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Genotypes affecting the condensation and transmission of heterochromtic B chromosomes in the mealybug *Pseudococcus affinis*

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Abstract. In male mealybugs (Pseudococcide: Homoptera) the set of chromosomes of paternal origin becomes heterochromatic (H) and genetically inactive in early embryogenesis. During spermatogenesis the two sets segregate and only the meiotic products with the euchromatic (E) set form sperm. Individuals of *Pseudococcus affinis* (Maskell) may carry a B chromosome (B) which is usually heterochromatic. During prophase I of spermatogenesis, however, the B becomes even less condensed than the E set and usually segregates with the E set. We have previously shown that natural populations of *P. affinis* contain genotypes that can reduce the rate of transmission (k) of Bs from more than 0.9 to less than 0.1. We now demonstrate that these genotypes suppress k either by enhancing or by preventing the decondensation of the B, which in turn affects the position of the B on the metaphase plate and its segregation. We also demonstrate that radiation-induced fragment of the B, and a piece of a B which has been translocated onto a piece of an H chromosome, retain their characteristic pattern of condensation and thus that the condensation of the B is not controlled by one or a few *cis*-acting centers or loci.

Introduction

The mealybug *Pseudococcus affinis* (Maskell) (=*obscurus* Essig) possesses supernumerary B chromosomes (Bs) which are usually transmitted without reduction during spermatogenesis, and thus exhibit a strong "meiotic drive" (Nur 1962). The high rate of transmission (k) of the Bs is possible because of the unusual chromosome system (the lecanoid system) of the species. In this system females develop from embryos in which both sets of chromosomes remain euchromatic (E), while in embryos destined to become males the set of chromosomes of paternal origin becomes heterochromatic (H) and genetically inactive (Brown and Nelson-Rees 1961). During spermatogenesis the two sets segregate to opposite poles and only the products of meiosis containing the E set develop into functional sperm (Hughes-Schrader 1948). Prior to spermatogenesis, the B is heterochromatic, but during prophase I (PI) of spermatogenesis it becomes even less condensed than the E set [negatively heteropycnotic (Hp^-)] and this change in condensation apparently makes it possible for the B to segregate with the E set, and to be transmitted to over 90% of the offspring (Nur 1962).

Recently, we have demonstrated that isofemale lines of *P. affinis* differ in their ability to maintain the B under laboratory conditions and that this difference is due to the presence of genotypes that affect the rate of transmission of the B in males (Nur and Brett 1985, 1987). In this report we describe the behavior during spermatogenesis of one particular B on different genetic backgrounds and demonstrate that these backgrounds affect the condensation (appearance) of the B, which in turn, affects the position of the B in the cell, and the rate of transmission of Bs. We also describe the behavior of radiation-induced fragments of Bs and that of a translocation between a B and a regular chromosome.

Materials and methods

The material used in this study was derived from the isofemale lines used previously to study the genetic control of the rate of transmission of Bs by males (Nur and Brett 1985, 1987). The four lines used were selected from about 30 isofemale lines established with females collected at Berkeley and Davis, California, and represent lines with two extreme types of B transmission: those in which the B was transmitted at a relatively high k (>0.9) and has been maintained at a mean frequency of more than four Bs per individual [lines (L)-60 and L-72] and those in which the B was transmitted at significantly lower rates (0–0.8) and in which the B was lost rapidly (L-119 and L-230). The history of these lines is described in Nur and Brett (1987).

Most of the males examined cytologically had one B and originated from the same crosses as the males whose rate of transmission was measured in series II of Nur and Brett (1985). However, additional males of each type were also examined from later series to confirm the results.

The production of translocations between a B and an H chromosome [*T(B;H)s*] involved the use of a line with Bs that carried the body color mutation *pale* (*p*) which is recessive in both sexes and changes body color from pinkish-brown to cream. The translocations were produced by irradiating young males with about 2400 rep from a ^{60}Co source. The irradiated males carried the recessive mutation *p* on the chromosome set of maternal origin and the wild-type allele p^+ on the set of paternal origin. The irradiated males were expected to have two to four Bs, and the males were irradiated when they were in the late first or early

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second instar stage. Among the offspring of the irradiated males and females without Bs, about 4% were wild type in color and some of these were fixed for cytology. Because mealybug males normally do not transmit chromosomes of paternal origin, it was expected that these wild-type offspring would carry a translocation in which the p^+ allele was translocated onto either an E chromosome or a B. For cytological analysis males were fixed in a mixture of chloroform: 95% ethanol: glacial acetic acid (4:3:1), stained in alcoholic-HCl carmine (Snow 1963), squashed in Hoyer's mounting medium and examined and photographed using phase contrast optics.

Results

The types of male examined

Previously, we have demonstrated (Nur and Brett 1985, 1987) that the rate of transmission of the B by males of L-60 and L-72 is high (mean $k > 0.8$). However, in the presence of an E set (of maternal origin) from either L-119 or L-230, k was significantly lower, and in some of these males $k < 0.1$. In this study, we compared the behavior of the B during spermatogenesis in males whose E set was derived from one of these four lines, in order to determine how an E set of either L-119 or L-230 reduces the k of the males carrying it. The study compares the behavior of the same Bs (from line 72) in males: (1) with both chromosome sets from line 60 (L-60); (2) with an E set from L-119 and an H set from L-60; (3) with an E set from L-230 and an H set from L-60; and (4) with both sets from L-72. Information about the lines and about the crosses used to produce the males are rpesented in Nur and Brett (1985). The males of the first and fourth types will be referred to as the L-60 and L-72 males, and those with the E set from L-119 and from L-230 as the RT-1 and RT-2 (reduced transmission) males, respectively.

The lecanoid type of spermatogenesis

Spermatogenesis of what is now termed the lecanoid type was first described by Schrader (1921, 1923) from three mealybug species, including *P. affinis* [which was first referred to as *P. maritimus* (Ehrhorn) and later as *P. obscurus* Essig]. Additional details were added by Hughes-Schrader (1948), Brown (1959), Nur (1962) and Nelson-Rees (1963). The main features of spermatogenesis of the lecanoid type are: during PI homologous chromosomes do not pair and the first division is mitosis-like and involves the separation of sister chromatids. During metaphase of the second division (MII) the chromosomes of the E set and of the H set form two groups that lie side by side and form what has been termed a "double metaphase plate" (Brown 1959). Then during anaphase II (AII) the E and the H sets move to opposite poles.

It has been suggested (Nur 1962) that the formation of the double metaphase plate depends on the arrangement of the two sets in metaphase I (MI), during which the chromosomes of the H set are clumped together and the chromosomes of the E set form an incomplete circle around them (Fig. 1). This arrangement is maintained during anaphase I (AI) and telophase I (TI), but during prophase II (PII) the semicircle formed by the E set straightens and this leads almost directly to the formation of the double metaphase plate of MII (Figs. 11 and 12 in Nur 1962). This interpretation is consistent with the observation that in the mealybug *Cataenococcus olivaceus* (with $2n = 38$), in which the small chromosomes could have formed a complete circle around the H set, the circle is incomplete (Nur et al. 1987).

Spermatogenesis in males with a high k

The behavior of the B during spermatogenesis in males with a high k was described by Nur (1962). During early PI the B is heterochromatic and is associated with the H set (Fig. 1a–c). Prior to MI the B becomes either isopycnotic or Hp^- (less condensed than the E set) and this change in condensation apparently causes the B to move away from the H set and subsequently to segregate with the E set in AII. As a result, the B is usually transmitted to over 90% of the offspring.

The behavior of the B in L-60 males which came from series II of Nur and Brett (1985, 1987) was fairly similar to that previously described from males with a high k (Nur 1962). In early PI the B appeared to be as condensed as the H set, and the change from being heterochromatic (Hp^+) to being Hp^- took place in late PI. In L-60 males from a line established from the crosses leading to series VII of Nur and Brett (1987), however, the B appeared slightly less condensed than the H set in early PI (Fig. 1a–c) and was more clearly Hp^- in late PI (Fig. 1d, e). During MI the B appeared Hp^-, and was present at the periphery of the metaphase plate, usually in the gap between the two ends of the semicircle formed by the E chromosomes (Fig. 1f, g). The behavior of the B in later stages was similar to that described by Nur (1962).

The behavior of the Bs in an L-72 male

Several males from L-72 were examined but only one had a favorable cytology and was studied in detail. In this male most of the primary spermatocytes had six Bs and the rest had five. In most respects, the behavior of the Bs was similar to that in the L-60 males and to that described by Nur (1962). This male demonstrated, however, that when several Bs are present, the change in the Bs from Hp^+ to Hp^- usually does not occur simultaneously in all the Bs of the same cell.

During early PI the H set and the Bs were condensed to about the same degree and appeared together as a group (Fig. 2a). During late PI, however, some of the Bs were less condensed and were present among the E set. Thus, the change in the Bs which made them resemble the E set was associated with a tendency of the Bs to leave the H set and to be associated with the E set. By MI all the Bs were no longer associated with the H set and most appeared either isopycnotic or only slightly less condensed than the E set. Some of the Bs were present around the H set, usually in the gap in the incomplete circle formed by the E set, but some were at some distance from the E and H sets (Fig. 2e–g). Whether the outlying Bs represent those that decondensed precociously is not known. During AI one or more Bs often lagged in their division, but the fate of these laggards could not be determined.

The segregation of the Bs in AII could not be determined directly. It was sometimes possible, however, to learn about the segregation of the Bs by examining two sub-

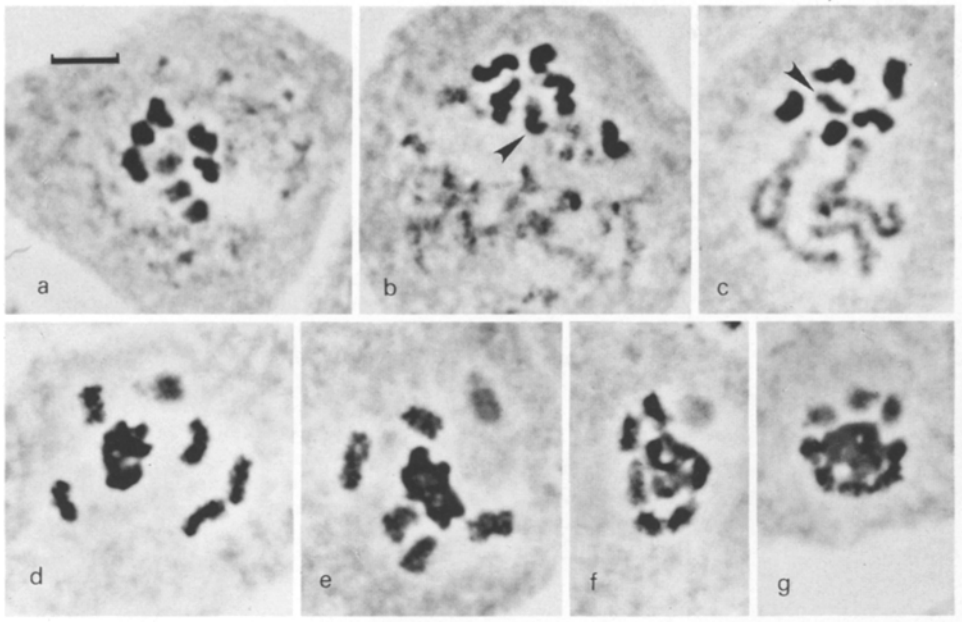

Fig. 1 a–g. The behavior of B chromosomes (Bs) during spermatogenesis in males from line 60. The bar represents 5 μm. **a** Premeiotic interphase. The three Bs are smaller and slightly less condensed than the set of five heterochromatic (H) chromosomes (of paternal origin). **b** Early prophase I. The B (*arrowhead*) is as condensed as the H set. **c** Mid prophase I. The B (*arrowhead*) is slightly less condensed than the H set. **d, e** Late prophase I. The H set is clumped at the *center*, and the euchromatic (E) set and the B are at some distance from it. The B is slightly less condensed than the E set and is thus negatively heteropycnotic (Hp^-). **f, g** Metaphase I. The B in **f** and the three Bs in **g** are Hp^- and are not as close to the H set as the E set

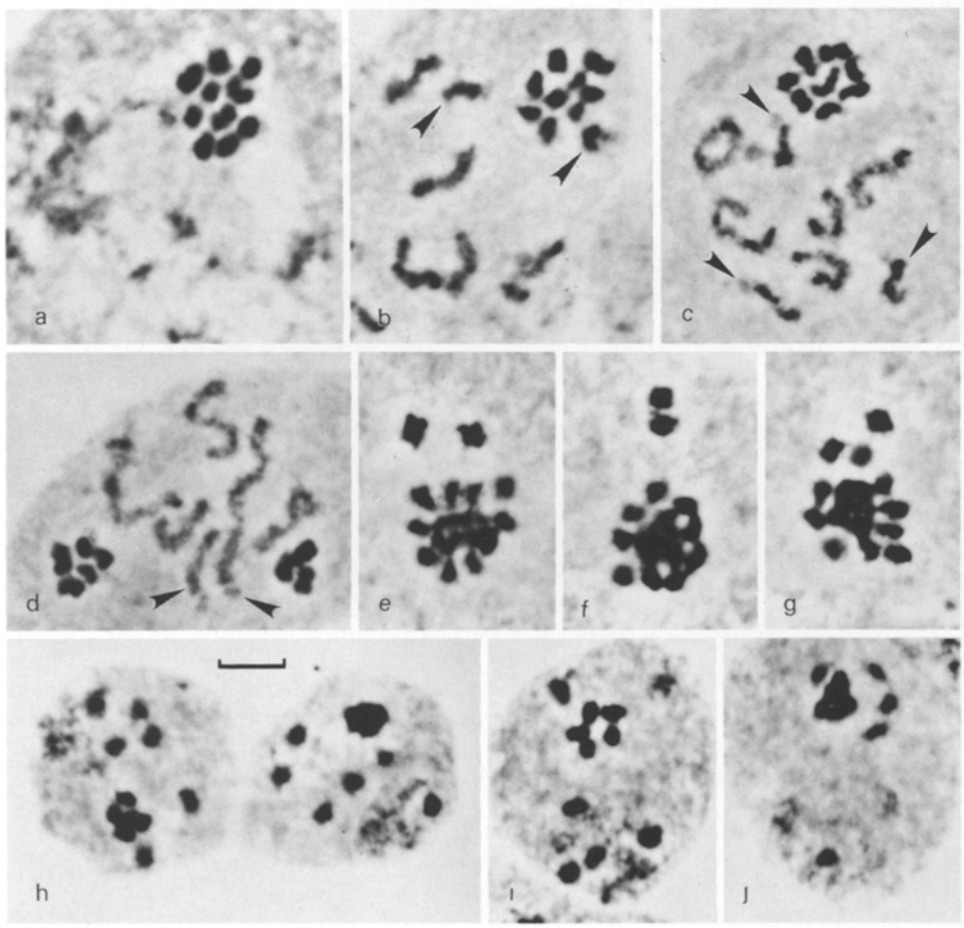

Fig. 2 a–j. The behavior of the B chromosomes (Bs) during spermatogenesis in a male from L-72. The bar represents 5 μm. **a** Premeiotic interphase. A group of 10 or 11 heterochromatic (H) chromosomes (the H set and 5 or 6 Bs). **b–d** Prophase I. Each cell contains condensed (Hp^+) and decondensed (or partially condensed) Bs which exhibit a satellite at one end. In **b** 4 Bs are condensed and are associated with the H set and 2 (*arrowheads*) are partially decondensed, and appear to be moving towards the euchromatic (E) set. In **c** 3 condensed Bs are associated with the H set and 3 uncondensed Bs (*arrowheads*) are associated with the E set. In **d** the condensed Bs are associated with the H set and 2 uncondensed Bs are associated with the E set. **e, f, g** Metaphase I. The H set is at the center and 11 chromosomes (the E set and 6 Bs) either surround the H set or are at some distance from it. **h–j** Four binucleate spermatids each with a condensed group of 5 H chromosomes, a nucleus containing the diffuse E set and either 5 or 6 Bs. The Bs which were not incorporated into the nucleus containing the E set are less condensed than the chromosomes of the H set and are not closely associated with them

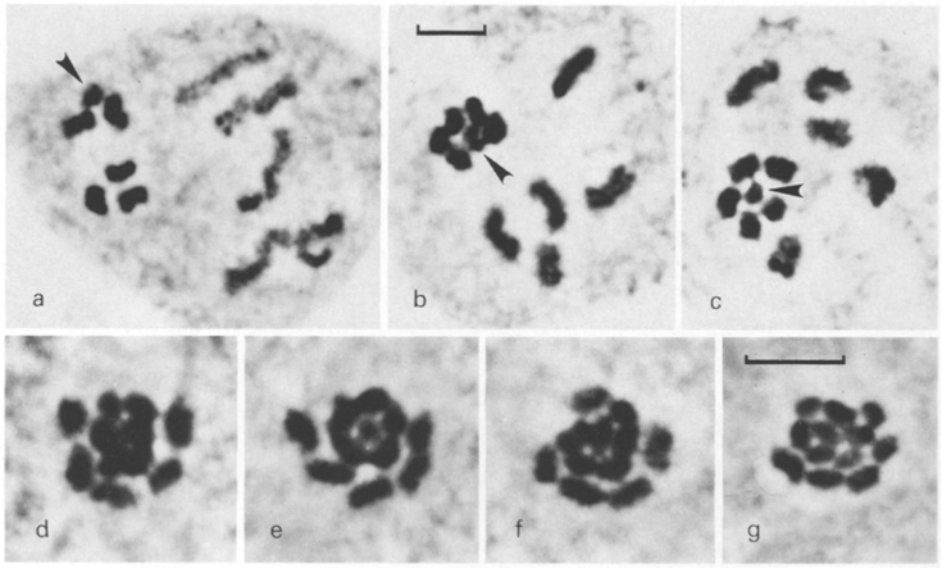

Fig. 3a–g. The behavior of a B chromosome (B) (*arrowhead*) during spermatogenesis in RT-2 males [with an euchromatic (E) set from L-230 and an heterochromatic (H) set from L-60]. The bars represents 5 μm. In **d–g** the chromosomes are enlarged 40% more than in **a–c. a–c** Prophase I. **d–g** Metaphase I. The B(s) is as condensed as the H chromosomes and is associated with them. In **a–f** each cell contains one B, and in **g** two Bs

sequent stages, during which the spermatids are first binucleate (with one E and one H meiotic product) and then following the fusion of pairs of sister binucleate spermatids, they become quadrinucleate (Hughes-Schrader 1948). The ability to use the early spermatid stages to determine the segregation of the B in AII is based on the observation that when the B segregates with the E set, it usually persists for a while as a distinct body (Fig. 2h–j; Nur and Brett 1985, Fig. 4).

In the binucleate spermatids of the L-72 male examined, the boundary of the nuclei containing the E set was often unclear. It was clear, however, that in most of the spermatids, the Bs which did not segregate with the E set were less condensed than the chromosomes of the H set and were not closely associated with them (Fig. 2h–j). The k of the L-72 male examined could not be determined accurately, but was estimated to be about 0.5. This rate is much lower than the rate of $k > 0.9$ observed in L-72 males with one or two Bs in controlled crosses. It is consistent, however, with previous results which indicate that k decreases with the increase in the number of Bs (Nur 1969).

The behavior of the B in the RT-2 males

The RT-2 males examined carried the E set of L-230 and usually had a reduced k. During PI the B was usually as condensed as the H set (Fig. 3a–c) and remained associated with it. During MI the behavior of the B varied somewhat, but in many cells the B remained heterochromatic and was associated with the H set on the MI plate (Fig. 3d–g). In general, the frequency of cells in which the B(s) remained associated with the H set in MI was negatively correlated with the mean ks of the males in the series from which they were obtained. Thus, in the RT-2 males of series II of Nur and Brett (1985) in which the mean k was 0.56, the B was associated with the H set in about half of the cells in MI, while in the males from series V, in which k was less than 0.1, the B was associated with the H set in almost all the cells in MI. These observations suggest that the low k exhibited by many of the RT-2 males, especially those from series V, was the result of the failure of the B to become Hp and to move away from the H set prior to MI.

One RT-2 male with one B (1B male) from series II had many early quadrinucleate spermatids that were unusually favorable for analysis (see Fig. 4 in Nur and Brett 1985), and thus, it was possible to determine the presence or absence of the B in the two E nuclei of 100 quadrinucleate spermatids: in 46 spermatids, one B was associated with each of the two E nuclei, in 11, one B was associated with one of the two E nuclei, and one with one of the H nuclei, and in 43 one B was present near each of the two H nuclei, and thus in these spermatids the B failed to segregate with the E set in both of the secondary spermatocytes. An analysis of the frequencies of the three types of quadrinucleate spermatid indicates that the segregation of the B in the two sister secondary spermatocytes which fused to form the quadrinucleate spermatids was not independent. Thus, with independent segregation, the expected numbers of the three types are 26.5, 50.0 and 23.5, and the difference between these numbers and those observed (46, 11 and 43) is highly significant ($\chi^2 = 60.95$, 1 *df*, $P < 0.001$). These data are consistent with the proposal made earlier that the events that occur during the first meiotic division, such as the position of the B on the metaphase plate, have a strong influence on the segregation of the B during AII. The data also indicate, however, that the segregation of the B during AII is not completely determined by the events which take place during the first meiotic division.

The behavior of the B in the RT-1 males

The RT-1 males carried the E set of L-119 (see Materials and methods) and had a reduced k (Nur and Brett 1985). The behavior of the B in the RT-1 males differed from that in the other lines studied in at least three ways: (1) in five out of the nine RT-1 males with many analyzable cells in PI, more than 10% of the primary spermatocytes lacked a B, and in the nine males the mean loss of the B prior to meiosis was about 12%. In contrast, there was no evidence for a similar loss of Bs in L-60 and RT-2 males

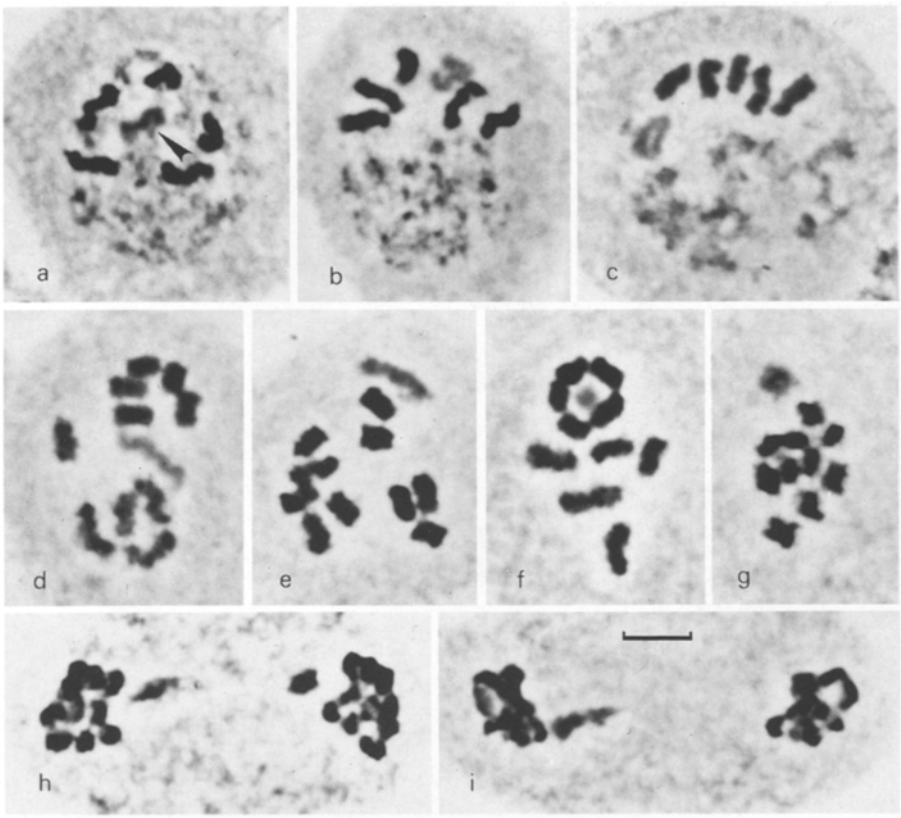

Fig. 4a–i. The behavior of a B chromosome (B) (*arrowhead*) during spermatogenesis in RT-1 males [with an euchromatic (E) set from L-119 and an heterochromatic (H) set from L-60]. The bar represents 5 μm. **a–c** Early prophase I. The B is less condensed than the H set. **d, e** Late prophase I. The chromosomes of the H set appear longer and less clumped than in males from the other lines. The B is longer than the chromosomes of either the E or the H set (Hp^-). **f** Late prophase I. The B appears to have been trapped by the H set. **g** Early metaphase I. The B is Hp^-. **h, i** Telophase I. The B lags in its division and the two daughter chromosomes have segregated to different poles in **h** and to the same pole in **i**

with one to three Bs. (2) In the RT-1 males the B was already Hp^- in very early PI (Fig. 4a–c), and throughout PI the B usually appeared longer and more lightly staining than in the L-60 males, or in the males of any other line. Moreover, in many cells in PI the B was even longer than the chromosomes of the E set (Fig. 4d, e). Thus, the E set of L-119 caused the B to decondense precociously, and to a greater degree than in the other lines. (3) During AII and TII the B sometimes lagged in its division (Fig. 4h, i), and in some of the cells the two division products of the B apparently segregated into the same daughter nucleus (Fig. 4i). This non-disjunction of the B accounts for the observation that in controlled crosses involving RT-1 males with 1 B, more than half the males transmitted 2 Bs to at least 1 daughter in 20 (Nur and Brett 1985, 1987).

In addition to affecting the behavior of the B, the E set of L-119 apparently also affected the behavior of the H set. Thus, in males from most of the lines the H set was usually clumped during early PI and the H chromosomes appeared as fairly round and compact bodies (Fig. 1a–c, Fig. 2a–c). In early PI in the RT-1 males, however, the H chromosomes appeared longer and less clumped than in other males (Fig. 4a–c). This modified appearance of the H set was observed in eight of the nine RT-1 males from series II which had cells in early PI, and was correlated with the less condensed appearance of the B in PI.

The behavior of a (B;H) translocation and B fragments

The origin of a male with a translocation between a B and an H chromosome [*T(B;H)*] and with fragments of Bs (B fragments) is described in Materials and methods. The behavior of the *T(B;H)* was studied in a male that apparently had, in addition to one intact set of five E chromosomes, one large *T(B;H)*, four H chromosomes, a B which may have been smaller than a normal B, and zero, one or two small fragments which probably represented parts of a B(s) (Fig. 5). The characterization of the karyotype of the male is based on the distinct behavior of the three types of chromosome in early spermatids, and the behavior of the chromosomes in the other stages is consistent with this characterization.

In cells in PI, five of the chromosomes were euchromatic and the remaining chromosomes and the fragments were Hp^+ (Fig. 5a, b). In MI several cells had seven chromosomes around the H set, of which two were Hp^- (Fig. 5c, d). The larger of the two Hp^- chromosomes was often present at some distance from the H set and clearly represented the B, while the smaller Hp^- element was always apposed to the H set and apparently represented the B component of the *T(B;H)*. Some of the cells also exhibited one or two fragments which were usually Hp^- and were present near the periphery of the H set and thus were apparently B fragments. In many of the cells in AII and TII the *T(B;H)* was stretched between the E and H sets (Fig. 5e) and in some of the cells the *T(B;H)* remained attached to the two sets even after TII (Fig. 5h). In most cells, however, the *T(B;H)* eventually segregated either with the E set (Fig. 5f) or with the H set (Fig. 5g). The chromosomal constitution of the male could be determined most clearly in binucleate spermatids, because at this stage the E, the H and the B chromosomes differed in their condensation and could be identified. An examination of cells at this stage indicated that the B and the B fragments were Hp^- and that each of these sometimes segregated with the E and sometimes with the H set (Fig. 5f, g).

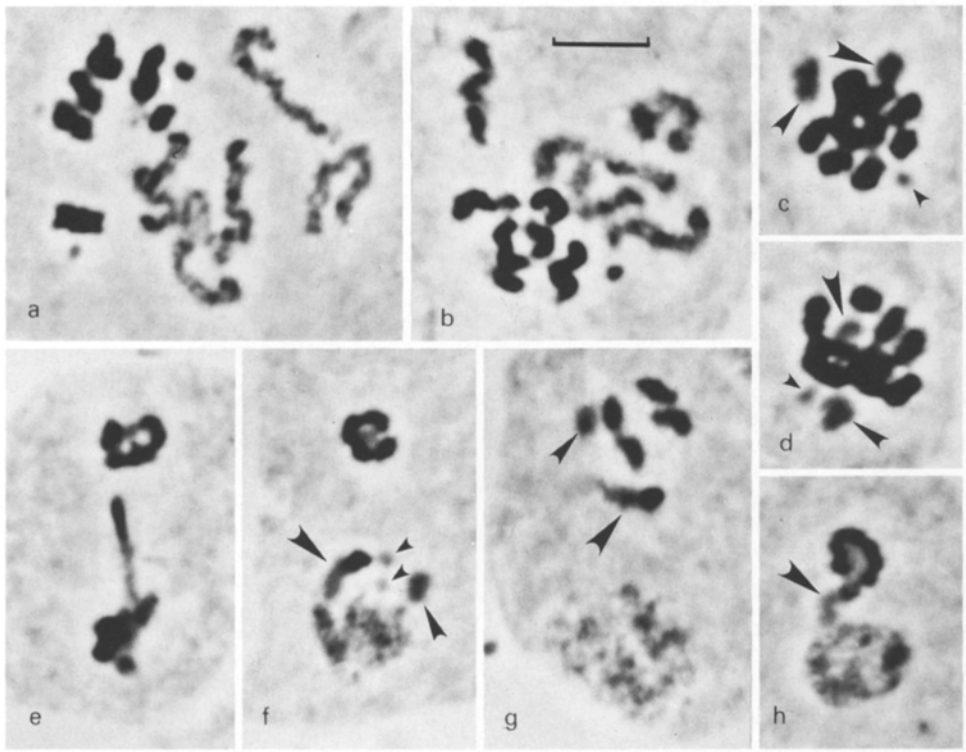

Fig. 5a–h. The behavior of B chromosome (B) fragments and a B–H translocation [*T(B;H)*] during spermatogenesis. The male carried five intact euchromatic (E) chromosomes, four heterochromatic (H) chromosomes, a *T(B;H)* (*large arrowhead*), a B (*mid-sized arrowheads*) and one or two fragments of a B (*small arrowheads*). The bar represents 5 μm. **a, b** Prophase I. The B, the B fragment(s) and the *T(B;H)* are heterochromatic (Hp^+). **c, d** Metaphase I. The H chromosomes are at the center and the five E chromosomes are at the periphery. The B, the B fragments and the B component of the *T(B;H)* are negatively heteropycnotic (Hp^-), while the rest of the *T(B;H)* is Hp^+ and is associated with the other four H chromosomes. **e** Telophase II. The *T(B;H)* is stretched between the four H chromosomes (*above*) and the E set (*below*). **f–h** Binucleate spermatids. The four H chromosomes above and the diffuse E set *below*. The B component of the *T(B;H)* and the B fragments are Hp^-. The *T(B;H)* is still connected to both the E and the H meiotic products in **h**, segregated with the B and the H chromosomes in **g**, and with the B, the B fragments and the E set in **f**. In **g** it can be seen that the H set consists of four H chromosomes and the H component of the *T(B;H)*

Discussion

The control of condensation

The results of the preceding section demonstrate that parts of B chromosomes which are either present as fragments or are translocated onto a regular chromosome will become decondensed and Hp^- at the same time as intact Bs. Moreover, in the *T(B;H)* the change in condensation occurred in the B part, but not in the non-B part. Thus, the degree of condensation of the B is apparently not regulated by one or two *cis*-acting control centers, as has been postulated for the X chromosome of the mammalian female (reviewed in Gartler and Riggs 1984) and for mammalian autosomes (Bianchi 1982). This conclusion, however, is not unexpected, because it has previously been shwon that in mealybugs, radiation-induced fragments behave in a similar way during both the heterochromatinization of the set of paternal origin and the reversal of this process. Thus, following paternal irradiation all the fragmented and rearranged chromosomes of paternal origin became heterochromatic in male embryos (Brown and Nelson-Rees 1961; Nelson-Rees 1962). Moreover, in certain tissues the entire H set, including the fragments, then became euchromatic and genetically active (Nur 1967). It has also been shown that in translocations between E and H chromosomes the two types of chromatin maintained their distinct identity (Nur 1970). The results of the present study, however, demonstrate for the first time that one part of a translocation can undergo a *change* in condensation without affecting or being affected by the other part.

The biochemical changes involved in both heterochromatinization and its reversal are poorly understood (Gartler and Riggs 1984), but even less is known about the nature of negative heteropycnosis. It is clear, however, that because at least some of the changes in condensation involved the B, but not the H set, the heterochromatin in the B must be in some way different from that of the H chromosomes. One difference between the B and the regular chromosomes of *P. affinis* involves repetitive DNA sequences. Thus, while both types of chromosome carry repetitive DNA, there is apparently very little homology between the sequences carried by the two types of chromosome (Klein and Eckhardt 1976).

Another possible difference, this time between the B and the H set, is in the degree to which the DNA of the two types of heterochromatin is methylated. Thus, the percentage of 5-methylcytosine (m^5C) in the DNA of *P. affinis* was found to be higher in males than in females, and higher in females without Bs than in females with Bs (Scarbrough et al. 1984). On the assumption that the E sets of the males and of females with and without Bs were methylated to the same extent, it was estimated that the percentage of

the cytosines which were methylated was about 1.25–1.30 in the E set, about 1.5–2.0 in the H set, and less than 0.75 in the Bs. The higher percentage of m^5C in males (where an entire set of chromosomes is heterochromatic) is consistent with the results of several other studies which have found that the percentage of m^5C in heterochromatin is higher than in euchromatin (Doerfler 1983). In contrast, the lower percentage of m^5C in the DNA of the Bs was unexpected. It may make it possible, however, for the B and the H set to react differently to certain biochemical changes, or signals.

On the basis of the preceding discussion it is clear that lines L-119 and L-230 (which provided the E set to the RT-1 and RT-2 males, respectively) carry transmission-reducing genes (TRGs) that affect the condensation of chromosomes. Several such genes have been described previously in other organisms (Baker et al. 1976; Smith et al. 1985), and at least two genes in *Drosophila melanogaster* apparently affect the condensation of heterochromatin but not of euchromatin. One was identified by the mutation *Su-var(2)1*^{01} which suppresses position-effect variegation (Dorn et al. 1986). This mutation is dominant over the wild-type allele and is associated with an increase in the acetylation of the histone H4.

A second gene affecting the condensation of heterochromatin was identified by the mutation *mus-101*^{tsi}, which is temperature sensitive (Gatti et al. 1983) and at the restrictive temperature (29° C) is a recessive lethal. After a short (2–4 h) exposure of larvae homozygous for the mutation to 29° C, the *Y* chromosome in most of the neuroblasts in prophase and metaphase appeared elongated and undercondensed, and after a longer exposure the heterochromatic parts of the *X* and the autosomes also appeared undercondensed. The mutation apparently does not affect euchromatin, and Gatti et al. (1983) have proposed that the locus codes for a protein which is necessary for the condensation of heterochromatin but not of euchromatin.

Overall, the effect of *mus-101*^{tsi} on the condensation of heterochromatin resembles that of the E set of L-119, because both lead to the undercondensation of constitutive heterochromatin. The effects of the two differ, however, in at least two important respects. (1) While the former affects the condensation of heterochromatin during mitosis, the effects of the TRGs of L-119 are apparently restricted to male meiosis; in somatic cells of both males and females the Bs are clearly heterochromatic (Nur and Brett, unpublished observations). (2) While *mus-101*^{tsi} apparently does not affect chromatin which is already condensed (Gatti et al. 1983), the TRGs of L-119 apparently do affect such chromatin. The present results also indicate that the effect of the TRGs of L-119 on constitutive heterochromatin (the B) was much more pronounced than on facultative heterochromatin (the H set).

The relationship between condensation and the position of the B in the cell

On the basis of the preceding discussion, it is clear that *P. affinis* contains genotypes or genes that affect the behavior of the B during spermatogenesis. Moreover, it is likely that the change in the behavior of the B, such as the change in its position in the cell relative to that of the two sets, depends on the condensation state of the B, because this is almost certainly the basis of the differences in the behavior of the E and the H set during spermatogenesis.

Overall, the degree to which the B was condensed was negatively correlated with the distance between the B and the H set. Thus, when in MI the B was as condensed as the chromosomes of the H set it was usually associated with it (Fig. 3). In contrast, when the B was about as condensed as the E set it was present outside the H set at about the same distance from the H set as the chromosomes of the E set (Fig. 2). Moreover, when it was Hp^- it was usually further away from the H set than the chromosomes of the E set (Figs. 1, 2, 4 and also Fig. 10 of Nur 1962). A similar relationship between the condensation of the B and its position was also observed in late PI and in the early spermatids, but apparently not in early PI.

The strong correlation between the degree to which in *P. affinis* the chromosomes are condensed and their relative position in the cell is clearly unusual, and we do not know of any similar case. It has been known for a long time that in many animal and plant species the position of chromosomes on the metaphase plate may depend on their relative size: the large chromosomes are often present at the periphery of the plate and the small chromosomes occupy the center (White 1973). It is unlikely, however, that the position of the Bs depends mostly on their size, because B fragments and intact Bs usually occupied similar positions.

At present the most likely explanation of why in mealybugs and related groups the state of condensation of the chromosomes has such a pronounced effect on their behavior is that these chromosomes are holokinetic (Hughes-Schrader 1948), i.e., the attachment sites of the spindle or chromosome fibers are apparently distributed over the entire length of these chromosomes. Thus, it may be reasonable to assume that a change in the condensation of the chromatin is more likely to affect chromosome behavior and movement of holokinetic chromosomes than a similar change in the degree of condensation of chromosomes with a localized centromere (such as the heterochromatinization of the mammalian X chromosome).

Evolutionary considerations

The available information about the nature of the Bs in *P. affinis* indicates that they reduce the fitness of individuals carrying them (and are thus "parasitic") and that they are maintained only because they exhibit meiotic drive (Nur 1969; Nur and Brett 1987). Natural populations of this species, however, contain genotypes, such as the TRGs present in L-119 and L-230, that can greatly reduce *k* and can eliminate the Bs from laboratory populations (Nur and Brett 1985). Thus, the presence of TRGs in natural populations raises the question of why such genotypes have not become more common, permitting the mean frequency of the Bs in some populations to exceed 2.5 Bs per individual (Nur 1969). The most likely explanation for the low frequency of the TRGs is that in these populations the TRGs have pleiotropic detrimental effects. The results of this study are consistent with this explanation, because the atypical appearance of the H set during PI in most of the RT-1 males suggests that the TRGs affect not only the condensation of the B, but also that of the regular chromosomes, especially that of the H set.

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