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THE SYNAPTONEMAL COMPLEXES OF *CAENORHABDITIS ELEGANS*: PACHYTENE KARYOTYPE ANALYSIS OF HERMAPHRODITES FROM THE RECESSIVE *him*-5 AND *him*-7 MUTANTS

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SUMMARY

The *him*-5 and *him*-7 mutants (high incidence of males) of *Caenorhabditis elegans* both showed increased rates of X chromosome non-disjunction (16 % and 3 %, respectively) but *him*-7 also had a high frequency of autosomal non-disjunction (34 %). Synaptonemal complex (SC) karyotype analysis revealed a haploid chromosome number of six in each strain. Alterations in *him*-7 nuclear morphology were observed but there were no aberrations in SC structure that could account for the increased frequency of autosomal non-disjunction. However, the frequency of X-chromosome non-disjunction occurred at predicted rates on the basis of the number of disjunction regulator regions (DRRs) present on the SCs. The observation that the levels of X-chromosome non-disjunction were not influenced by the increase in the frequency of autosomal non-disjunction supports the notion that the X chromosome is subject to separate controls during meiosis. The *him*-7 mutant is nested within the *rad*-4 map region on linkage group V, however, SC analysis did not reveal the physical position on the chromosome because of synaptic adjustment.

INTRODUCTION

The free-living nematode *Caenorhabditis elegans* has six chromosomes present in each haploid nucleus ($n = 6$; Nigon & Brun, 1955; Goldstein & Slaton, 1982) and because of their small size (1–2 μm in length) standard light-microscopic analysis cannot differentiate individual chromosomes. In order to characterize the chromosomes, it is advantageous to select a stage in the cell cycle at which time the chromosomes are extended to their maximum length (14–15 μm). For this reason, the pachytene stage of meiotic prophase is analysed and the synaptonemal complexes (SC), which are formed between homologously paired chromosomes, are examined. The SC has at least two distinct functions: mediation of recognition patterns of homologous chromosomes and regulation of proper disjunction of homologues after prophase (Westergaard & von Wettstein, 1970). If the homology is altered, the pairing process and subsequent disjunction of the chromosomes may be affected. Therefore, a change in the frequency of non-disjunction may be related to a change in the structure of the SC or an SC-associated structure.

Production of male offspring in *C. elegans* is a consequence of X-chromosome non-disjunction so that hermaphrodites have the chromosomal complement of

Key words: synaptonemal complex, karyotype analysis, *Caenorhabditis elegans*.

5AA,XX and males are 5AA,XO (Nigon & Brun, 1955). In the wild-type population, males are produced only rarely (0·2%; Hirsh *et al.* 1976), yet there are recessive mutants that yield a high incidence of males (termed *him*; Hodgkin *et al.* 1979). These mutants are believed to have arisen after a point mutation, which may have a resultant effect on the localized structure of the SC. Such an alteration of the SC structure would explain the increased frequency of X-chromosome non-disjunction observed in the *him*-5 and *him*-7 mutants. In a previous study (Goldstein & Slaton, 1982), an alteration of the SC in non-disjunction mutants was not observed. However, changes in distribution of an SC associated structure, termed a disjunction regulator region (DRR) (Goldstein, 1984*a,b,c*) were noted. The presence of the DRR is related to the frequency of occurrence of X-chromosome non-disjunction.

The present study represents the first SC pachytene karyotype analysis in *C. elegans* utilizing three mutations (*him*-5, *him*-7 and data from *rad*-4) along a single chromosome (linkage group V) and seeks to identify the interspersion (as genetically determined; Hartman & Herman, 1982) of *him*-7 within the *rad*-4 region (see Fig. 2).

MATERIALS AND METHODS

The individuals studied were 4- to 5-day-old hermaphrodites of *C. elegans*. The worms were obtained from the *Caenorhabditis* Genetics Center and were processed for electron microscopy as previously described (Goldstein & Slaton, 1982). Hermaphrodites were also selected after a few generations of growth and analysed in the same fashion.

The pachytene karyotypes of three early–mid pachytene nuclei were reconstructed from electron micrographs of serial sections from *him*-5 and *him*-7 (Tables 1, 2). One pachytene nucleus from each of three different worms from each strain was reconstructed. In addition, seven complete pachytene nuclei were inspected (but not photographed) in the electron microscope in order to determine the number of DRRs. From the 10 nuclei analysed from each strain, the number of DRRs observed in *him*-7 was either 3 or 4 while in *him*-5 it was either 2 or 3, with an average number of DRRs per nucleus for *him*-5 was 2·5 and for *him*-7 it was 3·5 (which differ slightly from Tables 1, 2, which depict a sample size of only three nuclei).

RESULTS

The structure of the synaptonemal complexes (SC) observed in the pachytene nuclei of *him*-5 and *him*-7 mutants of *C. elegans* are identical to the SCs found in the wild-type, i.e. the SC is a tripartite structure composed of two lateral elements and a striated central element (Goldstein & Slaton, 1982; Goldstein, 1982). The six SCs can be followed along the entire length of the bivalents (Tables 1, 2; Fig. 1A,B) and the lengths of individual SCs range from 2·5 to 14·2 μm in *him*-5 and 3·1 to 14·1 μm in *him*-7. The total karyotype length in *him*-5 was 43 μm and was similar to wild-type while in *him*-7 the length was 57 μm, which is 50% greater than wild-type. There was good reproducibility of these values in the three nuclei reconstructed from *him*-5 and *him*-7, as shown in Figs 3A,B and 4A,B. In common with other strains of *C. elegans* analysed, the XX bivalent (SC no. 1) maintains a standard relative percentage length of the total karyotype (6%) and pairs synchronously with the autosomes during

Table 1. *Pachytene karyotype analysis of the synaptonemal complexes of the* him-7 *hermaphrodite of* C. elegans

SC	Nucleus no. 1 (L)*	(%)	Nucleus no. 2 (L)	(%)	Nucleus no. 3 (L)	(%)	Average (L)	(%)
1	3·1	5·5	3·6	6·1	3·4	6·1	3·4	6·0
2	9·2	16·2	6·4†	10·9	6·7	12·1	7·4	13·0
3	9·7†	17·1	8·5	14·5	10·1†	18·2	9·4	16·5
4	10·5†	18·5	12·3†	20·9	10·7†	19·3	11·2	19·6
5	11·0†‡	19·4	13·9†‡	23·6	11·8†‡	21·3	12·2	21·4
6	13·1	23·1	14·1†	23·9	12·7†	22·9	13·3	23·4
Total	**56·6**		**58·8**		**55·4**		**56·9**	
Nucleus vol. (μm^3)	20·0		24·2		25·3		23·1	
Nucleolus vol. (μm^3)	4·4		4·6		4·8		4·6	
% vol. nucleolus	22·0		19·0		18·9		19·9	
No. of DRR†	3·0		3·0		4·0		3·3	

* Length in μm.
† Disjunction regulator region.
‡ Nucleolar organizer region found on this bivalent.

Table 2. *Pachytene karyotype analysis of the synaptonemal complexes of the* him-5 *hermaphrodite of* C. elegans

SC	Nucleus no. 1 (L)*	(%)	Nucleus no. 2 (L)	(%)	Nucleus no. 3 (L)	(%)	Average (L)	(%)
1	2·8	6·6	2·5	5·5	2·5	6·0	2·6	6·0
2	5·3	12·6	6·3	13·9	4·2	10·1	5·3	12·3
3	5·4	12·9	6·5†	14·3	4·6	11·0	5·5	12·8
4	6·7†	16·0	7·3†	16·1	5·2†‡	12·5	6·4	14·9
5	9·1†‡	21·7	9·7†‡	21·4	11·0‡	26·3	9·9	23·1
6	12·7	30·2	13·0	28·8	14·2	34·1	13·3	30·9
Total	**42·0**		**45·3**		**41·7**		**43·0**	
Nucleus vol. (μm^3)	15·5		14·7		15·4		15·2	
Nucleolus vol. (μm^3)	6·1		6·3		5·9		6·1	
% vol. nucleolus	39·4		42·9		38·3		40·2	
No. of DRR†	2·0		3·0		2·0		2·3	

* Length in μm.
† Disjunction regulator region.
‡ Nucleolar organizer region found on this bivalent.

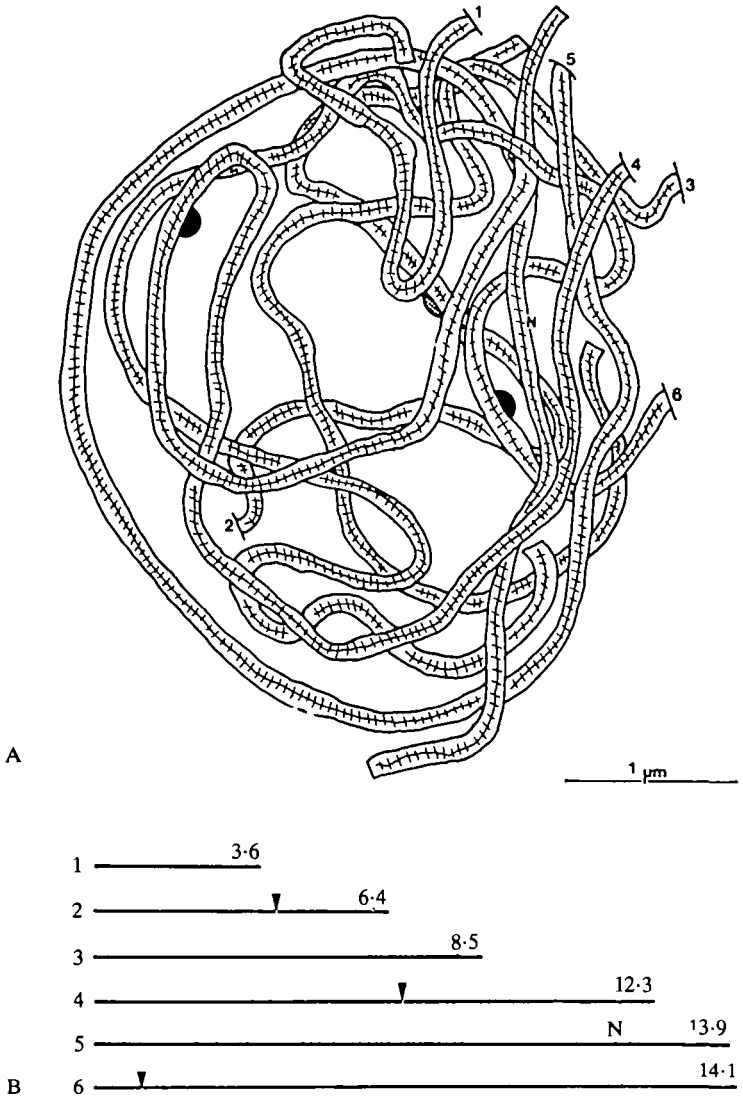


Fig. 1. A,B. Reconstruction from serial sections of the SCs from nucleus no. 2 of the *him*-7 *C. elegans* hermaphrodite. There is no bouquet arrangement of the chromosome ends that are attached to the nuclear envelope. The unattached end of each bivalent is numbered according to its relative SC length, as in B. The nucleolus organizer is labelled N in both figures on SC no. 5. B. A karyotype drawing representing the numbered SCs with position of DRRs indicated by arrowheads. A: bar, 1 μm.

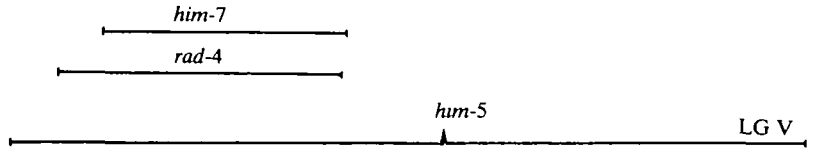
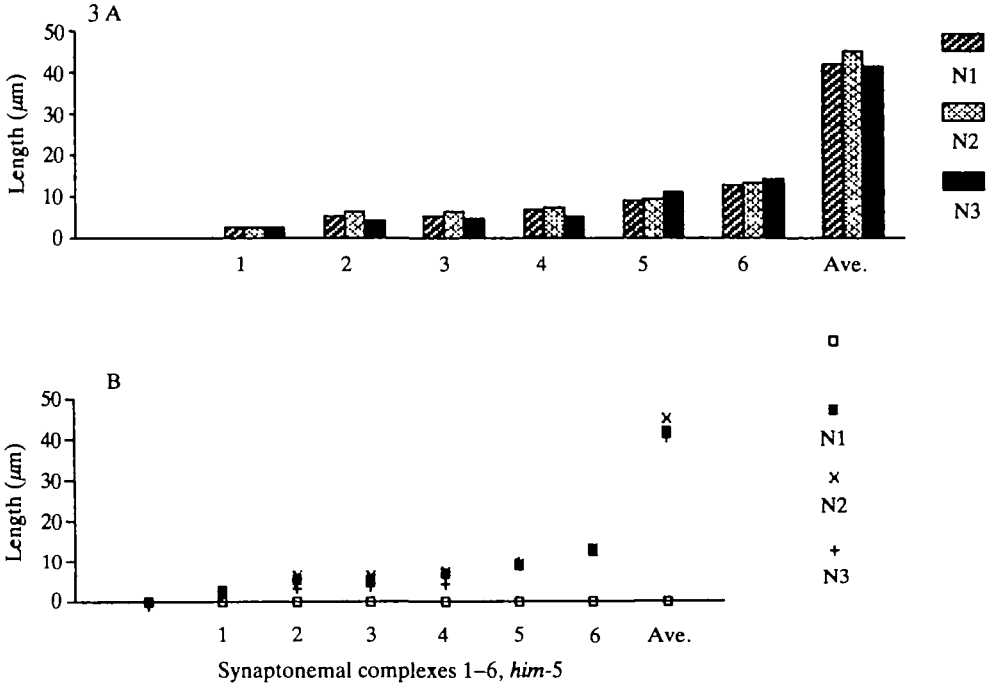
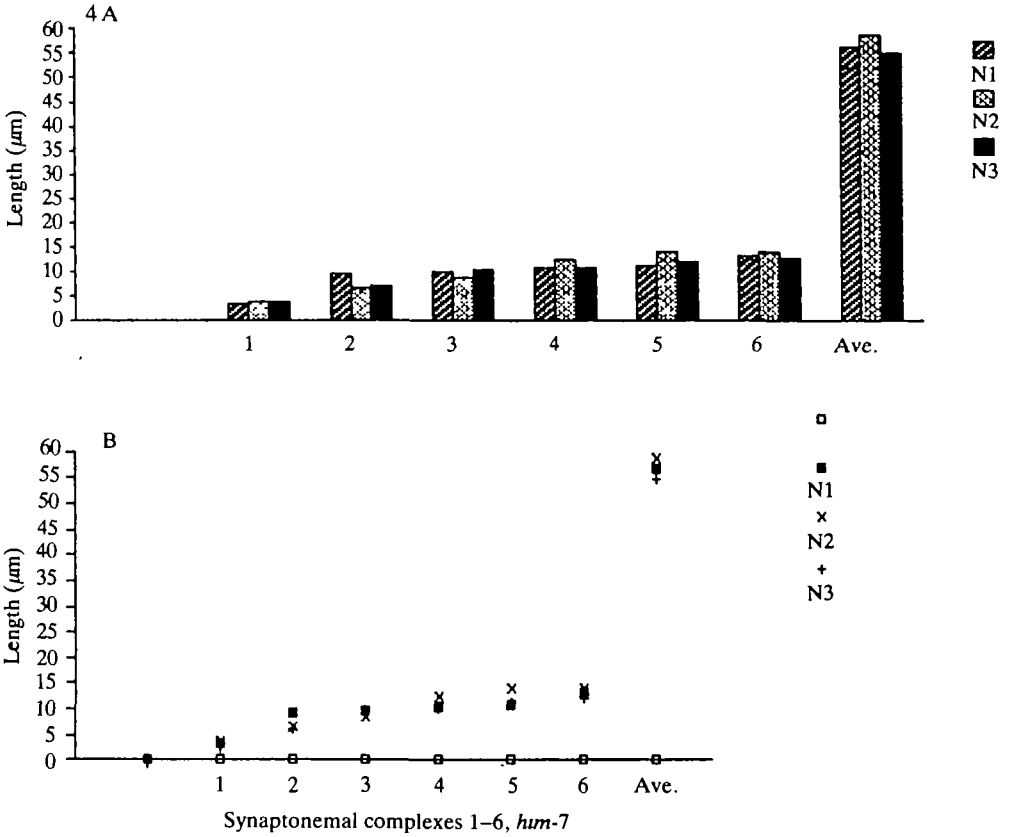


Fig. 2. The partial genetic map of linkage group V (LG V) of *C. elegans* depicting the relative positions of the genetic markers used in the present study.

Figs 3, 4. Reconstruction data from *him*-5 (Fig. 3) and *him*-7 (Fig. 4) nuclei, respectively, are highly reproducible, as depicted in these graphs of data from three nuclei in each strain (from Tables 1, 2). Figs 3A, 4A: a comparison of each SC from each nucleus. Figs 3B, 4B: marked points analysis that shows overlapping of SC values.



Synaptonemal complexes 1–6, *him*-5



Synaptonemal complexes 1–6, *hum*-7

pachytene (Goldstein, 1982). Also, the SCs are randomly attached at only one end to the nuclear envelope (NE) while the other end is free in the nucleoplasm (Fig. 1A). Since attachment of the ends of SCs is random, there is no bouquet formation at pachytene, which is different from many other organisms but similar to the situation found in other nematodes (Goldstein, 1981). Unpaired axial cores cannot be observed at zygotene (Goldstein, 1981), thus the possibility of bouquet formation at that stage cannot be answered. Only two of the SCs could be consistently identified in different nuclei: (1) the XX bivalent, which is SC no. 1 in *him*-5 and *him*-7, comprises 6·0 % of the total karyotype and is similar to many other strains in which the XX bivalent has been identified (Goldstein, 1984*a*); (2) the bivalent carrying the nucleolar organizer region (NOR) was in both strains on SC no. 5 and, in five out of six nuclei reconstructed, the NOR was also associated with a disjunction regulator region (DRR) (Tables 1, 2). The NOR was near the end of the SC (85 %) that was attached to the NE. In *him*-7, one NOR was at 87 % from the attached end of the SC (Fig. 1B) while the other NOR was at 18 % from the unattached end. This ability of either end of the SC to attach to the NE has been observed in other nematodes (Goldstein, 1981, 1982).

An SC-associated structure, the disjunction regulator region, has been previously described in other strains of *C. elegans* (Goldstein, 1984*a,b,c*, 1985*a,b*) and is also present on the SCs of *him*-5 and *him*-7. There was an average of 2·3 DRRs in *him*-5 while in *him*-7 there were 3·3 such structures present in the pachytene nuclei (Tables 1, 2). These values correlate with a 16 % frequency of X-chromosome non-disjunction in the former and a value of 3 % in the latter (Fig. 5A,B).

Each *him*-5 nucleus has a large nucleolus that occupies 40 % of the nuclear volume (Table 1) and this is similar to the value for the wild-type (Goldstein & Slaton, 1982). The relative nucleolar volume (20 %) in *him*-7 is only half that of the wild-type, which is a result of the doubling in nuclear volume compared with the wild-type. In addition, nucleonemata are characteristically observed in *him*-7 but rarely present in any other *C. elegans* strain.

DISCUSSION

Alterations in chromosome structure may result in alteration in SC structure owing to the association of non-homologous regions. Such alterations include changes in lateral (LE) (Zickler & Sage, 1981) and central element (CE) structure and SC formation (e.g. in non-homologous regions the CE is not formed although adjacent homologous regions have normal CE; Goldstein & Triantaphyllou, 1980). It is the initial specificity requirement of pairing regions that regulates the assembly of the SC (Moens, 1973). However, a second-phase pairing at later stages of pachytene has been described (Rasmussen & Holm, 1980; Moses & Poorman, 1981) whereby SC formation is possible even in non-homologous regions and it is this non-specificity that accounts for reports of SC formation in haploid organisms (Gillies, 1974). Thus, Moses & Poorman (1981) describe a process called 'synaptic adjustment' that accounts for the integration of inversion and duplication loops (that are

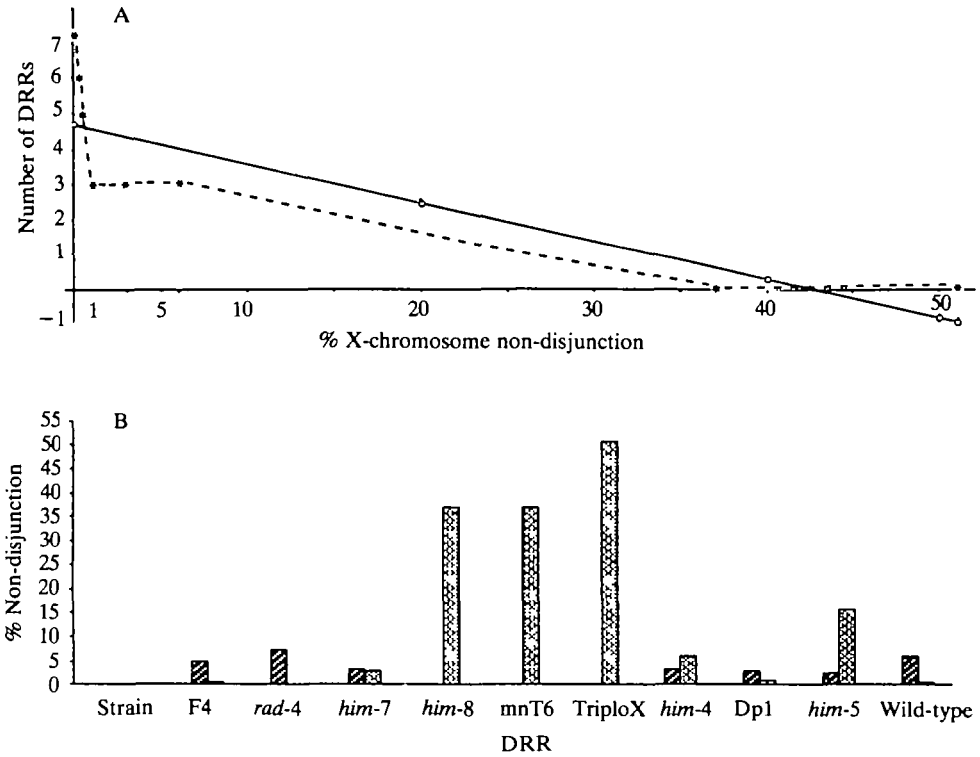


Fig. 5. A. Linear regression analysis (continuous line) and least means square of data from Goldstein (1985*a*). The asterisks represent the real data points. The coefficient of determination is 75 %, the coefficient of correlation is 86 % and $P < 0{\cdot}01$. B. The % X-chromosome non-disjunction is plotted against the occurrence of DRRs. In *him*-8, another recessive *him* mutant, there is 37 % X-chromosome non-disjunction correlated with zero DRRs, while in the wild-type there is 0·2 % X-chromosome non-disjunction correlated with six DRRs.

initially void of SC) into the chromosomal axes and permits formation of SCs even along extended lengths of the bivalent that are non-homologous (or heterologous).

Studies in nematodes have shown that alteration in SC structure following a duplication may be recognized even in a situation where the entire SC structure has been reduced to a bipartite form as a result of the absence of the CE (Goldstein & Triantaphyllou, 1978). However, a duplication mutant (Dp1) in *C. elegans* was analysed (Goldstein, 1985*a*) and the expected duplication loop was not observed after the SC karyotype was characterized, possibly because the duplication loop was only 1 μm in length and the differences in the chromosomal axes may have been accommodated by twisting the extra lengths of the lateral elements. Apparently, pachytene proceeds very quickly in *C. elegans* (Goldstein, 1985*b*) and the process of synaptic adjustment cannot be viewed in action but the results of the process are seen. Thus, aberrations in the bivalent may not be manifested as changes in SC structure. Synaptic adjustment also occurs in yeast whereby a duplication could not be visualized (Moens & Ashton, 1985) and in the mouse insertion Is (7;1)40 H so

that quadrivalent formation was eliminated (Mahadevaiah *et al.* 1984). In the present study, the actual position of *him*-7 on the SC and its nested relationship within *rad*-4 could not be delineated since all regions along the SCs appeared normal. This may be the result of synaptic adjustment.

Changes in the pachytene nucleus may be indicative of the range of metabolic problems inherent to *him*-7, e.g. the pachytene karyotype is 50% longer than in the wild-type, the nucleus has a greater volume and the nucleolus has a volume half that of the wild-type (Table 1). The *him*-7 mutant also affects the entire chromosomal complement so that inviable zygotes are produced at a frequency of 34% due to autosomal non-disjunction and males arise at a frequency of 3% after X-chromosome non-disjunction (Hodgkin *et al.* 1979). However, the frequency of X-chromosome non-disjunction is independent of the cause of autosomal non-disjunction because it is specifically influenced by the presence of disjunction regulator regions.

The biological modulator produced by the DRR facilitates regular disjunction of the X chromosome and statistical analysis of results from 85 nuclei (from 10 different strains) (Fig. 5A,B) shows a good correlation between the frequency of occurrence of X-chromosome non-disjunction and the number of DRRs (Goldstein, 1984*a,b,c*, 1985*a,b*). In *him*-7, there was an average of 3·3 such structures per pachytene nucleus, which is consistent with a frequency of 3% X-chromosome non-disjunction. Thus, the separate control of the X chromosome during meiosis, as predicted by Hodgkin *et al.* (1979), is not affected by the high rate of autosomal non-disjunction that is characteristic of *him*-7.

The pachytene nucleus of the *him*-5 mutant is similar to that of the wild-type in nuclear and nucleolar volume, and SC karyotype length. Autosomal non-disjunction occurs at the same rate as X-chromosome non-disjunction (Hodgkin *et al.* 1979); so, in *him*-5 the point mutation responsible for the *him* phenotype, may affect the segregational properties of all the chromosomes. However, the presence of 2·3 DRRs per pachytene nucleus was consistent with a 16% frequency of X-chromosome non-disjunction (Goldstein, 1984*b,c*), which still suggests separate control of the X chromosome and that the similar value obtained for the rate of autosomal non-disjunction is coincidental. With increasing age, regulatory mechanisms are different in *him*-5 than most other *C. elegans* strains, so that the rate of X-chromosome non-disjunction decreases instead of increasing (Hodgkin *et al.* 1979). This has been supported by the observation of an increase in the number of DRRs in aged *him*-5 individuals, which is consistent with a decrease in the rate of X-chromosome non-disjunction (Goldstein & Curis, unpublished data).

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