RAPID CHANGE OF CHROMOMERIC AND PAIRING PATTERNS OF POLYTENE CHROMOSOME TIPS IN *D. MELANOGASTER*: MIGRATION OF POLYTENE-NONPOLYTENE TRANSITION ZONE?

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ABSTRACT

The high variability of chromomeric patterns in near-terminal regions of polytene chromosome arms has been explored in a number of races, strains and hybrids of *Drosophila melanogaster*. Traditional explanations for tip differences between strains (differential compaction of chromatin, somatic or germinal deletion) are examined and, in the light of the reported observations, rejected. The range of polytene tip variability and rates of change in wild races are greater than has been supposed: strains formerly considered to be terminally deleted appear to gain terminal bands; others, formerly considered normal, appear to have lost them. Strains with high cell-to-cell tip variability are also described. Cell-to-cell variations, as well as much of the observed rapid changes in tip appearance, are probably due to heritable differences in the location of an abrupt transition zone between polytene and nonpolytene chromatin. A quantitative relationship between the amount of certain subterminal bands present and the frequency of tip association of nonhomologous chromosomes is shown and its possible significance for chromosome pairing discussed.

THE tips of salivary gland polytene chromosomes of *Drosophila melanogaster* show a variability that, when considered in concert with radiation studies, is somewhat paradoxical. Apparently capped by telomeres, the extreme tips are rarely, if ever, deleted or even simply added on to by natural or artificial means (MULLER and HERSKOWITZ 1954; ROBERTS 1975). Nevertheless, tips described, drawn or photographed relative to the series of polytene chromosome maps drawn by C. B. BRIDGES and his son, P. N. BRIDGES, in the period from 1938 to 1941 have changed appreciably within a period of 40 years (several hundred fly generations). The so-called terminal deficiency, *Df(1)260–1* of DEMEREC and HOOVER (1936), for example, no longer has the appearance originally described. Instead, several additional faint bands that do not appear homologous to distal *X* chromosome material now can be seen to cap the rearrangement (ROBERTS 1969, 1976).

Suspecting that this is no isolated phenomenon, I have searched for differences in chromomeric patterns among wild-type strains held for many years in major stock centers. In several cases, I have found alterations in tip appearances in

strains bearing the same designation, hence of recent common ancestry. Other changes between replicate cultures of the same strain have arisen within a few months in our laboratory. (All differences described here arose, of course, in the absence of known mutagenic treatment.)

I will show here that Oregon-RC strains originally described by BRIDGES (LINDSLEY and GRELL 1968) as terminally deficient in the right arm of chromosome *2*, now have "extra" bands, which, had they been polytenized at the time, should have been easily observed by BRIDGES. As discussed below, observed variations in tip morphology have been attributed in the past to a variety of causes including mutation, mechanical fracture during slide preparation and variable compaction of near-terminal bands. I have observed, however, striking morphological variability of certain chromosome tips within individual larvae. Such observations can best be explained by assuming cell-to-cell differences in the proximal-distal position of a rather abrupt transitional zone between high and low degrees of polyteny. Much interstrain variation in tip appearance is explicable as a consequence of heritable, semi-stable differences in the location of this transition zone.

MATERIALS AND METHODS

The stocks and races of *D. melanogaster* studied were obtained from the major Drosophila stock centers. Most were obtained from Bowling Green State University, Bowling Green, Ohio [for example, Urbana-S(BG)] and some were obtained from the California Institute of Technology, Pasadena California. The original Urbana-S(CT) strain showed a very high frequency of terminal associations of chromosome tips *X*, *2L* and *3R*. In order to preserve this unusual property, it was subdivided into a number of sublines. Within a period of one year, it had evolved into Urbana-S(CTB) (with the original strain properties) as well as an Urbana-S(CTA) line, in which only chromosome tips *X* and *2L* still paired frequently. Stocks were maintained at 20°.

Chromosomes were prepared according to the method of LEFEVRE (1976). Salivary glands were dissected into a fixative of 45% acetic acid, stained for a minute in 2% lactic acetic orcein and squashed under a siliconized coverslip. They were photographed with a Zeiss phase contrast microscope using a Planapochromat 100/1.3 oil immersion phase objective. Original magnification before enlargement was 1000–1200 diameters.

RESULTS AND DISCUSSION

A summary of the results of this study may be seen in Table 1. Some of the tip variability listed in Table 1 is illustrated in Figure 1. Most variations will be considered in detail during the following analysis of their nature.

The high variability of Drosophila polytene chromosome tips from strain to strain has been noted since the publication of BRIDGES' standard maps. Initially, there was no reason for BRIDGES to doubt that what was seen included all that was there. Consequently, the apparent absence of terminal bands of the right arm of chromosome *2* strains isolated in Oregon and Sweden led BRIDGES to designate them as homozygous for terminal deletions transmitted through the germ line: *Df(2R)Ore-RC* and *Df(2R)Swedish-b(c)*. BRIDGES noted no pheno-

TABLE 1

Polytene chromosome tips observed in various populations of D. melanogaster

Strain or race	Length of chromosome tip	
	X	*2R*
Oregon-RC(BG)	L	L-S (Variable)
Oregon-RC(CT)	L	S
Oregon-R(BG)	L	S
Oregon-R(BG-iso 70)	S+	S
Urbana-S(BG)	S	S
Urbana-S(CT)	S-L (Variable)	S
Wageningen	L	S
Swedish-C	L	S
Hikone-R	L	S
Samarkand	L	S
Crimea	S	S
Amherst-3	S	L
Florida-9	S	L
Canton-S(CT)	L	S+

L = long; S = short.

type effects of either supposed deficiency (LINDSLEY and GRELL 1968, pp. 303, 431).

An alternative explanation that some tip deficiencies are mere artifacts of the process of squashing terminally adherent tips, was then proposed. Following the report of HINTON and ATWOOD (1941) that the tips of various chromosomes tend to adhere to one another in strain-specific patterns, GOLDSCHMIDT and KODANI (1943) postulated that in addition to "truly terminal deficiencies" there is a class of "pseudo-deficiencies and translocations . . . pulled off under mechanical stress after sticking to another tip." Such rearrangements, they maintained, are distinguishable from true deficiencies by not being present in all nuclei (as one might expect of a germinally transmitted rearrangement). A direct test by stretching chromosomes adhering by their tips with a micromanipulator, however, failed to substantiate the GOLDSCHMIDT-KODANI hypothesis: chromosome ends were tenaciously held together by chromatin that stretched and (usually) broke without removing terminal bands (HINTON 1945).

KODANI (1947) proposed another explanation for tip variability: bands that appear to be absent in certain strains are actually present but are compressed into a single thick polytene band. That is, in strains where the maximum number of bands can be discerned, a thick, compressed band has elongated like an accordion, revealing two or more fainter bands.

In sum, the hypotheses that have been favored as explanations for variations in tip morphology are: (1) rearrangements, especially terminal deficiencies that are transmitted through the germ line; (2) pseudo-deficiencies, artifacts of force applied to terminally adherent salivary polytene chromosomes during squashing; (3) variable compaction of a constant amount of polytene chromatin into

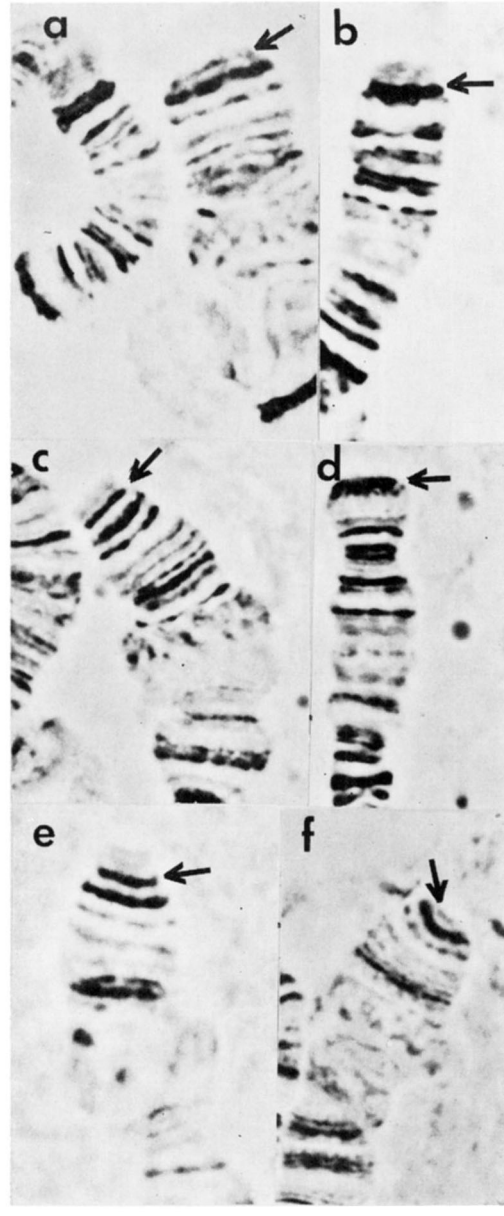

FIGURE 1.—Race and strain variations in length of the *X* and *2R* polytene chromosomes tips. (a) Short *X* and asynaptic (short) *2R* chromosome tips of the Amherst race. (b) Typical long *2R* chromosome tip of Amherst race. (c) Long *X* and, (4), short *2R* chromosome tips of Samarkand race. (e) Long *X* chromosome of Hikone race. (f) Very short *X* chromosome tip of Urbana-S (BG) Stain. Note virtual absence of polytene bands distal to 1A6 in this *X* chromosome. For orientation, arrow on all *X* chromosomes in these Figures indicates bands 1A5–6. Arrow on all *2R* chromosomes indicates bands 60F2–3.

one or several bands. We will argue for the greater prevalence of another mechanism, that of strain-specific variation in the location of the transition zone between polytene and nonpolytene chromatin. In order to build a case for the latter mechanism, it is essential to examine, critically, the several alternate explanations for variations in tip morphology.

The observations reported here, are, for the most part, of the euchromatic tip of the *X* chromosome and the right arm of chromosome *2(2R)*. These regions are of particular interest because they have been most often reported as varying in length from strain to strain. Furthermore, at least on occasion, both *X* and *2R* have been described as terminally deleted.

Hybrids between strains differing in tip chromomere pattern are particularly useful for testing the hypothesis that "extra" bands in one strain may habitually collapse, accordion-like, to yield a single, darker band in another strain (variable compaction hypothesis of KODANI, 1947). A number of studies have shown that there is a strong correlation between the width and density of a chromatin band and its DNA content (*cf.*, KEYL 1965). In hybrids, somatic pairing facilitates identification of homologous chromomeres. Moreover, chromomeres of different strains with, possibly, different degrees of compaction are best compared when both are in the same nucleus under identical staining conditions. In the comparison of chromomeres of hybrids that follows, we will assume that somatic pairing provides an accurate guide to band homology and that visual estimates of either the essential similarity or of striking differences in the DNA content of polytene bands are not misleading.

Homozygotes and inter-strain hybrids are compared in Figure 2. Consider, first, the Oregon strains, diagnosed as terminally deleted in chromosome *2R* by BRIDGES (LINDSLEY and GRELL 1968, p. 303). Figure 2(g) shows the *2R* tip of a hybrid between Ore-RC and Ore-R(iso-70). Although the last heavy bands (60F2–3) are of the same approximate density in both homologues, the Ore-RC strain now has three or four bands beyond 60F3, some of which are of appreciable density and quite unlike the two thin bands depicted on the standard map of *2R* (BRIDGES and BRIDGES 1939). These bands do not appear to be telescoped into the last heavy bands of the Ore-R homologue (Figure 2g, right), which appears to end at 60F3. The *X* chromosome from the same hybrid shows clearly (Figure 2f) that the 1A5–6 bands are of equal density in both *X* chromosomes. The more distal *X* bands are much heavier on the right (Ore-RC) side than on the left, where only a very faint terminal band is visible. The variable compaction hypothesis predicts, in general, that chromosomes with "long" tips (maximum number of chromomeres) will end with faint bands, while homologous chromosomes with "short" tips should end in heavy bands. This prediction is not met. Instead, the short *X* chromosome tips of heterozygotes (see below) or of homozygotes (Figures 1a, 1f, 2c) have terminal 1A5–6 bands of lower than typical density.

Further evidence against the variable compaction hypothesis may be gathered from hybrids between Swedish-C and Crimea strains. The Swedish-C *X* chromosome tip is consistently "long" (Figure 2a, homozygote), while the Crimea *X* chromosome is quite "short" (Figure 2c, homozygote) compared with BRIDGES'

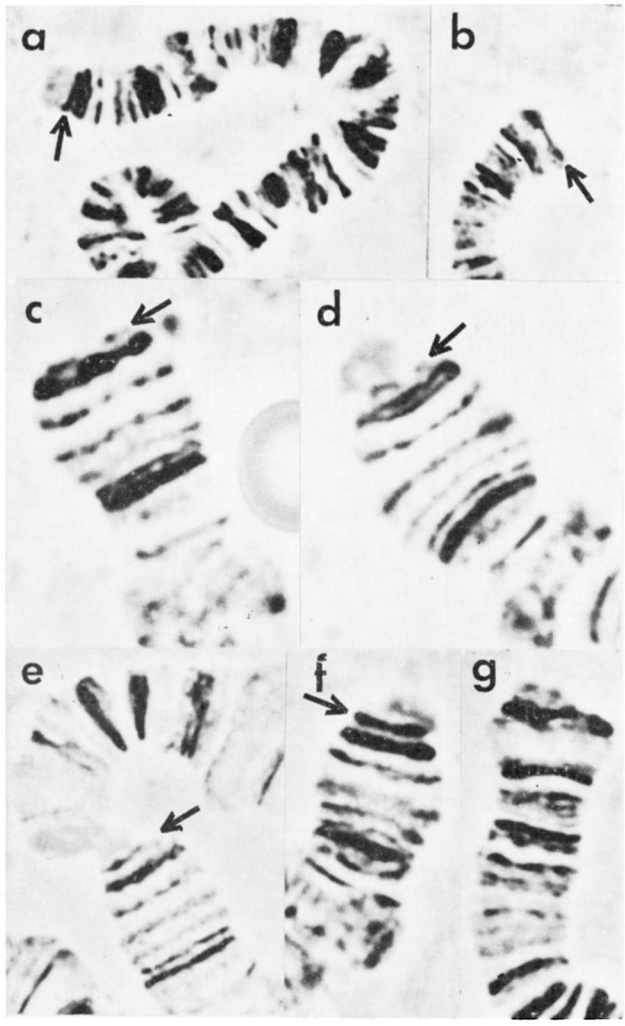

FIGURE 2.—Homozygotes and inter-strain hybrids compared. (a) Swedish-C (long) *X* chromosome tip. (b) Swedish-C (short) *2R* chromosome tip. (c) Crimea-(short) *X* chromosome tip. (d) Hybrid, Swedish-C/Crimea. Somatically paired *X* chromosomes of Swedish-C (left) and Crimea (right) races. There is clearly more chromatin in and distal to 1A5–6 in the Swedish polytene chromosome. (e) Hybrid, Urbana-S (BG)/Urbana-S(CT). The terminal adhesion to the *2L* chromosome involves the Urbana-S(CT) chromosome. Compare Urbana-(BG) strand with homozygote (Figure 1f). (f), (g) Hybrid, Ore-RC/Ore-R. Shows "extra" chromatin in the Ore-RC strand of the *X* (f-right) and of the *2R* (g-left) chromosomes. (See text for details.)

(1938) reference map. The Swedish-C *X* chromosome matches BRIDGES' map, while its *2R* chromosome (Figure 2b) still has the appearance described by BRIDGES as *Df(2R)Swedish* (*i.e.*, "missing" bands 60F3–4). But the Crimea *X* chromosome apparently lacks bands 1A1–4. The hybrid between the Crimea and Swedish stocks (Figure 2d) shows even more clearly that the last visible band

on the Crimea *X* chromosome is 1A5–6. The additional bands (1A1–4) corresponding to the left (Swedish) strand are not compacted with the low density 1A5–6 band of the Crimea strain for there is, if anything, less chromatin there! The final hybrid, of Urbana strains from the Cal. Tech (CT) and Bowling Green (BG) stock centers, shows essentially the same phenomenon. The *X* tip from Urbana (BG) consistently ends at 1A5–6 (Figure 1f, homozygote; note low density of bands 1A5–6), while the *X* from Urbana(CT) is of conventional length, *i.e.*, matches BRIDGES' (1938) map. The hybrid *X* chromosome shown (Figure 2e) in terminal association with *2L* has the "long" *X* of Urbana (CT) somatically paired with the "short" *X* of Urbana (BG) (right); there is, however, a greater density to the 1A5–6 bands on the long chromosome than the ("terminal") 1A5–6 bands on the short. The extra chromatin of the long chromosomes cannot be simply compressed into the low density 1A5–6 bands of the short *X* of Urbana(BG) (Figure 2e). Therefore, differential compaction or telescoping of near-terminal bands as postulated by KODANI (1947) cannot account for these variations in tip chromomeres. The variations described and illustrated here appear to be due to true differences in the amount of polytene chromatin present in comparable regions of different strains.

Is it likely that terminal bands have simply been deleted by mutations that have been fixed in the germ lines of certain strains, but not others? [Such was the opinion of BRIDGES and of HINTON (1945)] Considerable evidence has accumulated, however, that simple removal of the telomere followed by stabilization of the terminally deleted chromosome is a very rare event in Drosophila, if it ever occurs at all (MULLER and HERSKOWITZ 1954; ROBERTS 1975). Therefore, chromosomes that seem to end abruptly, short of the "terminal" bands of the standard polytene chromosome maps must not be automatically classified as terminally deleted.

Can forces generated during chromosome squashing remove terminal bands with sufficient regularity to account for strain differences? Although it is possible that material may, very occasionally, be removed from chromosome tips as was postulated by GOLDSCHMIDT and KODANI (1943), HINTON (1945) has provided evidence to the contrary. HINTON was unable to remove bands from terminally adhered arms by stretching the arms with a micromanipulator until they broke. Careful evaluation of our material supports HINTON's contention. Figure 3(d) shows tip adhesions between chromosomes *2R* and *3R* in the Ore-RC(BG) strain where one-half to two-thirds of nuclei of a salivary gland have a "long" *2R*, carrying three or four bands more than the balance of *2R* tips. In Figure 3(d), it is unlikely that material has been removed because adhesion is to the side of the tip; yet *2R*, in this case is short. Cases may also be found where terminal adhesions have been preserved through the squashing process: in Figure 3(f), for example, the *X* chromosome terminates as in Figure 3(c), while chromosome *2R* terminates as in Figure 3(d) or (e); the zone of adhesion or "joint" between the two chromosome tips is clearly visible but the "extra" bands of chromosome *2R* visible in Figures 3(a-c) simply are not visible despite the impossibility of bands being physically removed from the "joint."

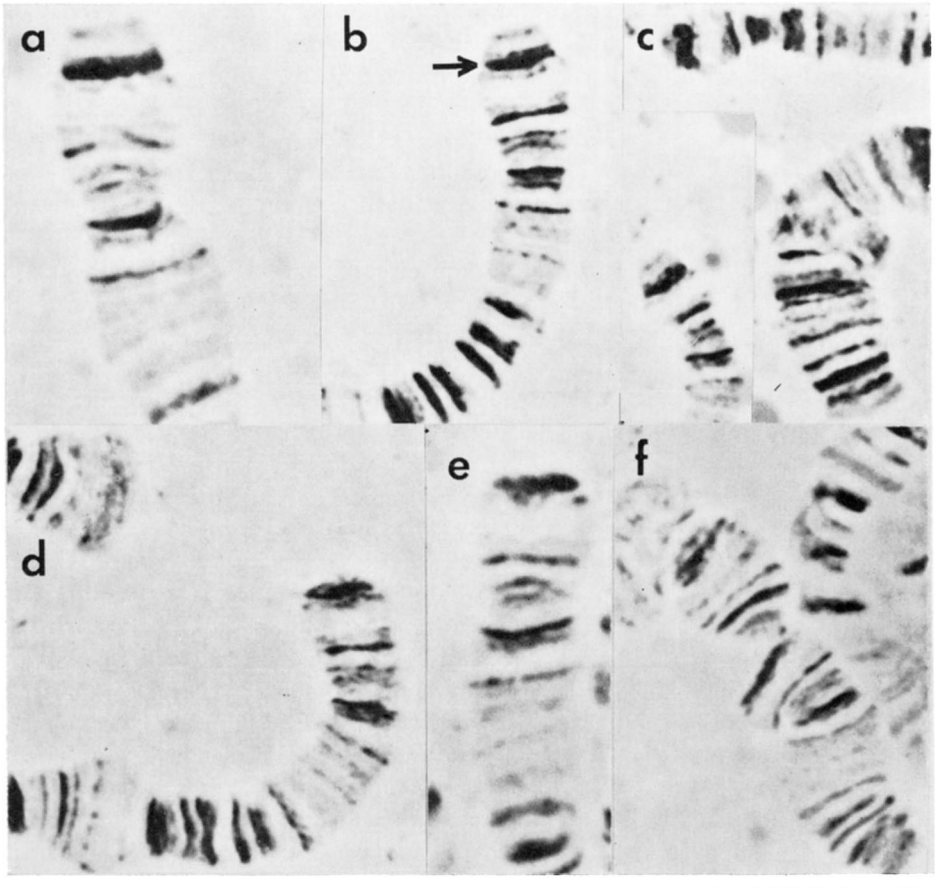

FIGURE 3.—Variability of the tip of chromosome *2R* within a single Ore-RC(BG) larva. Note presence of "long" (a-c) and "short" (d-f) forms in roughly equal proportions. Comparison of the *X* chromosome (Figure 3c) with the *X* chromosome of Figure 3f shows that bands are not pulled from *2R* in "short" forms of chromosome *2R* (see DISCUSSION for details).

Furthermore, although excessive force applied during squashing can, at times, increase asynapsis, there is no indication that asynaptic strands of chromosome *2R* tend to lose their "extra" bands (Figure 3c). From many examples of this nature, we can, in this strain, safely conclude that it is not possible to account for the regular appearance of "short" *2R* chromosomes as artifacts of mechanical removal of bands.

These conclusions are reinforced by the circumstances of the variable appearance of the *X* chromosome in another strain, Urbana-S(CTA) (Figure 4, a-c). In this strain, the *X* chromosome may terminate with virtually no chromatin visible beyond 1A5–6 (Figure 4a), with a fraction of the terminal bands drawn on BRIDGES' (1938) map (Figure 4b), or with chromatin sufficient to form an approximation of the complete terminal sequence, 1A1–4 of BRIDGES map (Figure 4c). Note, here, that the stretched terminal adhesion between the *X* and *2L*

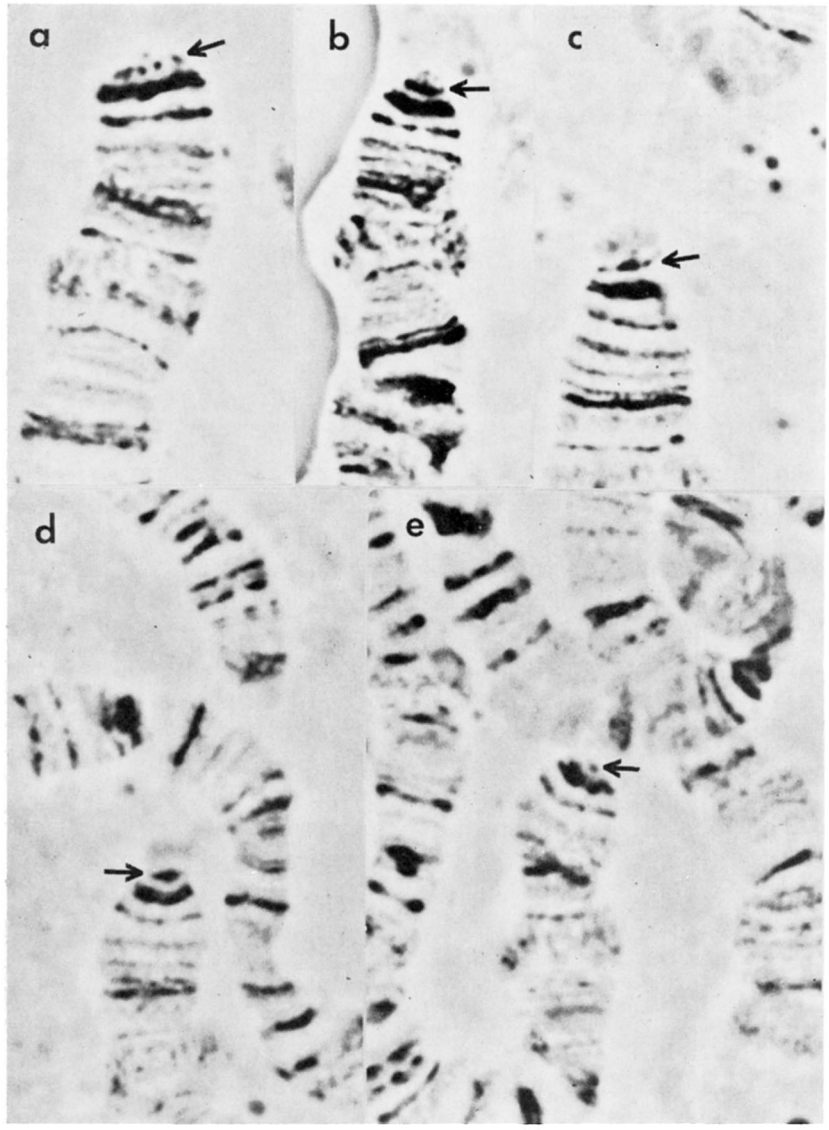

FIGURE 4.—Typical variability of the *X* chromosome tip in two strains of Urbana-S(CT). (a), (b) and (c) are from a single larva of the Urbana-S(CTA) strain. It is apparent that the amount of DNA in polytene "bands" distal to 1A5–6 is quite variable, ranging from almost none in (a), to the typical appearance for the region in (c). The terminal association (TA) between the *X* and *2L* chromosomes (c) is typical for this strain, which has undergone an alteration in the tip of chromosome *3R* associated with fewer TA's involving chromosome *3R* (see Figure 5). (d) and (e) are from a single larva of Urbana-S(CTB) strain. In this strain, TA's involving the "long" chromosome *3R* are common. Figure 4(d) shows that the separated TA of the *X* and *3R* chromosomes has not pulled bands 1A1–4 from the *X* chromosome. Figure 4(e) shows a TA of the *2L* (left), *3R* (right) and *X* (below) chromosomes. Bands 1A1–4 have not been pulled away, yet are missing, and bands 1A5–6 are reduced. Failure of polytenization is a more likely explanation of such variability than mechanical loss of bands (see text).

chromosomes has not removed much, if any *X* chromosome material. Note, too, that the terminal adhesion between *X* and *3R* chromosomes in the Urbana-S (CTB) strain (Figure 4d) has not removed the terminal bands of the *X* chromosomes. In striking contrast, however, stand the terminal bands of the *X* chromosome engaged in terminal adhesion with autosomes *2L* and *3R* (Figure 4e). Virtually no tension has been applied to the *X* chromosome; yet, only a meager wisp of chromatin may be seen between the *X* chromosome tip and the two autosomal tips. Not only are the terminal bands visible in Figures 4(b), (c) and (d) missing in the *X* chromosome of Figure 4(e), but 1A5–6, normally a heavy doublet, is only a fraction of its normal width and density. A short *X* chromosome terminally adherent to *2L* and *3R*, which could not have suffered any loss through removal of bands, indicates that the terminal chromomeres, in those frequent cases when the *X* chromosome is short, are not being removed.

Table 1 lists the variations in morphology of the *X* and *2R* chromosome tips found in geographic races and accumulated, perhaps, over periods of allopatry of 10^2 to 10^6 years. It also includes strains derived from a single race but maintained as stock isolates for half a century or less. For the most part, races can be identified by their characteristic chromosome tips; this suggests that such population changes in semi-stable cytological traits as these should be considered evolutionary changes. The apparent lack of direction suggests selective neutrality and drift, but selection may, in fact, be acting on the region (see below). While Amherst and Florida races have long chromosome *2R* polytene tips, most Eurasian races have short chromosome *2R* polytene tips resembling the Swedish and Oregon races originally considered by BRIDGES to be terminally deleted (LINDSLEY and GRELL 1968).

How long these racial differences persist is problematic. It is now apparent that significant changes in polytene tip morphology can occur within intervals ranging from a few decades to a few months. For example, both Ore-R and Ore-RC strains were described by BRIDGES (1938) as terminally deleted. As shown above (Figure 3), about half the salivary gland nuclei of any larva of the Ore-RC(BG) strain now have several "extra" bands beyond 60F3. Although Ore-R(CT) and Swedish-C still match BRIDGES' description (ends abruptly at 60F3), Ore-RC(CT) sometimes has a faint band visible beyond 60F3. The Urbana-S strain was described by BRIDGES in the 1930's as having normal polytene chromosomes (LINDSLEY and GRELL 1968). Yet the *X* chromosome of the Urbana-S(BG) strain in 1978 is consistently as short (Figure 1f) as the *X* chromosome of the Crimea strain (Figure 2c), a condition that would surely have been noted by BRIDGES had it been present in the 1930's. Although one might conceive of a near-terminal (interstitial) deletion being fixed in the Urbana-S(BG) strain over a 50-year period, it is difficult to conceive of deleted bands reappearing in that time interval, as would have to be the case if BRIDGES' classification of the right arm of chromosome *2* of the Ore-RC strain as terminally deleted were correct. Moreover, (as mentioned with regard to the Ore-RC strain) "long" *2R* chromosomes now have more (and different) bands than the faint, dotted 60F4,5 delineated by BRIDGES. (These may be seen in

Figures 1b [Amherst], 2g, and 3a-c [Ore-RC]). The prevalence of short *2R* chromosome tips among the various races and strains in Table 1 adds further support to the belief that there is no deletion of terminal *2R* bands in Oregon or Swedish strains; the "short" *2R* (like the "short" *X*) is merely one pole of the normal range of variability within the species.

If morphological changes of the type described here were found in only one to two strains, while others remained constant, one might conceivably raise the issue of "contamination," *i.e.*, migration of flies from one strain to another. We have sought to minimize this possibility by studying and comparing many strains. A further strong argument against migration as a prominent cause of these tip chromomere variations can be made from the frequent differences between strains in the appearance of more than one chromosome tip (migration, of course, would tend to even out appearances). Furthermore, tip changes have occurred in our laboratory, as described below, under conditions in which contamination was excluded. Another strong argument against contamination as a source of tip changes can be made from the nature of the alterations. It is useful to recall, at this point, that *Df(1)260–1* has "acquired" additional bands (ROBERTS 1969, 1976) since it was described as a terminal deletion by DEMEREC and HOOVER (1936). There is no possibility of "contamination" in this case. In the discussion of this interesting deficiency (ROBERTS 1976), I suggested that either *Df(1)260–1* has acquired new bands by translocation or transposition in the intervening 30 years or that the faint bands now capping the deficient *X* chromosome were initially overlooked. We now favor the latter point of view. It now seems probable that the additional bands were overlooked because they were truly invisible, *i.e.*, nonpolytene, at the time of the original description, in 1936. The evidence for this assertion follows.

The key to the problem of the causes of rapid tip evolution would seem to be the strains with high tip variability. We have examined dozens of larvae of the Urbana-S(CTA), Urbana-S(CTB) and the Ore-RC(BG) strains. Within a single larva of the two former strains, one may observe, intermingled in adjacent nuclei in roughly equal proportions, both long and short forms of the *X* chromosome tip; similarly, in the latter strain, one may observe both long and short forms of chromosome *2R* (Figure 3). Figure 4 shows that in the same larva of the Urbana-S(CTA) strain various nuclei may have bands 1A1–4 represented strongly (Figure 4c), weakly (Figure 4b) or very weakly (Figure 4a). Similarly, in another larva of the Urbana-S(CTB) strain, bands 1A1–4 may be represented either strongly (Figure 4d) or not at all (Figure 4e). In Figure 4e, not only are bands 1A1–4 absent from view, but 1A5–6, usually a heavy doublet, is reduced to the vanishing point! [Similar degrees of reduction of 1A5–6 may be seen in Urbana-S(BG) (Figure 1f) and Crimea (Figure 2d).]

The most probable explanation for these observations is that the proximal-distal location of the transition zone between polytene and nonpolytene (or, at least, a low grade of polytene chromatin) can vary from nucleus to nucleus in certain strains. According to this interpretation, the polytene-nonpolytene transition zone is at its most proximal (between 1A6 and 5) in nuclei, such as illus-

trated in Figure 4e, but is progressively more distal in Figure 4a (1A5–1A4), Figure 4b (1A4–3) and Figures (c) and (d) (transition zone at or beyond 1A1). Similarly, in the Ore-RC strain, one may see long or short tips of 2R in approximately half the salivary nuclei of any given larva (the actual proportions observed range from one-third to two-thirds of nuclei with short forms of chromosome *2R* in any single Ore-RC(BG) larva). Typical variability of chromosome *2R* within salivary glands of a single larva is illustrated in Figure 3.

We have shown that the bands in question are not, when seemingly absent, present as highly compacted chromatin. Nor have the bands been removed by germinally transmitted terminal deletions or by the mechanics of squashing in somatic cells. Differences in chromatin content of long and short tips are real, but are not a consequence of physical removal of bands. Somatic mutation must be considered, but it is not a likely explanation either: somatic deletions, to account for short tips, would have to occur at an unbelievably high rate, yet be confined to the tip of a single chromosome arm to yield the equal proportions of intermingled nuclei with long and short tips observed in the highly variable strains just described. The only reasonable explanation consistent with the sum of observations outlined above is that long and short tips result from cell-to-cell variations in the number of chromomeres reaching high enough degrees of polyteny to be visible with the light microscope.

With most of the race and strain differences listed in Table 1, cellular control of the location of the zone of polyteny appears to be sufficiently stringent so that most of the observed nuclei of the several larvae of each strain that were studied showed the same chromosome tip morphology. Exceptions to complete consistency were noted, however. For example, although most Amherst nuclei have a short *X* chromosome and a long chromosome *2R* tip, the short asynaptic chromosome *2R* near the *X* chromosome in Figure 1 is an interesting, but infrequent, exception. It would probably be more accurate to describe the Urbana-S(CT) and Ore-RC(BG) strains as highly variable, respectively, for the *X* and chromosome *2R* tips, with other strains showing low variability rather than no variability. Since the cell-to-cell tip variability of the two highly variable strains encompasses the range of variability observed between races of *D. melanogaster*, it is parsimonious to attribute most of the racial variation to the same cause: differential polytenization.

Admittedly, as one compares ever more distantly related strains or races, the possibility of tip mutation by chromosomal rearrangement increases. Interstitial deletions are not likely to be a frequent cause of changes in tip morphology, for such deletions near the telomere are exceedingly rare even in the presence of a heavy flux of X irradiation (ROBERTS 1975). It is possible, however, that some of the cytological differences between distantly related strains result from translocations between the tips of nonhomologues. The presence of shared DNA sequences on otherwise nonhomologous tips (see below) suggests that such exchanges are possible. Although this was the first hypothesis entertained as an explanation for tip alterations (ROBERTS 1969), I have found no compelling evidence for the occurrence of such rearrangements in the present material. Never-

theless, this possible source of tip variation must be kept in mind, especially in the light of the interesting observation illustrated by LEFEVRE 1976) that a fourth chromosome tip translocated by X rays to a distal position became less compact.

A tacit assumption of BRIDGES and many others who have considered specific *D. melanogaster* chromosomes shorter than some standard to be deficient has been that the salivary chromosome remains polytene out to its very tip or telomere. We now know that this is not true of the proximal heterochromatic region of the *X* chromosome. There, a substantial segment (one-third of the mitotic length) remains unpolytenized (RUDKIN 1965). If, as proposed here, strain differences in chromosome tip appearance are due, for the most part, to what is or is not polytene, then considerable autonomy of replication is required in order to get the striking differences in the amounts of terminal polytene chromatin observed in hybrids (Figure 2). Such autonomy has already been demonstrated: the DNA of polytene chromosomes is organized into a large number of autonomous replicons (PLAUT, NASH and FANNING 1966). A model in which the transition from polytene to nonpolytene zones is mediated through the use of branch points has been proposed; if such a zone exists at the tip, the number of free ends available for DNA duplex fusion would be minimized (LAIRD *et al.* 1973). In sum, if transition from high to low levels of polyteny occurs (as outlined above) at the chromosome tip as well as at the base (as has already been documented), one may resolve the apparent paradox of high morphological variability of the region tightly linked to the required telomere.

Tip association and chromosomal pairing relations

In his detailed study of the associations of polytene chromosome ends referred to above, HINTON (1945) described changes in the frequencies of terminal association (TA) of certain chromosome arms over a period of three years. Crosses between high TA and low TA lines revealed, at first, dominance of Ore-R over Swedish-b and later, no dominance, the hybrids being intermediate. Reciprocal crosses revealed no maternal or cytoplasmic effects. With a series of crosses, HINTON substituted another genome for all but the very tip of the *X* chromosome and was able to show that control of a specific heritable TA pattern is localized to the involved tip. This finding is consistent with the autonomy of replicons mentioned above and with our observations, discussed below, that manipulation of the number of polytene strands of an arm determines its TA frequency.

We have been able to confirm and extend HINTON's (1945) observations, using different strains. Female larvae of the Urbana-S (CT) strain have TA's in approximately 80% of squashed nuclei (300 observed). These associations involve, chiefly, various combinations of *X*, *2L*, and *3R* chromosomes: *X–2L* (22%), *X–3R* (25%), *X–2L–3R* (34%), and *2L–3R* (9%). A minority of associations involved the other arms, *2R* and *3L*: *X–2R* (7%); *2L–2R* (less than 1%); *X–3L* (3%).

In contrast with Urbana-S(CT), the Urbana-S(BG) strain exhibits only 5% TA's (most often *2L* with *2R*). When hybrids were made between the high and

low strains, the F_1 Urbana-S(CT)/Urbana-S(BG) larvae exhibited 50% TA's. In the hybrids, the "long" *X* chromosome tip from the Urbana-S(CT) parent was usually the only *X* chromosome in a TA (see Figure 2e). Bands 1A1–4 of the Urbana-S(CT) strain were frequently observed stretched between *X–2L* and *X–3R* tips that had been squashed slightly apart. It appears, therefore, that the presence (presumably due to extra polytenization) of the 1A1–4 bands of the Urbana-S(CT) *X* tip predisposes it to initiate or maintain terminal associations. A reasonable explanation of these observations is that the more strands present carrying the same base sequences (*i.e.*, the more polytene the tip), the greater the frequency of pairing events that involve DNA of this region. It would be desirable, however, to test the effects of an intermediate level of these near-terminal sequences on the frequency of terminal associations. Fortunately, this is possible in males of the Urbana-S(CT) strain.

Additional support for the hypothesis that there is a relationship between the relative numbers of *X* bands 1A1–4 present and the number of TA's involving the *X* chromosome comes from examination of male nuclei. In males of the Urbana-S(CT) strain, the percentage of nuclei with TA's is only 58% (160 nuclei observed), a decline of one-fourth from the (2*X*) female. Even more significantly, of these, instead of 81% in the female, only 30% involve the *X* chromosome! The proportion involving associations between chromosome *2L* and *3R* rose (from 9% in females) to 60% in males. The sharp decline in *X* chromosome pairing events in males suggests that halving the number of terminal *X* bands available for pairing decreases the frequency of observed TA pairing events involving these regions. This relationship holds at a third quantitative level, for in the Urbana-S(BG) strains where bands 1A1–4 are "absent" (again, not visible because presumably nonpolytenized) pairing events involving the *X* chromosome are virtually abolished. (Table 2.)

The high frequency of TA's (80%) in the Urbana-S(CT) strain approaches the frequency and specificity of synapsis of homologues (in spite of the short length of the somatically paired segment). We may, perhaps not unreasonably, attempt to extrapolate from insights gained from these manipulations of somatic pairing to the nature of the variables governing chromosome pairing in general. It seems unlikely from observed rapid changes in pairing relations (HINTON 1945; below) and from the sharp drop in TA's when the number of *X* chromosome strands is halved that the protein component of chromatin is most signifi-

TABLE 2

Relationship of the X *chromosome terminal association (TA) frequency to proportion of* X *chromosome bands 1A1–4 present*

Strain	Sex	Number of nuclei scored	Ratios of tip bands present	% Nuclei with TA's	% TA's involving *X* tip
Urbana-S(CT)	female	300	++++	80%	81%
Urbana-S(CT)	male	160	++	58%	30%
Urbana-S(BG)	female	110	±	5%	1%

cant in determining this specific pairing. We propose, therefore, that the higher the representation of certain classes of repeated DNA sequences in one chromosome that are (nearly) isosequential with DNA sequences of another chromosome, the greater the probability of the two chromosomal regions initiating and maintaining specific pairing, whether synaptic or somatic.

Support for the notion that the DNA sequences especially important for pairing initiation and maintenance are homologous repeat units comes from studies of cloned Drosophila DNA. DNA homologous to a tandemly repeated 3-kilobase base unit of a cloned DNA segment (Dm356) has been found by *in situ* hybridization on the tips of all five major chromosome arms of the Oregon-R strain (RUBIN 1977). A radioactive probe made from cDm356 labels the extreme tips of all five major arms and the thin fibers often seen connecting splayed tips—the very regions involved in TA's. There is some indication, too, that different repeat units may be found at the ends of different chromosome arms (RUBIN 1977).

Terminal associations, therefore, of (mostly) nonhomologous chromosomes appear to be quantitatively dependent on the presence of limited numbers of specific homologous sequences on the tips. The pairing relationships may be quantitative either because the probability of collision of homologous repeated sequences in an initial pairing contact is thereby increased, or because pairing configurations may be better held by multiplying the weak hydrogen bonds of DNA base pairing, or both. We propose that these pairing relations are revealed by the behavior of terminal associations of (for the most part) nonhomologous chromosomes, but are not limited to them; rather, they probably apply generally to chromosome pairing.

Unfortunately, it is impossible to make a generalization about the relationship between tip length and the frequency of terminal associations that covers all strains. There are many strains with long *X* chromosome tips, for example, which have low frequencies of TA's. Furthermore, high replication of the 1A1–4 sequences is not even an absolute requirement for the establishment and maintenance of terminal associations within the Urbana-S(CT) strain (see Figure 4e). Nevertheless, the quantitative relationships referred to above hold within the Urbana-S strain: Urbana-S(CT) males and Urbana-S(BG) females with progressively fewer 1A1–4 bands visible at the *X* chromosome tip have drastically reduced pairing of their *X* chromosomes with other tips, as compared with Urbana-S(CT) females. In all probability, as we study ever more distantly related populations, we are observing the interactions of two primary variables in the formation of these terminal associations: changes in the qualitative nature of base sequences and changes in the quantity of homologous DNA, both of which influence the frequency of pairing events. It remains to demonstrate the speed with which one or both of these variables can change.

Following the original study of the high TA Urbana-S(CT) line, a number of separate cultures were started from single pair matings. The F_1 generation was checked cytologically and all sublines showed the high TA characteristics involving the *X*, *2L* and *3R* chromosomes. Within a year, however, one of these lines, Urbana-S(CTA) showed virtually no involvement of the *3R* chromosome in

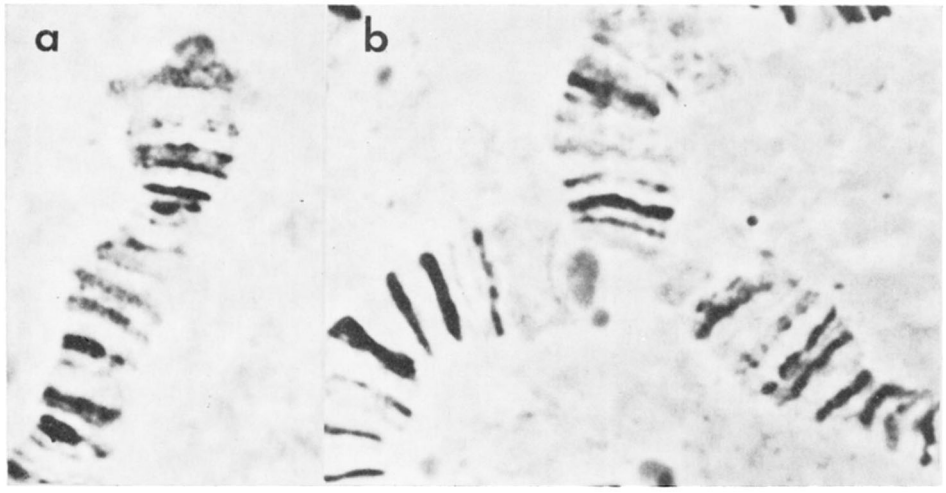

FIGURE 5.—(a), (b), Heterozygote, Urbana-S(CTA)/Urbana-S(CTB). In the course of a year, the "A" line lost the propensity for TA's involving chromosome *3R*. Cytological comparison of the hybrid with both strains reveals fewer bands at the tip of the "A" strain (Figure 5(a), left) and a tendency for associations of chromosome *3R* to involve the "extra bands" of the "B" strain (Figure 5(b): *2L*, left; *X*, top; *3R*, right with "*B*" chromosome tip paired with the *X*).

TA's, although the Urbana-S(CTB) line continued to behave like the original. As might be expected, the proportion of nuclei showing TA's dropped from 81% to 56% in the A line: there, *X–2L* associations (Figure 4c) comprise the majority (48%) with 6% *X–2R* and only 2% *X–2L–3R*. Accompanying these decreases in *3R* chromosome pairing were changes in the morphology of the tip of *3R*. These changes are best seen in the hybrids, Urbana-S(CTA)/Urbana-S(CTB). Again, there is "extra" material (Figure 5a) and this "extra" chromatin (beyond (100F.5?) belongs to the high TA Urbana-S(CTB) strain. In terminal associations involving the hybrid, it is the additional polytene material that typically associates with the tip of the *X* chromosome (Figure 5b). (Not unexpectedly, when the Urbana-S strain was reordered from the Caltech stock center after a lapse of four years, the frequency of nuclei with TA's was found to have dropped to 22%!).

These observations reinforce previous arguments and extend to other arms the generalization that strain-specific differential replication of near-terminal DNA is probably the chief explanation of tip variability. The behavior of the tip of the *3R* chromosome provides another argument against rearrangement as a cause of these temporal changes. Large deletions are almost invariably selectively disadvantageous and, consequently, are extremely rare in natural populations (reviewed in ROBERTS 1976). Therefore, it is unlikely that a true deletion of the large size illustrated in Figure 5 could have originated and become fixed in the Urbana-S(CTA) strain within a year's time. It is more likely that these changes are due to mutational or other alterations in the control circuit of somatic DNA replication.

Although near-terminal bands may disappear from view for months or even years, they frequently reappear—albeit in an altered form. This suggests that the retention of at least some near-terminal DNA sequences is selectively advantageous. Several possibilities come to mind. Near-terminal chromomeres may play an essential role in chromosome pairing or in nuclear membrane associations; they may include genes vital to embryonic development or to certain tissues (certainly bands distal to 1A5–6 and to 60F3 do not seem essential to salivary gland function, judging from their lack of polytenization in many thriving strains); or they may be inseparably linked (crossing over in this region is extremely rare) to other vital loci or structures—the telomere, for example. Contributing to the high morphological variability of near-terminal bands may be any number of standard mutational mechanisms—acting on a region where, at least for a substantial proportion of the included DNA sequences, stabilizing selection appears relaxed.

The near-terminal bands so often involved in terminal associations of nonhomologues often appear attenuated or puffed. (LEFEVRE 1976). It is uncertain whether this is purely fortuitous or selectively advantageous. Attenuation of tip DNA may provide a greater opportunity for homologous DNA sequences on different arms to contact one another and pair. It seems likely that a pairing mechanism so effective as to occasionally cause terminal associations of nonhomologues in 80% of nuclei has a more general significance than the somatic pairing of polytene chromosome tips. As shared repeated sequences concentrated at the tips of chromosomes or as specific repetitive sequences confined to homologues and concentrated in distal regions, "sticky DNA" such as that of near-terminal bands described above may play a critical role in the synapsis of meiotic chromosomes.

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