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Constructing balancer chromosomes for genetic screens in *Drosophila hydei*

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Summary. We used a screen for maternally generated late embryonic lethals as a new method for the isolation of inversions that are suitable for the balancing of mutations in *Drosophila hydei*. The recovery of several inversions by this method demonstrates that female meiosis in *D. hydei* apparently differs from meiosis in female *D. melanogaster*, since in *D. hydei* the defective chromosomes which are generated by a single crossing-over within a paracentric inversion can be recovered via the egg nucleus. In addition, the classic method of crossing-over suppression was used in order to isolate more inversions and to improve the balancing capacities of inversions. We succeeded in constructing chromosomes that allow the balancing of mutations on nearly the whole genome of *D. hydei*. We discuss here whether or not this method is suited for application to other organisms.

Key words: *Drosophila* – Balancers – Inversions – Translocations – Meiosis

Introduction

Mutants are extremely useful in analyzing development and differentiation. Therefore, species that allow genetic probing of developmental processes such as *Arabidopsis thaliana* (Meyerowitz 1989), *Caenorhabditis elegans*, *Drosophila melanogaster*, or *Mus musculus* (Wilkins 1986) are the favorite objects of such studies. However, sometimes a species without these benefits provides substantial advantages for studying morphogenesis and cellular differentiation. In such cases it might be a reasonable investment to develop the tools needed for a genetic approach. Balancer chromosomes are needed for the recovery and maintainance of mutations without "visible" phenotypes, such as lethal and sterile mutations, but their construction is not always achieved by simple and rapid approaches. Recently, genetic tools have been created for *Caenorhabditis elegans* (Brenner 1974), and currently, efforts are undertaken to achieve the same for the zebra fish *Brachydanio rerio* (Kimmel 1989).

The Y chromosome of *Drosophila* is of central importance for spermatogenesis (Hackstein 1987, 1991) and, in addition, it forms giant lampbrush loops in the nuclei of primary spermatocytes. These lampbrush loops were discovered in *D. melanogaster* (Meyer et al. 1961), but the morphology of such loops in *Drosophila hydei* – a species without elaborated genetics – was so favorable that successful studies on the genetics of the Y chromosome were initiated (Hess 1965, 1967; Hackstein et al. 1982, 1991).

The peculiar morphology of the different lampbrush loops offered the chance to recover genes that regulate the expression of Y chromosomal fertility genes. A first screen for such mutations on the X chromosome of *D. hydei* was undertaken by Lifschytz (1974, 1975). However, systematic screens for such genes on the whole genome could not be effected because of the lack of suitable balancer chromosomes and marker genes. Therefore, we decided to explore the possibilities for the construction of inverted chromosomes in *D. hydei* that permit the balancing of lethal or sterile mutations.

A limited number of mutants with morphological deviations was available from earlier genetic studies (Spencer 1949; Gloor 1971), while others were recovered incidentially in the course of our genetic screens for Y chromosomal mutations. However, the number of useful marker genes on the different chromosomes was low and, consequently, the screening for crossing-over suppressors promised only limited success (cf. Roberts 1976).

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Therefore, we tested an alternative method which does not require multiple marker genes on each of the chromosomes.

Materials and methods

Drosophila hydei flies were raised as described earlier (Hackstein et al. 1982). The wild-type stock *Tübingen* was used, and in addition, the following mutants: Recessive mutations (Gloor 1971): *st "scarlet"*: eye color mutation on chromosome 2; thin layer chromatography of extracts of flies reveals that it is not homologous to the *D. melanogaster st*, rather it is homologous to *cd*. The true *st* homologue is on chromosome 4, called "*cn*".
px plexus: defective wing veins, chromosome 3
ht heart: thorax heart-shaped, chromosome 4
sca scabrous: rough eye, on the chromosome 5; most likely homologous to the *D. melanogaster mutation*
ss^{Anp} *spineless Antennapedia*: homeotic mutation proximal on chromosome 2 (Gloor and Kobel (1966)
Dominant mutations, homozygous lethal; if not otherwise stated, recovered by Hackstein in earlier screens:
Sm Scutum abnormal, most likely allelic to *Sp* (Gloor and Kobel 1966); chromosome 2
Al Aristaless: arista reduced in size, chromosome 3
D Dichaete-like: wings held out, chromosome 3
Apt Apterous: wingless, homozygous viable, chromosome 3
Th Thorax dark colored: black thorax, chromosome 4
N^{IV} *Notch-4*: wing margins notched or indented, chromosome 4
Cl Clipped: distal wing margin notched; chromosome 4
Stw Straw: yellowish pigmentation of the body, homozygous viable; chromosome 5 (Gloor 1971)
Z zebra: light pigmented patches on thorax. Exibits position effect variegation although the breakpoints are in the euchromatin (cf van Breugel 1988); chromosome 5

The homozygous lethal dominant mutations were used to balance the inversions. Alternatively, newly induced lethal or male-sterile mutations were used for balancing.

Aged (fertile) males were irradiated with 9,500 or 12,500 rads of X-rays, respectively (for details see Hackstein et al. 1982).

Late third instar larvae were used to make squash preparations of polytene salivary gland chromosomes, in order to identify the breakpoints of chromosomal aberrations. For the preparation of squash preparations see Ashburner (1989).

Eggs that did no hatch within 24 h after oviposition were dechorinated using a commercial sodium hypochloride solution and inspected to determine whether or not they contained segmented, lethal embryos.

Results

The rationale

If a single crossing-over occurs within an inversion carried by a heterozygous female, duplications, deletions, acentric fragments and dicentric chromatids can be generated (Fig. 1). If the defective chromosomes – especially those carrying large deletions – are included into the egg nucleus, embryonic lethality among the progeny can be the consequence. Because in *D. melanogaster* large deletions (in a heterozygous condition) frequently cause lethality at later stages of embryonic development (Garcia-Bellido and Moscoso del Prado 1979), Garcia-Bellido

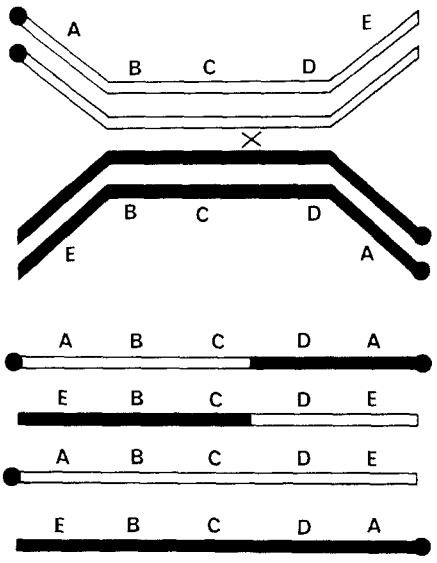

Fig. 1. Crossing-over within a paracentric inversion generates both acentric fragments and dicentric chromatids. After fusion with a "normal" spermatozoon, zygotes are formed that are heterozygous for large deletions or monosomic, respectively

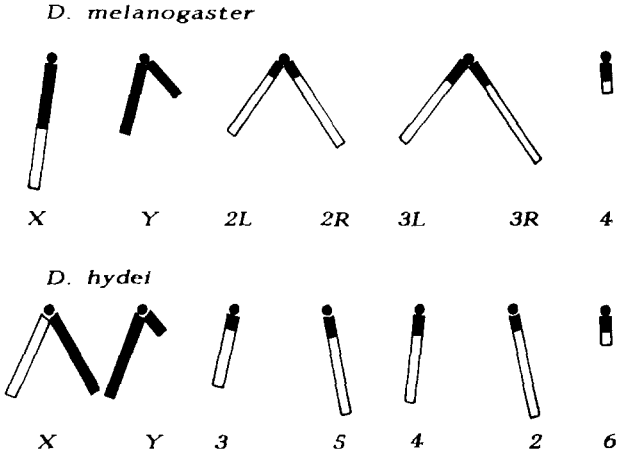

Fig. 2. Schematic drawing of the metaphase chromosomes of *D. hydei* and *D. melanogaster*. Homology is based on the banding pattern of salivary gland polytene chromosomes (Berendes 1963) and the apparent homology between mutations with visible phenotypes (cf. Gloor 1971). The large, metacentric autosomes of *D. melanogaster* can be interpreted as Robertsonian fusions of the acrocentric autosomes 2 to 5 of a common ancestor of both species. However, both species are only distantly related. For the evolution of *D. hydei* see Wasserman (1982). *Open bars* indicate euchromatin, *black bars* heterochromatin. It cannot be overlooked that the "acrocentric" autosomes of *D. hydei* bear a tiny, heterochromatic, short arm

speculated whether or not a screen for maternally generated, late embryonic lethals (LELs) could allow the recovery of inversions – in principle without the need of any marker gene (A. Garcia-Bellido, personal communication). Because crossing-over is absent in male *Drosophila*, only females heterozygous for an inversion should

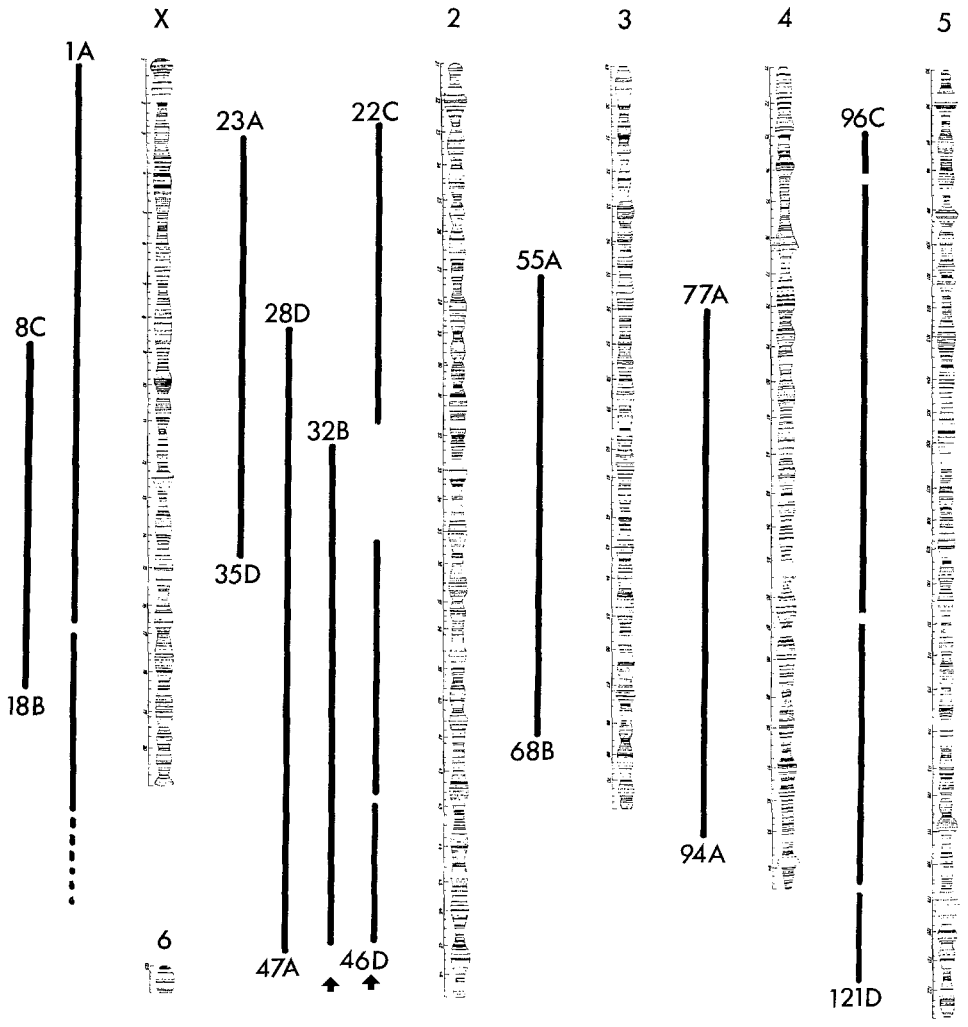

Fig. 3. Chromosomal rearrangements of *D. hydei* that were used to screen for male-sterile mutations (Lifschytz 1974, 1975; Hackstein et al. 1990). *Bars* indicate the inverted chromosomal segments. Chromosome numbers are according to Berendes (1963). Beginning from the *left*, the rearrangements are: *In (1)* f^3, f^3 1A-8B/18B-8C/18C-20D (Gloor 1971); *In (1 LR)* w^{m3}, w^{m3} h^1 NOR/16D3,4-1A h^s. h^1 NOR/17A1,2 NOR (van Breugel 1970); *In (2) Lewontin, Dl* (Gloor 1971) 21A-22D/35D-23A/36A-48C; *In (2) Sb, Sb* 21A-28C/47A-28D/47B-48C; *In (2) Ya e, T(2;5), Ya e, (Yellow abdomen ebony)* 32B-46D inserted at 121D; *In (2) Ya e -1, T(2;5), Ya e* 21A-22B/31C-22C/31D-35C/47B-48C, insertion into chromosome 5 most likely as in the ancestral rearrangement *Ya e. In (3) RA, RA (Rauhes Auge)* 49A-54D/68B-55A/68C-70D; *In (4) Th ht, Th ht* 71A-77A/94A-77B/94BC; *In (5) V10, Stw sca* 95A-96C/121D-118D/110D-118D/98A-110D/98A-96C/121D-122D; *Arrows* indicate the complex rearrangements; translocation to chromosome is not shown

generate late embryonic lethals in their progeny. In contrast, deletions occurring in the progeny of translocation-bearing flies are the results of meiotic segregation and, consequently, both sexes should generate LELs. Therefore, reciprocal crosses should allow the discrimination between inversions and translocations.

However, the autosomes of *Drosophila hydei* are acrocentric (Fig. 2), and therefore only paracentric inversions (i.e., inversions restricted to one chromosome arm, not including the centromere) are expected to be formed after irradiation. In the case of a paracentric inversion, a single crossing-over between the inversion breakpoints generates acentric and dicentric fragments (Fig. 1), which are normally exclosed from the egg nuclei because they are eliminated or deposited in the polar nuclei – at least in *Drosophila melanogaster* (Roberts 1976). Therefore, only if female meiosis in *D. hydei* allows the recovery of monosomic embryos or of the fragments of breaking dicentric chromosomes, can lethal embryos be expected among the progeny of mothers that are heterozygous for a paracentric inversion.

Meiosis in female D. hydei is different from D. melanogaster

We tested the predictions using flies that were heterozygous for the paracentric inversion *In (2) Lewontin, Dl* (cf. Gloor 1971; breakpoints, see Fig. 3). Het-

erozygous females produced embryos with a considerable fraction of LELs (more than 10% of the deposited eggs), while heterozygous males did not produce any LELs (Table 1). In *D. hydei*, such LELs can be easily recognized by the presence of non-hatching eggs which turn brown around their micropyle. Unfertilized eggs from wild-type flies or nullo-X embryos (generated in an attached-X stock) that die early during embryonic development do not become brown. Thus, in contrast to *D. melanogaster*, the products of single crossing-overs within the limits of a paracentric inversion are apparently included into the egg nucleus and – after fertilization by wild-type sperm – embryos develop that die after segmentation.

Screening for matroclinous LELs

Mature wild-type males were irradiated with approximately 12,500 rads of X-rays. Subsequently, these males were crossed with females homozygous for the recessive marker genes *st/st*; *px/px*; *ht/ht*; *sca/sca*, in order to permit the chasing of the irradiated chromosomes carrying a putative inversion. A total of 1,850 F_1 females was individually crossed to homozygous, multiply-marked males. Approximately 850 females produced LELs. In the case of ten strains it was possible to identify the chromosome responsible for the formation of LELs after setting up four lines with only one chromosome each in the heterozygous condition. Heterozygous males were tested for the generation of LEL progeny. A preliminary cytological inspection of the salivary gland chromosomes confirmed the expectations from LEL formation (Table 1). Most useful were inversions with a dominant, visible morphological trait caused by a mutation in one of the breakpoints, i.e., *In(2)Sb* and *In(3)RA*.

Table 1. Chromosome aberrations cause the production of late embryonic lethals (LELs)

No.	Mat. LEL[a]	Pat. LEL[b]	Cytology[c]	Marker[d]	Induction
1	+	–	In(1)	–	R5
2	+	–	In(2)	Sb	R5
3	+	–	In(3)	RA	R5
4	+	–	In(5)	–	R5
5	+	+	Df(5)	–	R5
6	+	+	T(1;4)	–	R5
7	+	+	T(1;5)	–	R5
8	+	+	T(3;4)	WW	R5
9	+	+	T(3;5)	–	R5
10	+	+	T(3;5)	–	R5
11	+	–	In(2)	Dl	Gloor 1971
12	+	+	In(2)	Ya e	x
13	+	+	In(2)	Ya e-1	x
14	+	–	In(4)	Th ht	y
15	(+)[e]	–	In(5)	Stw sca	y

[a] Females produce brown eggs
[b] Males induce formation of brown eggs in F_1
[c] Preliminary classification
[d] Marker genes – in the case of R5 most likely associated with one breakpoint. *Sb*, *Stubble*; *RA*, *Rauhes Auge*; *WW*, *Wavy wing*; *Dl*, *Delta*; *Ya e*, *Yellow abdomen, ebony*; *Th ht*, *Thorax dark colored, heart*; *Stw sca*, *Straw, scabrous*
[e] Decreased number of LELs most likely because the multiple inversion (cf. Fig. 3) suppresses crossing-over more effectively than a single inversion
x: X-ray induced, preliminary classified as *In (2)*, detailed analysis revealed *T(2;5)*. *In (2) Ya e-1* was recovered after irradiation of *Ya e* as a suppressor of crossing over with ss^{Anp}.
y: induced by X-rays (9,500 rad) as suppressors of crossing-over
Induction R5: irradiation with 12,500 rads of X-rays
Consistently, females heterozygous for large paracentric inversions produce LELs, while males of the same constitution do not cause patroclinous LELs. Flies of both sexes produce LELs if carrying a translocation

Improving the balancing capacities of inversions

Because in our first experiment we did not recover chromosomal aberrations that were suited for balancing chromosomes 4 and 5, we used a screen for suppressors of crossing-over. On chromosome 4, we had the closely linked, dominant markers *Th* (*Thorax dark colored*) and N^{IV} (*Notch-4*). At a distance of approximately 30 map units the recessive mutation *ht* (*heart*) could be localized. We irradiated males carrying the markers *Th ht* or N^{IV} *ht*, respectively, with 9,500 rads of X-rays, and crossed them with wild-type females. A total of 1,400 F_1 females – heterozygous for the marker genes – was crossed to *ht/ht* males and screened for crossing-over suppression. Four lines could be established that effectively suppressed crossing-over between the markers (less than 0.1% exchange). Three lines carried stable inversions of chromosome 4. Females heterozygous for each of the inversions produced LELs while males did not.

On chromosome 5, the markers *Stw* and *sca*, exchanging with a frequency of more than 50%, were available. In contrast to the results with chromosome 4, we did not succeed in recovering stable crossing-over suppressors in the first two screens with a total of more than 2,500 lines. In a third experiment, we recovered a chromosome that reduced the crossing-over between *Stw* and *sca* to less than 15%. Heterozygous females gave rise to large numbers of LEL progeny. After two additional irradiations and screens for crossing-over suppression, exchange between both marker genes could be completely eliminated. At the same time, production of LELs was drastically reduced. Cytological analysis revealed a multiply-inverted chromosome, which apparently reduced the incidence of crossing-over.

The same procedure was also applied to the translocation *"In (2)Ya e"*. On the basis of a preliminary cytological analysis, this aberration had been regarded as a complex inversion. However, both males and females

heterozygous for the aberration produced brown eggs in the progeny, arguing for a translocation. This expectation could be verified (Fig. 3). Two rounds of irradiation and screening for suppression of crossing-over with the most proximal second chromosome marker ss^{Anp} yielded *In(2) Ya e-1*, which effectively balances the whole length of chromosome 2. Because of the presence of a translocation in this rearrangement, both sexes cause embryonic lethality among the progeny.

Application

Together with the large inversions $In(1)f^3$, *In(3)RA*, and *In(4)Th ht 1* the above-mentioned chromosomal rearrangements should allow the balancing of almost the entire genome (Fig. 3). Although there was no possibility of testing the effectiveness of balancing mutations directly, the chromosomes indicated in this figure were used to screen more than 16,500 chromosomes for male-sterile mutations; 365 male sterile mutants were recovered and analyzed (Hackstein et al. 1990). Thus, our balancers proved their usefulness in an extended experiment. The application of genetic probing to study spermatogenesis in *D. hydei* was a very useful approach, since the advantages of *D. hydei* for a cytological analysis of nearly all stages of spermatogenesis made it possible to make substantial advances in understanding spermatogenesis in *Drosophila* (Hackstein 1991).

Discussion

Our study demonstrates that screening for LELs provides a feasable method for the recovery of chromosomal aberrations. Also, paracentric inversions could be isolated because in *D. hydei*, the defective chromosomal products of female meiosis are not eliminated as in other organisms such as *D. melanogaster* or *Zea mays* (McClintock 1933, Roberts 1976). Since we did not investigate female meiosis in *D. hydei* cytologically, we have no direct confirmation about the formation of aneuploid egg nuclei or nuclei harboring fragments of dicentric chromosomes. However, the results of the reciprocal crosses with either a male or female parent carrying a well-defined inversion clearly demonstrate that functional egg nuclei must contain defective chromosomes. Our experiments also reveal the limitations of this method. Since the background "noise" is rather high (at least at the high doses of X-rays used in this study), one must perform reciprocal crosses with nearly half of the lines in order to eliminate undesired complex rearrangements, reciprocal translocations and deletions. A second limitation lies in the problems of chasing the putative inversion, because only inversions that cannot undergo crossing-over with the marker gene can be traced through the generations. The most favorable situation is met if one of the breakpoints causes a visible, dominant phenotype (i.e., *In(2)Sb* and *In(3)RA*). The frequency of dominant mutations with visible phenotypes is rather high (roughly 2–3 per 10,000 F_1 flies), and nearly half of such mutations appear to carry chromosomal aberrations. The screening for matroclinous LELs in the progeny of such flies makes possible a rapid and easy method of filtering out suitable putative inversions. In addition, the production of LELs allows us to monitor the improvement of the balancing capacities of an inversion after repeated irradiations, since the incidence of LELs decreases with increasing balancing properties.

In conclusion, our study demonstrates the feasibility of constructing effective balancer chromosomes for *Drosophila hydei* – a species without elaborated formal genetics. It is not possible to predict whether or not this method will work in other organisms, since a number of limitations has already been demonstrated. First, monosomy or large deletions of the genome of the zygote must cause lethality of embryos. Second, the products of a single crossing-over within an inversion must be included into the egg nucleus. Third, the crossing-over frequency should be different in male and female. In species that meet these requirements, the generation of matroclinous (or patroclinous) embryonic lethals could provide a feasible method for the recovery and diagnosis of inversions and translocations – even if only a very limited number of mutants is available.

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