Cytogenetic Analysis of Segregation Distortion in *Drosophila melanogaster*: The Cytological Organization of the Responder (Rsp) Locus

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ABSTRACT

The segregation distortion phenomenon occurs in *Drosophila melanogaster* males carrying an SD second chromosome and an SD* homolog. In such males the SD chromosome is transmitted to the progeny more frequently than the expected 50% because of an abnormal differentiation of the SD*-bearing sperms. Three major loci are involved in this phenomenon: SD and Rsp, associated with the SD and SD* chromosome, respectively, and E(SD). In the present work we performed a cytogenetic analysis of the Rsp locus which was known to map to the centromeric heterochromatin of the second chromosome. Hoechst- and N-banding techniques were used to characterize chromosomes carrying Responder insensitive (Rspi), Responder sensitive (Rsp+) and Responder supersensitive (Rspi+) alleles. Our results locate the Rsp locus to the h39 region of 2R heterochromatin. This region is a Hoechst-bright, N-banding negative heterochromatic block adjacent to the centromere. Quantitative variations of the h39 region were observed. The degree of sensitivity to Sd was found to be directly correlated with the physical size of that region, demonstrating that the Rsp locus is composed of repeated DNA.

SEGREGATION distortion in *Drosophila melanogaster* is a typical case of meiotic drive. This phenomenon occurs in males heterozygous for an SD (Segregation Distorter) second chromosome and an SD* homolog. The offspring of these males are almost exclusively composed of individuals which received an SD chromosome from their fathers (Sandler, Hiraizumi and Sandler 1959). This is the consequence of the dysfunction of SD*-bearing sperms (Hartl, Hiraizumi and Crow 1967) and is associated with an abnormal chromatin condensation of sperm and generally aberrant spermiogenesis (Nicoletti 1968; Peacock, Tokuyasu and Hardy 1972; Tokuyasu, Peacock and Hardy 1977). Genetically the distorted segregation is due to the interaction between three major genetic elements: Sd (Segregation distorter), whose presence confers the distorting property to the SD chromosome, Rsp (Responder), responsible for the sensitivity of the SD* chromosome and E(SD) (Enhancer of SD) (Ganetzky 1977; Brittnacher and Ganetzky 1983; Sharp, Hilliker and Holm 1985), which appear to have a drive ability independent of Sd (Sharp, Hilliker and Holm 1985). SD maps to the region 37D2-6 of the salivary gland chromosome map (Brittnacher and Ganetzky 1983), Rsp is located in the centric heterochromatin of 2R, proximal to all the known heterochromatic loci (Ganetzky 1977; Hiraizumi 1981; Hiraizumi, Martin and Eckstrand 1980; Sharp, Hilliker and Holm 1985; Brittnacher and Ganetzky 1988) and E(SD) lies in the centric heterochromatin of 2L (Brittnacher and Ganetzky 1983; Sharp, Hilliker and Holm 1985).

In addition, different allelic states of Rsp are known which show different degrees of sensitivity to the Sd locus (Martin and Hiraizumi 1979; Hiraizumi and Thomas 1984; Temin and Marthas 1984; Lyttle, Brittnacher and Ganetzky 1986). Finally, Ganetzky (1977) suggests that both Sd and Rsp loci may be dispensable functions. (For a more complete view of the topic, see Hartl and Hiraizumi 1976; Crow 1979; Sandler and Golic 1985.)

One of the problems in studying the segregation distortion phenomenon is that the Rsp locus is not easily approachable by conventional genetic analysis because of its heterochromatic location. Heterochromatin does not undergo meiotic recombination. Furthermore, since the bulk of heterochromatin is included in the chromocenter, cytogenetic analysis of this material by polytene chromosome analysis is restricted to the more distal segments of β-heterochromatin. Early cytological observations of aceto-orcein-stained neuroblast chromosomes have permitted the study of some heterochromatic functions of *Drosophila melanogaster*. Recently, high resolution chromosome banding techniques such as Hoechst (Gatti, Pimpinelli and Santini 1976; Gatti and Pimpinelli 1983; Pimpinelli et al. 1985) and N-banding (Pimpinelli, Santini and Gatti 1976) have made it possible

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We dedicate this work to the memory of Larry Sandler who was one of the founders of this area of genetics, a source of intellectual inspiration and our unforgettable friend.

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to differentiate mitotic heterochromatin of *Drosophila* into several regions with different cytological features. That has permitted the elaboration of a cytogenetic map of mitotic heterochromatin (S. Pimpinelli, S. Bonaccorsi, P. Dimitri and M. Gatti, unpublished data) comparable to the polytene chromosome map of euchromatin. The cytogenetic analysis of heterochromatin by chromosome rearrangements has yielded significant insight into the physical size and location of several heterochromatic loci (Gatti and Pimpinelli 1983; Pimpinelli et al. 1985; reviewed by Pimpinelli et al. 1986). In the present work we have studied the cytogenetic organization of the *Rsp* locus using the above-mentioned approach, in order to obtain some insight into its nature.

To this aim, chromosomes, from natural and laboratory populations, which show different sensitivities to *Sd*, were analyzed by banding techniques. The results show that the *Rsp* locus does correspond to a specific heterochromatic region with peculiar cytological features. Moreover, the extent of the *Rsp* sensitivity appears to be related to the physical size of this region.

**MATERIALS AND METHODS**

**Chromosomes:** All the genetic markers are described in Lindsley and Grell (1968).

*cn bw* is the standard *Sd* "*Rsp*" second chromosome.

*Tokyo-bw* is a *Sd* "*Rsp*" chromosomes, constructed by recombination between *Tokyo* and *cn bw* chromosomes (Hiraiumi, Martin and Eckstrand 1980).

*SD-Roma* was collected in Roma, Italy, by Nicoletti and Trif.springa (1967).

*SD-72* and *SD-5* were collected in Madison, Wisconsin, by Sandler, Hiraiumi and Sandler (1959).

*SD-NH2* was collected in Japan.

Wild *SD* chromosomes were collected and characterized for sensitivity to *Sd* by Temin and Marthas (1984). The *k* values are shown in Table 1. The *k* value of some of these chromosomes were also measured with the *B10-4* test (Lyttle, Brittnacher and Ganetzy 1986), a test which is specific for variation at the *Rsp* locus. Other chromosomes will be described in the text.

**Nomenclature:** The cytological analysis was performed on larval neuroblast chromosomes using sequential staining with the fluorochrome Hoechst 33258 and N-banding techniques (Gatti, Pimpinelli and Santini 1976; Pimpinelli, Santini and Gatti 1976; Gatti and Pimpinelli 1983) as subsequently described. The nomenclature of the heterochromatic regions of the second chromosome was elaborated according to the work of Gatti and Pimpinelli (1983) and is referred to in the cytogenetic map of *Drosophila melanogaster* mitotic heterochromatin (S. Pimpinelli, S. Bonaccorsi, P. Dimitri and M. Gatti, unpublished data).

Using the second chromosome of the Oregon-R strain, 12 different heterochromatic regions are resolved by sequential Hoechst- and N-banding procedures. These regions are designated h35 to h46. The *cn bw* chromosome that has been used to synthesize *Rsp* deficiencies and duplications shows an additional region in 2Rh with respect to the second chromosome of the Oregon-R strain. This region was designated 42B (Figure 5).

**Mitotic chromosome preparation:** Neutral ganglia of third-instar larvae were dissected in saline (0.7% NaCl) and transferred to a hypotonic solution (0.5% sodium citrate) for 10 min at room temperature. After 10–20 s of fixation in a fresh mixture of acetic acid, methanol and deionated *H*2O (5:5:5:5:1), ganglia were placed into a small drop (3–4 µl) of 45% acetic acid on siliconized 22 mm2 coverslip and then squashed after lowering a slide onto the coverslip. After freezing the slide on dry ice, the coverslip was removed with a razor blade and slides were air dried at room temperature.

**Hoechst staining:** The slides were rehydrated, stained with Hoechst 33258 (0.5 µg/ml) for 10 min and rapidly washed. Rehydration, staining and washing procedures were performed using 0.15 M NaCl-0.03 M KCl-0.01 M Na phosphate buffer (pH 7). The slides were air dried and then mounted in 0.16 M Na phosphate-0.04 M Na citrate (pH 7). Hoechst banding was examined under a Zeiss fluorescence microscope equipped with 200 W mercury light source for incident illumination. Fluorescence was observed using UG5+BG3 excitation filters, FT460 dichroic mirror and LP475 barrier filter (ultraviolet-violet excitation range).

**N-banding:** The slides were incubated in 1 M NaH2PO4 buffer (pH 4–4.2) for 15 min at 87°, washed in water and stained for 20 min in 1:25 dilution of Giemsa (Merck) in PBS (pH 7). After air drying at room temperature the slides were mounted in euparal and viewed using phase contrast Zeiss microscopy.

**Microphotography:** All microphotographs were taken with Kodak Pan X film. To compare Hoechst- and N-banding staining sequentially performed on the same prometaphase chromosomes, photomicrographs taken after each step were printed both on normal paper (Ilfochrom) and on transparent Kodalith onto film. Transparent prints were overlaid on the normal ones and the different staining exactly compared.

**RESULTS**

A Hoechst 33258 stained metaphase spread containing the standard *cn bw* *Rsp* second chromosome is shown in Figure 1a. In Figure 1b the *cn bw* *Rsp* chromosome sequentially stained with H33258 and N-banding techniques is shown. With H33258 the heterochromatin of this chromosome shows a banding pattern with several regions characterized by different degrees of fluorescence. When stained by the N-banding technique, the same heterochromatin shows five darkly stained regions: one of these corresponds to the centromeric region, another is located in the left arm and three are in the right arm (Figure 1b). Thus, by the combination of Hoechst- and N-banding techniques, a map on the *cn bw* chromosome heterochromatin was obtained which includes 13 segments, h35–h46, as diagrammed in Figure 5a.

**Cytogenetic organization of the *Rsp* locus:** To map the *Rsp* locus, we characterized cytologically two different heterochromatic rearrangements, both of which have been induced by X-rays on the standard *Rsp* *cn bw* chromosome. One of the two, (c70), is a heterochromatic free duplication (Figure 2a) synthe-
sized from the $ln(2LR)\,lt^{e70}\,Rsp'$ $cn\,bw$ chromosome which retains full sensitivity to the $Sd$ action (BRITTNACHER and GANETZKY 1988). The other one, ($Rsp'^{a}$), is a homozygous viable and fertile chromosome (Figure 2b) which is insensitive to $Sd$ (GANETZKY 1977). Comparing the cytology of both rearrangements to that of the $cn\,bw$ chromosome, it is clear that $Rsp'^{a}$ carries a complete deficiency of a very proximal, brightly Hoechst-fluorescent block of $2R$ heterochromatin ($h39$) (Figures 2b and 5d), which is still present in the free duplication $e70$. Thus, the cytological analysis of two different rearrangements induced on the $cn\,bw$ chromosome allows us to conclude that the $Rsp$ locus maps to a specific Hoechst-bright, N-banding-negative region of $2Rh$ close to the centromere. This is designated the $h39$ region on the $cn\,bw$ chromosome heterochromatin map (Figure 5a).

To get more information about the organization of the $Rsp$ locus, we analyzed several $SD\,Rsp'$ chromosomes and also the standard $Rsp'$ $Cy$ chromosome (HARTL 1975). This analysis demonstrated that all the $Rsp'$ chromosomes lack the $h39$ region (Figures 3 and 5d). This established a strong correlation between the phenotype of insensitivity and the absence of $h39$. These data gain a particular significance when coupled with the cytology of the $Rsp''$ Tokyo-$bw$ chromosome.

FIGURE 1.—Fluorescence and sequential N-banding pattern of the standard $cn\,bw\,Rsp'$ chromosome. a. Example of $h33258$-stained metaphase of $D.\,melanogaster$ male with numbered autosome pairs. b, the standard $cn\,bw\,Rsp'$ second chromosome sequentially stained with $h33258$ (above) and N-banding (below). The numbers indicate the different heterochromatic regions which are diagrammed in Figure 5. $X = X$ chromosome; $Y = Y$ chromosome; $c =$ centromere; $2L$ and $2R =$ second chromosome left arm and right arm, respectively.

FIGURE 2.—Fluorescence and sequential N-banding pattern of $Dp(2;F)\,e70$ and $Df(2R)\,Rsp'^{a}$. a. The free duplication $e70$ (see also insert) and the $cn\,bw\,Rsp'$ from which the duplication has been synthesized. The arrows mark the $Rsp$ region ($h39$). b. The $Rsp'^{a}$ chromosome derived from the standard $cn\,bw$ chromosome. The $Rsp'$ chromosome lacks the $h39$ region; as a consequence, the centromeric N-band ($h38$) and the most proximal one ($h40$) on the right arm are collapsed.

Some in Tokyo-$bw/cn\,bw$ individuals (Figure 4). Tokyo-$bw$ carries of heterochromatic inversion $ln(2LR)\,h37-38;\,h40-41$. In addition Tokyo-$bw$ does show a $Rsp$ region ($h39$), which is larger than that present in the $cn\,bw$ sensitive chromosome (see diagram in Figure 5b). Therefore, the sensitivity of $Rsp$ to $Sd$ appears to be related to the size of the $h39$ region and the insensitivity to the absence of this region.

This notion is supported by our analysis of several chromosomes, recently collected from natural populations (TEMIN and MARTHAS 1984), which show allelic variation at the $Rsp$ locus (Table 1). In the TEMIN and MARTHAS study a continuum of sensitivity was observed. A chromosome was genetically defined as $Sd^*\,Rsp'$ if it was sensitive to three different $SD$ chromosomes and with a $k < 0.65$ in a test with the suicide chromosome $R(cn)10$, in $Sd\,Rsp'/Sd^*\,Rsp'$ males. If was defined as $Sd^*\,Rsp''$ if highly sensitive to the 3 $SD$ chromosomes and if it was distorted by the suicide chromosome, such that $k \geq 0.65$, for $Sd\,Rsp'/Sd^*\,Rsp'$ males. It was defined as $Sd^*\,Rsp''$ if it was insensitive to the $SD$ testers and if it caused suicide behavior (self distortion), such that $k < 0.40$, approximately. A more refined delineation of some of these chromosomes was obtained using the $B10-4$ test (LYTLE, BRITTNACHER and GANETZKY 1986), which is specific for variation at the $Rsp$ locus itself.

The degree of sensitivity (Table 1) and the banding pattern of the chromosomes (Figure 5) were classified independently in double blind experiments and were found to be strongly correlated.

Three chromosomes ($C171,\,C55$ and $C115$) show a heterochromatic banding pattern similar to that of $Rsp'\,cn\,bw$. According to TEMIN and MARTHAS (1984),
C115 is Rsp', while C55, C171 chromosomes appear to be borderline between Rsp'' and Rsp' categories. However, with the B10-4 test, Lyttle, Brittnacher and Ganetzky (1986) classified C55, C115 and C171 chromosomes as Rsp'. Five chromosomes (M107, M65, C38, M10 and MM27), which are Rsp'', show a h39 region which is larger than that present in the cn bw chromosome and are cytologically identical to Tokyo-bw. One of these (M107) has been classified as Rsp'' by Lyttle, Brittnacher and Ganetzky (1986). The most probable explanation for this result is that M107 carries some genetic modifier(s) of segregation distortion, which maps to chromosome sites different from Rsp'. We also found that three Rsp'' chromosomes, MM33, MM204 and MM153 (Tables 1 and 2), recently collected from another natural population (R. Temin and M. Marthas, personal communication), have a very large h39 region but lack the inversion (Figures 5c and 7). This argues strongly against the possibility that the inversion per se was necessary for the initial generation of the supersensitive chromosomes and/or the maintenance of the integrity of the Rsp'' region. SD*Rsp' chromosomes isolated from natural populations (C25-3, C111, MM113 and C182) show a normal N-banding pattern. However, they contain, in the same location of the h39 region, a heterochromatic block as large as h39, which differs in that it is dully fluorescent (Figures 5e and 6). The extensive cytogenetic analysis of the Y chromosome (Gatti and Pimpinelli 1983) has shown that rearrangements that
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TABLE 1
k values for Sd Rsps/wild SD+ and Sd Rsps/wild SD* males
(TEMIN and MARTHAS 1984)*

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>SD-5 bw</th>
<th>SD-Roma</th>
<th>R(cn)10</th>
<th>R-1 bw</th>
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</thead>
<tbody>
<tr>
<td>M65</td>
<td>1.00</td>
<td>0.99</td>
<td>0.83</td>
<td>0.96</td>
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<tr>
<td>C38</td>
<td>1.00</td>
<td>0.92</td>
<td>0.80</td>
<td>0.85</td>
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<tr>
<td>M107+</td>
<td>1.00</td>
<td>0.95</td>
<td>0.76</td>
<td>0.86</td>
</tr>
<tr>
<td>M10</td>
<td>0.98</td>
<td>0.66</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>MM27</td>
<td>0.96</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM113</td>
<td>0.95</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM33</td>
<td>0.93</td>
<td>0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM204</td>
<td>0.94</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C171-</td>
<td>0.99</td>
<td>0.97</td>
<td>0.64</td>
<td>0.98</td>
</tr>
<tr>
<td>C35'</td>
<td>1.00</td>
<td>0.95</td>
<td>0.65</td>
<td>0.97</td>
</tr>
<tr>
<td>C115'+</td>
<td>0.97</td>
<td>0.68</td>
<td>0.63</td>
<td>0.72</td>
</tr>
<tr>
<td>MM113</td>
<td>0.56</td>
<td>0.47</td>
<td>0.38</td>
<td>0.47</td>
</tr>
<tr>
<td>C182</td>
<td>0.63</td>
<td>0.49</td>
<td>0.11</td>
<td>0.53</td>
</tr>
<tr>
<td>C25-3</td>
<td>0.83</td>
<td>0.55</td>
<td>0.30</td>
<td>0.46</td>
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<tr>
<td>C111</td>
<td>0.67</td>
<td>0.49</td>
<td>0.39</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* The k values refer to the proportion of progeny with the Sd-bearing homolog from the cross Sd Rsps/wild SD+ or Sd Rsps/wild SD* males × en bw females and have been published by TEMIN and MARTHAS (1984). C and M indicate chromosomes isolated from a natural population in California and in Madison, Wisconsin, respectively.

* Using the B10-4 test, which is specific for variation at the Rsps locus, M107+ appears to be Rsps' (LYTTEL, BRITTNACHER and GANETZKY 1986).

* k values calculated by PIMPINELLI and DIMITRI; the number of progeny and the number of tested males are shown in Table 2.

* k values calculated by R. TEMIN and M. MARTHAS (personal communication).

* Using the B10-4 test, C171, C35 and C115 chromosomes have been classified as Rsps' (LYTTEL, BRITTNACHER and GANETZKY 1986).

break within Hoechst- and N-banded regions do not alter the intensity of staining. Thus, it is unlikely that the decreased fluorescence of h39 of SD+Rsp' chromosomes is due to a partial deficiency. Rather, because H33258 brightly fluorescent chromosome spots correspond to AT-rich regions (GATTI, PIMPINELLI and SANTINI 1976), we suggest that the region present in SD+Rsp' is not as AT rich as the "canonical" h39 and contains instead another type of DNA with different base composition. Therefore, these chromosomes can formally be regarded, genetically and cytologically, as Rsps deleted. In this regard it is worth noting that all the SDRsp' chromosomes invariably show a fusion of the h38 and h40 N-banded regions, indicating the complete deletion of h39. This brings out an interesting difference between the SDRsp' and SD+Rsp' chromosomes. It appears that a complete deficiency of the Rsps region, h39, could be selected by the Sd element in the SD chromosomes. If such selection exists, this suggests the intriguing possibility that the h39 region in SD+Rsp' chromosomes, which does not contain at least a "canonical" Rsps locus, could be important for the generation of Rsps' chromosomes in natural populations.

![Diagram](image_url)

**FIGURE 5.**—The cytogenetic map of different Rsps allele. The en bw chromosome heterochromatin is subdivided in thirteen different regions defined by the H33258 and N-banding staining patterns. The banding patterns of the other Rsps alleles are very similar except for the h39 region which shows variation in size and in fluorescent properties. The dark areas correspond to bright regions; the cross-hatched areas, to moderately fluorescent regions; the open areas, to non-fluorescent regions. The non-fluorescent regions, except for h42A, correspond to the N-bands, a, Sd"Rsps/cn bw-like; b, Sd"Rsps" Tokyo-bw-like; in these chromosomes a pericentric inversion-lm(2LR)h37-38; h40-41 is present; c, Sd"Rsps"; d, Sd Rsps; this class also includes Rsps10 and Sd"Rsps'Cy chromosomes; e, Sd"Rsps'.
DISCUSSION

The nature of the \textit{Rsp} locus: Previous genetic data established two important features of the \textit{Rsp} locus: (1) a heterochromatic location in \textit{2Rh} (GANETZKY 1977; HIRAIZUMI, MARTIN and ECKSTRAND 1980; HIRAIZUMI 1981; SHARP, HILLIKER and HOLM 1985) and (2) the existence of different allelic state (MARTIN and HIRAIZUMI 1979; HIRAIZUMI and THOMAS 1984; TEMIN and MARTHAS 1984; LYTTLE, BRITTNACHER and GANETZKY 1986).

The present results permit a precise cytological mapping and insights into the nature of this peculiar heterochromatic locus. We show that \textit{Rsp} sensitivity correlates with the physical size of h39, a specific heterochromatic region of \textit{2Rh} and that \textit{Rsp} insensitivity corresponds to the absence of the same region. Therefore the \textit{Rsp} locus not only maps to h39 but appears to be coextensive with that region. In this context we want to stress that cytologically the \textit{Rsp}\textsuperscript{i} allelic state is equivalent to the loss of the \textit{Rsp} region, as suggested by the genetic analysis of GANETZKY (1977). Since deletion of the \textit{Rsp} locus does not seem to be associated with any phenotypic effect, other than insensitivity, our data give further support to the hypothesis that \textit{Rsp} could be a dispensable genetic element (GANETZKY 1977). The particular cytogenetic features of the \textit{Rsp} locus that we describe here imply that it is made up of an array of repeated DNA sequences, whose copy number determines the degree of sensitivity to \textit{Sd}. From a qualitative point of view, we do not have any basis for discrimination, at the cytological level, between a moderately or highly repeated class of DNA. However, since the Hoechst staining seems to be a general indicator for AT-rich chromosome regions, the fluorescence brightness of the sensitive h39 region suggests that \textit{Rsp} DNA is rather AT rich. These cytogenetic observations are in agreement with recent genetic and molecular evidences on the nature of \textit{Rsp} LYTTLE (1988) found that the \textit{Rsp} locus can be split, by chromosome rearrangements, into two still sensitive parts and Wu \textit{et al.} (1988) isolated a specific class of AT-rich repetitive DNA whose abundance correlates with \textit{Rsp} sensitivity.

\textbf{On the \textit{Rsp} action:} Prior to discussion on the possible role of the \textit{Rsp} locus in segregation distortion,
we wish to point out the main genetic and physiological aspects of this phenomenon as it is understood so far.

During spermatogenesis in an SD/SD⁺ male the Rsp locus, after an interaction with Sd, becomes a dominant gametic lethal. Lethality is correlated with a failure of the chromatin condensation and individualization of the Rsp-bearing sperms, along with the lack of the normal lysine-rich to arginine-rich histone transition (KETTANEH and HARTL 1976; HAUSHEITZ-JUNGEN and HARTL 1982). It is important to note that spermatogenesis in Drosophila melanogaster takes place in a syncytium and that postmeiotic gene activity is not required in males (MULLER and SETTLES 1927; OLIVIERI 1965; MCCLOSKEY 1966; HENNIC 1967; LINDSLEY and GRELL 1969).

Sd dosage effect on male fertility has been also observed. Males with two different SD chromosomes (KETTANNEH and HARTL 1980) or with two doses of the same Sd allele (BRITTNACHER and GANETZKY 1983) show a reduction in fertility. In such males the reduction in fertility is related to the presence of dysfunctional sperms with an abnormal phenotype similar to that observed in SD/SD⁺ male sperms (PEACOCK, TOKUYASU and HARDY 1972; KETTANEH and HARTL 1980).

Three main models have been put forward to explain segregation distortion. The models of HARDT (1973) and HIRAIUMI, MARTIN and ECKSTRAND (1980) predict a physiological role of Rsp in normal spermogenesis. The third model of GANETZKY (1977) seems to be more consistent with the available experimental data, included the data presented here. This model consists of three main points: (1) Sd and Rsp do not have any normal functions, (2) the Sd product is limited in amount, and (3) the binding of Sd product to Rsp is the event that leads to dysfunction.

The open question is how the binding of Sd product to Rsp leads to sperm dysfunction; that is, how Rsp become a dominant gametic lethal. On the basis of the cytogenetic organization of the Rsp locus described here, we suggest a possible explanation of the role played by Rsp in segregation distortion. Sd might produce a protein or RNA with high affinity for the Rsp region, which per se could directly induce sperm dysfunction, as suggested by the experiments with SD/SD(Rsp) males. Then, the Rsp region binds to the Sd product by some tereiation mechanism and drives the product through the sperm development. This, in turn, would generate a concentration of the Sd product in postmeiotic cells where Rsp is present, causing an asymmetric sperm dysfunction. The quantitative aspects of this interaction would depend on both the amount of Sd product and the number of Rsp-binding sites, i.e., the amount of repetitive Rsp DNA. To complete our view, we want to point out that our hypothesis it is also consistent with the possibility that the Sd product-Rsp DNA complex is indeed responsible for the dysfunction. We stress that one important feature of this model is that it does not require any postmeiotic transcriptional activity. Moreover, it is possible to reconcile some apparently contradictory results previously obtained on the quantitative aspects of the Sd product. On one hand, experiments with males carrying two Rsp alleles with different degrees of sensitivity to Sd show that these loci can be independently influenced by a single Sd as if the Sd product was in excess (LYTTLE, BRITTNACHER and GANETZKY 1986). On the other hand, three different sets of data support the view that the Sd product is made in limited amounts. First, it has been shown that addition of extra copies of Rsp to SD/SD⁺ males results in increase in viability of the Rsp⁻-bearing sperms. This could suggest that the extra copies dilute the Sd effect on each Rsp (LYTTLE, BRITTNACHER and GANETZKY 1986). Second, males carrying an Sd and two Rsp copies do not show any drastic reduction in fertility, as one would expect from the relative sensitivities (LYTTLE, BRITTNACHER and GANETZKY 1986). Third, multiple copies of Sd have an additive effect on a single Rsp target (LYTTLE 1986).

The present model reconciles all these results on the basis of a limited Sd product amount by assuming that, when two different Rsp alleles are both present in Sd males, there is a differential distribution of the Sd product which is proportional to the relative size of the two Rsp loci; this would result in a proportional increase in the survival probability of the Rsp-bearing sperms and consequently also in general dilution of the Sd effect.

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