

Review article.

Kinetochores Underlies Karyotypic Fission Theory: Possible Legacy of Symbiogenesis in Mammalian Chromosome Evolution

NEIL B. TODD

*Carnivore Genetics Research Center, 26 Walnut Place, Newtonville,
MA 02460-1819, USA, Tel. +1-617-527-1161, E-mail. NEILBTODD@cs.com*

Received December 4, 2000; Accepted December 16, 2000

Abstract

This paper is a rejoinder to Godfrey and Masters' criticism of Kolnicki's interpretation of implications of karyotypic fissioning theory for speciation. It is argued that karyotypic fissioning should not be dismissed as a cause of speciation in *Muntiacus* or any other mammalian taxa.

Keywords: Evolution, mammals, *Muntiacus*, chromosomes, karyotypic fissioning, pericentric inversion, centromere-kinetochores

The numerical and morphological analysis of karyotypes began early in the 20th century propelled by the "chromosome theory of heredity". Many investigators noted that genetically similar species differed significantly in their chromosome complements with diploid number, size and morphology (e.g. whether mediocentric or acrocentric) varying greatly. Such observations have stimulated a nearly century-long debate on the nature of individual rearrangements and the overall direction of change. Recent interpretations of some such taxa include, e.g., carnivores (Todd, 1970), artiodactyls (Todd, 1975, 1985), primates (Guisto and Margulis, 1981) and lemurs (Kolnicki, 1999).

Kolnicki's analysis of the Lemuridae (1999) has precipitated a reappraisal of the whole matter of karyotypic fissioning and, ultimately, led to consideration of underlying cell biological mechanisms (Kolnicki, 2000; Kolnicki, in preparation). Kolnicki has found that all extant karyotypes of the five lemur families "are most parsimoniously explained as the product of four karyotypic fissionings, two primary and two secondary, followed by pericentric inversions". Modern lemur species vary in diploid number from 20 to 70. To invoke fusion to explain these differences would require approximately 100 independent steps to derive karyotypes of the Lepilemuridae ($2n = 20-38$), Daubentoniidae ($2n = 30$), Lemuridae ($2n = 44-62$), Cheirogaleidae ($2n = 46, 66$) and Indriidae ($2n = 40-70$). Kolnicki suggests that a primary fissioning event became fixed in ancestors to daubentonids, indrids and a lineage common to lemurids and cheirogalids. This was followed by a second, more recent fissioning that isolated lemurids from cheirogalids. The ancestral lepilemurids, unaffected by this earliest primary fissioning, most likely experienced their own subsequent, independent fissioning.

M.J.D. White (1973) postulated that most chromosomal evolution proceeded through fusions from high ancestral numbers to low derived numbers, arguing that no ready mechanism for fission was known and that fissioning was a "preposterous" notion because it would require many independent, low probability, individual mutational events. However, while fusion mechanisms have been documented, they likewise are individual mutational events and generally so complicated that they offer no better an explanation for much of the karyological diversity that may be observed. Furthermore, when the overall distribution of diploid numbers is viewed in relationship to known paleontological and zoogeographic evidence, an opposite explanation is suggested, viz. that diploid numbers were primitively low and have risen in derivative lineages. The most dramatic example of this is found in marsupials in which South American and Australian forms share very similar $2n = 14$ karyotypes (Hayman et al., 1971), whereas the higher numbers found on both continents are clearly dissimilar. $2n = 14$ karyotypes, although in altered forms, may also be discerned in some placental mammals. Thus, the original formulation of the theory of karyotypic fissioning assumed that a $2n = 14$ condition was shared, at least, by the common ancestor of metatherians and eutherians (Todd, 2000). But, largely because of the general acceptance of White's objections, the assumption that $2n = 14$ was primitive has been ignored despite several exhaustive statistical analyses suggesting otherwise (Imai, 1978; Imai and Maruyama, 1978; Imai and Crozier, 1980) and karyotypic fissioning has been adopted in only a few instances to explain evolutionary relationships in mammals (Todd, 1970, 1976, 1985; Guisto and Margulis, 1981; Kolnicki, 1999).

Recent cell biological studies of kinetochore-centromeres have greatly

altered and enhanced understanding of chromosome behavior. Literature assembled by Kolnicki (2000) makes clear the explanation of karyotypic fissioning lies in the fundamental reproductive modes of kinetochore-centromeres. In recognition of many new studies on the underlying nucleic acid and protein composition and activities of these regions of the chromosomes (their attachment to the mitotic-meiotic spindle and, hence, their role in karyotype behaviour), Kolnicki has explained not merely the relative number and morphology of chromosomes but has incorporated the following discoveries of cell biologists. 1) Complete sets of dicentric chromatids (where each chromatid has an extra kinetochore-centromere) can be synthesized during gametogenesis. This occurs when the rate of disk-shaped, synchronously-produced kinetochores exceeds the rates of development (reproduction) of the rest of the cell in which the kinetochore-centromeres reside; 2) kinetochore protein dephosphorylation regulates dicentric chromatid segregation via a well-defined "checkpoint". The breakage of dicentric chromosomes between chromosome pairs, a phenomenon well-known from clinical studies, generates acrocentric derivatives from mediocentric parental chromosomes. Meiotic "checkpoints" regulate chromosome disjunction to form, abruptly (from a geological-evolutionary perspective), fissioned karyotypes. The basis of mitotic and meiotic checkpoints is tension applied by natural microtubule attachment of the chromatids (mitosis) or chromosomes (meiosis) to both poles (or tension initiated by a micromanipulation needle) leading directly to dephosphorylation of special kinetochore proteins (kinases, presumably Mad and Bub). Chromatid (or chromosome) separation is inhibited until chromosomes are properly aligned such that tension is distributed equally to both poles. Kinetochore proteins that are misattached remain phosphorylated. These phosphorylated proteins inhibit anaphase movement until every kinetochore protein is dephosphorylated on all chromosomes. The revelation of how mechanical force (in this instance tension on the centromere-kinetochore via its attached spindle microtubules) is transduced to chemical signals (dephosphorylation of specifically aligned proteins) is a discovery comparable to that of light transduction by chlorophyll molecules). Taken together the cell biological work underlies the explanation of common behavior of all chromosomes and their kinetochore-centromeres in a cell. This coordinated behavior of all chromosomes in a given cell negates M.J.D. White's accusation that fission theory is preposterous because it requires many independent, random mutational events. Kinetochore-centromere reproduction is neither random, nor exceedingly rare. And, importantly, the reproductive activity of kinetochore-centromeres on one chromosome are not independent of those on other chromosomes in a cell.

In their recent commentary Godfrey and Masters (2000) acknowledge Kolnicki's breakthrough and the power of her reanalysis to explain major

changes in animal karyotypes. They especially recognize how the theory sheds light on the observation of the occurrence of "very different diploid numbers in closely related species" However, they raise two criticisms. First, they question the generality of fissioning theory to explain most mammalian karyotypes and, in particular, remark that "Kolnicki's attempt to link karyotypic fissioning and speciation is weak and unnecessary" and claim "There are many examples of karyotypic differences among closely related species [that] simply can not be explained via karyotypic fissioning". They offer the Indian muntjac ($2n = 6/7$) and its close relative, the Chinese muntjac ($2n = 46$) whose phenotypic differences are largely trivial and who produce viable (if sterile) hybrids. Second, they reject any direct association of fissioned karyotypes with speciation and erroneously characterize the theory as suggesting that the spread of deleterious pericentric inversions through populations triggers speciation. "The chances of fixing a deleterious rearrangement, the effects of which are strong enough to present a significant barrier to gene flow, are extremely low without the aid of a prolonged and severe bottleneck". Rather, they conclude that any such chromosomal changes would be eliminated by natural selection. Fissioning, as discussed elsewhere in detail (Todd, 1970), preceded or followed by pericentric inversions in chromosomally heteromorphic populations may lead to reduced fertility and/or sterility between certain animals of a previously panmictic population. But, such fissions and/or inversions are not necessarily deleterious vis-a-vis the immediately antecedent population in which they arise. Only for certain heteromorphic chromosomal combinations do they lead to problems of segregation. Under these circumstances an impediment to gene flow between populations may be erected by a narrow band of hybrids. However, despite any potential or real reproductive deficiencies, these hybrids may be viable, competitive and somatically well adapted. This is particularly relevant in mammals where survival strategies are often based, pre-eminently, on individual behavioral responses (behavioral lability rooted in learning capacity). There is no reason to suppose that such individuals will simply perish allowing the unmitigated effects of natural selection to prevail. Rather, they may persist and become one more obstacle to gene flow. If the inevitable outcome is to produce two reproductively isolated forms, then this may be called speciation. If one wishes to stipulate that speciation is only effected when pre-mating isolation has evolved, the argument tends to degenerate into one of definitions. Since speciation is a concerted process, the question may be when the point of no return has been reached and two immiscible populations exist. A precise answer may be very difficult to find but may lie somewhere after fissioning and other interacting transformations, but before pre-mating isolation.

A further purpose of this commentary, using Godfrey and Masters' example of

Muntiacus is to challenge the assertion that karyotypic fissioning has not played a role in speciation in this genus. The genus *Muntiacus*, small, hardy Asian deer, is variously described as encompassing some seven or eight species and more subspecies (Amato et al., 1999). However, the taxonomy is still in a state of flux and the "ifs" and "wheres" of species boundaries often remain vague. Considering that the genus has a multimillion square mile range, the present meager sampling can hardly be considered as representative. Further selected sampling may well result in very useful, if not surprising, findings. Despite recent research, the karyological and zoogeographic evidence is still inadequate to reach any definitive assessment. Thus the question is, perhaps, best reformulated in the following way. Does karyotypic fissioning underlie the observed karyological diversity and to what extent may this diversity lead to speciation?

There is general consensus that *M. muntjak* and *M. reevesi* are distinct species and they certainly have strikingly different karyotypes, $2n = 6/7; 8/9$ and $2n = 46$, respectively. There are several other putative species and subspecies that cluster with *M. muntjak*, viz. *M. rooseveltorum*, $2n = 6/7$ (Amato et al., 1999); *M. crinifrons*, $2n = 8/9$ (Yang et al., 1997a); *M. gongshanensis*, $2n = 8/9$ (Shi and Ma, 1988); *M. feae*, $2n = 12/13/14$ (Soma et al., 1983). This variation has been subjected to several analyses that posit a high diploid number as ancestral with fusions of one kind or another explicative of the current diversity. The arguments are often tortured and unsatisfying in some regards (Yang et al., 1997b; Wang and Lan, 2000). Fusions, of whatever sort, are singular and inherently "messy" events. If they are invoked to explain reduction of a karyotype from $2n = 46$ to single and low double digit levels, one would expect the "landscape" to be littered with the debris of such disruptions. Yet, there is nothing between the low cluster and a "pristine" $2n = 46$. The likelihood of future findings in this void is probably good and, if realized, would have important bearing on the question of directionality. For the moment, this deficiency, and the absence of any significant information on clinal distributions of karyotypic variability frustrate a final resolution.

Notwithstanding the limited data currently available, a logical scenario based on fissioning may be constructed to explain the observed chromosomal diversity in *Muntiacus*. This throws up some very interesting morphological and numerical expectations which are startlingly consistent with constraints imposed by the theory of karyotypic fissioning.

In the putative $2n = 14 = 12M + XX/XY$ primitive karyotype, an X-autosome translocation-fusion was incorporated. Such a transformation serves as an impediment, if not to fissioning itself, to the incorporation of fissioned autosomes. In turn, this may lead to the possibility of the accumulation of further reciprocal, centric and/or tandem fusions. If and when this X-autosome translocation-fusion dissociates, fissioning again becomes unrestricted. The

dissociation of the X-autosome translocation is not without consequences that may be detectable in surviving lineages. The most important of these is the conversion of the X chromosome from the primitive "simplex" to an acrocentric. Simultaneously, one autosomal pair will also be represented as derived acrocentrics. Thus, a karyotype of $10M + 2A + XX/XY$ (where the X is acrocentric) may be anticipated. Fissioning of this karyotype would yield $20A + 2A + XX/XY = 24$ which, when all acrocentrics are converted to mediocentrics through pericentric inversion, will be $22M + XX/XY$ (with acrocentric X). This is precisely the expected karyotype which by fissioning would yield $44A + XX/XY$ (with acrocentric X), the condition found in *M. reevesi*.

Fig. 1 presents a rough outline of the sequence of postulated events. From a common ancestral stock that was characterized by the incorporation of an X-autosome translocation-fusion, a population in which this was "dissolved" was isolated. The subsequent histories of these two derivatives followed very different courses. While one accumulated various fusions and other rearrangements to yield a cluster of forms in the $2n = 6-14$ range, the other underwent two successive fissionings to yield $2n = 46$. All of these transformations may lead to reduced hybrid fertility and, ultimately, to speciation. Yet, none appear to have been sufficiently disruptive to preclude viable hybridization. Hence, while the karyological differences may be great, the genomic differences are slight. The implication of this inference is that temporal separation is not great and that chromosomal differences, among them fissioning, are relatively recent and the principal impetus to speciation.

If it is supposed that any fusion (reciprocal, centric, tandem) is a singular, independent event, there are in the order of magnitude $22!-6!$ (1.124×10^{21}) paths by which a $2n = 46$ karyotype might be reduced to $2n = 14$. Despite a lamentable paucity of karyotype samples, that no condition between these two extremes has yet been detected is noteworthy. This, provisionally, is best construed to suggest that some mechanism other than fusion has operated to produce the observed differences. While the case for fusions underlying a reduction from $2n = 14$ to the various lower diploid numbers found in *Muntiacus* spp. is defensible, to extend this mechanism to explain a reduction from $2n = 46$ strains credulity. Since M.J.D. White's prejudice favoring independent fusions in chromosomal evolution is now difficult to support, its usefulness may be questioned. As Kolnicki (1999) has observed, the origin of the centriole-kinetosome and centromere-kinetochore is probably an endocellular symbiont (a motile eubacterium, perhaps a spirochete). These "... tend to reproduce out of synchrony from their hosts even in co-evolved eubacterial symbiotic associations ...". Karyotypic fissioning becomes equivalent to kinetochore reproduction. This kind of whole-karyotype chromosomal mutation process can then be "... understood as rapid centromeric residual reproduction of a once foreign ... genome, entirely consistent with a 'symbiogenetic' ... concept of eukaryotic cell

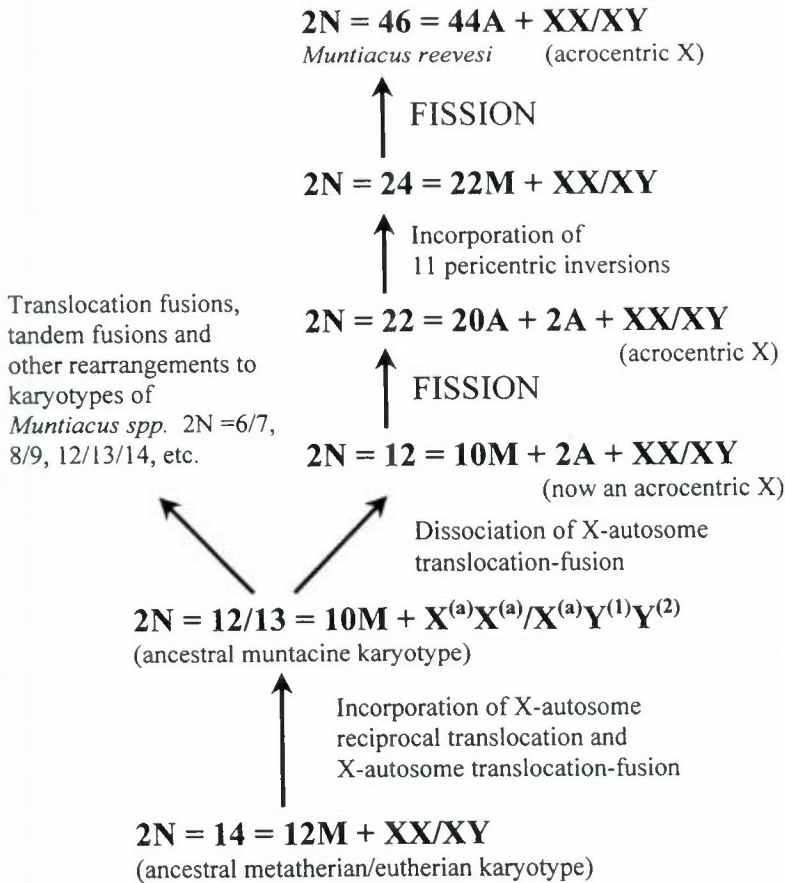


Figure 1. A telegraphic scheme representing major transformations in the evolution of *Muntiacus* karyotypes. M = mediocentric autosome; A = acrocentric autosome; X(a) = X-autosome translocation-fusion. While the generalizations are valid, data for certain crucial, detailed analyses are not yet available. See text for further discussion.

evolution" (Kolnicki, 1999, p. 137). Thus, a further, detailed assessment of the *Muntiacus* case, based on a karyotypic fission model, should be attempted as soon as additional data can be accumulated. And, attention should now be focused on other aspects of fission theory such as factors facilitating insertion and fixation of fissioned chromosomes in a population. Clearly, something appears to favor the incorporation of fissioned (acrocentric) chromosomes and their subsequent conversion to mediocentrics through pericentric inversion. The impetus for these processes probably relates more to chromosome segregation

than to any attributes of the "somatic" animal. While mammal populations are, of course, subject to natural selection, their karyotypes seem to respond to a special selective regime that operates with considerable autonomy, probably largely modulated by exigencies of mitosis and meiosis. Beside the tendency of kinetochore-centromeres to reproduce synchronously, examination of other aspects of this "special selection" should be vigorously pursued.

REFERENCES

- Amato, G., Egan, M.G., Schaller, G.B., Baker, R.H., Rosenbaum, H.C., Robichaud, W.G., and DeSalle, R. 1999. Rediscovery of Roosevelt's barking deer (*Muntiacus rooseveltorum*). *Journal of Mammalogy* **80**: 639-643.
- Godfrey, L.R. and Masters, J.C. 2000. Kinetochore reproduction theory may explain rapid chromosome evolution. *Proceedings of the National Academy of Sciences* **97**: 9821-9823.
- Guisto, J.P. and Margulis, L. 1981. Karyotypic fission theory and the evolution of old world monkey's and apes. *Bio Systems* **13**: 267-302.
- Hayman, D.L., Kirsch, J.A.W., Martin, P.G., and Waller, P.F. 1971. Chromosomal and serological studies of the Caenolestidae and their implications for marsupial evolution. *Nature* **231**: 194-195.
- Imai, H.T. 1978. On the origins of telocentric chromosomes in mammals. *Journal of Theoretical Biology* **71**: 619-637.
- Imai, H.T. and Maruyama, T. 1978. Karyotype evolution by pericentric inversion as a stochastic process. *Journal of Theoretical Biology* **70**: 253-261.
- Imai, H.T. and Crozier, R.H. 1980. Quantitative analysis of directionality in mammalian karyotype evolution. *American Naturalist* **116**: 537-569.
- Kolnicki, R.L. 1999. Karyotypic fission theory applied: Kinetochore reproduction and lemur evolution. *Symbiosis* **26**: 123-141.
- Kolnicki, R.L. 2000. Kinetochore reproduction in animal evolution: Cell biological explanation of karyotypic fission theory. *Proceedings of the National Academy of Sciences* **97**: 9493-9497.
- Kolnicki, R.L. Kinetochore reproduction (=karyotypic fission) theory: Divergence of lemur taxa subsequent to simple chromosomal mutation. In preparation.
- Shi, L.M. and Ma, C.X. 1988. A new karyotype of muntjac (*Muntiacus* sp.) from Gongshan County in China. *Zoological Research* **9**: 343-347.
- Soma, H., Kada, H., Mitayoshi, K., Suzuki, Y., Heckuichal, C., Manannop, A., and Vatanardmya, B. 1983. The chromosomes of *Muntiacus feae*. *Cytogenetics and Cell Genetics* **35**: 156-158.
- Todd, N.B. 1970. Karyotypic fissioning and canid phylogeny. *Journal of Theoretical Biology* **26**: 445-480.
- Todd, N.B. 1975. Chromosomal mechanisms in the evolution of artiodactyls. *Paleobiology* **1**: 175-188.
- Todd, N.B. 1985. Significance of a diploid number of 20 in the peccary *Catagonus wagneri*. *Journal of Heredity* **76**: 310.

- Todd, N.B. 2000. Mammalian evolution: Karyotypic Fission Theory. In: *Environmental Evolution*, 2nd edition. Margulis, L., Matthews, C. and Haselton, A., eds. MIT Press, Cambridge MA, London England.
- Wang, W. and Lan, H. 2000. Rapid and parallel chromosomal number reductions in muntjac deer inferred from mitochondrial DNA phylogeny. *Molecular and Biological Evolution* **17**: 1326-1333.
- White, M.J.D. 1973. *Animal Cytology and Evolution*. Cambridge University Press.
- Yang, F., O'Brien, P.C., Wienberg, J., and Ferguson-Smith, M.A. 1997a. Evolution of the black muntjac (*Muntiacus crinifrons*) karyotype revealed by comparative chromosome painting. *Cytogenetics and Cell Genetics* **76**: 159-163.
- Yang, F., O'Brien, P.C., Wienberg, J., and Ferguson-Smith, M.A. 1997b. A reappraisal of the tandem fusion theory of karyotype evolution in Indian muntjac using chromosome painting. *Chromosome Research* **5**: 109-117.