Expansion of this cell population may initiate a cascade of events that leads to immunodeficiency. Other uninfected cells, including B cells, T cells, and other hematopoietic cells, also proliferate in this disease (*2, 3, 5, 9*). The cyclophosphamide treatment was probably also cytostatic for these cells.

This model of pathogenesis may be relevant to other immunodeficiency syndromes. For example, if this mouse model of immunodeficiency shares some aspect of its pathogenesis with human AIDS, and especially if a critical target cell population is proliferating in human AIDS, this type of therapy may be considered for prevention and treatment of human AIDS. A detailed comparison of the pathogenesis of both diseases will be necessary to test the validity of this approach in humans. The recent isolation of a simian immunodeficiency virus variant that induces a severe gut lymphoproliferative disorder with loss of T and B lymphocytes (*24*) suggests that a link may exist, as in MAIDS, between lentivirus-induced immunodeficiency and cell proliferation.

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Evidence for Biased Gene Conversion in Concerted Evolution of Ribosomal DNA

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Concerted evolution is the production and maintenance of homogeneity within repeated families of DNA. Two mechanisms—unequal crossing over and biased gene conversion—have been the principal explanations of concerted evolution. Concerted evolution of ribosomal DNA (rDNA) arrays is thought to be largely the result of unequal crossing over. However, concerted evolution of rDNA in parthenogenetic lizards of hybrid origin is strongly biased toward one of two parental sequences, which is consistent with biased gene conversion as the operative mechanism. The apparent gene conversions are independent of initial genome dosage and result in homogenization of rDNA arrays across all nucleolar organizer regions.

ALTHOUGH CONCERTED EVOLUTION of DNA sequences (the homogenization of repeated units within individuals and populations) has been documented for several gene families (*1*), the proposed mechanisms, which include unequal crossing over (*2*) and biased gene conversion (*3*), remain controversial. The debate over the relative importance of these mechanisms primarily has involved theoretical, rather than empirical, studies (*2–4*; but see *5, 6*). Unisexual hybrids provide a unique opportunity to study interchromosomal mechanisms of concerted evolution because they contain entire arrays of repeated gene families that initially are fixed within chromosomes but differ between chromosomes (*6*). Moreover, unisexual hybrid lineages provide an opportunity to distinguish between unequal crossing over and biased gene conversion because the predicted patterns of variation produced by these two mechanisms differ in these "permanent hybrid" lines.

We have investigated the tandemly repeated ribosomal RNA genes and their associated spacer regions (rDNA) within a group of unisexual parthenogenetic lizards and their sexual relatives, which together comprise the *Heteronotia binoei* complex (*7*). We conducted this study to determine whether concerted evolution occurs in parthenogenetic lineages, and, if so, to determine which of the proposed mechanisms of concerted evolution is consistent with the observed patterns of variation.

The *H. binoei* complex consists of several cryptic sexual species and a large number of parthenogenetic lines of hybrid origin (*7, 8*). The two sexual species known to be involved in the origination of the parthenogens have been designated CA6 and SM6, pending their formal description (*8*). These two species apparently hybridized in the past, and backcrosses to each of the sexual species produced many independent, triploid, parthenogenetic lineages (*8*). Each of these lineages contains one haploid set of chromosomes from one of their sexual ancestors and two from the other (that is, 1CA6/2SM6 or 2CA6/1SM6). Unlike most other unisexual vertebrates, there is clear evidence from allozyme studies for multiple origins of these parthenogenetic lineages (*8*).

Parthenogenetic species that arise through hybridization are usually fixed heterozygotes at all loci that are diagnostic in the parental species (*9*). In *Heteronotia*, each of the parental haploid genomes contains one nucleolar organizer region (NOR), which contains the rDNA cluster, so each triploid lineage should contain three NORs inherited from the two parental species at the time of lineage formation. Some lineages

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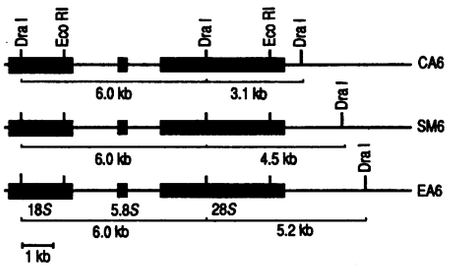

Fig. 1. Restriction map of the ribosomal DNA array of three sexual species of the *H. binoei* complex, showing the lengths of the diagnostic Dra I fragments (*10*).

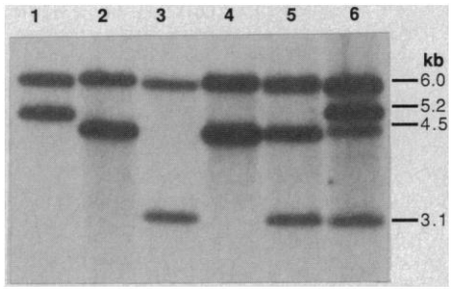

Fig. 2. Autoradiogram of Southern blot produced from a gel of *Heteronotia* genomic DNA cleaved with Dra I and probed with a 28S rDNA probe (*10*). The three sexual species are represented in lane 1 (EA6), lane 2 (SM6), and lane 3 (CA6). Three triploid parthenogens are represented in lanes 4 and 5 (2CA6/1SM6) and lane 6 (CA6/EA6/SM6). The triploid in lane 4 has lost the CA6-diagnostic fragment, despite the presence of two doses of the CA6 genome.

would inherit two NORs from CA6 and one from SM6, whereas others would inherit two NORs from SM6 and one from CA6. However, if concerted evolution occurs in parthenogens, heterozygosity of rDNA may be lost. In the absence of concerted evolution or loss of rDNA cistrons, the triploid, asexual *Heteronotia* lineages should contain rDNA sequences of both parental sexual species.

If the rDNA arrays of the parthenogens are evolving in a concerted fashion, different patterns of variation would be expected as a result of unequal crossing over or biased gene conversion. If unequal crossing over, essentially a stochastic process, is the primary mechanism, each parental rDNA sequence should become fixed in separate parthenogenetic lineages (*2*) with a probability related to the starting frequency of the rDNA array. In other words, fixation of the CA6 rDNA genotype should be more common among lineages with two copies of the CA6 genome, whereas fixation of the SM6 rDNA genotype should be more common among lineages with two copies of the SM6 genome. However, if biased gene conversion is the operative mechanism, either the CA6 or the SM6 genotype should consistently become fixed, regardless of the starting frequencies of the two rDNA arrays (*3*).

We examined restriction site variation within the rDNA array of members of the *H. binoei* complex (*10*) and found diagnostic Dra I sites in the intergenic spacer region for three sexual species of *Heteronotia* (CA6, SM6, and a third species, EA6, which was not thought to be involved in the origin of the triploid parthenogens) (Figs. 1 and 2). We used this diagnostic marker to screen 157 individuals of *Heteronotia* (Table 1), including 109 parthenogens that represent approximately 40 lineages descended from distinct hybridization events, as estimated from previous studies based on the geographic distribution of our samples (*8*). The identities of all individuals were determined by karyotypic examination; the morphology of chromosome 6 (which contains the NOR) is diagnostic in each of the three parental species (*11*).

Dra I sites diagnostic of CA6 and SM6 rDNA were found in 56 parthenogens, including one individual that appeared to contain rDNA from all three sexual species (Fig. 2) (*12*). However, 53 triploids contained only the SM6 rDNA genotype (Fig. 2). No individuals were found with only CA6 rDNA. Among the parthenogens with both CA6 and SM6 rDNA, the number of SM6 rDNA repeats exceeded the number of CA6 rDNA repeats in 51 of 56 individuals, on the basis of densitometric comparisons of the diagnostic fragments on autoradiograms (*13*).

To determine whether the CA6 NORs had been lost or replaced in the parthenogens that are fixed for SM6 rDNA, we examined the chromosomes of two of these triploids using the silver-staining method to detect transcriptionally active NORs (*14*) and in situ hybridization with a biotinylated rDNA probe (Fig. 3) (*15*). In these individuals, three NORs were detected (one on each of the parental sixth chromosomes), and all three were active. It therefore appears that the CA6 NORs were converted to SM6-like sequences, not lost from the genome.

The rDNA restriction data are sufficient to reject the hypotheses that fixation is

Table 1. Ribosomal DNA genotypes of two karyotypic classes of triploid, parthenogenetic *Heteronotia* (*12*).

rDNA genotype	Karyotype (*n*)	
	2CA6/1SM6	1CA6/2SM6
CA6	0	0
SM6	32	21
CA6 + SM6	41	14
CA6 + EA6 + SM6	1	0

equally probable for either genotype ($P < 0.001$) or that the probability of fixation is directly proportional to the frequency of the parental NORs ($P < 0.001$) (*16*), two predicted outcomes of a stochastic process such as unequal crossing over. In contrast, the data are compatible with biased gene conversion in favor of the SM6 genotype. The possibility remains that unequal crossing over is not a stochastic process in these lizards; selection or a localized high rate of recombination within the SM6 sequence could produce biased unequal crossing over, although no recombinant sixth chromosomes have been found among C-banded karyotypes of the parthenogens (*17*). An appropriate test of biased unequal crossing over would be to sequence several

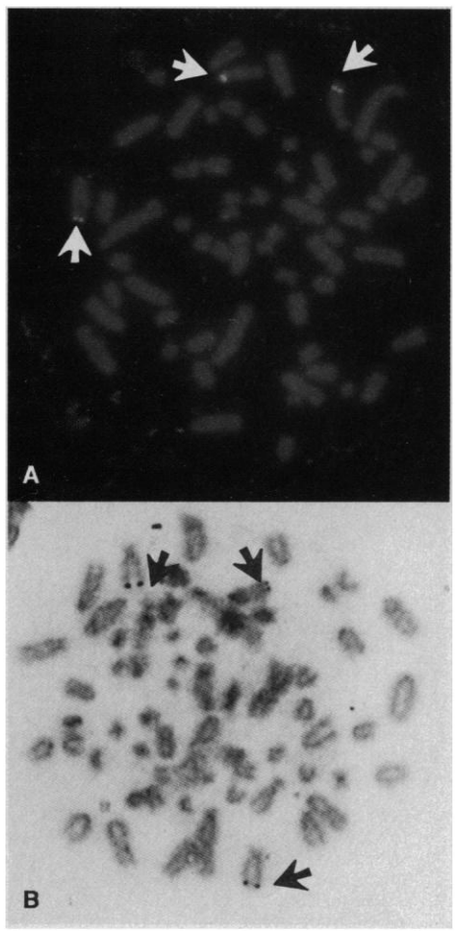

Fig. 3. (**A**) In situ hybridization with an rDNA probe of chromosomes of a triploid parthenogenetic *Heteronotia* (1CA6/2SM6) in which only SM6 rDNA occurs. Hybridization of the probe indicates the presence of rDNA at all three chromosomal locations of NORs (arrows). The acrocentric NOR-bearing chromosome was inherited from the CA6 parent, and the submetacentric NOR-bearing chromosomes from the SM6 parent. (**B**) Silver-stained chromosomes of the same individual shown in (A). The dark staining (arrows) indicates that all three NORs are active.

tandem repeats from the CA6 NOR in parthenogens with both SM6 and CA6 rDNA. If biased unequal crossing over is operating, most tandem repeats should be of one parental sequence, whereas biased gene conversion should produce interspersed mixtures of both sequences. In any case, there is strong, unidirectional bias in the concerted evolution of *Heteronotia* rDNA arrays in favor of the SM6 genotype.

The processes responsible for concerted evolution may differ between parthenogenetic and sexual species. Unequal crossing over could be restricted in parthenogens because of the requisite modifications to meiosis in these species (*18*). However, this study demonstrates that concerted evolution of rDNA can be driven by directional, rather than stochastic, processes, and that these directional processes do occur among (as well as within) chromosomes. It also supports the idea that some mutations in repeated gene families may spread rapidly through the genome ("molecular drive") (*4*), even in supposedly clonal organisms such as parthenogens. Finally, the concept of a "permanent hybrid" genome in parthenogenetic vertebrates (*9*) does not apply to repeated DNA sequences, which continue to evolve in a concerted fashion in these species.

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10. Genomic DNA was cleaved with Dra I and separated by electrophoresis on 0.8% agarose gels as described in D. M. Hillis and S. K. Davis, *Evolution* **40**, 1275 (1986). Southern (DNA) blots of the gels were hybridized with a nick-translated ^{32}P-labeled rDNA probe [pE2528, described in D. M. Hillis, S. K. Davis, *Mol. Biol. Evolut.* **4**, 117 (1987)]. Blots were hybridized with *Escherichia coli* carrier DNA for 18 hours at 65°C in 5× standard saline citrate (SSC), 0.5% SDS, 5× Denhardt's solution, and 2.5 mM KH_2PO_4. Two washes were performed for 30 min in 2× SSC, 0.2% SDS at 37°C, and two additional washes were conducted for 1 hour in 1× SSC, 0.1% SDS at 55°C. The map in Fig. 1 was determined by double digestion with Dra I and Eco RI, with the Eco RI sites used as reference points [the Eco RI sites are conserved throughout vertebrates; D. M. Hillis and M. T. Dixon, in *The Hierarchy of Life: Molecules and Morphology in Phylogenetic Analysis*, B. Fernholm, K. Bremer, H. Jörnvall, Eds. (Elsevier Science, Amsterdam, 1989), pp. 355–367]. In addition to the diagnostic Dra I sites shown in Fig. 1, some individuals have other Dra I sites in a fraction of their repeats that result in additional Dra I fragments.
11. Methods as described in (7).
12. The individual represented in lane 6 of Fig. 2 has the rDNA restriction patterns of all three sexual species. However, analysis of chromosomes and allozymes did not reveal any of the other markers diagnostic for the EA6 sexuals.
13. SM6 rDNA is expected to be more abundant in the triploids with two doses of the SM6 genome, even in the absence of biased gene conversion. If the comparison is restricted to the 2CA6/1SM6 individuals, in which CA6 rDNA should be approximately twice as abundant as SM6 rDNA if biased conversion does not occur, the SM6 rDNA was more abundant than the CA6 fragment in 36 of 41 individuals. In the single individual with all three diagnostic fragments, the relative concentration of the three fragments was EA6 > SM6 > CA6.
14. Silver staining was modified from C. Goodpasture and S. E. Bloom, *Chromosoma* **53**, 37 (1975). Approximately 200 μl of 2% gelatin (with 1% formic acid) was mixed with an equal volume of 50% silver nitrate on each slide. Slides were incubated for 6 to 9 min at 37°C, rinsed for 4 min in 5% sodium thiosulfate, and lightly counterstained in 2% Giemsa.
15. We performed in situ hybridization using as probe the I-19 rDNA cloned from *Mus musculus* by N. Arnheim, *Gene* **7**, 83 (1979). The plasmid was biotin-labeled, and hybridization followed the procedure in R. K. Moyzis *et al.*, *Chromosoma* **95**, 375 (1987), except that we used blaze-dried chromosome spreads to better visualize centromere position.
16. Chi-square tests were performed to test the hypothesis that fixation is equally probable for either genotype (expected: 2CA6/1SM6 individuals with CA6 genotype = 16, and with SM6 genotype = 16; 1CA6/2SM6 individuals with CA6 genotype = 10.5, and with SM6 genotype = 10.5; $\chi^2 = 53$, df = 3, $P < 0.001$) and the hypothesis that the probability of fixation is directly proportional to the frequency of parental NORs (expected: 2CA6/1SM6 individuals with CA6 genotype = 21.33, and with SM6 genotype = 10.67; 1CA6/2SM6 individuals with CA6 genotype = 7, and with SM6 genotype = 14; $\chi^2 = 74.47$, df = 3, $P < 0.001$).
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The Effect of the Floor Plate on Pattern and Polarity in the Developing Central Nervous System

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The effect of floor plate on cellular differentiation in the neural tube of quail embryos was examined. In the developing neural tube the floor plate, which consists of specialized neuroepithelial cells, is located in the ventral midline of the neural tube. When Hensen's node was extirpated the floor plate and notochord did not develop, and the normal differentiation of the ventral horn motor neurons and dorsal and ventral roots did not occur. When one side of the neural tube was deprived of notochord, the ventro-dorsal differentiation took place on both sides. However, when one side of the neural tube was deprived of the floor plate, the ventral horn motor neurons and dorsal and ventral roots did not develop on that side. These observations suggest that the floor plate influences motor neuron differentiation and acts as an intrinsic organizer to establish pattern and polarity in the developing nervous system.

SHORTLY AFTER THE CLOSURE OF THE neural tube, functionally distinct classes of neurons differentiate in specific locations within the neural tube (*1*). For example, motor neurons begin to differentiate in the ventral horn of the spinal cord and send their axons out by way of the ventral root. Subsequently, sensory neurons develop in the dorsal horn and sensory nerve fibers from the periphery enter the central nervous system (CNS) through the dorsal root. It is not clear which factors establish this basic pattern. Recent studies indicate that in rats the floor plate provides chemo-

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