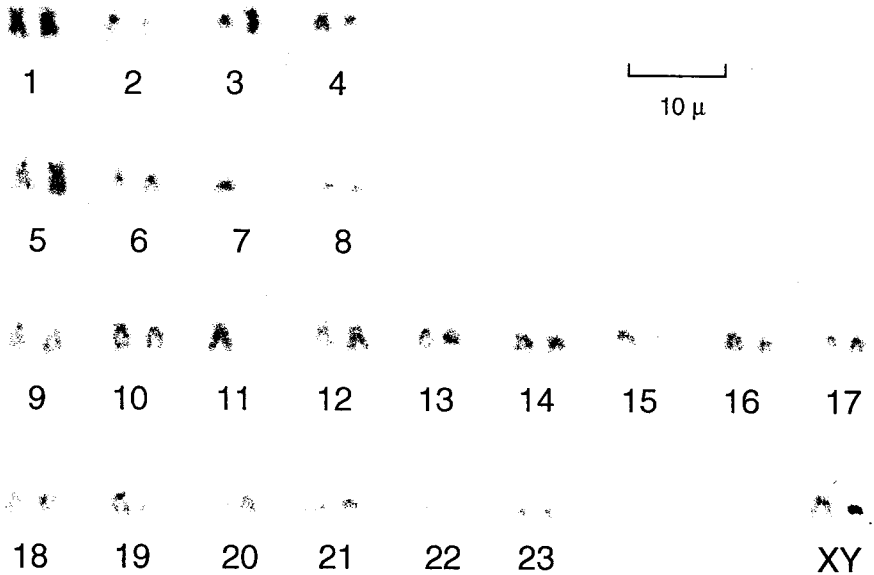


Figure 4. C-banding pattern of the black jackrabbit *L. insularis* (36544 female and 36545 male, National Mammal Collection, Instituto de Biología, Universidad Nacional Autónoma de México, from Espíritu Santo Island, Municipio La Paz, Baja California Sur, México, 24° 24.5' N, 110° 20.9' W). Numbers identify each chromosome pair.

DISCUSSION

Conventional karyotypes of the black jackrabbit (*Lepus insularis*) and a Mexican population of the black-tailed jackrabbit (*L. c. xanti*) are described here for the first time. Thus the $2n$ and FN of all species of jackrabbits occurring in Mexico are now reported. As expected, the Mexican populations of *L. californicus* and *L. insularis* both have the same diploid number present in other species of the genus *Lepus*. However, compared to previous findings on the FN of jackrabbits world wide (Azzaroli Puccetti *et al.*, 1996; Best, 1996; González and Cervantes, 1996; Robinson *et al.*, 1983; Uribe-Alcocer *et al.*, 1989), both species of jackrabbits have fewer autosomal arms. American populations of *L. californicus* have an FN = 88 (Best, 1996; Robinson *et al.*, 1983; Worthington and Sutton, 1966), whereas the Mexican specimens examined have an FN = 82. In addition, *L. insularis* displayed the lowest FN (80), having six pairs of uni-armed chromosomes rather than five, as in the black-

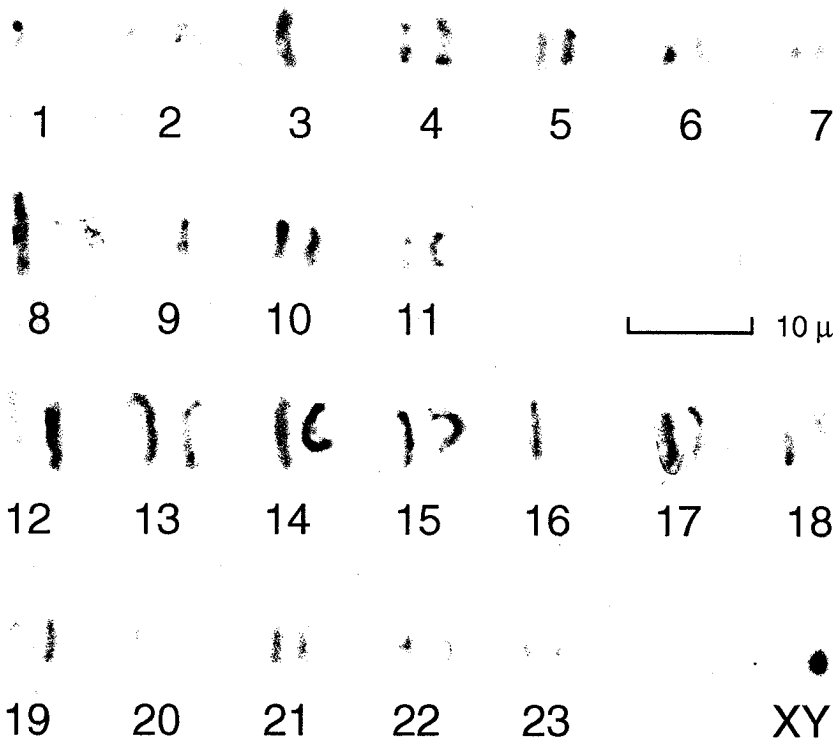


Figure 5. C-banding pattern of the black-tailed jackrabbit *L. californicus* (38409 female and 37142 male, National Mammal Collection, Instituto de Biología, Universidad Nacional Autónoma de México, from 60 km NW La Paz, Municipio La Paz, Baja California Sur, México, 24° 36.4' N, 110° 20.9' W). Numbers identify each chromosome pair. The X chromosome shows almost no heterochromatin.

tailed jackrabbit. Further research is needed to know if differences in FN between American and Mexican populations of *L. californicus* are due to the occurrence of non-Robertsonian events in the latter.

The X chromosome of *L. insularis* and *L. californicus* is medium-sized and submetacentric, as in other species of *Lepus* (Azzaroli Puccetti *et al.*, 1996; Robinson *et al.*, 1983; Uribe *et al.*, 1989). However, the Y chromosome of both species does not share the same morphology. The submetacentric Y chromosome of *L. californicus*

was similar to that of *L. callotis* (González and Cervantes, 1996). In contrast, the small telocentric chromosome of *L. insularis* is more in accord with reports for other species of jackrabbits, such as *L. habessinicus* from Ethiopia and *L. europeus* from central Europe (Azzaroli Puccetti *et al.*, 1996; González and Cervantes, 1996; Robinson *et al.*, 1983).

The differential staining procedures for G-bands and the pairing of homologous chromosomes between species did not give any evidence of Robertsonian events. That is, there is a wide correspondence with the G-banding patterns described for other jackrabbits of the world (Azzaroli Puccetti *et al.*, 1996; Robinson *et al.*, 1983). This part of our results supports the conservative nature of the karyotype of the genus *Lepus*. However, a pericentric inversion and loss of two euchromatic bands were identified in *L. insularis*. These types of non-Robertsonian rearrangements had not been observed previously in any species of *Lepus* (Robinson *et al.*, 1983). The inversion identified in this report may explain differences in the FN between the black jackrabbit and *L. californicus*. Similarly, interspecific differences in the morphology of chromosomes are explained by losses of euchromatic segments in the black jackrabbit. Therefore, the results show chromosome differentiation between these species of jackrabbits from Baja California Sur, Mexico, which may have evolved in the black jackrabbit during the Pleistocene after vicariant allopatric isolation. These data are the first evidence of karyotypic divergence within the genus *Lepus*.

The C-banding patterns observed in *L. insularis* and *L. californicus* were similar to that found in other jackrabbits (Robinson *et al.*, 1981, 1983; Stock, 1976). Constitutive heterochromatin occurs in small amounts in most chromosomes including the X, while the Y is heterochromatic. Conversely, each species of jackrabbit appears to possess different amounts of heterochromatin. The differences in amount and distribution of constitutive heterochromatin between *L. californicus* and *L. insularis* are clear. These attributes contribute to the chromosomal distinctiveness of each species. In contrast to rabbits (*Sylvilagus*), the evolutionary meaning of the differences among species of jackrabbits may not be clear (Robinson *et al.*, 1981, 1983).

The FN of *L. californicus* and *L. insularis* deviates from the pattern reported for other jackrabbits. In addition, there are differences between the differentially stained karyotypes of both species of jackrabbits due to non-Robertsonian events and differential amounts of heterochromatin. These results do not match other species of *Lepus* in that the karyotypic diversity within this genus is almost absent.

Lepus californicus and *L. insularis* had the typical diploid number of *Lepus*. However, they have fewer autosomal arms than other jackrabbit species what means a deviation from the conservative condition of the *Lepus* karyotype. In addition, *L. insularis* is chromosomally differentiated from *L. californicus*. This is also unusual, as expected from the conservative pattern of chromosome differentiation among species of *Lepus*. These results complement the morphological (Hall, 1981) and

morphometrical (Dixon *et al.*, 1983) data, and support the hypothesis that the two species are both distinct and closely related to each other. Presumably, *L. insularis* has been isolated from *L. californicus* for > 11,000 years, although the habitats they occupy are similar. Mammal populations of the islands of the Peninsula of Baja California typically are assumed to be descendants of a small number of effective colonizers (Lawlor, 1983).

This study may have important conservation implications for *Lepus* populations from the Peninsula of Baja California, particularly for *Lepus insularis*. This is a fragile species that has an insular distribution restricted to a small island and may soon face a high risk of extinction (Best, 1996; Chapman *et al.*, 1990). *Lepus insularis* represents a distinctive genetic lineage that must be protected and preserved. Further research on the biology of this species is recommended to plan conservation actions.

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