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Author(s): Joshua Laerm, John C. Avise, John C. Patton, Robert A. Lansman
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GENETIC DETERMINATION OF THE STATUS OF AN ENDANGERED SPECIES OF POCKET GOPHER IN GEORGIA

JOSHUA LAERM, Department of Zoology, University of Georgia, Athens, GA 30602; JOHN C. AVISE, JOHN C. PATTON, and ROBERT A. LANSMAN, Department of Molecular and Population Genetics, University of Georgia, Athens, GA 30602.

In the southeastern United States, *Geomys* pocket gophers are represented by 2 extant taxonomic species, *G. colonus* and *G. pinetis*. *G. colonus* was described in 1898 (Bangs 1898), but remained essentially unnoticed and unstudied until 1967 when a population within the historic range of *colonus* was "rediscovered." The first described range of *colonus* encompassed about 16 km^2 of coastal plain in Camden County, Georgia. The current population referable to "*colonus*" covers less than 200 ha, is estimated at less than 100 individuals (Ford 1980), and is presently listed as endangered by the State of Georgia (Odom et al. 1977).

The original description of *G. colonus* distinguished it from *G. pinetis* on the basis of darker pelage and minor cranial differences. We have examined over 1,500 museum skins of *pinetis* collected throughout its range, and found that pelage color is widely variable both within and among populations, and is not a satisfactory means for distinguishing *colonus* from *pinetis*, or *pinetis* populations from one another (ms. in prep.). We have also compared 18 body and cranial measurements between *colonus* and surrounding populations of *pinetis*, and found that *colonus* is not more distinct from *pinetis* than are populations of *pinetis* from each other. Williams and Genoways (1980) have also compared *pinetis* and *colonus* morphometrically, and concluded that there was probably no basis for the recognition of *colonus* as a distinct species. Because morphology may not be a reliable guide to distinguish *colonus* from *pinetis*, and because the basis for the original taxonomic description of *colonus* is thus suspect, we conducted the following molecular-genetic survey.

METHODS

We previously examined various molecular-genetic characteristics of a total of 171 live-trapped specimens of *Geomys pinetis* representing 24 populations distributed across the range of the species (Avise et al. 1979*b*). That paper, which did not include *G. colonus* and did not address issues of direct significance to population management, should be consulted for background information relevant to this report. Here, we examine the genetic composition of 5 specimens of *G. colonus*, and compare this information to

the data previously published for *G. pinetis*. Although the absolute size of our *colonus* sample is small, it does provide an adequate representation (>5%) of the entire species. Because *colonus* is protected, additional permits for larger samples could not be obtained.

We assayed genetically determined variation and differentiation in proteins encoded by 25 genetic loci. The proteins examined (and numbers of genetic loci encoding their production) were: lactate dehydrogenase (2 loci), malate dehydrogenase (2), glutamate-oxaloacetate transaminase (2), isocitrate dehydrogenase (2), 6-phosphogluconate dehydrogenase (2), nucleoside phosphorylase (1), phosphoglucoisomerase (1), phosphoglucomutase (1), tetrazolium oxidase (1), alcohol dehydrogenase (1), sorbitol dehydrogenase (1), xanthine dehydrogenase (1), α-glycerophosphate dehydrogenase (1), peptidase (2), esterase (3), albumin (1), and hemoglobin (scored as 1 locus). Proteins were electrophoresed on horizontal starch-gels according to standard procedures (Selander et al. 1971, Ayala et al. 1972), using muscle, liver, heart, plasma, and hemolysate as tissue sources. Heterozygosity estimates ($\bar{H}$, the mean proportion of individuals heterozygous per locus in a local population) were obtained by direct count.

Karyotypic variation in 4 individuals of each of 24 populations of *G. pinetis* and in 4 specimens of *colonus* was examined using standard bone marrow techniques (Baker 1973).

Mitochondrial DNA (mtDNA) nucleotide divergence was compared between 4 *colonus* and 87 *pinetis* from across the range of the species. Mitochondrial DNA from livers of individual animals was purified, and then cleaved separately with restriction endonucleases *Eco*RI, *Bam*HI, *Bst*EII, and *Hind*III. The cleavage fragments were electrophoresed on 1.1% agarose gels. *Hind*III digests of phage lambda DNA were coelectrophoresed as molecular weight standards. Details of these techniques are described elsewhere (Avise et al. 1979*a*,*b*).

RESULTS

Proteins

For the 25 loci examined, no allozymic variation ($\bar{H} = 0.00$) was found within *G. colonus*, or within most of the immediately surrounding populations of *G. pinetis* in Camden, Charlton, and Pierce counties, Georgia and Nassau County, Florida. In 1 or more other populations of *pinetis*, a total of 10 variable loci contributed to heterozygosity estimates ranging from 0.000 to 0.058 with overall unweighted $\bar{H} = 0.025$ per population (Avise et al. 1979*b*). Similar levels of variation have been reported in other species and genera of pocket gophers (Patton et al. 1972, Penney and Zimmerman 1976).

Electromorphs encoded by 2 loci, 6-phosphogluconate dehydrogenase (*PGD*) and albumin (*ALB*), exhibit pronounced macrogeographic variation in *pinetis* (Avise et al. 1979*b*, Fig. 1). ALB^{100} was fixed in *colonus* and in all "eastern" populations of *pinetis*, while ALB^{95} was the predominant electromorph in "western" *pinetis* populations. The *colonus* assayed were also monomorphic for PGD^{100}, the common electromorph in "eastern" *pinetis*. Distributions of electromorphs at *ALB* and *PGD* are presented in Fig. 1.

Genetic distances between populations across all assayed loci were summarized using Nei's (1972) $\bar{D}$ statistic. Small genetic distances ($\bar{D} < 0.001$) were observed between populations of *pinetis* in close geographic proximity, so we reduced our samples into 10 population groups for ease of representation and

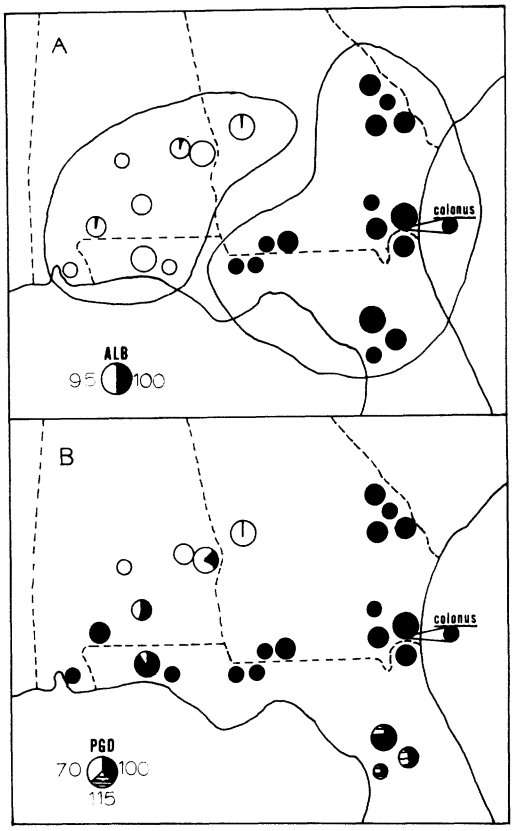

Fig. 1. Geographic distribution of electromorph frequencies of (A) albumin and (B) 6-phosphogluconate dehydrogenase in *G. colonus* and *G. pinetis*. Large circles represent samples $N \geq 12$; medium circles, $5 \leq N \leq 11$; small circles, $N \leq 4$. Heavy lines separate "eastern" and "western" forms of *pinetis* as defined by *ALB* and mtDNA analysis.

comparison against *colonus*. A sample of the more distantly related western species, *Geomys bursarius*, was also assayed and added for perspective. These data were clustered by the unweighted pair group method with arithmetic means (Sneath and Sokal 1973) to obtain a 2-dimensional distance phenogram (Fig. 2).

In protein-electrophoretic composition, *colonus* appears identical to populations of *G. pinetis* throughout eastern Georgia at the 25 assayed loci. The estimated genetic distance ($\bar{D} = 0.00$) between *colonus* and *pinetis* from several

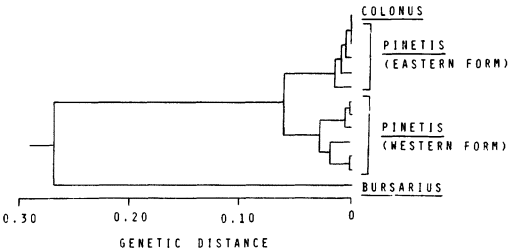

Fig. 2. Distance phenogram, derived from protein-electrophoretic data from 25 loci, in *G. colonus* and *G. pinetis*. A sample of *G. bursarius* is provided for perspective.

counties in eastern Georgia and from Nassau County, Florida is considerably less than the $\bar{D} = 0.06$ between apparently conspecific "eastern" and "western" populations of *pinetis* (Avise et al. 1979*b*). *G. bursarius* is genetically distinct ($\bar{D} = 0.28$) from both *pinetis* and *colonus*. All $\bar{D}$ values within and between populations of *colonus* and *pinetis* are well within the range characteristic of conspecific populations in other mammals (Avise 1974).

Karyotypes

The standard mitotic karyotype of *colonus* appears identical to that of other "eastern" *pinetis* populations, exhibiting 2N = 42 and fundamental number (FN) = 80. This karyotype in *pinetis* has previously been published (Williams and Genoways 1975). Minor karyotypic divergence was observed between "eastern" and "western" populations of *pinetis*, the latter of which exhibit 2N = 42, FN = 76.

Mitochondrial DNA

Recently, several laboratories have demonstrated that extensive mtDNA nucleotide sequence divergence can be detected within mammalian species (Upholt and Dawid 1977; Avise et al. 1979*a*,*b*; Brown et al. 1979). This work suggests that mtDNA evolves more rapidly than

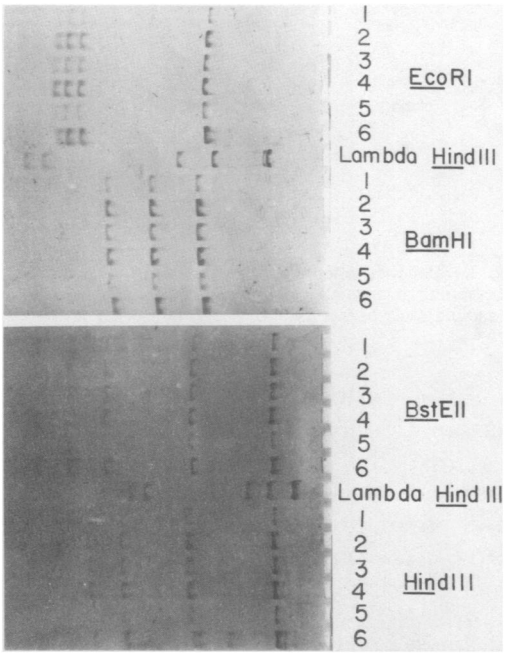

Fig. 3. Mitochondrial DNA digestion phenotypes produced by 4 restriction enzymes in (1) *G. colonus*, and (2–6) *G. pinetis* samples from Camden County, Georgia. *Hind*III digests of phase lambda DNA were coelectrophoresed as molecular weight standards. The *Hind*III digests of samples 4 and 6 contain additional bands which result from incomplete digestion.

unique-sequence nuclear DNA (Avise et al. 1979*a*, Brown et al. 1979) and that the use of restriction endonucleases to detect mtDNA sequence divergence provides a highly sensitive technique for assessing evolutionary relatedness between closely similar populations. We have previously shown that extensive mtDNA sequence diversity exists within *G. pinetis* (Avise et al. 1979*b*). We estimated the nucleotide sequence divergence, p, using the analysis suggested by Nei and Li (1979), in pairwise comparisons between *G. pinetis* individuals collected throughout the range of the species. It was shown that "eastern" and "western" forms differ by more than 3% ($p = 0.034$) in mtDNA sequence. Diversity within "eastern" populations is less extensive ($p = 0.005$), but still easily detectable.

We used 4 endonucleases to digest mtDNA from 4 samples of *G. colonus*, and compared the fragment patterns with those observed previously in *G. pinetis*. Our observation that the *G. colonus* mtDNA appears identical to the mtDNA seen in Camden County samples of *G. pinetis* ($p = 0.00$) is documented in Fig. 3. Since mitochondria are inherited through females, we conclude that the *G. colonus* individuals studied share a common maternal phylogeny with surrounding populations of *G. pinetis*.

DISCUSSION

Considering all available evidence, we reject the hypothesis that the gopher population presently recognized as *Geomys colonus* differs significantly in genetic composition from surrounding populations of *G. pinetis*. This conclusion is not an artifact of poor sensitivity in the genetic assays employed, as these same techniques indicated considerable genetic differences among geographic populations of *G. pinetis*. In particular, at least 2 major genetic stocks (lineages), previously unrecognized, exist in *pinetis*.

Therefore, "*colonus*" appears to represent no more than a slightly differentiated local population of the eastern form of *G. pinetis* and should be synonymized with it. Could this conclusion be incorrect? To "prove" that *colonus* does not differ from eastern *pinetis* is tantamount to proving a null hypothesis, an epistemological impossibility. It would always remain conceivable that some critical genetic characters determining species status of *colonus* do indeed exist but had not yet been examined (this argument would apply to any "conspecific" populations of *pinetis* as well). There-

fore, to be doubly safe, the Georgia Department of Natural Resources (DNR) has recently transplanted several *"colonus"* gophers to other localities where it is hoped they will continue to survive.

The results of our genetic survey of an endangered species are important for several reasons. Specifically, the Georgia DNR has for the past decade invested many thousands of man-hours and dollars in protecting *"colonus"* against human encroachment. With the recognition that *"colonus"* is not an unusually distinctive population of common *G. pinetis*, the Georgia DNR can turn more of its attention and finite resources to other truly endangered species. Any future programs of pocket gopher management should also profit from the recognition and delimitation of the 2 major genetic stocks of *pinetis* that we have discovered.

Our results are also important in a broader context. There has previously been almost no input from geneticists into endangered species programs. Many endangered "species" are poorly known genetically or otherwise, yet huge efforts are expended in their management. Conversely, many genetically distinct populations and species probably remain unrecognized because of their morphological similarity. Thus in many instances, comprehensive genetic information should help broaden and strengthen the basis for intelligent management decisions.

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AN INDEX FOR EVALUATING THE TEMPERATURE STABILITY OF A SUBNIVEAN ENVIRONMENT

PETER J. MARCHAND, Johnson State College, Johnson, VT 05656.

The energetic advantage of exploiting the subnivean environment during winter is considerable for small nonhibernating mammals of northern latitudes. Beyond the limits of physical thermoregulation, a reduction of body-air temperature differences by remaining under the snow cover is often the only means of maintaining a favorable energy balance with decreasing air temperatures. For many small mammals, the presence of an adequate snow cover is a critically important factor in their overwintering success. Thus, Formozov (1946:115) recognized that "the thermal insulating properties of snow have succeeded in playing a large role in the history of faunas."

The temperature stability of the subnivean environment is a function of snow density as well as depth. These extremely variable snowpack characteristics are influenced by a number of factors including antecedent weather conditions, vegetation type, and snowpack metamorphosis. While the thermal properties of snow are well understood (Mellor 1964, Yen 1965, Anderson 1976), a standard procedure for estimating the ecological value of a snow cover to subnivean animals (or plants) has been lacking.

Pruitt (1970) introduced the use of a snow index value, $SI = C(\Sigma\ TD)$, where C = snow cover of a plot expressed as a percentage of the plot covered, T = thickness (cm), and D = density of each discrete layer of snow. He then attempted to demonstrate a relationship between annual variations in snow index value and subnivean mammal population levels. The concept is potentially useful, but Pruitt's index implies a direct relationship between snow density and insulative value rather than an actual inverse relationship. Thus, his index is of little use for estimating the temperature stability of the subnivean environment. As a simple illustration using Pruitt's formula, an undisturbed snow cover of 40 cm thickness with a uniform density of 0.1 g/cm^3 would have the same snow index value (4.0) if it were compacted to a depth of 20 cm with a concomitant increase in density to 0.2 g/cm^3. The insulative value of these 2 snow covers would differ considerably because the thermal conductivity of the snowpack increases with both decreasing thickness and increasing density.

I propose the use of a snow thermal index