Chromosomal and mitochondrial DNA variation in four laboratory populations of collared lemmings (Dicrostonyx)

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Genetic differentiation among populations and speciation in Dicrostonyx is hypothesized to have resulted from early allopatric divergence in glacial refugia during the Wisconsin or sympatric processes uncorrelated with refugial isolation. We examined chromosomal and mitochondrial DNA variation in four laboratory colonies, representing three species, in a preliminary evaluation of these hypotheses. Chromosomal variation is extensive among populations, diploid numbers ranging from 38 to 50. Autosomal variation appears to be due primarily to Robertsonian rearrangements and additions of supernumerary chromosomes, and is geographically unpatterned. Sex chromosome morphology is geographically structured and correlated with proposed southern and northern refugia. Restriction fragment analysis of mitochondrial DNA revealed two ancient, divergent genotypic assemblages, corresponding to geographic distributions of sex chromosomes. Autosomal variation, and any resulting reproductive isolation, probably is recent and uncorrelated with refugial history, whereas divergence of sex chromosomes and disparate mitochondrial assemblages likely predate the Wisconsin.


Chez Dicrostonyx, la différenciation génétique entre populations et la spéciation roulent d’une divergence allopatrique dans les refuges glaciaires au cours du Wisconsinien, ou alors de processus ‘sympatiques’ sans corrélation avec l’isolement dans les refuges. En guise d’évaluation préliminaire de ces hypothèses, nous avons procédé à l’examen de la variation chromosomique et de la variation de l’ADN des mitochondries chez quatre colonies de laboratoire représentant trois espèces. La variation chromosomique est importante chez les populations et les nombres diploïdes varient entre 38 et 50. La variation autosomique semble due surtout à des rearrangements de type Robertson et à l’addition de chromosomes supplémentaires et elle ne semble pas suivre de pattern géographique particulier. La morphologie des chromosomes sexuels suit un schéma géographique et est en corrélation avec les refuges refugial boral et austral. L’analyse de l’ADN des mitochondries au moyen d’essais de restriction a révélé l’existence de deux associations génétiques anciennes divergentes correspondant aux répartitions géographiques des chromosomes sexuels. La variation autosomique, de même que tout isolement génétique qui peut en avoir découlé, est récente et n’est pas reliée à l’histoire des refuges, alors que la divergence des chromosomes sexuels et les associations mitochordonales varient probablement à une époque plus ancienne que la glaciation du Wisconsinien.

[Traduit par la rédaction]

Introduction

Collared lemmings (Dicrostonyx) are tundra-specific rodents renowned for their dramatic fluctuations in population size during which inbreeding probably is periodically intensive (Carothers 1980; Jarrell 1987; Stearneth 1978). Dicrostonyx torquatus once was considered to represent a single circum-polar species (Ognev 1948; Rausch 1963). However, karyo-

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TABLE 1. Composite mtDNA genotypes positions 1–16) and their frequency of occurrence in four laboratory populations of collared lemmings

<table>
<thead>
<tr>
<th>No.</th>
<th>Composite genotype*</th>
<th>Churchill (N = 8)</th>
<th>Arviat (N = 5)</th>
<th>Igloolik (N = 6)</th>
<th>Pearce Point (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
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<td>0.0</td>
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<td>0.400</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.167</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.333</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>BABABABABABBBB</td>
<td>0.0</td>
<td>0.0</td>
<td>0.333</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.167</td>
<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.444</td>
<td>0.0</td>
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<tr>
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<td>0.0</td>
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<tr>
<td>11</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.111</td>
</tr>
</tbody>
</table>

* Notes: Lemmings from Churchill and Arviat are D. rufusculus, and those from Igloolik and Pearce Point are D. groenlandicus and D. alaskanus, respectively.

Rausch (1972). In the Palaecarctic, chromosomal diversity in Dicrostonyx occurs through centric fusions, variation in number of supernumerary chromosomes, and, perhaps, pericentric inversions (Gileva 1983). An unusual mode of sex determination also has been reported for several taxa, with phenotypic females being either XX or XY (see Bull and Bultner 1981; Gileva 1987; Gileva et al. 1982; Gileva and Chebotar 1979; Malcolm et al. 1986). XY females have a XX-linked factor that apparently interferes with normal expression of male sex-determining genes. Males are XY with an unmodified X chromosome. The X-linked factor commonly appears as a polymorphism within populations of collared lemmings, and XY females are fully fertile with a reproductive output equal to that of XX females (Gileva 1987; Gileva et al. 1982).

Three species of the torquatus-group are sometimes recognized in arctic Canada, mainly on the basis of distinct karyotypes from single localities (summarized by Gileva 1983). The extent of chromosomal polymorphism and geographic distribution of karyotypes is largely unknown, however, and the distribution of these "cyspecies" is inferred from subtle morphological differences formerly used to characterize subspecies. Dicrostonyx lepismalis (2n = 47, Banks Island; Rausch 1977) purportedly occurs on Banks and Victoria Islands and the adjacent mainland in the northwestern arctic; D. richardsoni (2n = 42-44, Churchill; Malcolm et al. 1986; Rausch and Rausch 1972) occurs in continental Canada, west of Hudson Bay; and D. groenlandicus (2n = 46, Devon Island; Rausch 1973) occurs in the central and eastern High Arctic, Baffin Island, and west to Southampton Island (Ronack et al. 1982; see also Hall 1981).

Rausch (1977, 1980) hypothesized that chromosomal diversity and the evolution of reproductive isolation was due to allopatric differentiation in glacial refugia during the Wisconsinan (70,000-10,000 years ago) or earlier, in accord with the refugial model of MacPherson (1965). Given the level of polymorphism observed in a few populations and the geographic pattern of interpopulation karyotypic variation, however, isolation in glacial refugia does not appear to explain all the karyotypic variation observed in the species (Krohn 1982). Alternatively, Hoffmann (1981) and Modl (1987) observed that chromosomal variation in Dicrostonyx was also consistent with a model of sympatric chromosomal speciation (stasisaritc model; White 1968), uncorrelated with isolation or refugia.

Herein, we examine variation in chromosomes and mitochonferal DNA (mtDNA) within and among laboratory colonies of Dicrostonyx representing the three Canadian torquatus-group species, in a preliminary evaluation of these alternatives. If Dicrostonyx was geographically fragmented during the Wisconsinan, this pattern of fragmentation should be reflected in geographic partitioning of rapidly evolving, selectively neutral marker sets such as mtDNA (Avise et al. 1987). If chromosomal differentiation is recent and also occurred primarily during isolation in glacial refugia, then geographic patterns of chromosomal and mitochondrial variation should be correlated. Alternatively, if karyotypic variation is due to sympatric differentiation, then geographic patterning should be spatially random and uncorrelated with phylogeographic patterns of divergence in mtDNA.

Materials and methods

Lemming populations

Collared lemmings were taken from four captive populations established with animals from Pearce Point, N.W.T., (by R. Bonnycastle, and from Churchill, Manitoba, Arviat, N.W.T., and Igloolik, N.W.T. (by R. J. Brooks). Voucher specimens are deposited in the research collection of the Department of Mammalogy, Royal Ontario Museum.

Chromosomal analysis

Standard karyotypes were prepared from 29 individuals, as follows: Churchill (8), Northwest Territories (Arviat (6), Igloolik (4), Pearce Point (11)). Standard karyotypes were prepared using the iev bone marrow technique of Patton (1967), as modified by Lee (1969). Terminology regarding relationships of chromocenin arm ratios follows Patton (1967). Fundamental numbers (FN) were calculated as the number of autosomal arms (excluding the presumptive sex chromosomes).

Mitochondrial DNA analysis

For each of 28 specimens (Table 1), mtDNA was isolated from livers and kidneys immediately after death or within 7 days of storage in grinding buffer with high EDTA content (Lamnson et al. 1981). The isolation protocol followed a modification of those described in Brown (1980) and Lasantus et al. (1981). Approximately 2 g of tissue was chopped finely with crossed scalpels on a chilled petri dish.
dish, and then ground with 8–10 strokes in a Dounce homogenizer. The homogenate was centrifuged to pellet the intact nuclei and later the mitochondria. The mtDNA was released from the mitochondria by lysing with SDS, and was purified with two CsCl density gradients, each for 10 h at 43,000 × g in a Beckman TL-100 ultracentrifuge.

All mtDNA samples were digested with 16 restriction endonucleases: 15 six-base enzymes and 1 four-base enzyme. Fragments generated by these enzymes were end-labelled with 32P by nick translation. These were separated by electrophoresis in 1.2 or 1.5% agarose and 4% polyacrylamide gels, and visualized by autoradiography. We used a 1-kb ladder to size standards on all gels.

The net extent of nucleotide divergence between populations (d), corrected for within-population polymorphism, was calculated from fragment data using the method of Nei and Li (1979):

\[ d_x - d_y = (d_x - d_y)/2 \]

where \( d_x \) and \( d_y \) are the nucleotide diversity values in populations \( X \) and \( Y \), respectively, and \( d_{XY} \) is the average number of nucleotide substitutions per site between \( X \) and \( Y \).

Results

Karyology

Dicerostylon richardsoni

Churchill, Manitoba (2n = 47–45, FN = 48; Fig. 1A).–The diploid complement comprised 1–4 large submetacentric and (or) metacentric chromosomes, 1 or 2 large subtelocentric, 4 small metacentric elements, and 35–39 acrocentric chromosomes in a graded series from large to small. A few of the chromosomes defined herein as acrocentric had small areas of chromatid distal to the centromere and could alternatively be termed subtelocentric. Of the eight specimens examined, one male and one female had 2n = 43 with three large metacentric to submetacentric chromosomes, one male and four females had 2n = 44 with two large metacentric to submetacentric chromosomes, and one female had 2n = 45 with one large metacentric. As noted for specimens from this colony by Malcolm et al. (1986), the number of autosomal arms is invariant, regardless of diploid number. The two males had one large, obviously subtelocentric chromosome, whereas females had one or two large, obvious subtelocentrics. The length of the second arm of this chromosome appeared to vary widely in both sexes. Given that XY females are hypothesized to be present in this colony (Malcolm et al. 1986), we suggest that this element likely is the X chromosome. We could not positively identify the Y chromosome, but presume it to be a medium-sized to small acrocentric chromosome.

Arviat, Northwest Territories (2n = 46, FN = 48; Fig. 1B).–The diploid complement comprised, or large subtelocentric chromosomes, 4 small metacentric chromosomes and 40 or 41 acrocentric chromosomes in a graded series from large to small. No variation was observed among the six females examined, except that the number of large, obviously subtelocentric chromosomes varied from one to two. Based on similarity of this karyotype to that of individuals in Churchill, we presume that the large subtelocentric chromosome is the X, and that some XY females were present in this sample.

Dicrostonyx groenlandicus

Igloolik, Northwest Territories (2n = 38–44, FN = 48; Fig. 1C).–The autosomal complement comprised 8 large submetacentric to metacentric chromosomes, 4 or 5 small metacentric chromosomes, 10 medium-sized to large acrocentric chromosomes and 14–20 small acrocentric elements. Each female had an additional pair of large submetacentric chromosomes, whereas each male had one large and one medium-sized submetacentric. We presume that the large submetacentric chromosome is the X and that the medium-sized submetacentric element is the Y. No XY females were observed. Variation in diploid number among individuals was due entirely to variation in the number of small metacentric and acrocentric chromosomes present in the complement. Of the four individuals examined, one male had 2n = 38, 4 small metacentric and 14 small acrocentric autosomes; one female had 2n = 39, with 4 small metacentric and 15 small acrocentric elements; one male had 2n = 41, with 5 small metacentric and 16 small acrocentric chromosomes; and one female had 2n = 44, 4 small metacentric and 20 small acrocentric elements. The additional small metacentric and acrocentric elements present in some individuals are probably supernumerary (B) chromosomes, which are common in Palaeartic members of the torquatus-group (Gileva 1980; Gileva and Chebotar 1979). Based on comparison with other reported karyotypes of Dicrostonyx, the 2n = 38 karyotype with 4 small metacentric and 14 small acrocentric autosomes is likely to be the basic (or 'A') chromosome complement and the autosomal FN for the population is listed tentatively as 48 (excluding B chromosomes).

Dicrostonyx klangmiuak

Pearce Point, Northwest Territories (2n = 47–51, FN = 48, Fig. 1D).–The autosomal complement comprised 40 acrocentric chromosomes in a graded series from large to small and 5–8 small metacentric elements. In addition, three females and all five males had one large and one medium-sized submetacentric chromosome, whereas the other three females had two large submetacentric chromosomes. The large submetacentric chromosome is identified tentatively as the X and the medium-sized metacentric element as the Y. The length of the short arm of the Y chromosome appeared to vary among individuals. Thus, three of the females examined are presumed to be XX and the other three XY. The six females examined were laboratory offspring of mothers that produced an excess of daughters, and the ratio of presumed XY to XX females in our small sample is consistent with that expected among daughters of XY females (1:1; Gileva 1987; Gileva and Chebotar 1979). Variation in diploid number among individuals was due entirely to differences in numbers of small metacentric chromosomes and there was no obvious correlation of diploid number with pre-axillary sex chromosome constitution. Three females (IXX, XXY) had 2n = 47, with five small metacentric elements; one male and two females (IXX, IXY) had 2n = 48, with six small metacentric chromosomes; one male and one female (IXX) had 2n = 49, with seven small metacentric elements; and three males had 2n = 50, with eight small metacentric chromosomes. The small metacentric chromosomes

Fig. 1. Representative karyotypes of Dicrostonyx. (A) A female D. richardsoni (2n = 44, FN = 48) from Churchill, Manitoba. (B) A male D. richardsoni (2n = 46, FN = 48) from Arviat, N.W.T. (C) A female D. groenlandicus (2n = 39, FN = 44) from Igloolik, N.W.T., with one B chromosome. (D) A male D. klangmiuak (2n = 50, FN = 48) from Pearce Point, N.W.T., with four B chromosomes. 
Fig. 2. Relatedness of mtDNA clones found in four laboratory populations of Dicrostonyx, based on UPGMA cluster analysis of percent sequence divergence among clones. Lemmings from Churchill and Arviat are D. richardsonii and these from Igloolik and Peerce Point are D. groenlandicus and D. klungniutak, respectively. I and II designate the two clonal assemblages separated by the deep branch in the gene tree.

present, in addition to the four that are ubiquitous among all Dicrostonyx which have been karyotyped, are most likely supernumerary chromosomes. Therefore, we tentatively list the fundamental number of the autosomal complement as 48 (excluding B chromosomes).

Mitochondrial DNA variation

Based on enzymes that produced fragments in a suitable size range for detection on our gels, we estimate the mitochondrial genome size in collared lemmings to be about 16,700 bp. This value is typical of microtine rodents (Plante et al. 1989) and of vterebates in general (Moritz et al. 1988). The restriction enzymes we used produced an average of 87 fragments per individual, representing 425 recognized base pairs, or about 2.5% of the mitochondrial genome.

A total of 11 composite mtDNA genotypes (clones) was detected in the 28 collared lemmings we surveyed from the four populations (Table 1). Two major clonal assemblages are apparent (Fig. 2): lemmings from the southern sampling sites at Churchill and Arviat have variations on one type of mtDNA (clonal assemblage I), whereas animals from the northern sites, Igloolik and Pearce Point, have variants of another type (clonal assemblage II). Lemmings can be assigned unequivocally to these clonal assemblages by polyacrylamide gels detected by three restriction enzymes (BstI, HincII, and HindIII). In addition, six enzymes (FokI, SalI, XbaI, BglII, NcoI, and SacI) detect clones that are restricted to populations within either the northern or the southern assemblage.

Clonal diversity varies among populations; the Churchill sample is fixed for one clone, the Arviat sample has two clones which are both well represented, and the Igloolik and Pearce Point samples each have four clones. All clones are unique to single populations.

Discussion

Chromosomal variation

Variation within laboratory stocks of collared lemmings is ascribed to additions of supernumerary (B) chromosomes, Robertsonian translocations, and, in three of four populations, the presence of XY females. Supernumerary chromosomes previously were reported in several populations of D. torquatus from Siberia (summarized by Gileva 1983) and apparently are present in Dicrostonyx from the Arctic Coastal Plain of Alaska (G. H. Jarrel, personal communication). Thus, their presence in Canadian samples was not unexpected; however, identification of specific B chromosomes in this study is tentative and needs confirmation with C-banding (Gileva 1982). Polymorphism for apparent Robertsonian translocations occurred in the colonies from Churchill, as previously reported by Malcolm et al. (1986). Centric fusions are known in D. torquatus (Gileva 1980) and occurred in the derivation of the karyotype of D. klungniutak (Medv 1987); XY females are common in the torquatus-group (see Gileva 1987) and, in Canada, have been hypothesized to occur in D. richardsonii (Malcolm et al. 1986), based on sex ratios of laboratory offspring. Our identification of XY females was based on cytological recognition of the sex chromosomes, which should be confirmed by differential banding (particularly for D. richardsonii, where the Y could not be positively identified).

Each laboratory sample was chromosomally distinct. Karyotypes of D. richardsonii from Arviat and Churchill were similar, except for polymorphism for Robertsonian rearrangements at Churchill. The 'A' autosomal complement of collared lemmings from Pearce Point was entirely acrocentric and distinct from the karyotype of D. klungniutak reported from Banks Island (Rausch 1977), wherein there are two large metacentric autosomes (M. D. Enstrom, unpublished data). The 'A' complement from Igloolik contained eight large metacentric autosomes and was distinct from the all-acrocentric karyotype previously reported for D. groenlandicus from Devon Island in the High Arctic (Rausch 1977). The number of autosomal arms in karyotypes from the two samples of D. groenlandicus are identical and the difference in letter number might have resulted from centric fusions or fission. Great variation in karyotypes occurs within each of the 'cytotypes' recognized by previous authors, purportedly derived from single glacial refugia (a outlined by MacPher- son 1951). However, it is not yet established whether the hypothesis of chromosomal differentiation in Wisconsin refugia (Rausch 1977, 1980; Rausch and Rausch 1972), but not in laboratory populations, is supported by local differentiation uncorrelated with refugial isolation (Hoffmann 1981; Modi 1987). Whether individual structural rearrangements lead to speciation by disrup- ting meiosis in heterozygotes, as required by the stasipartizionation model, is not yet established. Indeed, even monobrachial fusions, previously proposed to result in speciation in other rodents (Baker and Bickham 1986; Capanna 1981), do not result in sterility in hybrid D. torgata (Gileva 1980). Delimitation of species boundaries solely on the basis of differences in diploid num- ber, without concomitant breeding studies, should be viewed sceptically.

The morphology of the sex chromosomes of D. richardsonii is distinct from that of D. groenlandicus and D. klungniutak. In D. richardsonii the X chromosome is large and subtelo- centric and the Y is presumed to be a medium-sized to small acrocentric chromosome, whereas in both D. groenlandicus and D. klungniutak the X is large and submetacentric and the Y is medium-sized and submetacentric. This difference is con- sistent with the proposed separate, southern periglacial origin for D. richardsonii (Van Wyndberhe and Enstrom 1993). Based on the pronounced level of mtDNA divergence among these taxa, however, this difference probably predates the Wisconsin.