

Comparative cytogenetics and the determination of primitive karyotypes

M. B. Qumsiyeh and R. J. Baker

Department of Biological Sciences and the Museum, Texas Tech University, Lubbock, TX

The field of comparative cytogenetics has advanced extensively with the development of techniques for differentially staining mitotic chromosomes. The information gleaned from such studies has been used by systematists in formulating more rigorous hypotheses concerning phylogenies of the groups under investigation, by cytogeneticists in deducing the number and types of chromosomal rearrangements incorporated in the course of evolution, and by population geneticists in discerning the forces affecting the fixation of chromosomal rearrangements. To formulate these hypotheses, accurate determination of the primitive karyotype (i.e., the karyotype proposed to have been possessed by the ancestor of the taxa examined) is critical. Chromosomal rearrangements that are modified from the primitive karyotype are derived character states and thus can be used like morphological attributes to characterize assemblages of the taxa examined. On the other hand, the fact that two taxa share a primitive condition cannot be used as a valid test of their relationship (Engelman and Wiley, 1977). Determination of the primitive karyotype and, by extension, of the derived karyotypes allows us, if chromosomal banding data are adequate, to infer the number and types of chromosomal rearrangements incorporated in the lineage.

Many cytogeneticists who have utilized chromosomal rearrangements as character states have failed to take into account the vast literature on constructing phylogenies based mostly on morphological data (see references). We will not attempt here to review the various arguments in that considerable literature; rather, we will summarize for the comparative cytogeneticist the pros and cons of various methods of utilizing chromosomal data in formulating phylogenetic hypotheses similar to those that use morphologic data. Methods applied to ontogenetic and fossil evidence are inapplicable to chromosomal data, which simplifies the task of comparing the various methods.

The most common tool for comparative cytogenetics, as evidenced by the number of papers in recent issues of *Cytogenetics and Cell Genetics*, is the use of G-banded (or R-banded) and C-banded chromosomes, and thus we will employ some of these stud-

ies as examples. Recent studies continue to document the correspondence between G-band (and, by inference, R-band) sequences and genetic homology of chromosomal segments (Lalley et al., 1978; Stallings et al., 1983, 1985; Sawyer and Hozier, 1986; Baker et al., 1987a). Thus, determination of G-band (or R-band) linkage groups can be very valuable in studying chromosomal evolution (Baker et al., 1987a). Various methods for uncovering ancestral character states have been utilized in the recent cytogenetic literature, and here we discuss the utility and limitations of each of these methods. We also analyze data from four gerbil genera (Qumsiyeh and Chesser, 1987) by each of the methods.

The concept of "common equals primitive" assumes that if a character is found in several taxa in the group investigated, it is primitive (Estabrook, 1977). This approach has been used for studies of chromosome evolution in Muridae (Viegas-Péquignot et al., 1983, 1985a), Gerbillidae (Benazzou et al., 1982a, 1982b, 1984; Viegas-Péquignot et al., 1982), Primates (Dutrillaux et al., 1981; Viegas-Péquignot et al., 1985; Rumpler et al., 1986), and Carnivora (Dutrillaux and Couetier, 1983; Couturier et al., 1986), among other taxa. Wiley (1981), Watrous and Wheeler (1981), and others have demonstrated that there is no logical correspondence between commonness and primitiveness; further, they have shown that common characteristics are not always primitive. For a good discussion of the inadequacy of the commonality principle on philosophical grounds, see Watrous and Wheeler (1981) and Wheeler (1981).

Although there are examples where primitive conditions may be common, such as in marsupials (Rofe and Hayman, 1985), in many cases there is no correspondence between commonality and primitiveness. It may be that the primitive character state was retained by only one or a few of the taxa examined and that all others share a derived state. An example of this is the presence of an inverted chromosome 2 as a derived state in 23 of the 29 species of *Peromyscus* examined by Stangl and Baker (1984). The proposed primitive condition of chromosome 2 and others also occurs in sister genera, such as *Reithrodontomys* and *Neotoma* (Hood et al., 1984; Koop et al., 1985). Another example occurs in phyllostomid bats, where the proposed primitive karyotype has been identified in only a single species, *Macrotus waterhousi*, and most species possess variously derived karyotypes (Baker and Bickham, 1980). In our analyses of four gerbil genera using the commonality principle (Fig. 1a), only two taxa are shown to have a more recent common ancestor by this method. Benazzou et al. (1984) presented a

Dr. Qumsiyeh's present address is Department of Pharmacology, St. Jude Children's Hospital, Memphis, TN 38101 (USA).

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Request reprints from: Dr. R. J. Baker, The Museum, Texas Tech University, Lubbock, TX 79401 (USA).

similar unresolved tree for some of these same genera. This tree also shows conflict in at least one character (fission in 32/8).

Another method is based on an a priori assumption regarding the direction of chromosomal evolution. Some authors have portrayed chromosomal evolution as resulting in a general decrease of diploid number by chromosome fusion and thus have considered high diploid numbers to be primitive (e.g., Freitas et al., 1983, 1984; Jotterand-Bellomo, 1984). The reverse, a general increase in diploid numbers through fission, is championed by others (Todd, 1967, 1970; Imai and Crozier, 1980). The method of assuming a direction for chromosomal evolution based on chromosome fission or fusion can be rejected on both philosophical and cytogenetic grounds. Proposing a specific criterion (e.g., fission) as a "tendency statement" represents a "descriptive generalization of low explanatory value" (Crisci and Stuessy, 1980). On cytogenetic grounds, both general types of rearrangements have been recorded in various organisms ranging from *Drosophila* to man.

A related method is to choose a taxon based on morphological primitiveness and assume that it retains a correspondingly primitive karyotype (e.g., Haiduk et al., 1981). Numerous studies have documented that morphological divergence may or may not correlate with chromosomal divergence (Baker and Bickham, 1980; Shields, 1982; Qumsiyeh, 1986a, b; Qumsiyeh and Chesser, 1987). It is also possible that no single species within a group investigated retains all the primitive chromosomal conditions. Analysis of the gerbil data by assuming fusions to be derived produces a tree (not shown) similar to that based on commonality in low resolution but requiring at least two homoplastic events (convergence in fusions 32/8 and 15/16).

The most widely accepted technique for phylogenetic reconstruction using morphological data is the outgroup method, which has been applied to chromosomal studies on phyllostomid bats (Baker et al., 1978, 1979), vespertilionid bats (Bickham, 1978, 1979; Baker et al., 1985), sigmodontine rodents (Koop et al., 1984, 1985; Rogers, 1983; Stangl and Baker, 1984), gerbils (Qumsiyeh, 1986a, b; Qumsiyeh and Chesser, 1987; Qumsiyeh et al., 1987), and murids (Baverstock et al., 1982, 1983; Baker et al., 1987b). In the outgroup method, determination of a character state as being primitive is made only if that state is shared with the outgroups by one or more of the ingroups. This methodology assumes, based on some independent data set, that ingroup taxa comprise a monophyletic group (Maddison et al., 1984). The choice of the outgroup(s) is thus critical. For chromosomal data, the outgroup has to be far enough removed that it cannot later be recognized as an ingroup taxon, but it cannot be so far removed that it shares little homology with any of the ingroup taxa. With these assumptions in mind, the outgroup method allows for construction of parsimonious phylogenetic trees based on properly hypothesized character transformation series (Watrous and Wheeler, 1981; Wiley, 1981; Maddison et al., 1984) and thus permits a more logical and testable method of analysis.

Data utilizing chromosomal rearrangements as character states are often more amenable to these kinds of analyses than gross morphological data. This is because chromosomal rearrangements appear as discrete events. Using sigmodontids and murids as outgroups, the same gerbil data discussed earlier document a common ancestry for the two *Meriones* species, as do the other two methods, but also provide further resolution at the

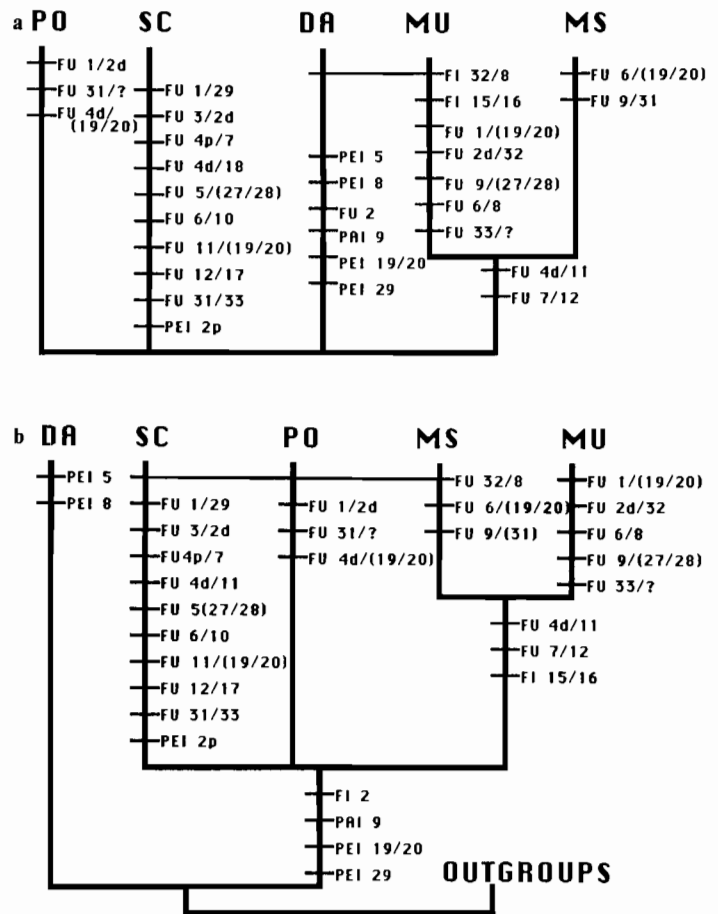


Fig. 1. Two methods of analyses of relationships for four genera of gerbils based on chromosomal rearrangements (Qumsiyeh and Chesser, 1987), using (a) the principle of "common equals primitive" and (b) the outgroup method; see text for discussion. Numbers refer to the standardized gerbil chromosome numbers (Qumsiyeh, 1986b). Other abbreviations are as follows: DA = *Desmodillus auricularis*, SC = *Sekeetamys calurus*, PO = *Psammomys obesus*, MS = *Meriones shawi*, MU = *M. unquiculatus*, FI = fission, FU = fusion, PAI = paracentric inversion, and PEI = pericentric inversion.

generic level (Fig. 1b). The outgroup method also documents two fission events (in chromosomes 2 and 15/16). The gerbil example discussed here documents that different methods of analyses produce different resolution levels (and may even show different branching patterns). More important from a cytogenetic viewpoint is that different methods of analyses produce different accounts of the kinds and number of chromosomal rearrangements incorporated during evolution of groups under study. Analyses of the gerbil data by the outgroup method produced a phylogenetic tree that is supported by independent electrophoretic and morphologic analyses. Similar support from independent data sets for trees generated by the outgroup method has been reported for phyllostomid bats (Arnold et al., 1982, 1983) and peromyscine rodents (Stangl and Baker, 1984).

Recent studies have documented that G-band sequence homology can be determined not only between genera but even between families of murid rodents (Koop et al., 1984; Baker et al., 1987b;

Qumsiyeh, 1986a, b) and between families of bats (Bickham and Baker, 1981; Qumsiyeh and Baker, 1985). This creates an ideal situation for chromosomal studies by allowing studies of chromosomal evolution in genera and families of mammals using closely related genera and families as outgroups. It should be emphasized that outgroup analyses must be performed before a determination of primitiveness. Thus character states are compared between the outgroup(s), and all members of the ingroup investigated and those character states found in the outgroup and in *any member* of the ingroup are presumed primitive. Choices between trees with different numbers of "steps" (chromosomal rearrangements) are performed by assuming minimum evolution or maximum parsimony (Farris, 1972). There are several computer packages available to choose parsimonious trees which are recommended when the number of character-state changes do not allow for directly choosing the most parsimonious tree (e.g., Farris, 1972; Swofford and Selander, 1981; Felsenstein, 1982; and references therein). However, even with the outgroup method, homoplasy can be underestimated, and a need may arise for an independent data set to choose between alternative hypotheses of

relationships that differ in a small percentage of homoplastic events (Qumsiyeh et al., 1987).

The conclusions from the above discussion are as follows: G-band (or R-band) sequences can be compared between species, genera, and even families of mammals, and these sequence homologies do indicate genetic homology. When employing such data for meaningful systematic and evolutionary studies, comparative cytogeneticists should attempt to use the outgroup method to determine primitive and derived character states and avoid such methods as "common equals primitive" and a priori assumptions regarding the direction of chromosomal evolution. Finally, confidence in chromosomal phylogenies can be increased and some problems with homoplasy resolved by using independent data sets, such as morphology, protein electrophoresis, protein sequencing, immunology, DNA hybridization, and/or DNA sequencing (Baker et al., 1987a; Qumsiyeh et al., 1987).

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