A theoretical approach to chromosome banding pattern analysis

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ABSTRACT

Based on a schematic model of karyotype evolution, a new methodology for G-, R-, or Q-banding pattern analysis was investigated. Banding pattern analysis essentially depends on the unidirectional alteration and the randomness of the exchange sites of the $\overline{AM}$ -inversion. In karyotypes that evolved by $\overline{AM}$ -inversion and Robertsonian rearrangement, two matching patterns appear; (1) tandem and (2) complementary matching patterns. The former is characteristic of a single lineage sharing the same $\overline{AM}$ -inversions, and the latter appears in different lineages sharing different $\overline{AM}$ -inversions, by which it is theoretically possible to detect the ancestral karyotype and to reconstruct the karyotype phylogeny (cladogram). In contrast, the evolutionary pathway cannot always be perceived if karyotypes evolve only by Robertsonian rearrangement. The tandem matching pattern does not always mean tandem fusion, but can be interpreted as 'tandem fission' by a combination of $\overline{AM}$ -inversion and centric fission. Tandem fusion and $\overline{MM}$ -inversion often cause entangled matching patterns, and thus they interfere with banding pattern analysis. Some methodological problems inherent in the conventional banding pattern analysis are highlighted, and suggested that such problems can be minimized by using the karyograph method. The methodology of banding pattern analysis proposed in the present paper will be applicable for matching the chromosome map of genetic markers among different species.

1. INTRODUCTION

Recent advances in chromosome banding techniques (G-, R-, or Q-bands) have provided a breakthrough in the field of evolutionary cytogenetics. For detailed reviews see Sumner (1990).

Chromosome banding is valuable for identifying homologous chromosomes in karyologically differentiated species, and especially for detecting chromosomal rearrangements that contributed to the karyotype evolution. A number of attempts have been made to reconstruct karyotype phylogenies of various taxa based on banding pattern analysis. For details see Qumsiyeh and Baker (1988).

As will be discussed later in detail, the conventional banding pattern analysis has some serious methodological problems concerning (1) detection of chromosomal rearrangements (i.e., the method of banding pattern matching), (2) the directionality of chromosomal rearrangements detected, and (3) determination of the

primitive (ancestral) karyotype of the examined taxon.

These problems are inherent in the experimental approach to banding pattern analysis. In this approach, each taxon investigated includes only a few (or at most a dozen) species obtained by chance, so that the karyological information for reconstructing karyotype phylogenies is often quite fragmentary. The primitive karyotype is consequently determined after complex (or often philosophical) arguments over issues other than chromosome banding. This is true even in the outgroup method. After all, chromosomal rearrangements are often detected comparing fragmentary karyotypes with a hypothetical primitive karyotype. This is really tautological, because determination of the primitive karyotype was the initial aim of the banding pattern analysis.

To remove such problems, this paper presents a theoretical approach for banding pattern analysis using a schematic model of karyotype evolution. With this model system, every ideal karyotype evolution including those of major chromosomal rearrangements can be visualized, and every karyotype derived from a primitive (starting) karyotype can be conserved there. Therefore, it will be simple to examine the effects of chromosomal rearrangements affecting the matching patterns of chromosome banding, and also to determine the best method for detecting the primitive karyotype based solely on chromosome banding.

2. A MODEL SYSTEM FOR BANDING PATTERN ANALYSIS AND TECHNICAL TERMS USED IN THE PRESENT PAPER

The series of G-, R-, or Q-banded karyotypes shown in Figure 1 indicate a hypothetical karyotype evolution altered by centric fission, centric fusion, or $A\bar{M}$ -inversion. I attempt to reconsider methodologies of banding pattern analysis based on these schematic karyotypes.

To simplify banding pattern analysis, each karyotype in Figure 1 is represented as a homomorphic haploid set (haploid karyotype), denoted Ko, K1, Ka2, Kb2, etc. Ko is the starting karyotype having two metacentric chromosomes in the broad sense ($\bar{M}$), i.e., $Ko=2\bar{M}$. In the same way, $K1=4A$, $Ka2=1A+3\bar{M}$, and $Kb2=4\bar{M}$, where A is an acrocentric chromosome. As mentioned later, C-bands (constitutive heterochromatin) are excluded from the present banding analysis. Therefore, A and $\bar{M}$ are roughly defined here as chromosomes with one and two euchromatic arms, respectively. For detailed definitions of A and $\bar{M}$ see Imai (1991).

Ka2, Ka3, Ka4, Ka4', and Ka5 are denoted here as being of Ka lineage. In the same way, Kb lineage includes Kb2, Kb3, Kb4, Kb4', and Kb5. Ka and Kb lineages share respectively three independent $A\bar{M}$ -inversions that occurred in Ka2 and Kb2. Of these karyotypes, odd numbers (K1, Ka3, Kb3, etc.) are acrocentric, and even numbered (Ka2, Kb2, etc.) are metacentric (rich).

In each karyotype mentioned above, chromosomes are arranged by size and

Fig. 1. A schematic model of karyotype evolution for chromosome banding analysis. Starting from $Ko=2\bar{M}$, haploid karyotypes ($K1$, $Ka2$, $Kb2$, $Kc2$, etc.) evolve by centric fission (fis), centric fusion (fus), or AM -inversion ($AM\ inv$). Stripes on each chromosome mean G-, R-, or Q-bands (or genetic markers if available). Chromosomal rearrangements are assumed to be fixed in each karyotype.

denoted as chromosomes 1, 2, 3, etc. or more simply Chrms 1, 2, 3, etc. Short and long arms of chromosomes are abbreviated as S and L, which corresponds with p and q in human chromosomes. By these definitions, the short and long arm of chromosome 1 in Ko is represented as 1S and 1L.

The major chromosomal rearrangements discussed here are the Robertsonian rearrangement (including centric fusion and centric fission) and AM -inversion (pericentric inversion changing A to $\bar{M}$). They are considered to have played a significant role in eukaryotic karyotype evolution. Tandem fusion and $\bar{M}\bar{M}$ - or $\bar{M}A$ -inversion (pericentric inversion changing $\bar{M}$ to $\bar{M}$ or $\bar{M}$ to A) are also discussed as examples of problematic rearrangements.

3. BANDING PATTERN ANALYSIS USING A SCHEMATIC MODEL OF KARYOTYPE EVOLUTION

In the hypothetical karyotype evolution shown in Figure 1, all karyotypes derived from the starting (ancestral) karyotype (Ko) are conserved, and their evolutionary pathways and contributed chromosomal rearrangements are given a

priori.

One of the serious problems of banding pattern analysis is that the available karyotypes are usually fragmentary. The crucial issue in banding pattern analysis is, therefore, how to detect the ancestral karyotype and chromosomal rearrangements based on such scarce information.

Taking these factors into consideration, banding pattern matching was performed in the present study using the following procedures;

- (1) C-bands (constitutive heterochromatin) were excluded from banding pattern matching.
- (2) Banding patterns were matched between every pair of karyotypes.
- (3) If a karyotype had metacentrics ($\overline{M}$), they were cut at the centromere and their short (S) and long (L) arms were regarded as independent acrocentrics (A).
- (4) Banding patterns were matched after arranging the centromere of each (acrocentric) chromosome upwardly.

Item (1) is of practical importance, because C-bands and others (G-, R-, or Q-bands) are substantially different. Constitutive heterochromatin blocks (C-bands) often show banding structure by G-band staining, which will cause mismatching.

To simplify descriptions of banding pattern analysis, karyotypes are classified into three evolutionary categories by the chromosome rearrangements involved: (1) $\overline{AM}$ -inversion and Robertsonian rearrangement, (2) Robertsonian rearrangement, and (3) tandem fusion or $\overline{MM}$ -inversion.

A) Banding pattern analysis in karyotypes evolving by $\overline{AM}$ -inversion and Robertsonian rearrangement

The results of banding pattern matching within Ka lineage and between Ka and Kb lineages are summarized in Figures 2 and 3. These schematic figures indicate that banding patterns always match tandemly in karyotypes belonging to a single lineage, but complementarily (i.e., partly overlapping) in those from different lineages. For example, in K1 and Ka3 (Fig. 2b), two small chromosomes (Chrms 3 and 1) of Ka3 tandemly match one large sized chromosome (Chrm 1) of K1. On the other hand, in Ka3 and Kb3 (Fig. 3c), four fragmentary chromosomes (Chrms 3 and 1 of Ka3, and Chrms 6 and 1 of Kb3) complement each other to form a closed matching group, of which the reconstructed banding pattern is identical with that of Chrm 1 of K1 (Fig. 3a).

It is noteworthy that in Figures 2 and 3 the reconstructed karyotypes are, in all cases except one (Ka3 and Ka5), identical to K1 (Figs. 2a and 3a). Ka3 appears as the ancestral karyotype of Ka3 and Ka5 (Fig. 2d), which is discussed later. However, it is reasonable to conclude that the common ancestor karyotype of the two arbitrarily given karyotypes is principally detectable by the banding pattern analysis at least in the karyotypes evolving by $\overline{AM}$ -inversion and Robertsonian

Fig. 2. Tandem matching patterns of chromosome banding in karyotypes from the same lineage (Ka) sharing the same AM-inversions. Note that karyotypes of the Ka lineage share three AM-inversions which occurred in Ka2 (see Fig. 1). More than two small chromosomes (or arms) of descendant karyotypes match 'tandemly' to one large chromosome (or arm) of an ancestral karyotype. **a.** The ancestral karyotype (K1) reconstructed by banding pattern matching between K1 and Ka3 (**b**), K1 and Ka5 (**c**), Ka3 and Ka5 (**d**), Ka2 and Ka4 (**e**), and Ka4' and Ka4 (**f**) is identical to K1=4A. Note that short and long arms always join tandemly in the metacentrics induced by AM-inversion (Ka2 and Ka4 in **e**), but different arms of different metacentrics join tandemly in metacentrics induced by centric fusion (Ka4' in **f**). **g.** An ancestral karyotype (K1') reconstructed by assuming that metacentrics of Ka4' were induced by AM-inversion. When only Ka4' and Ka4 are available, it is difficult to determine which of matching patterns **f** or **h** is correct because metacentrics can theoretically be induced by AM-inversion as well as by centric fusion. Arrowheads indicate chromosomal sites where AM-inversion and/or centric fission have occurred. S and L with numerals (e.g., 1S and 1L in Ka4) mean short and long arms of each chromosome (Chrm 1) in each karyotype (Ka4). For numbering of chromosomes see Fig. 1. For a better understanding of the matching pattern, chromosomes of either of the two karyotypes compared are vertically striped.

rearrangement, of which the theoretical backgrounds are discussed later.

B) Banding pattern analysis in karyotypes evolving by Robertsonian rearrangement

Ko, K1, and Kc2 karyotypes are connected only by centric fission or centric fusion (Fig. 1), where these rearrangements are assumed to have been fixed in a population (or species). In this case, it is difficult to determine their evolutionary pathway unconditionally. The reason is as follows. As Ko and Kc2 (metacentric

Fig. 3. Complementary matching patterns of chromosome banding in karyotypes from different lineages (Ka and Kb) sharing different $\overline{AM}$ -inversions. Note that karyotypes of Ka and Kb lineages share three different $\overline{AM}$ -inversions which occurred independently in Ka2 and Kb2 (see Fig. 1). More than two chromosomes in each of the two karyotypes compared are complementary. a. The ancestral karyotype reconstructed by comparing Ka5 and Kb5 (b), Ka3 and Kb3 (c), Ka4 and Kb2 (d), Ka4 and Kb4' (e), and Ka4' and Kb4' (f) is identical with K1=4A. As already pointed out in Fig. 2, short and long arms always join tandemly in the metacentrics induced by $\overline{AM}$ -inversion (Ka4 and Kb2 in d and e), but different arms of different metacentrics join tandemly in those induced by centric fusion (Ka4' and Kb4' in e and f).

karyotypes with $2\overline{M}$) can theoretically be induced from acrocentric karyotypes having 2A by $\overline{AM}$ -inversions as well as by centric fusion, at least three alternative matching patterns are possible (Figs. 4b, 4d, and 4f). Each matching pattern suggests a different ancestral karyotype, i.e., K1=4A in Fig. 4a, Kx=2A in Fig. 4c, and Ky=2A in Fig. 4e. Thus, three alternative evolutionary pathways (Figs. 4g, 4h, and 4i) are theoretically possible. In short, the evolutionary pathway among Ko, K1, and Kc2 is potentially bidirectional and practically unsettled (Fig. 4j).

These difficulties will be partly removed if centric fusion and fission are polymorphic. For example, Ka2, Ka3, and Ka4' karyotypes (which were assumed to be monomorphic in Fig. 1) are regarded in Figure 5 as three extremes in Robertsonian polymorphism. Robertsonian polymorphism can be subdivided into "fission polymorphism" (left half in Fig. 5), "fusion polymorphism" (right half in Fig. 5), and "fission-fusion polymorphism" (Fig. 5). Ka2 (=1A+3 $\overline{M}$) is the

Fig. 4. Three alternative banding pattern matches (**b**, **d**, and **f**) expected from the metacentric karyotypes (K_0 and K_{c2}) that evolved only by Robertsonian rearrangement. **b**, **d**, and **f** indicate, respectively, that both K_0 and K_{c2} were derived from $K_1=4A$ (**a**) by centric fusions; that K_0 was induced from $K_x=2A$ (**c**) by $A\bar{M}$ -inversion, and that K_{c2} originated from $K_y=2A$ (**e**) by $A\bar{M}$ -inversion. As there is no information to determine which of these three possibilities were actually realized (**g**, **h**, **i**), the evolutionary pathways of K_0 , K_1 , and K_{c2} are virtually bidirectional (**j**).

starting karyotype and Ka_3 ($=7A$) is the endproduct in fission polymorphism, whereas Ka_3 is the starting karyotype and Ka_4' ($=1A+3\bar{M}$) is one of the endproducts in fusion polymorphism. There are a number of intermediate (polymorphic) karyotypes between these extremes. To simplify the description, the chromosome numbering of Ka_3 (i.e., Chrms 1, 2, ..., 7) is adopted as the standard in Figure 5, by which the metacentric chromosomes (Chrms 1, 2, and 3 in Fig. 1) of Ka_2 are revised here as Chrms 1·3, 2·4, and 5·7.

If Chrms 1·3, 2·4, and 5·7 of Ka_2 alter into acrocentrics by centric fission sequentially, six intermediate karyotypes are to be expected (four of them are demonstrated in Fig. 5). The metacentrics in these intermediate karyotypes consist of either of the metacentrics (Chrms 1·3 or 2·4 or 5·7) involved in the starting karyotype (Ka_2). In other words, the arm combination of metacentrics are fixed in the fission polymorphism.

On the other hand, metacentrics in the fusion polymorphism are characterized by a remarkable heterogeneity in arm combinations (i.e., heterobrachial). The starting karyotype Ka_3 has seven acrocentrics ($7A$) (Fig. 5). If centric fusion occurs at random in two arbitrarily selected acrocentrics in Ka_3 , the total number

Fig. 5. Arm combinations of metacentrics in Robertsonian polymorphism. Robertsonian polymorphism can be divided into fission (left half), fusion (right half), and fission-fusion. The arm combinations of metacentrics are fixed in the fission polymorphism (i.e., identical with those involved in Ka2), whereas the fusion polymorphism is characterized by the frequent appearance of heterobrachial metacentrics. In fission-fusion polymorphism, karyotypes showing fission polymorphism will be recognized as if they were induced by centric fusion because of heterobrachial metacentrics. Chromosome numbering of Ka3 is temporarily used in this figure as the standard for describing these polymorphic karyotypes.

of karyotypes having one, two, or three heterobrachial metacentrics is estimated as ${}_{7C_2} + {}_{7C_2 \cdot 5C_2} + {}_{7C_2 \cdot 5C_2 \cdot 3C_2} = 861$ (six of them including Ka4' are demonstrated in Fig. 5).

If centric fission and fusion occur simultaneously, fission-fusion polymorphism will be recognized as fusion polymorphism because of the heterobrachial metacentrics.

C) Banding pattern analysis in karyotypes evolving by tandem fusion or $\overline{MM}$ -inversion

Ka4'' (Fig. 6b) is a karyotype induced from Ka3 (Fig. 6a) by three independent tandem fusions. Ka4'' matches tandemly to Ka3 and also to Ka5 (Fig. 6c). However, tandem fusion often causes some entangled matching patterns (Figs. 6d-6g), which will endanger the banding pattern analysis.

Fig. 6. Entangled matching patterns of chromosome banding resulting from tandem fusion. Ka4'' derived from Ka3 by three tandem fusions matches tandemly to Ka3 (a) and Ka5 (c), but entangled matching patterns appear elsewhere, such as K1 (d), Kb3 (e), Kb5 (f), and Kz (g). Kz is the outgroup karyotype to Ka and Kb lineages (see Fig. 11). Question marks refer to chromosomes with unique banding patterns. Chromosomes of Ka4'' are vertically striped.

$\overline{MM}$ -inversion is also a hazard of banding pattern analysis, because it jumbles banding patterns as does tandem fusion. An example is demonstrated in Ka3' (Fig. 7b), which was induced from Ka2 (Fig. 7a) by three independent $\overline{MM}$ -inversions. Although these $\overline{MM}$ -inversions are detectable by comparing Ka3' with Ka2, unsound matching patterns accompanying acentric fragments appear if Ka3' is compared with K1 (Fig. 7c) or Ka3 (Fig. 7d). The matching conditions deteriorate in karyotypes which are karyologically distant from Ka3', e.g., Ka5 (Fig. 7e) and Kb5 (Fig. 7f). It is notable that over half the chromosomes of Kb5 failed to find their partners (see question marks in Fig. 7f). They will, in practice, be recognized as unique chromosomes.

A possible karyotype phylogeny reconstructed by the present banding pattern analysis is summarized in Figure 8 (compare with Fig. 1), of which the theoretical bases follow.

Fig. 7. Unsound matching patterns of chromosome banding originated from $\overline{MM}$ -inversion. Ka3' (b) induced from Ka2 (a) by three $\overline{MM}$ -inversions was compared with K1 (c), Ka3 (d), Ka5 (e), and Kb5 (f). As banding patterns were jumbled by $\overline{MM}$ -inversion drastically, acentric fragments (c and d) or unmatched chromosomes with unique banding patterns (question marks in e and f) appear frequently.

Fig. 8. A karyotype phylogeny reconstructed by banding pattern analysis. Solid arrows mean pathways of karyotype alteration determined. Broken arrows are potential pathways resulting from either centric fusion or centric fission due to the bidirectionality of Robertsonian rearrangement, or illusory pathways resulting from an assumption that tandem matching is cytological evidence for

4. THEORETICAL BASES FOR BANDING PATTERN ANALYSIS

A) *Principles of banding pattern analysis*

The success of banding pattern analysis fundamentally depends on the detectability and unidirectionality of chromosomal rearrangements that contributed to karyotype evolution.

Both $\overline{AM}$ -inversion and Robertsonian rearrangement are detectable by the method of banding pattern matching adopted in this paper (Figs. 2 and 3). On the other hand, tandem fusion and $\overline{MM}$ -inversion remarkably disturb banding pattern analysis by unsound matching patterns and the appearance of acentric fragments or unique chromosomes (Figs. 6 and 7).

With regard to directionality, $\overline{AM}$ -inversion is, in a statistical sense, exclusively unidirectional. Namely, acrocentrics (A) can alter into metacentrics ($\overline{M}$) by $\overline{AM}$ -inversion, but the reverse alteration by $\overline{MA}$ -inversion is negligibly rare by both theoretical and experimental means (Imai and Maruyama, 1978; Imai, 1991). In contrast, Robertsonian rearrangement is potentially bidirectional, because it includes centric fusion and fission which are directionally opposite.

Under these restrictions in chromosomal alterations, banding pattern analysis ultimately depends on the unidirectional alteration of $\overline{AM}$ -inversion. Karyotypes evolving by $\overline{AM}$ -inversion and centric fission (Ka and Kb lineages in Figs. 1 and 8) can therefore be analyzed. In other words, their ancestral karyotypes and contributed chromosomal rearrangements can be detected, then their evolutionary pathways can be reconstructed (see solid arrows in Fig. 8).

Centric fusion is detectable only when $\overline{AM}$ -inversion information is conserved in the compared karyotypes, e.g., Ka4' and Kb4' (Figs. 1, 3f, and 8). If karyotypes are connected only by Robertsonian rearrangement (e.g., Ko, K1, and Kc2 in Fig. 1), it is difficult to determine their evolutionary pathways (Fig. 8) because of its bidirectionality (Fig. 4).

Tandem fusion and $\overline{MM}$ -inversion disturb banding pattern matching more seriously than has been considered, because they often cause entangled matching patterns (Figs. 6, and 7).

tandem fusion. The directionality of the Robertsonian rearrangement can be determined only when the compared karyotypes conserve information about $\overline{AM}$ -inversion. The evolutionary pathways of Ko, K1, and Kc2 are therefore unsettled (see Fig. 4), and thus K1 will be recognized as the ancestral karyotype of Ka and Kb lineages. Tandem matching patterns can be expected by $\overline{AM}$ -inversion and centric fission. Kz is the outgroup karyotype to Ka and Kb lineages (see Fig. 11). Ko' is the ancestral karyotype reconstructed by the banding pattern analysis between Kz and karyotypes of Ka and Kb lineages, by which the evolutionary pathway of Ko, K1, and Kc2 can be fixed (also see Fig. 11). Genuine tandem fusion and $\overline{MM}$ -inversion often cause entangled or unsound matching patterns, which are represented by solid arrows with gaps. For detailed karyotypes see Fig. 1.

B) Theoretical bases for $\overline{AM}$ -inversion

$\overline{AM}$ -inversion information is recorded on chromosomes as the localization of the centromere, though it is lost outwardly (cryptic) in A chromosomes induced by centric fission.

If karyotypes evolve toward increasing chromosome number by $\overline{AM}$ -inversion and centric fission, the size of the chromosomes in descendant karyotypes inevitably decreases (fragmentation of chromosomes denoted as tandem fission by Imai, 1988). This is the reason for the tandem matching pattern found in the Ka lineage (Figs. 1 and 2). The latent information of $\overline{AM}$ -inversion in the small sized A chromosomes of more descendant karyotypes (e.g., $Ka3=7A$ or $Ka5=14A$) is detectable only with reference to the large sized chromosomes of more ancestral karyotypes such as $K1=4A$ (see arrowheads in Figs. 2b and 2c).

As mentioned in the previous section, $Ka3$ is recognized as the ancestral karyotype of $Ka5$ (Fig. 2d). It is difficult to reconstruct more ancestral karyotypes ($Ka2$ or $K1$) based on $Ka3$ and $Ka5$, because karyological information

Fig. 9. Directionality of $\overline{MM}$ -inversion (a and b), and examples of misreading of the complementary matching (c and d). In $Ka2$ and $Ka3'$, if $Ka2$ is assumed as the standard, then $Ka3'$ is interpreted to have been derived from $Ka2$ by $\overline{MM}$ -inversions (a), and *vice versa* (b). It is difficult to determine which of the two alterations actually occurred. $\overline{MM}$ -inversion is thus bidirectional. In $Kb3$ and $Ka5$, the exchange sites of some $\overline{AM}$ -inversions which occurred independently in Ka and Kb lineages were very close by chance (see small bars in d). Each of the four complementary matching groups in d will be misread as two different matching groups (c). Such mismatched chromosomes have a minor deletion or extra euchromatin (small arrows in c) or will be recognized as unique chromosomes without banding homologies (question marks in c).

that Chrms 3 and 1, 4 and 2, and 7 and 5 in Ka3 were once linked, was lost at the time Ka3 was induced from Ka2 by centric fission (see arrowheads in Fig. 2d).

The randomness of the breakage sites in $\overline{AM}$ -inversion suggests that all $\overline{AM}$ -inversions will have different breakage sites. This is the reason for the complementary matching patterns found in karyotypes between Ka and Kb lineages (Fig. 3), where the ancestral karyotype reconstructed is always K1=4A. It is an important characteristic that the reliability of K1 as the ancestral karyotype can be reconfirmed repeatedly by comparing karyotypes chosen arbitrarily from Ka and Kb lineages (e.g., Ka5 and Kb5 in Fig. 3b, Ka3 and Kb3 in Fig. 3c, etc.). If breakage sites of independent $\overline{AM}$ -inversions that occurred in karyotypes of different lineages are very close by chance (e.g., see small bars on Kb3 and Ka5 in Fig. 9d), a series of mismatches accompanying partial deletion or addition of euchromatin (see small arrows in Fig. 9c) may be inevitable.

C) Theoretical bases for tandem fusion

It is generally believed that the tandem matching pattern is cytological evidence for tandem fusion (e.g., Indian muntjac, Shi et al., 1980). Indeed, in the present study, Ka4'' tandemly matches to Ka3 and also Ka5 (Fig. 6c). However, the same matching pattern also appears in karyotypes evolving by tandem fission (i.e., combination of $\overline{AM}$ -inversion and centric fission in Fig. 2). Tandem fusion and fission is, therefore, bidirectional.

This problem can be removed partly by C-banding pattern analysis. The tandem matching pattern resulting from genuine tandem fusion will accompany (in most cases but not always) an insertion of C-bands (Figs. 10a and 10b), whereas C-bands never appear in tandem fission (Figs. 10c and 10d). As chromosomes with C-band insertion are negligibly rare (less than 1%) in mammals (Imai, 1991),

Fig. 10. Identification of tandem fusion (a and b; Ka4''/Ka3) and tandem fission (c and d; K1/Ka3) by C-banding pattern analysis. Tandem fusion (a) often accompanies C-band insertion (b), but no C-bands can be expected by $\overline{AM}$ -inversion and centric fission (that is, tandem fission)(c).

tandem fusion may have contributed less to the mammalian karyotype evolution than was previously supposed. The evidence that a tandem fusion was found in a taxon does not necessarily guarantee its predominancy in all other taxa, where we need a quantitative or statistical approach.

D) Uncertainty as to the origin of $\overline{M}$ and A chromosomes

$\overline{M}$ chromosomes are theoretically inducible by centric fusion as well as $\overline{AM}$ -inversion. For example, $Ka4'$ is the former and $Ka4$ is the latter (Fig. 1). These two karyotypes are detectable by their matching patterns. Namely, in $\overline{M}$ chromosomes induced by $\overline{AM}$ -inversion, the short and long arm of each chromosome always joins tandemly (e.g., $Ka2$ and $Ka4$ in Fig. 2e), whereas different arms of different chromosomes join tandemly if $\overline{M}$ chromosomes are induced by centric fusion ($Ka4'$ in Fig. 2f).

However, if only $Ka4$ and $Ka4'$ are available, it is difficult to exclude the alternative possibility that $\overline{M}$ chromosomes of $Ka4'$ were induced by $\overline{AM}$ -inversion (Fig. 2h). In the last case, the ancestral karyotype reconstructed ($K1'$, Fig. 2g) and the genuine karyotype ($K1$, Fig. 2a) are quite different.

$\overline{M}$ chromosomes are also expected to arise from $\overline{MM}$ -inversion. Because of the bidirectionality of $\overline{MM}$ -inversion, it is difficult to determine the original karyotype based on banding pattern analysis. For example, in $Ka2$ and $Ka3'$ (Figs. 7a and 7b), two alternative matching patterns are possible (Figs. 9a and 9b). The former suggests that $Ka2$ is the original karyotype and $Ka3'$ is derived, but vice versa in the latter. There is no logical reason to exclude the latter possibility unconditionally.

A chromosomes are theoretically inducible by centric fission, $\overline{MA}$ -inversion, or tandem fusion. As mentioned above, both $\overline{MA}$ -inversion and tandem fusion contribute less frequently to the eukaryotic karyotype evolution. Thus, centric fission is the only available mechanism for inducing A chromosomes. In other words, if a karyotype includes acrocentrics, it is an indirect evidence supporting that those acrocentrics were induced by centric fission.

E) Theoretical bases for the karyological outgroup

$K1$ is the most ancestral karyotype which can be traced back by the method mentioned above (Fig. 8), where Ko (the genuine starting karyotype in Fig. 1) appears as if it is derived from $K1$ by centric fusion. This may be why acrocentric karyotypes (K_A) are often considered ancestral in eukaryotic karyotype evolution. If so, the simple question arises, that is, is it difficult to detect karyotypes more ancestral than $K1$? The answer is, that it is theoretically possible.

One example is shown in Figure 11. A new acrocentric karyotype Kz ($=8A$; Fig. 11a) is compared with $Ko=2\overline{M}$ (Fig. 11c), $K1=4A$ (Fig. 11d), $Kc2=2\overline{M}$ (Fig. 11e), $Kb3=8A$ (Fig. 11f), and $Ka5=14A$ (Fig. 11g) (for details of each karyotype

It must be further stressed that if a karyotype is connected with a batch of ingroup karyotypes only by Robertsonian rearrangement, it is not appropriate as the outgroup karyotype (e.g., Ko or Kc2 to Ka and Kb lineages in Fig. 8).

These series of banding pattern analyses assume that the basic banding pattern of Ko' is conserved perfectly in all descendant karyotypes, that is, there is no de novo formation or loss of chromosomal bands. This assumption will become most important for cytologists to resolve in the future, especially for comparing banding patterns between karyologically distant species at higher taxonomic categories such as family or order.

5. METHODOLOGICAL PROBLEMS INHERENT IN CONVENTIONAL BANDING PATTERN ANALYSIS

Recent publications that addressed banding pattern analysis can be classified into three categories, i.e., those that investigated (1) chromosomal polymorphisms in populations of a single species, (2) micro karyotype evolution in closely related species, and (3) macro karyotype evolution at the family or order level.

Robertsonian polymorphisms of European feral mice (*Mus domesticus*, Gropp et al., 1972; Corti et al. 1986), shrews (*Sorex araneus*; Searle, 1988), and humans belong to the first group. The abundance of heterobrachial metacentrics indicates that these cases are definitely centric fusion polymorphisms. On the other hand, Japanese raccoon dogs (Wada and Imai, 1991) evolved by centric fission polymorphism, because of the lack of heterobrachial metacentrics.

Karyotypic evolution by $\overline{AM}$ -inversion and addition of the constitutive heterochromatin (C^+) found in *Peromyscus* (Stangle and Baker, 1984) is an example of the second category. Because of the exclusive unidirectionality of $\overline{AM}$ -inversion, the cladogram they proposed may be appropriate.

The third category seems, however, to be problematic. Two typical examples of alvicoidea rodents and primates were analyzed using the karyograph method and are discussed below.

A) *G-banding pattern analysis of alvicoidea rodents*

A total of 22 species belonging to 8 genera were examined by Modi (1987), of which 18 species are here represented on the karyograph (Imai and Crozier, 1980) with abbreviated species names (Fig. 12). For definition of acrocentric (A) and metacentric ($\overline{M}$) chromosomes see Imai (1991). In the figure, haploid karyotypes are distributed in a sigmoid shape in the area between $AN=18-55$ and $n=9-32$, where AN and n mean haploid arm and chromosome numbers, respectively.

Modi (1987) adopted *Peromyscus* karyotype ($K=24A$) as the outgroup (PER; the open pentagram in Fig. 12), and assumed $K=27A+1\overline{M}$ as the ancestral karyotype of alvicoids (ALV; the solid pentagram) based on the modal hypothesis proposed by Matthey (1973). Among 141 chromosomal rearrangements he identified, the major types were centric fusion (27%), tandem fusion (14.2%), tandem fission (5.7%), pericentric inversion ($\overline{AM}$ - or $\overline{MM}$ -inversion, 12.8%), and paracentric inversion (AA - or $\overline{MM}$ -inversion, 8.5%). Besides, some deleterious rear-

Fig. 12. Karyotype distribution of alvicoidea rodents on the karyograph. Karyological data are based on Modi (1987). K_A and K_M border lines mean border lines by acrocentric (K_A) and metacentric (K_M) karyotypes. Arrows indicate the direction of shift of karyotypes on the graph by centric fission, centric fusion, tandem fusion, or A-M-inversion. Abbreviations of species: **PER** (*Peromyscus* as the outgroup of alvicoids, open pentagram), **ALV** (the ancestral karyotype of alvicoids proposed by Modi, solid pentagram), **MOR** (*Microtus oregoni*), **MOE** (*M. oeconomus*), **MME** (*M. mexicanus*), **MAG** (*M. agrestis*), **MTO** (*M. townsendii*), **MRI** (*M. richardsoni*), **MCA** (*M. californicus*), **MOC** (*M. ochrogaster*), **MPI** (*M. pinetorum*), **MLO** (*M. longicaudus*), **DTO** (*Dicrostonyx torquatus*), **SCO** (*Synaptomys cooperi*), **LSI** (*Lemmus sibiricus*), **NAL** (*Neofiber alleni*), **LCU** (*Lagurus curtatus*), **CCA** (*Clethrionomys californicus*), **CGA** (*C. gapperi*), **PIN** (*Phenacomys intermedius*).

rangements such as addition (10.6%) or deletion (7.8%) of euchromatin were observed, and 44 chromosomes identified as unique had no G-banding homology with any of the 27 standard autosomes.

The methodological problems of Modi's paper are as follows.

(1) The ancestral karyotype of alvicoids (ALV) was not reconstructed directly by G-banding pattern matching between every pair of alvicoidea karyotypes examined.

(2) Chromosomal rearrangements of alvicoidea karyotypes were identified by standardizing the G-banding pattern of *Peromyscus* karyotype. However, there are no concrete reasons for the G-banding pattern of *Peromyscus* (the outgroup karyotype) having been conserved perfectly since alvicoids and *Peromyscus* have differentiated.

(3) The tandem matching pattern does not always mean tandem fusion as discussed in the previous section. The insertion of C-bands (Figs. 10a and 10b)

requires verification. Tandem matching patterns without C-bands insertion rather suggest tandem fission (that is, a combination of $\overline{AM}$ -inversion and centric fission) than tandem fusion (Figs. 10c and 10d).

(4) As additions and deletions of euchromatin are extremely deleterious, it is unreasonable to assume that they would have contributed significantly to alvicoid karyotype evolution. A simple misreading of complementary matching (Figs. 9c and 9d) may be the alternative interpretation.

(5) The frequent appearance of unique chromosomes may result from modification of the G-banding pattern by $\overline{MM}$ - or paracentric inversions (Figs. 7e and 7f). The G-banding homology of each arm is not complete in alvicoid karyotypes, because many chromosomes were identified as homologous but still have a minor modification of the G-banding pattern due to unspecified rearrangements.

The frequency of the rearranged chromosomes per karyotype when plotted against the karyological distance by means of karyonumber ($KN=n+AN$; Imai et al., 1983) increases as the distance increases even in closely related species such as *Microtus* (solid triangles in Fig. 13). These results thus most probably indicate a methodological limitation of G-banding pattern analysis employed by Modi (1987).

Such problems will be partly removed by using the karyograph method (Fig. 12). Note that karyotypes plotted on the karyograph conserve the numerical

Fig. 13. Correlation between karyological distance and frequency of rearranged chromosomes per karyotype in alvicoid rodents. The rearranged chromosomes mean that they are identified as homologous but have minor modifications in banding patterns due to unspecified chromosomal rearrangements. The karyological distance is represented by the haploid karyonumber ($KN=n+AN$) based on the karyograph in Fig. 12. Abbreviations and symbols, see Fig. 12. Note that the frequency of rearranged chromosomes per karyotype increases even in closely related species (*Microtus*, see solid triangles) from the primitive ancestral karyotype of alvicoids (ALV).

information on chromosome rearrangements which have been recorded in karyotypes either as chromosome number (centric fusion, centric fission) or as arm number ($A\bar{M}$ -inversion) over the course of evolution. Although the karyograph method is not always useful for reconstructing the karyotypic phylogeny of a given taxon stringently, the distribution pattern of karyotypes on the karyograph provides valuable information on general tendency of the karyotype evolution of a given taxon.

The sigmoid pattern of alvicoid karyotypes on the karyograph (Fig. 12) may be best interpreted by assuming that their chromosomes have evolved *as a whole* toward increasing chromosome number and arm number by centric fission and $A\bar{M}$ -inversion. The major arguments of this alternative are summarized as follows: (1) *Microtus oregoni* (MOR) is the karyologically most primitive species in this group. It is difficult to induce the karyotype of MOR from that of *Peromyscus* (PER) (the outgroup assumed by Modi) or ALV (the ancestral karyotype of alvicoids assumed by Modi) because of a strong unidirectionality of $A\bar{M}$ -inversion (Imai and Maruyama, 1978). The only possibility is to assume a predominant occurrence of tandem fusion. If this is the case, metacentrics of MOR should have interstitial C-bands as shown in Fig. 10b, which is against the C-banding analysis. (2) The karyotypes of *M. oeconomus* (MOE) and *Dicrostonyx torquatus* (DTO) are not simple products from those of PER or ALV by centric fusion, because G-banding homology is not perfect between these two groups, i.e., the frequencies of modified chromosomes per karyotype by unknown factors are almost 100% (Fig. 13). An alternative is to assume that the three karyotypes (MOE, DTO, and MOR) are evolving to the right and upward along the K_M border line, where $A\bar{M}$ -inversion predominates over centric fission. (3) The acrocentric-rich karyotypes of majority of alvicoids (K_A) were induced by fission burst from some metacentric karyotypes (K_M) comparable with those of MOE or DTO (but not necessarily the same with them). Some of the centric fusions detected by Modi in this group may be secondary derivatives for eliminating C-bands after centric fission. (4) Some members of *Microtus* (MOC, MCA, MPI, and MLO) have started to evolve by $A\bar{M}$ -inversion after the fission burst. (5) Karyotype evolution and morphological evolution are basically independent phenomena. So that morphologically primitive species may not necessarily have primitive karyotypes.

B) R-banding pattern analysis of primates

Dutrillaux (1988) proposed a hypothetical karyotype $K_{PRI}=27A+1\bar{M}$ as the ancestral karyotype of primates based on the detailed R-banding pattern analysis of more than 100 living species.

Fourteen primate species are here selected from the materials used by Dutrillaux, which include Cercopithecoidea (4 species), Hominoidea (2), Lorisiformes (3), Lemuriformes (2), and Platyrrhini (4), and their karyotypes are plotted on the

Fig. 14. Karyographic representation of the ancestral karyotype of primates proposed by Dutrillaux (1988) (PRI, solid pentagram), and those estimated by the karyograph method (solid symbols) based on 14 living primates (open symbols). Abbreviations of species: LRF (*Lepilemur ruficaudatus*), LLE (*L. leucopus*), AGE (*Ateles geoffroyi*), SAI (*Saimiri sciureus*), CAL (*Cebus albifrons*), ATG (*Aotus trivirgatus griseimembra*), GAL (*Galago alleni*), GDE (*G. demidovii*), GCR (*G. crassicaudatus*), HSA (*Homo sapiens*), PTR (*Pan troglodytes*), MFA (*Macaca fascicularis*), and PCR (*Presbytis cristatus*), CAE (*Cercopithecus aethiops*). Karyotypes of these species were based on the atlases of mammalian chromosomes by Hsu and Benirschke (1967–1975), and a paper by Rumpler et al (1985). Note that the ancestral karyotypes of primates estimated by the karyograph (solid symbols) do not converge on the one proposed by Dutrillaux (PRI, solid pentagram).

karyograph, with the values $AN=20-49$ and $n=10-31$ (Fig. 14). I attempt to reconstruct the ancestral karyotype of primates based on these 14 living primates and the chromosomal rearrangements detected by Dutrillaux in his paper.

Let K_i be a karyotype represented at the point (AN_i, n_i) on the karyograph. K_i shifts on the graph by chromosome rearrangements changing either the arm number (AN_i) or the chromosome number (n_i) such as $\overline{AM}$ -inversion (to the right; AN_i+1), $\overline{MA}$ -inversion (to the left; AN_i-1), centric fusion (downward; n_i-1), or centric fission (upward; n_i+1). On the other hand, K_i does not shift by reciprocal translocation, $\overline{MM}$ -inversion, or paracentric inversion. If a karyotype K_j is derived from K_i by one $\overline{AM}$ -inversion and one centric fusion, the predicted point of K_j on the graph (AN_j, n_j) is represented as $(AN_j=AN_i+1, n_j=n_i-1)$. As the shift of karyotypes on the graph is theoretically reversible, the point of K_i can relatively be described by standardizing K_j as $(AN_i=AN_j-1, n_i=n_j+1)$. This

means that K_i is derived from K_j by one MA-inversion and by one centric fission. In other words, the ancestral karyotype K_i can be estimated by the karyograph method based on the derived karyotype K_j and the reverse mutations of chromosomal rearrangements identified.

According to the cladogram by Dutrillaux (1988), the karyotype of *Cebus albifrons* (CAL) would have been induced from the hypothetical ancestral karyotype of primates (K_{PRI}) by 3 $\overline{AM}$ -inversions, 5 centric fusions, 1 modification of heterochromatin, 1 other translocation, and 1 complex or unspecified rearrangement. The first two rearrangements are valuable for the karyograph method. The karyotype of CAL (K_{CAL}) is $K_{CAL}=18A+9\overline{M}$ (Hsu and Benirschke, 1975), where $AN=18+2\times 9=36$ and $n=18+9=27$. K_{CAL} is plotted at the point (36, 27) on the karyograph (Fig. 14; open triangle labelled CAL). When the ancestral karyotype of primates estimated by K_{CAL} is denoted as K_{PRI}' , K_{PRI}' can theoretically be distant from K_{CAL} by 3 $\overline{MA}$ -inversions and 5 centric fissions on the karyograph. Now, $K_{PRI}'=31A+1\overline{M}$ can be derived, and it is located at the point (33, 32) (Fig. 14; solid triangle labelled CAL). Likewise, K_{PRI}' estimated from each of the 14 species mentioned above are superimposed on the karyograph (see solid circles, triangles, squares, etc. in Fig. 14). If the arguments mentioned above are adequate and the chromosomal rearrangements identified by Dutrillaux are correct, $K_{PRI}'=K_{PRI}$ is predictable in all cases examined, i.e., K_{PRI}' should be distributed in a narrow area centered around K_{PRI} on the karyograph.

The results obtained are, however, $K_{PRI}'\neq K_{PRI}$ in all cases. Note that K_{PRI}' scatters diversely between $AN=20-40$ and $n=16-34$ on the karyograph (compare solid and open symbols in Fig. 14). Indeed, the low numbered karyotypes of *Lepilemur ruficaudatus* ($K_{LRF}=2A+11\overline{M}$) and *L. leucopus* ($K_{LLE}=10\overline{M}$) are really difficult to induce from that of K_{PRI} ($=27A+1\overline{M}$) as is the case in *Microtus* examined in the previous section. These results suggest strongly that both the hypothetical ancestral karyotype of primates ($K_{PRI}=27A+1\overline{M}$) and the karyotype phylogeny reconstructed by Dutrillaux might have some problematic details. The frequent appearance of unsound rearrangements such as complex or unspecified rearrangements, reverse inversions, convergent inversions, and also the concept of population evolution would suggest some serious methodological limitations inherent in the R-banding pattern analysis employed by Dutrillaux. An alternative argument that chromosomes of primates evolved as a whole toward increasing chromosome number by centric fission and $\overline{AM}$ -inversion with local reduction of chromosome number by centric fusion was previously presented by Imai and Crozier (1980, see their Figs. 14 and 15).

The banding pattern analysis is certainly valuable for analysing either chromosomal polymorphisms in populations of a single species or micro karyotype evolution in closely related species in a genus. It may not, however, always be suitable for macro karyotype evolution at the family or order level, where the karyograph method appears to provide a better alternative. The methodology of

banding pattern analysis proposed in the present paper should also be useful for matching the chromosome map of genetic markers among different species.

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