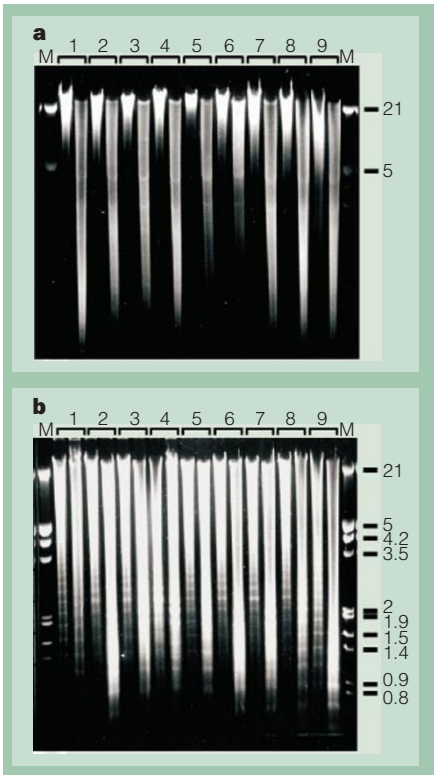


**Figure 1** Genome methylation analysis in interspecific hybrids by *Hpa*II (left lanes) and *Msp*I (right lanes) digestion. **a,** Hybridization between *Mus spretus* and *M. musculus*. Lane 1, male *M. spretus*; 2, male *M. musculus* × *M. spretus* $F_1$; 3, male *M. musculus* × *M. spretus* $F_1$; 4, male *M. musculus* × *M. spretus* $F_1$; 5, male *M. musculus* × *M. musculus*; 6, female *M. musculus*; 7, female *M. musculus* × *M. spretus* $F_1$; 8, female *M. musculus* × *M. musculus*; 9, female *M. spretus*. **b,** Hybridization between horse and donkey (mule) and donkey and horse (hinny). Lane 1, female horse; 2, female mule; 3, female mule 2; 4, male donkey; 5, male horse; 6, female hinny; 7, female donkey; 8, female hinny; 9, male horse. In all matings, the female is indicated first. Each lane corresponds to an individual animal. M, marker.

In addition, we assessed cytosine methylation by *in situ* nick-translation on the metaphase chromosomes of mouse $F_1$ hybrids and parental mice. For this analysis, mice of the *Mus musculus* strain Cremona were used. Because this strain has an aberrant chromosome number ($2N = 22$ instead of 40), it is possible to discriminate between the chromosomes derived from *Mus musculus* Cremona and *Mus spretus* in $F_1$ hybrids (Fig. 2). This analysis indicated that no major change had occurred in genome-wide demethylation or in centromere expansion. Finally, we were unable to detect amplification of the L1 retrotransposon by Southern-blot analysis (results not shown).

These results do not appear to agree with those obtained by O'Neill *et al.*[1] in their analysis of interspecific hybrids between several different species of macropodid marsupials. However, their findings are unequivocal and supported by earlier cytogenetic investigations of hybrids between additional macropodid species[8]. Genome-wide undermethylation, retrotransposon activation and chromosome extension may therefore be specific to interspecific hybridization in marsupials, or perhaps they occur only in macropodid marsupials.

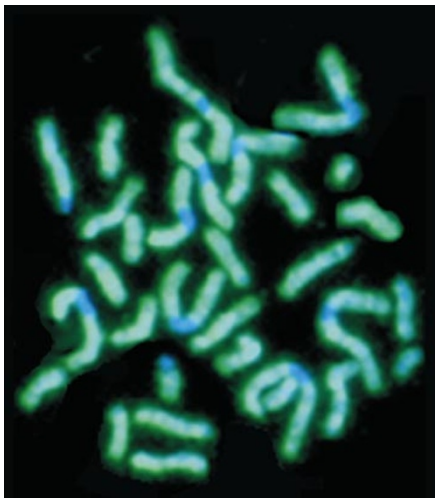

**Figure 2** Methylation analysis of *M. musculus* Cremona × *M. spretus* chromosomes. Metaphase spread from bone marrow was digested with *Hpa*II followed by *in situ* nick-translation. Staining patterns were identical with *Msp*I. There was no detectable difference in labelling intensity between *M. spretus* and *M. musculus* euchromatin. The faint labelling of *M. musculus* compared with *M. spretus* centromeric heterochromatin reflects the low frequency of *Msp*I/*Hpa*II sites in *M. musculus* major satellite DNA.

In any case, our results argue against the idea that such profound alterations in genome organization of interspecific hybrids are common events in placental mammals. Marsupials and placental mammals diverged about 130 million years ago[9], so the functional role of methylation may have changed between the two subclasses. For example, marsupial and placental mammals show pronounced differences in their processes of X-chromosome inactivation[9], in which the role of methylation is thought to be important[10].

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*O'Neill* et al. *reply* — The absence of global methylation changes in eutherian interspecific hybrids compared with their parents, observed by Roemer *et al.*, sharply contrasts with our own studies of interspecific hybrids between various species of kangaroo. We observed hybrid-specific undermethylation, retroelement activation and genome remodelling[1], and suggested that these events occurring together may bring about rapid karyotypic change.

We agree with Roemer *et al.* that marsupials and eutherians are likely to have diverged from one another in their reliance on and use of the epigenetic information conveyed by DNA methylation. Although these events may be specific to marsupial or even macropod interspecific hybrids, there is evidence that eutherian genomes may be subject to at least some degree of the same sort of hybrid dysgenic perturbations. Interspecific hybrids of the genus *Peromyscus* (deer mice) do not show whole-genome changes in methylation, as determined by digestion with *Msp*I and *Hpa*II (R.J.W.O'N. *et al.*, unpublished observations), yet they exhibit disruptions in imprinted gene expression associated with allele-specific undermethylation[2]. The mechanism underlying the loss of imprinting in these hybrids remains unknown, but there is a subtle change in methylation in this eutherian hybrid cross[2]. Digestion of genomic DNA with *Msp*I and *Hpa*II may be too blunt an instrument to reveal subtle changes (less than 20%) in methylation.

Our main finding is a link between DNA methylation, retroelement activity and genome rearrangement. The dramatic perturbations of methylation and genome structure that we observed in kangaroo hybrids may be an extreme example of dysgenic changes that occur on a broader scale in many organisms.

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