Am J Hum Genet 31:264–280, 1979

Heterochromatin and Satellite DNA in Man: Properties and Prospects

GEORGE L. GABOR MIKLOS[1] AND BERNARD JOHN

SUMMARY

In reviewing the properties of heterochromatin and satellite DNA in man, it is clear that the human genome does not readily lend itself to experimental tests of the postulated functions for satellite DNA. Since the spectrum of known structural properties of vertebrate and invertebrate satellite DNAs are broadly overlapping, an alternative avenue is to experimentially manipulate the heterochromatin of an organism, and then evaluate the generality of the results. When this is done in *Drosophila melanogaster,* the one organism where such an experimental approach is indeed possible, the results provide no support for most of the popular hypotheses concerning satellite DNA function. They do, however, reveal an important effect on the meiotic system, namely that the position of crossover events can be markedly altered in the presence of heterochromatin known to be rich in satellite DNAs. This effect is not peculiar to *Drosophila,* since supporting data are readily available from natural situations in both mammals and grasshoppers. In all such cases, the effects are most easily discernible where the heterochromatic blocks are substantial in size, and non-centric in location, situations which do not apply in man. The human system, however, offers other potentials. The ubiquity of naturally occurring heterochromatic polymorphisms, coupled with the extreme sensitivity of the human genome to perturbation, offers some scope for assessing the possible somatic effects of alterations in the amount of satellite DNA.

PRELUDE

"Respect for fact is more difficult for the human mind than the invention of theories."
Bertrand Russell

This brief review has three aims: (1) to outline the data on heterochromatin and satellite

Received December 27, 1978.

[1] Both authors: Department of Population Biology, Research School of Biological Sciences, The Australian National University, Canberra, A.C.T., Australia.

DNA in man, (2) to compare the situation in humans with that in *Drosophila melanogaster*, and (3) to evaluate the *Drosophila* data in terms of relevant situations in other animals.

THE HUMAN CONDITION

Although there is difficulty in defining the term heterochromatin with precision [1, 2], we will operationally equate constitutive heterochromatin in man with C-banded material [3]. In these terms, some 20% of the human genome is heterochromatic in character, and most is procentric in location (fig. 1*a*). The human Y chromosome is exceptional in having a relatively large heterochromatic segment in its long arm.

The total amount of satellite DNA in man is about 4%, which is much smaller than the amount of heterochromatin [4, 5, 6]. Thus most DNA in human heterochromatin is not satellite DNA as such. There is considerably more repetitive DNA present than the 4% represented by satellite DNA, since some 23% of human DNA reassociates by a C_0t of 1.0. However, it is still not clear how much of this is located in the procentric heterochromatin [7, 8, 9].

While equilibrium centrifugation of human DNA in cesium chloride reveals no obvious buoyant density satellites (fig. 1*b*), the use of silver or mercury ions does resolve a number of cryptic satellites (fig. 1*c*). Originally four such satellites (denoted *I-IV*) were isolated, characterized and localized by in situ hybridization to the heterochromatic blocks [10–19]. As techniques improved, so also did the satellite map [20], though even now some of the details remain controversial. Thus Thiery et al. [21] are of the opinion that satellites II and III may well turn out to be the same. While no sequence has yet been published for any of the satellite DNAs of man, it is clear that at least some of them are very simple. For example, satellite II has a complexity of less than 10 base pairs [5, 6].

Within the limits of the in situ hybridization technique, six chromosomes have C-bands which appear to lack all four of the conventional satellites, namely autosomes 2, 3, 4, 6, 8, and 11. Those chromosomes with high concentrations of one or more satellites in their C-bands include 1, 9, 13, 14, 15, 20, 21, 22, and the Y. All other chromosomes have intermediate amounts which fall below the thresholds used by Gosden et al. [20], and from which figure 2 has been constructed. The two chromosomes which carry most satellite DNA are 9 and the Y. Perhaps the most striking anomaly is 16 which has a large C-block, but only carries satellite *II*, and its amount is below the threshold used in preparing figure 2. In addition to the four conventionally recognized satellites, Manuelidis [5, 6] has isolated and mapped yet another satellite fraction using the dye Hoechst 33258 (*H*, fig. 2).

Restriction endonuclease cleavage of human DNA has also revealed a variety of repeated sequences. One of these, a 340-base pair Eco R1 restriction fragment, has been mapped (*R*, fig. 2). In general, this particular sequence is concentrated in those C-bands devoid of satellites I-IV (chromosomes 1, 3, 7, 10, and 19). Two additional Eco R1 fragments, respectively 176 and 352-base pairs in length, have been isolated [22]. These show a similar buoyant density to satellite IV, but differ from one another, as well as from IV, in their strand separation behavior in alkaline cesium chloride gradients.

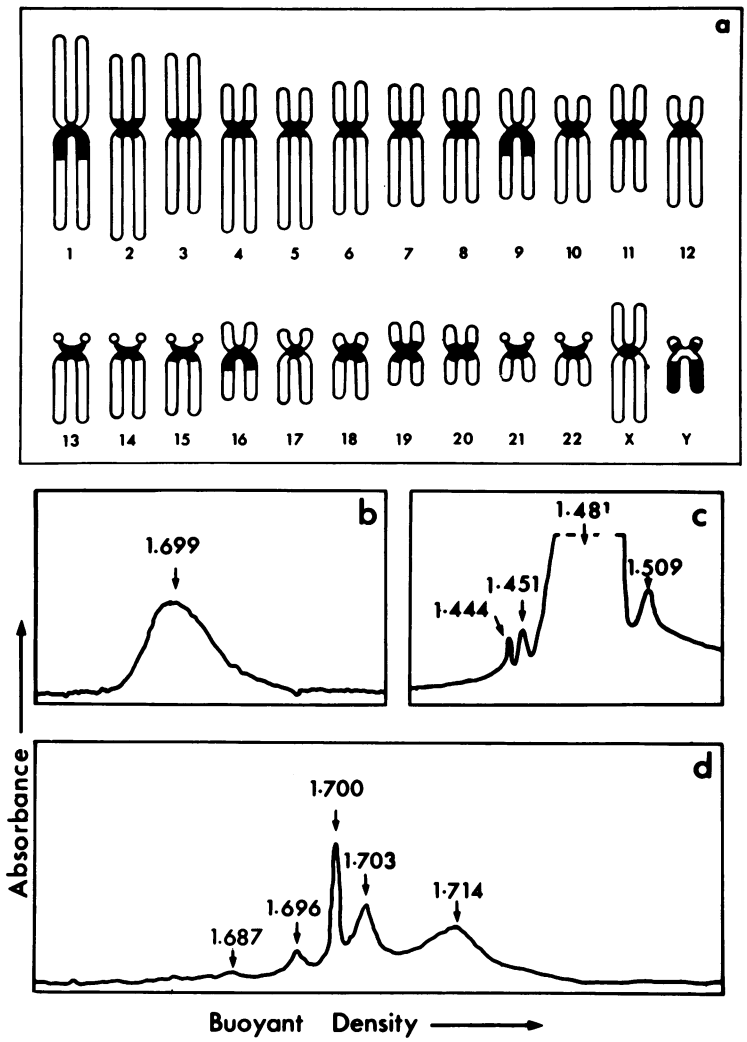

FIG. 1.—Cytological and biochemical characteristics of human heterochromatin. (*a*) C-band positive regions of the human karyotype [32]. (*b*) Analytical equilibrium density gradient centrifugation of total native human DNA in neutral cesium chloride [27]. (*c*) Analytical ultracentrifugation of total human placental DNA in a cesium sulphate gradient in the presence of silver ions [12] demonstrating the presence of satellites I (*1.444*), II (*1.451*), and III (*1.509*). (*d*) Analytical ultracentrifugation of human hydroxyapatite-isolated rapidly renaturing C_0t 1 DNA in neutral cesium chloride showing the five renatured repetitious DNA families [27].

Restriction digests have also revealed sequences which have specificity for the Y chromosome [23, 24, 25]. One of these, a 3500-base pair Hae III fragment, shares sequence homology with satellite III, but differs from it by the spacing of Hae III sites. Partial fingerprint analyses of cRNA transcribed from this fragment reveal a simple sequence, probably derived from an oligonucleotide such as AGUGG [23].

Finally, human heterochromatin is heterogeneous in containing both satellite and

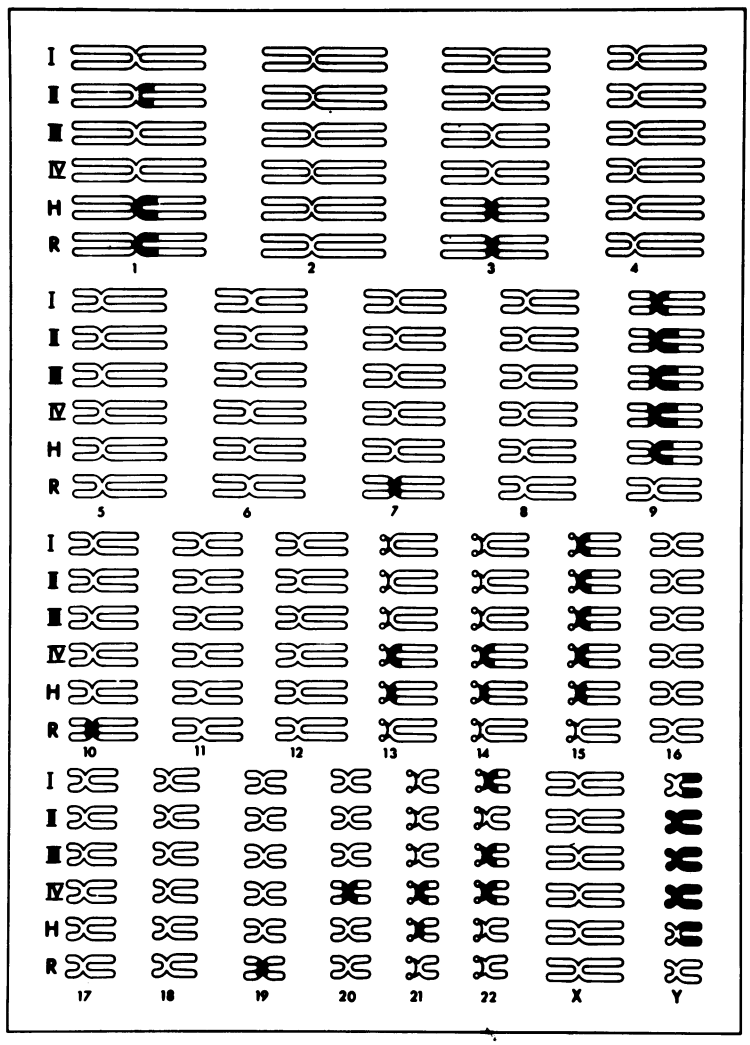

FIG. 2.—Satellite maps of man based on in situ hybridization data. Every chromosome is divided into equal sized segments, each corresponding to one-half the length of chromosome 21. Only major sites of hybridization to such segments are shown. Six sets of data are presented for each chromosome. The first four (*I-IV*) are from hybridization of cRNA made from each of the four major satellites. The threshold values we use correspond to those in figure 3 of Gosden et al. [20]. They are 1.5 grains/segment for satellites *I*, *II*, and *III*, and 2.0 grains/segment for satellite *IV*. The remaining two sets of data are from hybridization of the AT-rich satellite isolated using Hoechst 33258 (*H*) and from "nick translated" DNA from the 340-base pair EcoR1 restriction fragment (*R*). For the two latter cases we have used arbitrary cutoff points of 10 grains/segment (*H*) and 20 grains/segment (*R*) respectively [5, 6].

non-satellite sequences, and this is most clearly revealed by a study of reassociation kinetics coupled with in situ hybridization [7, 15, 26, 27, 28]. Thus Marx et al. [8, 9] have described five renatured families with satellite-like properties in the human genome (figs. 1*d* and 3*c*), and have shown that some of these are interspersed with

non-satellite sequences, though not always to the same extent. Six native components (fig. 3*e*), in addition to satellites I-IV, have also been identified using complementary RNA in buoyant density gradients [29, 30], but these have not as yet been mapped. Figures 3*a*, *b*, *e*, *f*, *g*, and *h* summarize the buoyant densities of the native DNA fractions isolated from the human genome, whereas figures 3*c* and *d* show those fractions obtained by reassociation kinetics. As is evident from these figures, the reassociation families of one study do not always agree with those of another.

In summary, the human genome has about 20% of its DNA as C-banded heterochromatin, and this consists of both simple sequence and more complex satellite sequences together with satellite-like sequences. These can be interspersed with non-satellite types and, in general, these sequences localize to the procentric heterochromatin. It is of some interest, therefore, that this heterochromatin is remarkably polymorphic, since chromosomes 1, 3, 4, 9, 13, 14, 15, 16, 21, 22, and the Y all show an essentially continuous pattern of variation for the size of their C-bands [31–39]. The Y chromosome's polymorphic size difference in heterochromatin is mirrored by differences in the size of the restriction peak deriving from the Hae III fragment [40]. However, other C-band polymorphisms are not necessarily related to satellite content.

QUO VADIMUS?

These studies in man, like comparable ones in numerous other organisms, have failed to yield positive results on what should, after all, be their primary objective,

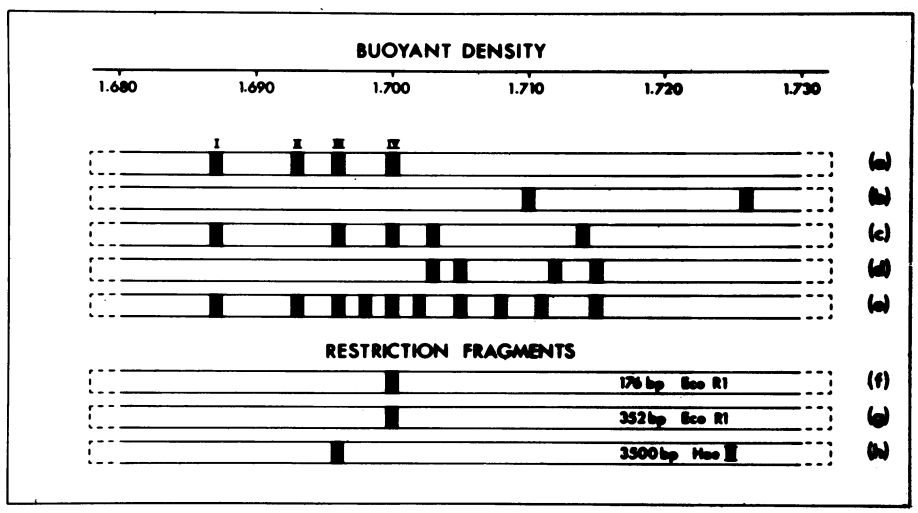

FIG. 3.—A summary of the buoyant densities of various satellite or repetitive fractions in the human genome. (*a*) The four conventional satellites, *I-IV* [28]. (*b*) Two satellites isolated using silver-cesium sulphate gradients and derived from a placental nucleolar fraction [28]. (*c*) Five reassociated repetitious families isolated using hydroxyapatite [27] and [8, 9]. (*d*) Four reassociated repetitious families isolated from the "fast" and "intermediate" fractions of human DNA [7]. (*e*) Ten native DNA components of varying repetitiveness identified by an improved RNA/DNA gradient hybridization technique [29, 30]. (*f*) 176-base pair EcoR1 monomer [22]. (*g*) 352-base pair EcoR1 monomer [22]. (*h*) 3500-base pair HaeIII Y-specific restriction fragment [23, 24]. Note: we have not included the Hoechst-satellite or the 340-base pair EcoR1 fragment isolated by Manuelidis [5, 6], since their buoyant densities were not given.

namely, the *functional* aspects of human heterochromatin and satellite DNA. Indeed much of the current research on satellite DNA appears to be directionless from the point of view of function. This stems, in no small part, from the fact that the problems posed by this class of DNA intrude into so many diverse fields. As a result, hypotheses have been proposed which could have been excluded in terms of data already available at the time of their proposal. The authors in question were either unaware of the data or chose to ignore it.

To illustrate this, we will, in the next section, consider some examples involving the experimental manipulation of satellite DNA in *D. melanogaster*. Here the relative amount of heterochromatin in the genome is about 30% (fig. 4*a*) which is relatively close to the amount in man. The heterochromatin of *Drosophila* includes four major buoyant density satellites (fig. 4*a*) which are easily resolved in actinomycin-cesium chloride gradients (fig. 4*b*). The sequences of three of these are simple [41, 42, 43]. The bulk of the heterochromatin of *Drosophila* consists of satellite sequences [42] and, although major genes can be identified within this heterochromatin, they are present in low frequency relative to the euchromatin [44].

At first sight it may seem that an organism as simple as a fly would be unlikely to reveal anything of relevance concerning the heterochromatin and satellite DNAs of man, primarily because of the extreme biological differences between them. It is worth emphasizing, therefore, that the level of morphological and physiological complexity of the phenotype bears no consistent relationship to the level of the biochemical complexity of the satellite DNAs within the chromosome. Some of the satellite

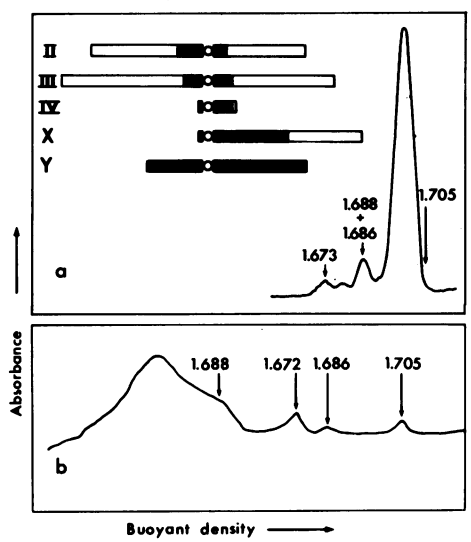

FIG. 4.—Cytological and biochemical characteristics of *D. melanogaster* heterochromatin. (*a*) Haploid male karyotype illustrates the distribution of heterochromatin (shown *solid*) and buoyant density profile of DNA from diploid larval brains showing the obvious satellites visible in neutral cesium chloride [41]. (*b*) Analytical ultra-centrifugation profile of four satellites of *D. melanogaster* in an actinomycin-cesium chloride gradient. The buoyant densities refer to those in neutral cesium chloride [43].

sequences in man are certainly as simple as those in *Drosophila*. Other mammals too can have very simple satellite sequences. The kangaroo rat, *Dipodomys ordii*, for example, has three satellites with variations on the sequences AAG, GGGTTA, and ACACAGCGGG respectively [45]. Similarly, one *Drosophila* satellite has a complexity of 365-base pairs [46], which is considerably greater than the 172-base pairs found in the α-satellite of the African Green Monkey [47], or the 235-base pair monomer of the mouse satellite [48, 49].

Thus from the point of view of investigating the possible functional significance of satellite DNA, it appears immaterial in a structural sense what system one chooses to examine. What is critical is to use an organism where the DNA can be experimentally manipulated. At the present time, *D. melanogaster* is one of the few organisms available for such an experimental approach, though, as we shall see, supporting data is available from natural populations.

HETEROCHROMATIN AND PAIRING

The Drosophila Data

It has been claimed that the organization of satellite DNAs within the heterochromatin of *D. melanogaster* is critical for chromosome recognition events in both meiotic and somatic cells [42, 43]. However, a number of authors have argued, from existing data, that heterochromatin is neither a sensible nor a viable candidate for such a function [1, 20, 50, 51, 52]. The problem can, however, be tackled directly. If satellite DNA is indeed crucial for chromosome pairing in *Drosophila*, then deletion of satellite sequences ought to lead to pairing problems. One can test this prediction by considering meiotic behavior in individuals where the heterochromatin content, and hence the satellite content, has been experimentally modified.

The male and female of *D. melanogaster* differ fundamentally from each other in the character of their meiotic mechanisms. The male, which has an achiasmate meiosis, has no genetic recombination, whereas the female does. We shall therefore deal separately with the two sexes. We also need to emphasize that there is no definable zygotene-pachytene stage in the male meiosis, and that this stage is only obtainable with difficulty in the female. Consequently, in the four examples that follow, pairing patterns in the male have had to be assessed by examining metaphase-I configurations, while in the female it has been necessary to rely on genetic segregation data.

Male Meiosis

Figure 5*b* illustrates an experiment in which, relative to the control (fig. 5*a*), virtually all the heterochromatin of the right arm of one homolog of chromosome *II* has been deleted, while the two arms of the partner metacentric have been detached. Metaphase-I pairing in this system is invariably complete. The separate free arms, *IIL* and *IIR*, each pair with their homologous arms, even though the right arm of the metacentric is devoid of satellites (fig. 5*b*). From this example, and others like it, one can confidently conclude that satellite sequences are not necessary for homolog recognition in any of the autosomes (II, III, IV) at male meiosis [53].

If it is indeed euchromatic homology which is of paramount importance for meiotic

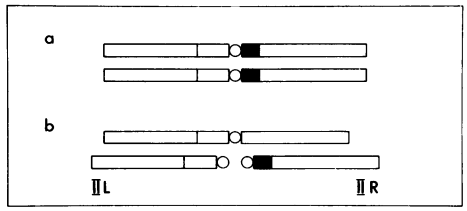

FIG. 5.—Structure of an experimental genome in *D. melanogaster* carrying a modified pair of second autosomes. (*a*) Normal II. The heterochromatin of the left arm (*IIL*) is *stippled*, that of the right arm (*IIR*) is shown *solid*. (*b*) Modified II. One homolog carries a deletion for heterochromatin of the right arm. The other is represented by two detachments *IIL* and *IIR*, each of which carry their normal heterochromatic blocks [53].

pairing in the male, then two further predictions follow: (1) chromosomes with *altered euchromatic homology* ought to show pairing problems, and (2) chromosomes possessing *only heterochromatic homology* ought also to experience pairing difficulties.

These predictions are borne out by two additional examples [53]. When a normal chromosome IV is accompanied by one in which most of the euchromatin is from the X, these two elements do not pair even though they have identical satellite sequences. Alternatively, one can construct a fly which has two chromosomes with perfect heterochromatic homology but effectively lacking in euchromatin. When two such identical heterochromatic mini-chromosomes derived from autosome II are added to a normal complement, they neither pair with each other nor with any member of the normal karyotype. This applies even though they share satellite homology with the heterochromatic blocks of the normal II, as well as with each other.

Female Meiosis

A number of deletions have been produced in the X chromosome which erode its heterochromatin to varying levels but leave the euchromatin intact in amount. By the criterion of genetic segregation, such chromosomes appear to find their homologs and segregate normally from them irrespective of whether they are heterozygous or homozygous for such deletions. This fact has been known for over 40 years [54], is documented by excellent data, and applies in cases where at least 80% of the heterochromatin in question has been deleted [54, 55].

These four examples show us that by manipulating heterochromatin in an organism where that heterochromatin is predominantly satellite DNA, it is possible to demonstrate unequivocally that satellite DNA per se is not involved in meiotic homolog recognition in either males or females of *D. melanogaster*. An additional 15 experimental genotypes examined by Yamamoto and Miklos [55] and Yamamoto [53] reinforce this conclusion. Comparable data also exist showing a lack of involvement of heterochromatin in somatic chromocenter formation of this same species [55].

The Mouse Data

Let us now ask what happens in a mammalian situation where satellite sequences are altered in amount. *Mus musculus musculus* and *Mus musculus molossinus* are two subspecies of mouse both of which contain the same satellite but in quite different amounts. The latter has only about 60% as much satellite DNA as *M. m. musculus*

[56], and this 60% is not evenly spread over all the chromosomes as it is in *M. m. musculus* [57]. Thus hybrids between these two subspecies provide us with homologous chromosomes which have radically different amounts of the same satellite DNA, but in an essentially comparable genetic background. This allows us to ask, and answer, a similar question to the one we formulated for *Drosophila*. Is pairing, and hence homolog recognition, normal when there is such a large disparity of satellite DNA content between otherwise homologous chromosomes? The answer is again unequivocal—not only is there normal pairing and a normal meiosis, but the hybrids are fully fertile. We can thus conclude that, in the mouse, radical differences in satellite DNA content do not cause pairing problems. Neither do they cause infertility, which is a powerful argument against those who have claimed that one of the functions of satellite DNA differences is to act as a sterility barrier between diverging incipient species [58, 59, 60].

HETEROCHROMATIN AND CROSSING OVER

Two additional examples not only validate the *Mus* story but enable us to take the argument a stage further. Natural populations of the grasshopper, *Atractomorpha similis* are polymorphic either for differences in the length of the distal heterochromatic segments or, less commonly, for the presence or absence of them. At least six, and sometimes more than ten, polymorphic chromosome ends may be present in a single population. Laboratory-bred hybrids between populations known to differ with respect to heterochromatin block size, show regular synapsis at meiosis despite marked asymmetries in a majority of bivalents [61]. This situation is, therefore, a precise parallel of the one we outlined in the mouse with the one qualification that this time the heterochromatic segments in question are non-centric.

An even more telling tale is found in the Algerian hedgehog, *Aethechinus algirus*. Here two pairs of long submetacentrics have exceptionally large distal blocks of heterochromatin [62, 63] which consist of rapidly reannealing DNA [64]. At male meiosis, these blocks are regularly excluded from chiasma formation, and the homologous arms which carry them diverge from each other in diplotene-diakinesis bivalents, so that there is also a recombination-free euchromatic region adjacent to the blocks themselves. In extreme cases this leads to rod bivalents in which the single chiasma present is located in the euchromatic short arm (fig. 6*a*). Similar behavior has been reported in the European and Romanian hedgehogs [65], although in neither case have the relevant DNA analyses been carried out. By contrast, in the South East Asian hedgehog, *Hemiechinus megalotis*, where there are no large heterochromatic segments equivalent to those of the three other species, ring bivalents with two or three chiasmata are present in all the large members of the complement [65].

This situation in hedgehogs is instructive in two respects. First, it shows that the pattern of crossing over within a chromosome can be radically modified by the presence of large homozygous blocks of terminal heterochromatin which are rich in repetitive DNA. Second, it demonstrates that in a related species, where such blocks do not occur, this effect is absent. We stress at this point that conventional crossing over rarely occurs in heterochromatin [51]. Hotta and Stern [66] have recently shown that mouse satellite DNA is an inactive template for the pachytene repair synthesis which

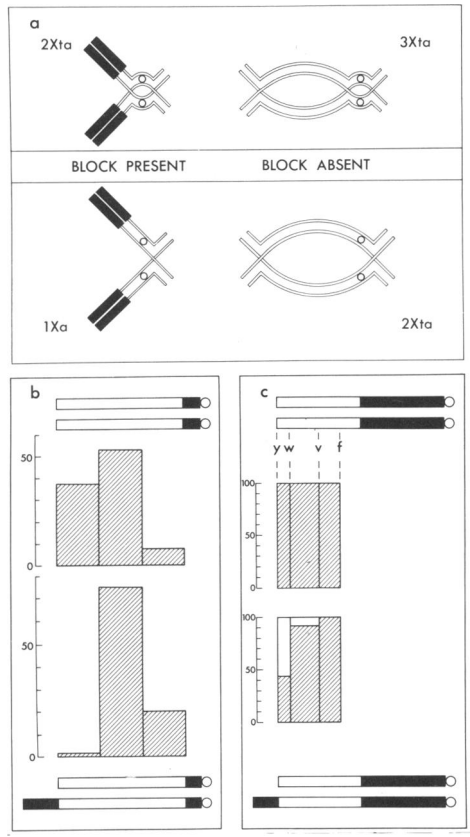

FIG. 6.—Effects of telomeric heterochromatin on chiasma characteristics of particular chromosomes. (*a*) Diakinesis bivalents in the hedgehogs, *Aethechinus algirus* (with heterochromatic blocks) and *Hemiechinus megalotis* (without heterochromatic blocks), illustrating an absence in crossing over in the blocks themselves and in the euchromatic segments adjacent to the blocks [62, 63, 65]. (*b*) The influence of a heterozygous terminal heterochromatic block on chiasma distribution in autosome 7 of *Atractomorpha similis*. Each distribution is based on 100 cells. Note the reduction in the chiasma potential of the euchromatic segment next to the block in the heterozygote compared to the chiasma distribution in homozygotes from the same population lacking the block [52]. The chromosome is divided into three intervals, *proximal, interstitial,* and *distal,* and the scale = the % of chiasmata falling in each interval. (*c*) Reduction in recombination in an experimentally constructed stock of *D. melanogaster* heterozygous for a telomeric block of heterchromatin (*bottom of figure*). The histograms refer to % recombination which is based on data from 11,605 progeny obtained from 106 cultures. The three intervals shown are $y-w^a$, w^a-v, and $v-f$ which have control map distances of ca. 2%, 30%, and 20% respectively, represented as 100% in each interval of the control chromosome (*top of figure*). Note the marked reduction in recombination in the $y-w^a$ interval next to the telomeric block of heterochromatin.

they have implicated in crossing over. As they themselves acknowledge, this finding does no more than add an aura of molecular respectability to what has been appreciated cytologically for many years. One of the few clear cases known to us where crossing over occurs in a C-band positive region is in the sex bivalent of the male Chinese hamster [67]. Here an interstitial chiasma is regularly formed between the distal segments of the short arms of the X and Y chromosomes. Although these segments are

regarded as heterochromatic, when C-banded they appear only slightly darker than the unpaired euchromatic segment on the long arm of the X. Further, these segments do not contain detectable amounts of the highly repetitive DNA which can be shown to be present in the strongly C-banded procentric blocks of heterochromatin present on both the X and the Y [68].

That the capacity of heterochromatin to reduce recombination in adjacent euchromatin is not an exclusive property of mammals can be confirmed by returning to the case of *Atractomorpha similis*. This grasshopper provides us with a natural polymorphism for the presence and absence of a telomeric block of heterochromatin known to consist of a cryptic satellite DNA [52]. When the pattern of recombination is compared between individuals homozygous for the lack of this block, and others from the same population which are heterozygous for it, there is a clear indication that the presence of this telomeric block leads to a severe reduction of chiasmata in the euchromatic segment next to the block (fig. 6*b*).

DROSOPHILA REVISITED

In *Drosophila* it is possible to mimic the situation in *Atractomorpha* under conditions where the genetic background is stringently controlled. Thus, if we construct a fly which has a telomeric block of heterochromatin consisting mainly of satellite DNA [69], we find a result similar to that in the grasshopper (fig. 6*c*). Recombination, as measured genetically, is severely reduced near the block, and the effect decreases with increasing distance from the block (Miklos, unpublished observations).

It should be stressed that in the three examples we have considered, the DNAs and their arrangements are radically different. In the hedgehog the DNA is highly repetitious as determined by C_0t analysis. In *Atractomorpha* the satellite is cryptic, while in *Drosophila* the heterochromatin is made up of a number of simple sequence DNAs. Despite the fact that the underlying repetitive sequence arrangements within the heterochromatic blocks in question are quite different, all three lead to a comparable reduction in recombination in neighboring euchromatin.

The *Drosophila* experiment is instructive in another sense, since it allows us to ask if recombination on other chromosomes is also altered in the presence of the experimentally constructed telomeric block. It transpires that there is indeed an increase in recombination values in the euchromatic regions near the centromeres of all the major autosomes [70]. Moreover, equivalent inter-chromosomal effects on chiasma formation occur in the presence of added heterochromatin in a number of natural situations in a variety of animals and plants [71].

Thus, alterations in the amount of heterochromatin in a single chromosome are not only capable of exerting an influence on the recombinational properties of that chromosome, but may, in addition, alter the cross-over potential of other members of the genome, too. In species where such effects occur, they could well provide a sufficient selective force to account for the presence of the heterochromatic blocks in question.

THE CENTROMERE EFFECT

Does the effect of satellite DNA on recombination also hold for centromerically located heterochromatin in *Drosophila*? If one experimentally deletes up to 80% of the centric heterochromatin of the X chromosome, one finds that as the amount of heterochromatin is reduced, so too is the amount of recombination in the proximal euchromatin [55]. This is unambiguous evidence for an inhibitory effect of the centromere per se. This experiment puts into clear perspective the early demonstrations [72, 73] that recombination decreases progressively when euchromatic sections are moved closer and closer to a centromere either by inversion or by translocation.

This leaves unresolved the question of whether the centric heterochromatin, like the telomeric heterochromatin, has an inhibitory effect on recombination in euchromatin near to it, or whether it acts simply as a passive spacer between the centromere and the proximal euchromatin. The indications are that heterochromatin indeed has an effect independent of the centromere, because recombination is still reduced at distances where the centromere effect is expected to be minimal [55].

Thus in situations where heterochromatin is located at the centromere, its effects on recombination can be confounded by effects exerted by the *centromere itself*. The precise outcome will then depend on the amount of heterochromatin and its effects, compared with the distance over which the centromere's own influence extends. Since, in man, virtually all of the heterochromatin is procentric, its amounts are small, and for the most part do not contain substantial quantities of satellite DNA, this is not a system that readily lends itself to demonstrating meiotic effects on recombination. Added to this, the pattern of chiasma distribution in human males shows a marked tendency for distal localization [74], which presumably reflects an underlying form of genotypic control.

That the overall pattern of recombination is indeed under genotypic control is well established [75], and such control regulates both pairing and crossing over. It may also simultaneously influence all members of a given complement, or else specifically influence individual chromosomes [76]. In this review, however, we have focused attention on two sources which may override conventional genotypic control: (1) influences exerted by a specific chromosome organelle, the centromere, and (2) influences determined by major inhomogeneities in DNA distribution, namely large blocks of heterochromatin which contain repetitive DNA.

By means of such influences, it is possible to see how a genome may be partitioned into zones within which the probabilities of recombination are quite different. Moreover, as far as the heterochromatic blocks are concerned, we have seen that their underlying repetitive sequence structure need not be uniform in character for them to be effective. Clearly what we now need to know is whether the heterochromatin needs to contain satellite DNA at all, or whether it is simply sufficient that, at the time of crossing over, any heterochromatinized section of DNA can inhibit recombination in its proximity. Additionally, whether repetitive regions which are interspersed throughout a chromosome arm, but are not recognizable cytologically as a discrete heterochromatic block, can have comparable effects, is not known.

There seems little doubt that heterochromatic influences on the meiotic system may have important adaptive or evolutionary consequences. In general, genes will tend to

be shielded from the effects of recombination when they are near heterochromatic blocks or centromeres, unless such situations can be neutralized genetically, as they evidently can in particular cases [77]. Thus, as the amount or location of heterochromatin on a chromosome changes, so also will the recombinational properties of that chromosome. Moreover, in at least some cases, such changes can be demonstrated on members of the genome other than those affected directly by the alterations in heterochromatin content [70, 71].

POSTLUDE

"And now, which of these finger-posts ought I to follow, I wonder?"

It was not a difficult question to answer as there was only one road and the finger-posts both pointed along it. "I'll settle it," Alice said to herself, "when the road divides and they point different ways."

Lewis Carroll

The existing hypotheses concerning the functions of satellite DNA usually leave the general reader with the impression of an insoluble morass. However, if one simply considers the hard data, as we have attempted to do in this article, it is soon apparent that the difficulties are more imaginary than real. There are indeed different finger posts, but many of them point unmistakably to an involvement of heterochromatin with the meiotic recombination mechanism.

It is true that we have made no attempt to consider possible somatic effects of heterochromatin and satellite DNA. Lubs [78], in discussing the possible significance of the known heteromorphisms in human chromosomes, draws attention to the fact that several statistically significant correlations have been demonstrated between these heteromorphisms and various clinical parameters. This suggests that certain variants may indeed influence development. Even so, no serious attempt has yet been made to examine this possibility in a systematic fashion in any organism.

There is an increasing awareness by molecular biologists of the importance of recombination. The discovery of "split genes" and the postulate by Gilbert [79] of the evolutionary importance of "gene pieces" embedded in a DNA matrix, have served to draw attention to the gap that exists between conventional molecular findings and their interpretation at an evolutionary, rather than a cellular, level. Perhaps the significance of intervening sequences, whether between or within genes, will also come to be seen as yet another strategy adopted by eukaryotes to provide an additional dimension of recombinational flexibility.

ACKNOWLEDGMENTS

We thank Sandy Smith, Ann Warrener, Maureen Whittaker, Graham Perceval and Barry Parr for illustrations, and the two Marilyns, Olsen and Miklos, for their patience in typing.

REFERENCES

1. COMINGS DE: The structure and function of chromatin, in *Advances in Human Genetics*, vol 3, edited by HARRIS H, HIRSCHHORN K, New York, Plenum Press, 1972, pp 237–431
2. JOHN B, MIKLOS GLG: Functional aspects of heterochromatin and satellite DNA, in *International Review of Cytology*, vol 58, edited by BOURNE GH, DANIELLI JF, New York,

Academic Press, 1979, pp 1–114
3. ARRIGHI FE, HSU TC: Localization of heterochromatin in human chromosomes, *Cytogenetics* 10:81–86, 1971
4. JONES KW: Repetitive DNA and primate evolution, in *Molecular Structure of Human Chromosomes*, edited by YUNIS JJ, New York, Academic Press, 1977, pp 295–326
5. MANUELIDIS L: Complex and simple sequences in human repeated DNAs. *Chromosoma* 66:1–21, 1978
6. MANUELIDIS L: Chromosomal localization of complex and simple repeated human DNAs. *Chromosoma* 66:23–32, 1978
7. SAUNDERS GF, SHIRAKAWA S, SAUNDERS PP, ARRIGHI FE, HSU TC: Populations of repeated DNA sequences in the human genome. *J Mol Biol* 63:323–334, 1972
8. MARX KA, ALLEN JR, HEARST JE: Characterization of the repetitious human DNA families. *Biochim Biophys Acta* 425:129–147, 1976
9. MARX KA, ALLEN JR, HEARST JE: Chromosomal localizations by *in situ* hybridization of the repetitious human DNA families and evidence of their satellite DNA equivalents. *Chromosoma* 59:23–42, 1976
10. CORNEO G, GINELLI E, POLLI E: Isolation of the complementary strands of a human satellite DNA. *J Mol Biol* 33:331–335, 1968
11. CORNEO G, GINELLI E, POLLI E: Repeated sequences in human DNA. *J Mol Biol* 48:319–327, 1970
12. CORNEO G, GINELLI E, POLLI E: Renaturation properties and localization in heterochromatin of human satellite DNAs. *Biochim Biophys Acta* 247:528–534, 1971
13. CORNEO G, ZARDI L, POLLI E: Evolution of human satellite DNAs on a methylated albumin Kielsguhr chromatographic column: isolation of satellite DNA IV. *Biochim Biophys Acta* 269:201–204, 1072
14. SCHILDKRAUT CL, MAIO JJ: Fractions of HeLa DNA differing in their content of Guanine and Cytosine. *J Mol Biol* 46:305–312, 1969
15. SAUNDERS GF, HSU TC, GETZ MJ, SIMES EL, ARRIGHI FE: Locations of a human satellite DNA in human chromosomes. *Nature* [*New Biology*] 236:244–246, 1972
16. JONES KW, CORNEO G: Location of satellite and homogeneous DNA sequences on human chromosomes. *Nature* [*New Biology*] 233:268–271, 1971
17. JONES KW, PROSSER J, CORNEO G, GINELLI E: The chromosome location of human satellite DNA III. *Chromosoma* 42:445–451, 1973
18. JONES KW, PURDOM IF, PROSSER J, CORNEO G: The chromosomal location of human satellite DNA I. *Chromosoma* 49:161–171, 1974
19. EVANS HJ, GOSDEN JR, MITCHELL AR, BUCKLAND RA: Location of human satellite DNAs on the Y chromosome. *Nature* 251:346–347, 1974
20. GOSDEN JR, MITCHELL AR, BUCKLAND RA, CLAYTON RP, EVANS HJ: The location of four human satellite DNAs on human chromosomes. *Exp Cell Res* 92:148–158, 1975
21. THIERY J-P, MACAYA G, BERNARDI G: Analysis of eukaryotic genomes by density gradient centrifugation. *J Mol Biol* 108:219–235, 1976
22. MAIO JJ, BROWN FL, MUSICH PR: Subunit structure of chromatin and the organization of eukaryotic highly repetitive DNA: recurrent periodicities and models for the evolutionary origins of repetitive DNA. *J Mol Biol* 117:637–655, 1977
23. COOKE HJ: Repeated sequence specific to human males. *Nature* 262:182–186, 1976
24. BOSTOCK CJ, GOSDEN JR, MITCHELL AR: Localization of a male-specific DNA fragment to a sub-region of the human Y-chromosome. *Nature* 272:324–328, 1978
25. COOKE HJ, MCKAY RDG: Evolution of a human Y-chromosome-specific repeated sequence. *Cell* 13:453–460, 1978
26. HSU TC, ARRIGHI FE, SAUNDERS GF: Compositional heterogeneity of human heterochromatin. *Proc Natl Acad Sci USA* 69:1464–1466, 1972
27. HEARST JE, CECH TR, MARX KA, ROSENFELD A, ALLEN JR: Characterization of the rapidly renaturing sequences in the main CsCl density bands of *Drosophila*, mouse, and human DNA. *Cold Spring Harbor Symp Quant Biol* 38:329–339, 1973

28. SAUNDERS GF: Human repetitious DNA. *Advances in Biological and Medical Physics*, vol 15, edited by LAWRENCE JH, GOFMAN JW, New York, Academic Press, 1974, pp 19–46
29. SANCHEZ O, YUNIS JJ: Analysis of the human genome by complementary RNA/DNA gradient hybridization and relaxed Cesium sulphate-silver ion density centrifugation. *Biochim Biophys Acta* 435:417–426, 1976
30. TSAI MY, YUNIS JJ: Identification of human DNA sequences complementary to chromosomal RNA with an improved RNA/DNA gradient hybridization technique. *Biochim Biophys Acta* 475:417–423, 1977
31. LUBS HA, RUDDLE FH: Chromosome polymorphism in American Negro and white populations. *Nature* 233:134–136, 1971
32. CRAIG-HOLMES AP, MOORE FB, SHAW MW: Polymorphism of human C-band heterochromatin I. Frequency of variants. *Am J Hum Genet* 25:181–192, 1973
33. CRAIG-HOLMES AP, MOORE FB, SHAW MW: Polymorphism of human C-band heterochromatin II. Family studies with suggestive evidence for somatic crossing over. *Am J Hum Genet* 27:178–189, 1975
34. GERAEDTS JPM, PEARSON PL: Flourescent chromosome polymorphisms: frequencies and segregations in a Dutch population. *Clin Genet* 6:247–257, 1974
35. GERAEDTS JPM, PEARSON PL, VAN DER PHLOEG M, VOSSEPOEL AM: Polymorphisms for human chromosomes I and Y. Feulgen and UV DNA measurements. *Exp Cell Res* 95:9–14, 1975
36. MULLER HJ, KLINGER JP, GLASSER M: Chromosome polymorphism in a human newborn population II. Potentials of polymorphic chromosome variants for characterizing the idiogram of an individual. *Cytogenet Cell Genet* 15:239–255, 1975
37. MULLER HJ, KLINGER HP: Chromosome polymorphism in a human newborn population—part I. *Chromosomes Today* 5:249–260, 1976
38. HOEHN H, AU K, KARP LE, MARTIN GM: Somatic stability of variant C-band heterochromatin. *Hum Genet* 35:163–168, 1977
39. EVANS HJ: Some facts and fancies relating to chromosome structure in man, in *Advances in Human Genetics*, vol 8, edited by HARRIS H, HIRSCHHORN K, New York, Plenum Press, 1977, pp 347–438
40. MCKAY RDG, BOBROW M, COOKE HJ: The identification of a repeated DNA sequence involved in the karyotype polymorphism of the human Y chromosome. *Cytogenet Cell Genet* 21:19–32, 1978
41. ENDOW SA, POLAN ML, GALL JG: Satellite DNA sequences of *Drosphila melanogaster*. *J Mol Biol* 96:665–692, 1975
42. PEACOCK WJ, APPELS R, DUNSMUIR P, LOHE AR, GERLACH W: Highly repeated DNA sequences: chromosomal localization and evolutionary conservatism, in *International Cell Biology*, edited by BRINKLEY BR, PORTER KR, New York, Rockefeller University Press, 1977, pp 494–506
43. BRUTLAG D, APPELS R, DENNIS ES, PEACOCK WJ: Highly repeated DNA in *Drosophila melanogaster*. *J Mol Biol* 112:31–47, 1977
44. HILLIKER AJ: Genetic analysis of the centromeric heterochromatin of chromosome 2 of *Drosophila melanogaster*: deficiency mapping of EMS-induced lethal complementation groups. *Genetics* 83:765–782, 1976
45. SALSER W, BOWEN S, BROWNE D, ADLI FE, FEDEROFF N, FRY K, HEINDELL H, PADDOCK G, POON R, WALLACE B, WHITCOME P: Investigation of the organization of mammalian chromosomes at the DNA sequence level. *Fed Proc* 35:23–35, 1976
46. MANTEUIL S, HAMER DH, THOMAS CA: Regular arrangement of restriction sites in *Drosophila* DNA. *Cell* 5:413–422, 1975
47. ROSENBERG H, SINGER M, ROSENBERG M: Highly reiterated sequences of SIMIANSIMIAN-SIMIANSIMIANSIMIAN. *Science* 200:394–402, 1978
48. SOUTHERN EM: Long range periodicities in mouse satellite DNA. *J Mol Biol* 94:51–69, 1975
49. HORZ W, ZACHAU HG: Characterization of distinct segments in mouse satellite DNA by

restriction nucleases. *Eur J Biochem* 73:383–392, 1977
50. HSU TC: A possible function of constitutive heterochromatin: the bodyguard hypothesis. *Genetics* 79:137–150, 1975
51. JOHN B: Myths and mechanisms of meiosis. *Chromosoma* 54:295–325, 1976
52. MIKLOS GLG, NANKIVELL RN: Telomeric satellite DNA functions in regulating recombination. *Chromosoma* 56:143–167, 1976
53. YAMAMOTO M: Cytological studies of heterochromatin function in the *Drosophila melanogaster* male: autosomal meiotic pairing. *Chromosoma*. In press, 1979
54. STURTEVANT AH, BEADLE GW: The relations of inversions in the X-chromosome of *Drosophila melanogaster* to crossing over and disjunction. *Genetics* 21:554–604, 1936
55. YAMAMOTO M, MIKLOS GLG: Genetic studies on heterochromatin in *Drosophila melanogaster* and their implications for the functions of satellite DNA. *Chromosoma* 66:71–98, 1978
56. RICE NR, STRAUSS NA: Relatedness of mouse satellite deoxyribonucleic acid to deoxyribonucleic acid of various *Mus* species. *Proc Natl Acad Sci* 70:3546–3550, 1973
57. DEV VG, MILLER DA, TANTRAVAHI R, SCHRECK RR, RODERICK TH, ERLANGER BF, MILLER OJ: Chromosome markers in *Mus musculus*: differences in C-banding between the subspecies *M. m. musculus* and *M. m. molossinus*. *Chromosoma* 53:335–344, 1975
58. CORNEO G: Do satellite DNAs function as sterility barriers in eukaryotes? *Evol Theory* 1:261–265, 1976
59. FRY K, SALSER W: Nucleotide sequences of Hs-α satellite DNA from kangaroo rat *Dipodomys ordii* and characterization of similar sequences in other rodents. *Cell* 12:1069–1084, 1977
60. JONES KW: Speculations on the functions of satellite DNA in evolution. *Z Morphol Anthropol*. In press, 1979
61. NANKIVELL RN: Karyotype differences in the crenaticeps-group of *Atractomorpha* (Orthoptera, Acridoidea, Pyrgomorphidae). *Chromosoma* 56:127–142, 1976
62. NATARAJAN AT, GROPP A: The meiotic behaviour of autosomal heterochromatic segments in hedgehogs. *Chromosoma* 35:143–152, 1971
63. GROPP A, NATARAJAN AT: Karyotype and heterochromatin pattern of the Algerian hedgehog. *Cytogenetics* 11:259–269, 1972
64. WILLEY AM, YUNIS JJ: Localization and characterization of the repetitive DNA of the Algerian hedgehog, *Erinaceus (Aethecinus) algirus* I. *Exp Cell Res* 91:223–232, 1975
65. GROPP A, CITOLER P, GEISLER M: Karyotypvariation und Heterochromatinmuster bei Igeln (*Erinaceus* und *Hemiechinus*). *Chromosoma* 27:288–307, 1969
66. HOTTA Y, STERN H: Absence of satellite DNA synthesis during meiotic prophase in mouse and human spermatocytes. *Chromosoma* 69:323–330, 1978
67. VISTORIN G, GAMPERI R, ROSENKRANZ W: Studies on sex chromosomes of four hamster species: *Cricetus griseus*, *Cricetulus griseus*, *Mesocricetus auratis* and *Phodopus scingorus*. *Cytogenet Cell Genet* 18:24–32, 1977
68. ARRIGHI F, HSU TC, PATHAK S, SAWADA H: The sex chromosomes of the Chinese hamster: constitutive heterochromatin deficient in repetitive DNA sequences. *Cytogenet Cell Genet* 13:268–274, 1974
69. NOVITSKI E: The genetic consequences of anaphase bridge formation in *Drosophila*. *Genetics* 37:270–287, 1952
70. SUZUKI DI: Inter-chromosomal effects on crossing over in *Drosophila melanogaster* II. A re-examination of X-chromosome inversion effects. *Genetics* 48:1605–1617, 1963
71. JOHN B: The cytogenetic systems of grasshoppers and locusts II. The origin and evolution of supernumerary segments. *Chromosoma* 44:123–146, 1973
72. BEADLE GW: A possible influence of the spindle fibre on crossing over in *Drosophila*. *Proc Natl Acad Sci USA* 18:160–165, 1932
73. MATHER K: Crossing over and heterochromatin in the X-chromosome of *Drosophila melanogaster*. *Genetics* 24:413–435, 1939
74. HULTÉN M: Chiasma distribution at diakinesis in the normal human male. *Hereditas*

76:55–78, 1974
75. REES H: Genotypic control of chromosome form and behaviour. *Bot Rev* 27:288–318, 1961
76. PARKER JS, JONES GH, TEASE C, PALMER RW: Chromosome-specific control of chiasma formation in *Hypochoeris* and *Crepis*, in *Current Chromosome Research*, edited by JONES K, BRANDHAM PE, Amsterdam, North Holland, 1976, pp 133–142
77. KLÁSTERSKÁ I, NATARAJAN AT, RAMEL C: Heterochromatin distribution and chiasma localization in the grasshopper, *Bryodema tuberculata* (Fabr) (Acrididae). *Chromosoma* 44:393–404, 1974
78. LUBS HA: Occurrence and significance of chromosome variants, in *Molecular Human Cytogenetics*, edited by SPARKES RS, COMINGS DE, FOX CF, New York, Academic Press, 1977, pp 443–455
79. GILBERT W: Why genes in pieces? *Nature* 271:501, 1978

The 24th annual meeting of *the Japan Society of Human Genetics* will be held in Tokyo, November 3–5, 1979. For information write to the General Secretary, Assoc. Prof. S. Takemura, Institute of Brain Research, University of Tokyo School of Medicine, 7-3-1, Hongo, Bunkyoku, Tokyo 113, Japan.