DNA SYNAPOMORPHIES FOR A VARIETY OF TAXONOMIC LEVELS FROM A COSMID LIBRARY FROM THE NEW WORLD BAT MACROTUS WATERHOUSII

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Abstract.—An effective method yielding taxon-specific markers from the genome of a single individual would be valuable for many types of scientific investigations, including systematic, forensic, conservation, and evolutionary studies. We explored the use of cosmid libraries, with insert sizes averaging 35 kb, to streamline the process of locating sequences of DNA that can serve as taxonomic markers from the specific to the ordinal levels. By screening approximately 2.6% of the leaf-nosed bat (Macrotus waterhousii) genome, we identified several potential DNA fragments that appear to be synapomorphic for a variety of taxonomic levels. A more thorough analysis of the markers documented that 17 Macrotus-specific clones represent three distinct DNA generic markers, whereas 30 microchiropteran clones represent multiple copies of a single family of repetitive DNA. The Microchiroptera taxon markers hybridize with representatives of most of the Microchiroptera families; however, no hybridization was detected for members of the superfamily Rhinolophoidea. These results demonstrate that cosmid libraries can be a valuable source for isolating taxon-specific markers from mammals even when the insert size is as large as 35 kb. [Cosmid library; DNA synapomorphies; genome; Macrotus waterhousii; phylogenetic screening; Rhinolophoidea; taxonomic levels.]

Genomes of more complex organisms are composed of billions of base pairs that contain sequences that range from being unique for individuals to some, such as ribosomal genes, that show considerable similarity among all forms of life (Gouy and Li, 1989). Although there is considerable potential for finding DNA markers that resolve all taxonomic levels, the problem is how to extract efficiently the desired levels of variation and shared character states from these billions of base pairs. An effective method that yields potential taxon-specific DNA probes across taxonomic levels would be valuable for many types of scientific investigations, including systematic, forensic, conservation, and evolutionary studies. We explored the use of cosmid libraries to streamline the process of locating sequences of DNA that can serve as taxonomic markers from the ordinal to the specific level.

We tested our approach by constructing a genomic cosmid library from the New World leaf-nosed bat, Macrotus waterhousii, a species whose specific, generic, familial, superfamilial, subordinal, and ordinal relationships are well resolved (Baker et al., 1989; Van Den Bussche, 1991; Koopman, 1993). Although there has been recent debate over the monophyly of the order Chiroptera (Baker et al., 1991a, 1991b; Pettigrew, 1991a, 1991b; Simmons et al., 1991), nearly all data, with the possible exception of some neural characters of the brain (Pettigrew, 1986) support the monophyly of Chiroptera (for review, see Simmons, 1994; Van Den Bussche et al., unpubl.). One assumption of our experimental design is that the groups in Figure 1 are monophyletic. It was our goal to locate clones that defined the taxonomic limits of the genus Macrotus, the family Phyllostomidae, the superfamily Noctilionoidea, the suborder Microchiroptera, and the order Chiroptera.

We examined 1,728 independent clones,
with an average insert size of approximately 35 kb, by probing with genomic DNA from 15 taxa to isolate clones that have potential to provide taxon-specific resolution. In a recent study (Van Den Bussche et al., 1995), these 1,728 clones were characterized for the presence of five repetitive elements known to be ubiquitous in the mammalian genome. These data will aid in understanding the nature of the clones that identify taxonomic limits so that this potential source of noise in the data can be eliminated.

**MATERIALS AND METHODS**

We first constructed a genomic library and plated random clones in an ordered arrangement for archival purposes and for hybridization. We then hybridized clones with genomic DNA probes from individuals representing a subset of each taxon (ingroups) and a subset of the outgroups. Clones that hybridized with all ingroup representatives for each taxon but not with the outgroups for that taxon were further examined as putative taxon-specific markers.

**Construction and Characterization of the Cosmid Genomic Library**

High-molecular-weight DNA was isolated from a male *M. waterhousii* collected from Cuba (Guantanamo Prov., Guantanamo Bay Naval Base, TK 32184). The library was constructed using the same methods as those used to construct human chromosome-specific cosmid libraries (Longmire et al., 1993). The characteristics of this library have been described (Van Den Bussche et al., 1995). In general, primary infection of *Escherichia coli* host strain DHαMCR yielded $7.4 \times 10^8$ independent recombinants. From this primary infection, 1,728 independent clones were chosen, grown, and archived into 96-well microtiter plates. A replica plater (Sigma Chemical Co.) was used to inoculate nylon membranes (Biodyne B 0.45 μm) with clones from the microtiter plates. Membranes were incubated at 37°C for 7 hr on LB agar containing kanamycin (30 mg/ml) and then transferred and incubated overnight at 37°C on LB agar containing kanamycin and chloramphenicol (170 mg/ml; Sambrook et al., 1989). DNA was fixed by placing the membranes sequentially on blotting pads soaked in 0.4 M NaOH (5 min), 0.5 M Tris/1.5 M NaCl, pH 7.5 (5 min), and 2× sodium citrate–sodium chloride (SSC) (5 min), followed by baking at 80°C for 2 hr.

**Isolation of Taxon-Specific Markers**

Genomic DNAs were isolated from six representatives of the microchiropteran family Phyllostomidae (*Macrotus waterhousii*, *M. californicus*, *Desmodus rotundus*, *Micromycteris hirsuta*, *Phyllostomus elongatus*, *Artibeus jamaicensis*), five additional microchiropteran families (Nectilionidae, Mormoopidae, Emballonuridae, Molossidae, Vespertilionidae), one representative of the suborder Megachiroptera (Pteropodidae), and representatives of the orders Dermoptera, Insectivora, and Primates. Tissues were obtained from the frozen tissue collection at The Museum at Texas Tech University. DNA isolation was performed using a modification of the technique of Longmire et al. (1991). (See the Appendix for a list of specimens used in various parts of this study.)

One microgram of each DNA sample was nick translated and used to probe the
M. waterhousei library. Following hybridization, the 1,728 clones were scored on a scale of 0 (=no detectable hybridization) to 3 (=a completely black spot on the autoradiograph for that clone; maximum detectable hybridization). Prior to hybridization, membranes were washed for 1 hr at 65°C in 0.1× SSC, 0.1% sodium dodecyl sulfate (SDS). Prehybridization was carried out at 65°C for 1 hr in 6× SSC, 40% formamide (Kodak), 1% SDS, 0.005 M ethylenediaminetetraacetic acid (pH 8.0), and 0.005 g/ml powdered milk. Membranes were hybridized overnight at 42°C in fresh prehybridization solution containing approximately 1 × 10⁶ cpm/ml probe. Probes were labeled with a (α²³⁵P) dCTP by nick translation; the nonincorporated label was removed by spin column chromatography (Sambrook et al., 1989). Prior to hybridization, probes were denatured for 10 min at 37°C in 0.1 M NaOH. Following hybridization, membranes were washed once for 15 min in 2× SSC, 0.1% SDS at room temperature and twice for 15 min in 0.1× SSC, 0.1% SDS at 50°C. Washed membranes were exposed at −80°C to Kodak XAR-5 film and two lighting plus intensifying screens.

Once putative taxon-specific clones were identified for each taxonomic level, we eliminated any clones that were identified by Van Den Bussche et al. (1995) as having tandemly repeated elements (microsatellites, etc.) that are ubiquitous among genomes. A series of Southern blot analyses (Southern, 1975) were performed to determine the complexity of collections of clones that were informative at either the generic or subordinal levels. Cosmid miniprep DNA was digested with EcoRI and electrophoresed on 0.8% agarose gels. The gels were blotted to nylon hybridization membranes and hybridized with a single clone from the informative collection. Any clone that produced detectable cross-hybridization with the first chosen informative clone (clone that was used as a probe) was classified as belonging to the same family of repetitive DNA. DNA from all other clones (those that did not cross-hybridize with the specific clone used as a probe) were used in a subsequent Southern blot analysis, and an arbitrarily chosen representative of this group was used to probe this membrane. As with the initial survey, all clones producing detectable hybridization were classified as belonging to a second family of repetitive DNA. This process was repeated until all clones were assigned to a specific family of repetitive DNA based on cross-hybridization experiments.

To evaluate the robustness of the putative taxonomic markers, a representative of the Macrotus- and Microchiroptera-specific clones were used as probes against slot blots of genomic DNA. In these experiments, we increased the phylogenetic breadth of the survey by including representatives of 21 genera of the family Phyllostomidae, all three families of the super-family Noctilioidea, eight families of the suborder Microchiroptera, five genera of the suborder Megachiroptera, one species of Dermoptera, two families of the order Primates, and one genus of each of the orders Rodentia and Insectivora. One microgram of genomic DNA from each of the above taxa was applied to hybridization membranes following the manufacturer's recommended procedure (Schleicher & Schuell, Keene, NH). Hybridization conditions were the same as those used in the first part of the experiment except that various stringency levels were explored, with hybridization conditions ranging from 40% formamide and 42°C to 50% formamide and 65°C. Based on the hybridization conditions, appropriate adjustments were made in posthybridization washes by varying both the temperature and salt concentration.

In situ Hybridization

Chromosomal preparations from M. waterhousei, M. californicus, and Artibeus jamaicensis were prepared from bone marrow after incubation with Velban (Baker and Qumsiyeh, 1988). In situ hybridization of a representative cosmid of the Macrotus- and Microchiroptera-specific repetitive DNA families was performed according to the method of Hamilton et al. (1990). A
TABLE 1. Total number and percentage of the 1,726 cosmids from the *Macrotrus waterhousii* library producing detectable hybridization with genomic DNA from 15 mammalian taxa.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>No. positive cosmids</th>
<th>% cosmids hybridizing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiroptera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microchiroptera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noctilionoidea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phyllostomidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. waterhousii</em></td>
<td>1,559</td>
<td>90</td>
</tr>
<tr>
<td><em>M. californicus</em></td>
<td>1,493</td>
<td>86</td>
</tr>
<tr>
<td>Desmodus</td>
<td>1,609</td>
<td>93</td>
</tr>
<tr>
<td>Micromertes</td>
<td>1,521</td>
<td>88</td>
</tr>
<tr>
<td>Phyllostomus</td>
<td>1,418</td>
<td>82</td>
</tr>
<tr>
<td>Artibeus</td>
<td>1,176</td>
<td>66</td>
</tr>
<tr>
<td>Mormooidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pteropus</td>
<td>1,301</td>
<td>75</td>
</tr>
<tr>
<td>Noctilionidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noctilus</td>
<td>991</td>
<td>57</td>
</tr>
<tr>
<td>Emballonuridae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccopteryx</td>
<td>885</td>
<td>51</td>
</tr>
<tr>
<td>Molossidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tadarida</td>
<td>1,329</td>
<td>77</td>
</tr>
<tr>
<td>Vespertilionidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myotis</td>
<td>955</td>
<td>55</td>
</tr>
<tr>
<td>Megachiroperta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pteropus</td>
<td>810</td>
<td>47</td>
</tr>
<tr>
<td>Dermoptera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cynocephalus</td>
<td>475</td>
<td>27</td>
</tr>
<tr>
<td>Primates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homo</td>
<td>521</td>
<td>30</td>
</tr>
<tr>
<td>Insectivora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crocidura</td>
<td>882</td>
<td>51</td>
</tr>
</tbody>
</table>

Minimum of 10 spreads were analyzed for each individual examined.

RESULTS

Characterization of the *M. waterhousii* Genomic Library

The pattern of hybridization of genomic DNA from the 15 mammalian taxa studied is provided in Table 1. Only 35 of the 1,728 cosmid clones screened in this study did not hybridize with any of the 21 (15 from the microchiropteran species plus 6 repetitive elements examined by Van Den Bussche et al., 1995) probes examined. Of these 35 clones (2.0% of those screened), 2 produced no bands when they were digested with the restriction endonuclease *EcoRI* and electrophoresed on an 0.8% agarose gel. These two clones apparently did not contain a cosmid or failed to grow to a density that allowed detection of the cosmid DNA with a standard miniprep procedure, reducing the actual number of cosmids screened to 1,726. The remaining 33 clones were visible on an ethidium-stained gel; when digested with *EcoRI*, each clone was verified as having the 6.7-kb DNA fragment characteristic of the sCos-1 vector as well as additional bands resulting from the insert DNA.

The insert size of 20 randomly selected cosmid clones ranged from 24.9 to 43.4 kb, with a mean insert size of 35.7 kb. Based on this mean insert size, the 1,726 clones represented $6.15 \times 10^6$ bp, or 2.6% of the *M. waterhousii* genome, assuming a genome size of $2.4 \times 10^9$ bp (based on a DNA content of 5.6 pg/cell; J. W. Bickham, pers. comm.). The total library of $7.4 \times 10^9$ primary clones thus is 11-fold representative for the *M. waterhousii* genome.

Taxon-Specific Clones

The hypothesized phylogeny for the taxa used in this study along with the total number of clones that are potential markers for each taxonomic category are presented in Figure 1. Based on cross-hybridization results, the 17 *Macrotrus*-specific clones represent three families comprised of 8, 7, and 2 clones, respectively. Of the 44 potential microchiropteran clones, 14 were eliminated because they contained other micro- or minisatellite repeats (Van Den Bussche et al., 1995). The remaining 30 clones cross-hybridized, indicating that these clones represent multiple members of a single family of repetitive DNA.

When clones representing each of the three families of *Macrotrus*-specific DNA were used to probe slot-blot membranes, detectable hybridization was seen only for *M. waterhousii* and *M. californicus* (Fig. 2). This same pattern was detected regardless of the stringency of hybridization and washes ranging from hybridization in 40% formaldehyde at 42°C to hybridization in 50% formaldehyde at 65°C and posthybridization washes in 0.1× SSC at temperatures ranging from 42°C to 65°C. Results from in situ hybridization demonstrated that these clones were localized in discrete blocks on
the chromosomes of *M. waterhousii* and *M. californicus* (Figs. 3a, 3b). In the slot-blot experiments, the level of hybridization was reduced in *M. californicus* compared with that seen in *M. waterhousii* (Fig. 2), and the lower abundance of these sequences in *M. californicus* also was observed in the in situ hybridization experiments. In *M. waterhousii*, this DNA hybridized with approximately 38 chromosomes at either one or both telomeres. However, in *M. californicus* hybridization was restricted to a single telomeric region on two small chromosomes (Figs. 3a, 3b).

When a representative of the Microchi-
Figure 3. Representative karyotypes of three bat species. The clones used as probes were labeled with biotin. Regions of hybridization fluoresce yellow (fluorescein) and unhybridized regions of the chromosome fluoresce red (propidium iodide). (a) Macrotus waterhousii, in situ hybridized with one of the Macrotus-specific clones. (b) Macrotus californicus, in situ hybridized with one of the Macrotus-specific clones. (c) Artibeus jamaicensis, in situ hybridized with a representative of a Microchiroptera-specific clone.
lionidae, Mormoopidae), whereas less intense hybridization was detected for representatives of the microchiropteran families of Natalidae, Furipteridae, Molossidae, and Emballonuridae. Representatives of the microchiropteran families Rhinolophidae, Hipposideridae, Megadermatidae, and Nycteridae produced no detectable hybridization. This clone also failed to hybridize with any representatives of the Megachiroptera, Primates, Insectivora, or Rodentia (Fig. 4). As with the Macrotrus-specific clones, similar patterns of hybridization were detected over the entire range of stringencies for hybridization and posthybridization conditions. The only notable variation in these results was that as the level of stringency was increased, the intensity of hybridization became lower in the nonphyllostomid microchiropterans. In situ hybridization of a representative of this family of DNA with the chromosomes of Artibeus jamaicensis revealed an interspersed pattern of hybridization on all chromosomes, with some chromosomal banding present (Fig. 3c).

**Discussion**

**Taxon-Specific Markers**

Although satellite DNA has been shown to be a useful phylogenetic marker for several taxa, including rodents (Hamilton et al., 1990, 1992), bats (Van Den Bussche et al., 1993), cetaceans (Arnason and Best, 1991; Arnason et al., 1992; Adegoke et al., 1993), primates (Durfy and Willard, 1990), falcons (Longmire et al., 1988), coral (McMillan and Miller, 1990; McMillan et al., 1991), and Drosophila (Bachmann et al., 1992), the isolation of these markers is usually time consuming and fortuitous. A more efficient method that screens the genome with the specific purpose of isolating taxon-specific markers would be valuable. Because other researchers have isolated stretches of DNA that are specific to species (Love and Deininger, 1992), subgenera (Hamilton et al., 1990), and genera (Longmire et al., 1988; Hamilton et al., 1992; Van Den Bussche et al., 1993), it should be possible to isolate, from a given genome, those stretches of DNA that identify that particular individual for a wide range of taxonomic groupings.

We have screened a cosmid library with the specific goal of identifying marker sequences that would be useful for addressing taxonomic questions at several levels. For all taxonomic categories examined, several cosmids produced hybridization patterns that were consistent with accepted monophyletic groupings (hybridized with all ingroups but not to any outgroups). How many of the potential taxonomic markers are simply copies of the
same repetitive element or family of repetitive elements, and how robust are these clones in resolving the proposed phylogenetic limits? To test these two questions, we examined the clones for informativeness at the generic and subordinal levels. Because this library was used in a previous study to examine the organization of repetitive DNA in the M. waterhousii genome (Van Den Bussche et al., 1995), we can identify which of the potential taxon-specific clones contain members of ubiquitous repeat families, such as microsatellites. There was a general trend toward the higher taxonomic levels for an increase in percentage of potential taxon-specific markers containing microsatellite clusters (Table 2). These data point out one of the difficulties in isolating taxon-specific markers. Microsatellite repeat clusters are ubiquitous in vertebrate genomes (Tautz and Renz, 1984; Stallings et al., 1991; Beckman and Weber, 1992; Janecek et al., 1993; Van Den Bussche et al., 1995) and therefore will hybridize across a broad array of taxa, giving false indication for taxon-specific boundaries that may not be the product of common ancestry.

**Generic Level Markers**

Seventeen cosmid clones contained stretches of DNA from the M. waterhousii genome that produced detectable levels of hybridization only with genomic DNA from its sister taxon, M. californicus. Based on cross-hybridization experiments, these 17 cosmids represent three unique families of repetitive DNA; representatives of each family do not cross-hybridize with representatives of the other two families, even under moderate levels of stringency for hybridization and posthybridization washes.

To test the efficiency of these three families of cosmids for identifying the genus Macrotus (which contains only two species), we hybridized these three probes with genomic DNA from 42 mammals representing 21 genera of phyllostomid bats, 11 microchiropteran families, and representatives of the Megachiroptera, Primates, Dermoptera, Rodentia, and Insectivora. The potential Macrotus-specific cosmids hybridized only with the genomic DNA from M. waterhousii and M. californicus (Fig. 2). Thus, screening of 2.6% of the genome of Macrotus resulted in isolation of three unique genus-specific markers.

**Subordinal Level Markers**

In contrast to the Macrotus-specific clones, in which three unique families of repetitive DNA were isolated, all 30 Microchiroptera-specific clones appear to belong to the same family of interspersed repetitive DNA (Figs. 3c, 4). In situ hybridization of representatives of this family of repetitive DNA with the chromosomes of Artibeus jamaicensis revealed that this family of repeats is interspersed and present on all chromosomes, which may be why this repeat was present in 30 of our 1,726 clones. Although in situ hybridization of this family produced some chromosomal banding, the banding pattern was not as discrete as seen with long interspersed repetitive elements (LINEs) and short interspersed repetitive elements (SINEs) in humans and deer mice (Korenberg and Rykowski, 1988; Baker and Wichman, 1990; Baker and Kass, 1994).

Hybridization of this family of repetitive sequences with genomic DNAs from 42 mammals demonstrates that these clones hybridize only with representatives of the suborder Microchiroptera (Fig. 4). However, under all conditions of hybridization, representatives of the Old World microchiropteran families Rhinolophidae, Hipposi-
deridae, Megaderidae, and Nycteridae produced no detectable hybridization (Fig. 4). There are three possible explanations for these results: (1) this DNA element in members of the superfamily Rhinolophoidea has diverged considerably from that of other microchiropteran taxa; (2) these four taxa do not form a monophyletic group within the other microchiropteran taxa; or (3) some genomic mechanism has drastically reduced or eliminated copy number of this family of repetitive DNA in these taxa. The four families, Rhinolophidae, Hipposideridae, Megadermatidae, and Nycteridae, are recognized as belonging to the microchiropteran superfamily Rhinolophoidea (Smith, 1976; Van Valen, 1979; Koopman, 1984; Pierson, 1986). Higher taxonomic relationships within the Microchiroptera are not well resolved and are controversial (Baker et al., 1991a). However, the absence of this repetitive element in representatives of these four families may document closer phylogenetic relationships of the remaining microchiropteran families to the exclusion of the superfamily Rhinolophoidea. Because elimination of a group of complex repetitive elements from a genome is more probable (especially if they occur in discrete blocks of heterochromatin) than the de novo evolution of such sequences, the presence of an element in a genome may be viewed as strong evidence for shared ancestry. However, at present it is unclear how often such repetitive elements can be eliminated, giving a false negative. The 30 clones isolated in this study provide evidence for shared ancestry of those microbat families that are positive for the repeat. However, the absence of this repetitive family in the rhinolophoids is more problematic. The observation that this family of repetitive sequences is distributed in an interspersed fashion on all chromosomes makes it less probable that all or most copies could be eliminated.

We examined only 2.6% of the M. waterhousin genome to locate this marker. Screening of a larger representation of the Macrotrus genus and the genome of other microchiropteran bats may be successful in isolating additional markers with phylogenetic information among families of Microchiroptera.

Previous molecular studies designed to elucidate the phylogenetic relationships among families of bats have failed to provide a robust number of synapomorphies documenting sister-group relationships (Baker et al., 1991a). The reason for this lack of resolution may be that several families of bats shared a common ancestor for a very short time before the lineages underwent a rapid radiation (Baker et al., 1991a). Screening large-insert-size libraries with genomic DNA from a taxonomically diverse group of organisms may provide resolution for higher taxonomic relationships by identifying large conservative stretches of DNA. Nonetheless, repetitive DNA has many problems for use in systematic studies because of the ubiquity of many of these DNA families in all eukaryotic organisms. Moreover, although in some DNA families all members share identical sequence structure because of a common evolutionary history and concerted evolution (Dover, 1982, 1986), members of other DNA families are able to evolve freely and the orthology of such fragments can cause problems in phylogenetic analyses (Tautz and Renz, 1984; Burton et al., 1986; Deininger and Daniels, 1986; Vassart et al., 1987; Deininger and Slagel, 1988; Deininger, 1989; Hutchison et al., 1989; Moyzis et al., 1989; Durfy and Willard, 1990; Janecek et al., 1993; Lee et al., 1996).

We have demonstrated that phylogenetic screening (Wichman et al., 1985) of a cosmid library with a broad taxonomic array of genomic DNAs can find markers with taxon-specific resolving power. However, before undertaking such a study there are issues to be considered, including the insert size to be employed in the construction of the library, the stringency used for hybridization and posthybridization washes, and the potential confounding effect that known families of repetitive DNA such as microsatellites, LINEs, SINEs, and various other transposable elements may have on the identification and isolation of taxon-specific markers. There are many different kinds of DNA libraries that can
be constructed from the genome of an individual. From the issues addressed here, the size of the vector insert appears to be the most important variable. The trade-off is that the smaller the piece of DNA inserted into a cloning vector, the more clones need to be screened to examine a reasonable percentage of the genome. However, as the insert size increases, there is an increase in the probability that other repetitive elements will obscure the segments of DNA that can resolve taxonomic boundaries at the desired level. We examined a cosmid library because if insert sizes of 35 kb could resolve higher taxonomic levels (family, superfamly, suborder, order) this would be valuable information for designing similar studies for isolating higher level taxon-specific sequences. Even with 35-kb insert sizes, this method appears to be effective in identifying clones of potential taxon-specific markers that can be expected to address issues for which such markers are applicable.

A drawback to this approach is that cosmid technology is not in widespread use among those laboratories in which studies of systematic relationships are conducted. In addition, screening several thousand cosmide clones by hybridization can be a rather labor-intensive and costly endeavor. Thus, the approach presented here will not be easily adopted by all laboratories and its applicability may be somewhat limited. However, clearly cosmide libraries represent a potentially wealthy source of taxon-specific markers to be used in appropriate systematic studies.

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REFERENCES


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APPENDIX
SPECIMENS EXAMINED

Collection (and deposition) locations are given for individuals from which genomic DNA was isolated (TK = Texas Tech.; NK = University of New Mexico).