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# CHROMOSOMAL VARIATION IN THE NEW ENGLAND COTTONTAIL, *SYLVILAGUS TRANSITIONALIS*

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The karyotype of the New England cottontail, *Sylvilagus transitionalis*, was first reported as having a diploid number (2n) of 52 and a fundamental number (number of autosomal arms) of 98 by Holden and Eabry (1970), for specimens collected in Connecticut. Wilson (1981) confirmed the 2n = 52 karyotype for *S. transitionalis* from New England, but indicated the fundamental number to be 94. Banded karyotypes presented by Robinson et al. (1983) from four specimens of *S. transitionalis* from Maryland and West Virginia were at variance with these earlier reports by having a 2n = 46. Robinson et al. (1983) suggested that previously reported karyotypes of *S. transitionalis* with 2n = 52 (Holden and Eabry, 1970; Wilson,

1981) resulted from incorrect species identification, or that *S. transitionalis* was chromosomally polymorphic. Robinson et al. (1984) considered the diploid number of *S. transitionalis* to be 46.

We investigated G- and C-banded patterns of *S. transitionalis* from northern and southern parts of the range of the species to determine whether two or more discrete chromosomal races, or cytotypes, exist in what is recognized as a monotypic species. We report here data confirming the existence of a 2n = 52 cytotype of *S. transitionalis*, the chromosomal rearrangements accounting for the polymorphism, and results of attempts to determine the geographic distribution of the two cytotypes. We define "cytotype" as a distinct chromosomal race of a putative species, a chromosomally characterized population; distinct cytotypes potentially represent the result of ongoing or recent speciation events.

Specimens of *S. transitionalis* were collected in New Hampshire, New York, Virginia, and West Virginia, in modified Stephenson box traps (Day et al., 1980). Animals were held in captivity from 1 week to 6 months allowing collection of blood samples for lymphocyte culture.

Lymphocyte culturing followed the procedure of the San Diego Zoo Research Department (O. Ryder, pers. comm.). Cultures were established from whole blood in the presence of either mitogen derived from pokeweed (*Phytolacca americana*) or 4$\beta$-phorbol 12-myristate 13-acetate (Sigma #P-8139; 0.25 ml in 5 ml medium) in $\alpha$-Minimum Essential Medium. Cultures were incubated for 48 h and were injected with 0.04 ml colcemid 40 min before harvest. Hypotonic treatment, fixation, and slide preparation were similar to methods described by Baker et al. (1982).

Slides were treated sequentially for G- and C-bands, providing unequivocal chromosome identification with both techniques. G-banding followed the technique of Seabright (1971). After selected metaphase spreads were photographed, slides were destained by washing for 2 min each in 1:1 xylene : absolute ethanol, absolute ethanol, 95% ethanol, and 50 ml 70% ethanol with 0.5 ml concentrated HCl. Slides were blotted with bibulous paper between each wash and rinsed in 70% ethanol before being air dried.

Chromosomes were C-banded by modification of the technique of Sumner (1972). Following destaining, slides were rinsed twice in 95% ethanol, dipped 5–8 times in 0.9% saline, immersed in a saturated solution of $Ba(OH)_2 \cdot 8H_2O$ at room temperature from 3 to 8 min, rinsed once in 0.9% saline, and immersed in 2× saline sodium citrate at 56°C for 4 h. Slides then were rinsed in tap water and stained from 30 to 45 min in 8% Giemsa (10 ml Giemsa, 0.8 ml 0.34% $KH_2PO_4$, pH 6.8). Chromosomes are numbered following Robinson et al. (1983) to facilitate comparison; in the discussion of chromosomal homologies, "p" and "q" refer to the short and long arms of biarmed chromosomes, respectively.

*Specimens examined.*—NEW HAMPSHIRE: Hillsboro Co.; Bedford, on Route 3, about 0.5 mi S Manchester Country Club, 0.2 mi from Merrimack River ($n$ = 2); Strafford Co.; Dover, Dover Industrial Park ($n$ = 3). NEW YORK: Westchester Co.; 0.4 mi N, 0.9 mi W Armonk, Louis Calder Conservation and Ecology Center of Fordham University ($n$ = 1). VIRGINIA: Bath Co.; 5.25 mi S, 1.5 mi W Hot Springs, Bald Knob ($n$ = 2). WEST VIRGINIA: Grant Co.; Dolly Sods Scenic Area (Monongahela National Forest), 0.96 km N, 0.35 km E Red Creek Campground, 39°02′28″N, 79°18′40″W, elevation 1,220 m ($n$ = 1).

Cottontails from New Hampshire and New York had 2n = 52 karyotypes, whereas *S. transitionalis* from Virginia and West Virginia had 2n = 46. The G-banded karyotype of the southern cytotype (Virginia and West Virginia) was indistinguishable from that reported by Robinson et al. (1983) for *S. transitionalis* from the same area. The diploid complement consists of 10 pairs of metacentric or submetacentric, 10 pairs of subtelocentric, and 2 pairs of acrocentric chromosomes (fundamental number = 84). The X chromosome is medium-sized and submetacentric; the Y is small and acrocentric. The G-banded (Fig. 1a) and C-banded (Fig. 1b) karytotypes of the northern cytotype, 2n = 52 (New Hampshire), consist of 7 pairs of submetacentric, 10 pairs of subtelocentric, and 8 pairs of acrocentric chromosomes, also resulting in a fundamental number of 84. Morphology of the X and Y chromosomes is the same as in the southern cytotype.

Our chromosomal data confirm the previously reported 2n = 46 karytotype of *S. transitionalis* in West Virginia (Robinson et al., 1983) and corroborate the existence of the 2n = 52 cytotype reported by Holden and Eabry (1970) and Wilson (1981). Clearly, there exist at least two cytotypes among populations traditionally referred to *Sylvilagus transitionalis*. These two cytotypes differ by a total of four rearrangements: three Robertsonian translocations and a paracentric inversion. The three Robertsonian rearrangements account for the difference in diploid number between the two cytotypes. Metacentric chromosomes 1, 2, and 4 of the southern cytotype are represented by six acrocentric chromosomes in the northern cytotype (Figs. 1a, b). Two fissions result in chromosomes 2p, 2q, 4p, and 4q of the northern cytotype. Centric fusion of *Lepus* acrocentric chromosomes 12 and 13 (Robinson et al., 1984), which resulted in biarmed chromosome 1 of the southern cytotype (Robinson et al., 1983), has not occurred in the 2n = 52 form. The single non-Robertsonian rearrangement distinguishing the two cytotypes is the paracentric inversion that occurred in the long arm of the fourth chromosome.

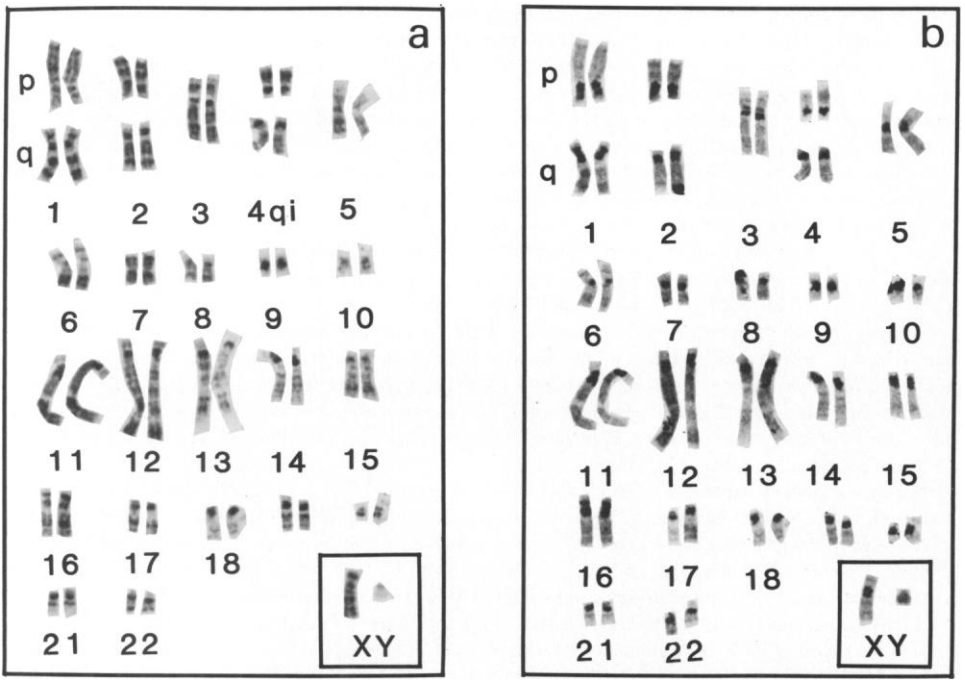


FIG. 1.—(a) G-banded karyotype of the northern cytotype (2n = 52) of *Sylvilagus transitionalis* from New Hampshire. Numbers correspond to chromosomes of the southern cytotype of *S. transitionalis* figured in Robinson et al. (1983); 4qi indicates an inversion in the long arm of chromosome 4 relative to the presumed ancestral leporid karyotype as found in *Lepus* (Robinson et al., 1984). (b) C-banded karyotype of *Sylvilagus transitionalis* from New Hampshire. This karyotype was prepared from the destained G-banded chromosomes shown in Fig. 1a.

Autosomal pairs 11 and 12 of the southern cytotype are markedly heterogeneous with respect to staining intensities of the constitutive heterochromatin (Robinson et al., 1983). This same phenomenon is seen in chromosomes 11, 12, and 13 of the northern cytotype (Fig. 1b). Such heterogeneity presumably results from differences in base composition of the DNA of these segments. The marked heteromorphism caused by interstitial addition of constitutive heterochromatin in certain acrocentric chromosomes of *S. nuttallii* (Robinson et al., 1984) is evident in chromosomes 11 and 13 of the northern cytotype (Fig. 2b). The significance of this heteromorphism, which is not exhibited in all individuals (Robinson et al., 1984), is unclear. The blocks of interstitial heterochromatin found in the northern cytotype probably represent the same features seen in the C-banded chromosomes of *S. nuttallii* and the southern cytotype of *S. transitionalis* reported by Robinson et al.(1984). Although it could be postulated that these heteromorphisms might cause meiotic problems, evidence from *Peromyscus* suggests that this is not the case, and that heterochromatic heterogeneity does not lead to speciation (Hale and Greenbaum, 1988). The euchromatic portions of these chromosomes are highly conserved, as is evidenced by their presence not only in the northern and southern cytotypes of *S. transitionalis*, but also in *S. nuttallii* and, almost unchanged, in the postulated ancestral leporid karyotype, found in *Lepus saxatilis* (Robinson et al., 1984).

Comparison of the *S. transitionalis* karyotypes with karyotypes of other species in *Silvilagus* (Robinson et al., 1983, 1984), with *Lepus* as the outgroup taxon, suggests that the two *S. transitionalis* cytotypes represent sister taxa. The presence of chromosomes 10 and 22 are synapomorphic character states uniting these taxa. In addition, *Lepus* chromosomes 14 and 18 are not in either of the *S. transitionalis* cytotypes (Robinson et al.,1983; this study). *Sylvilagus transitionalis* chromosomes 14, 19, and 21 correspond to 15, 21, and 23 of the *Lepus* genome (Robinson et al., 1983). These form the basis for several Robertsonian fusions in other lineages within *Sylvilagus* (Robinson et al., 1984). Autapomorphic characters of the 2n = 52 northern cytotype include chromosomes 2p and 2q, and 4p and 4q (Fig. 2). These chromosomes are the fission products of chromosomes 2 and 3, respectively, in the *Lepus* genome, where they are metacentric. Chromosomes 1p and 1q of the northern cytotype correspond to *Lepus* chromosomes 12 and 13.

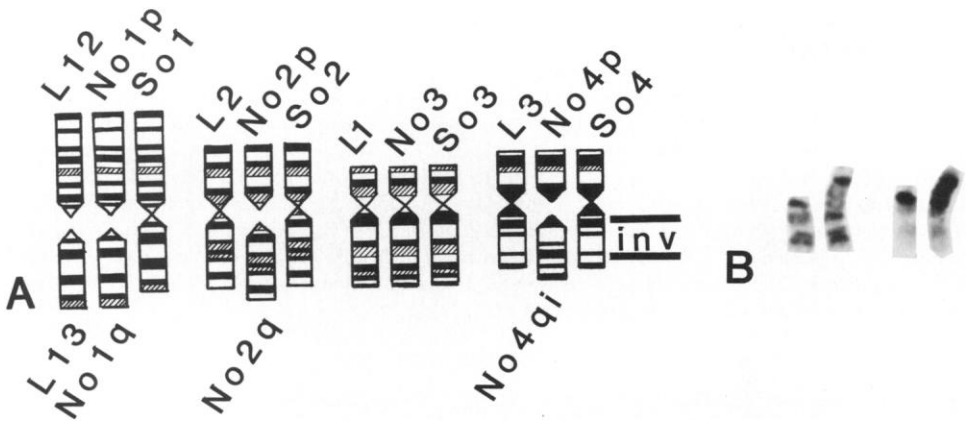


FIG. 2.—(a) Ideogram of G-banded chromosomes 1–4 of northern (No) and southern (So) cytotypes of *Sylvilagus transitionalis* with presumed ancestral condition found in *Lepus* (L; Robinson et al., 1984). The inverted segment of chromosome 4 in the northern cytotype is designated by "inv." (b) Autosomal pair 13 of the northern cytotype of *S. transitionalis* from New Hampshire, sequentially G- and C-stained. This autosomal pair shows marked heteromorphism caused by interstitial addition of constitutive heterochromatin in one of the homologues. The homologues shown are from the same metaphase spread.

Comparison of the cytotypes of *S. transitionalis* with the presumed ancestral karyotype of *Lepus* (Robinson et al., 1984) suggests that the 2n = 52 cytotype arose from centric fissions in an ancestral population with a presumptive karyotype of 2n = 48. The 2n = 46 southern cytotype is hypothesized to have evolved from the ancestral 2n = 48 cytotype by fusion of *Lepus* chromosomes 12 and 13 to form *Sylvilagus* chromosome 1 (Robinson et al., 1983, 1984). Single fission or fusion rearrangements are not thought to cause severe meiotic effects (Porter and Sites, 1986; White, 1978). Multiple fission or fusion events, however, could lead to fixation of individual rearrangements resulting from meiotic problems of disparate pairing (Baker and Bickham, 1986; Walsh, 1982; White, 1978).

Cottontails with the southern cytotype (2n = 46) are currently restricted to high elevation Appalachian habitats from at least Maryland, West Virginia, and Virginia, south presumably to northeast Georgia. Karyotypes of *S. transitionalis* from Pennsylvania, North Carolina, and farther south are unknown, but are also likely 2n = 46. These high-elevation habitats have changed little since the Wisconsinian (Critchfield, 1980). In contrast, cottontails with the northern cytotype have invaded the oak-chestnut (*Quercus-Castanea*) zone (Braun, 1950) and can be collected at lower elevations from New York to Maine. We suggest that the rearrangements resulting in the northern cytotype (2n = 52) became fixed sometime in the late Wisconsinian (ca. 12,500 years ago); animals with this cytotype spread northward with the oak-chestnut forest as the Wisconsinian ice shelf retreated (Critchfield, 1980; Davis, 1976; Leopold, 1967). The spread of cottontails with the 2n = 46 southern cytotype to lower elevations may have been hampered by competition with the ecologically similar *S. floridanus*.

Stock (1976) postulated that chromosomal evolution in leporids proceeded by centric fusion from the most conservative karyotype with 2n = 52 to the most derived karyotypes with 2n = 38, represented by *S. aquaticus* and *S. palustris* (Robinson et al., 1983, 1984). Robinson et al. (1984) considered the 2n = 48 karyotype of *S. bachmani* to be the highest number from a leporid outside the genus *Lepus*. Robertsonian fusions from a presumed ancestral leporid with 2n = 48 were proposed as the primary evolutionary force at work within *Sylvilagus* (Robinson et al., 1984). However, the presumed ancestral karyotype (2n = 48) is present in both *Lepus* and, *S. bachmani* (although *S. bachmani* has additional constitutive heterochromatin; Stock, 1976; Robinson et al., 1984). If this karyotype is primitive for the genus *Sylvilagus*, then Robertsonian fissions and fusions both must have occurred: fission from 2n = 48 to the 2n = 52 northern cytotype of *S. transitionalis*, and fusion resulting in the remaining karyotypes within *Sylvilagus*. The morphological similarity between the two cytotypes suggests that differentiation occurred recently, perhaps near the end of the Wisconsinian. It also is possible, as Stock (1976) postulated, that the *Sylvilagus* group includes the ancestral karyotype for all leporids.

To date, *S. transitionalis* represents the only chromosomally polymorphic taxon within the genus *Sylvilagus*. Recognition of the two chromosomal forms of *S. transitionalis* as distinct species may be warranted at this time, on the basis of the chromosomal differences documented herein and the lack of known hybrids between

the two cytotypes. Further studies of morphology and isozyme variability should clarify the taxonomic level at which these populations will be recognized.

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