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A strategy for generation and balancing of autosome: Y chromosome translocations

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We describe a method for generation and maintenance of translocations that move large autosomal segments onto the *Y* chromosome. Using this strategy we produced (*2;Y*) translocations that relocate between 1.5 and 4.8 Mb of the *2*nd chromosome.. All translocations were easily balanced over a *male-specific lethal 1* (*msl-1*) mutant chromosome. Both halves of the translocation carry visible markers, as well as *P*-element ends that enable molecular confirmation. Halves of these translocations can be separated to produce offspring with duplications and with lethal second chromosome deficiencies . Such large deficiencies are otherwise tedious to generate and maintain.

Introduction

Translocations are traditionally generated by randomly induced chromosome breakage. Use of the site-specific *FLIP recombinase* (*FLP*), which recombines *FLP recombination target* (*FRT*) sites, was pioneered by Golic and Lindquist,[1] and has been widely used for generation of deficiencies with defined end points.[1-3] This method also enables recombination between non-homologous chromosomes, but at a lower efficiency.[4] Translocations that move autosomal material onto the sex chromosomes allow predictable transmission of material now linked to the *X* or *Y* chromosome, making these particularly useful. We obtained insertions of *FRT* in *G9a*, situated at 1A1 on the tip of the *X* chromosome, for the purpose of generating *X;A* translocations. However, one of these insertions segregates with the *Y* chromosome, and in fact is inserted in a duplication of the *X* on the *Y* chromosome (*Dp(1;Y)y*$^+$). The duplicated region comprises 1A1–1B, and includes *y*$^+$. Using this *Y*-linked *FRT* site as one endpoint, we generated large translocations between the *Y* and *2*nd chromosomes with an efficiency similar to that for production of autosome to *X* translocations.[4] Both halves of these translocations carry visible markers, enabling maintenance by selection. Full translocations were easily balanced over the *male-specific lethal 1* (*msl-1*) mutation for maintenance without selection.

Results and Discussion

We obtained insertions of specialized *P*-elements, *P{RS3}* and *P{RS5}*, for the purpose of generating translocations as described in Golic and Golic[5] (see **Table 1** for stock descriptions). These elements have *FRT* sites flanking the 5′ exon or 3′ exon, respectively, of the mini-*white* gene. Flies carrying intact elements have dark red eyes. *FLP*-mediated recombination between the flanking *FRT* sites deletes one exon to generate reduced *P{RS3r}* and *P{RS5r}* elements, which retain a single *FRT* site and the 3′ (RS3r) or the 5′ (RS5r) exons of the mini-*white* gene. Flies with reduced elements have white eyes.

We used *P{RS3}* elements with insertion sites at cytological positions 22A2 and 25B1 on *2L*, and 56C6 and 57B1 on *2R* from the Drosophila Genetic Resource Center (DGRC, Kyoto, Japan). Two *P{RS5}* elements inserted in *G9a* at cytological position 1A1 on the tip of the *X* chromosome were also obtained. Although these elements were believed to be close to one another, the mini-*white* marker of one segregated with the *Y* chromosome (DGRC Stock # 125639). PCR confirmed that this stock had a *P*- element in *G9a*, as well as a copy of *G9a* lacking the insertion. This is consistent with insertion on *Dp(1;Y)y*$^+$, the *Y* chromosome present in the strain used to generate the original insertions, which carries 1A1–1B on the *Y* chromosome (personal communication, Cook K). This insertion has been renamed *Dp(1;Y)y*$^+$, *P{w[+mW.Scer\FRT.hs] = RS5}G9a[5-HA-2731] y*$^+$*/w*^{1118} and is now available from the Bloomington Drosophila Stock Center (BDSC # 3649). We performed matings to determine whether the *Y*-linked *FRT* site could recombine with autosomal sites. The Golic laboratory has demonstrated that the recovery of recombinants between non-homologous chromosomes is dramatically enhanced by a third *FRT* site.[4] In accord with these findings, we were unable to obtain *2;Y* translocations with 2 *FRT* sites (approximately 348 vials screened). We then generated flies carrying the *Y*-linked *P{RS5r}* element and 2 second chromosome *P{RS3r}* elements,

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Table 1. Full genotype and stock numbers of *Drosophila* strains used in this study

Description	Stock #, supplier	Genotype	Flybase Id #
P{RS5} on *Y*	43649 (BDSC) 125639 (DGRC)	Dp(1;Y)y[+], P{w[+mW.Scer\FRT.hs] = RS5} G9a[5-HA-2731] y[+]/w[1118]	FBab0010476; FBti0059095
P{RS3} at 22A2	124255 (DGRC)	w[1118]; P{RS3}CG18131[CB-6892–3]	FBti0059228
P{RS3} at 25B1	124291 (DGRC)	w[1118]; P{RS3}CG3036[UM-8026–3]	FBti0029115
P{RS3} at 56C6	124216 (DGRC)	w[1118]; P{RS3}CB-6775–3	FBti0058933
P{RS3} at 57B1	123609 (DGRC)	w[1118]; P{RS3}CB-5407–3	FBti0028557
3rd chromosome *FLP*	8705 (BDSC)	w[*]; wg[Sp-1]/CyO; P{w[+mC] = ovo-*FLP*.R}F1A	FBti0072900
X-linked *FLP*	8727 (BDSC)	P{w[+mC] = ovo-*FLP*.R}M1A, w[*]	FBti0072898
msl-1		*y w; msl1*^{1}/CyO	

Table 2. Recovery of translocations in this study

2nd chromosome *FRT* sites	# Vials scored	Translocations recovered (#)	Rate of recovery of translocations
22A2 and 56C6	140	22A2 (3)	2.14%
22A2 and 57B1	107	0	-
25B1 and 56B6	105	0	-
25B1 and 57B1	238	57B1 (1), 25B1 (1)	0.84%
All	590	5	0.85%

All dysgenic males carried a Y-linked FRT site and the indicated FRT sites on 2L and 2R. The rate of recovery of translocations was (# independently recovered translocations/ # vials scored) × 100.

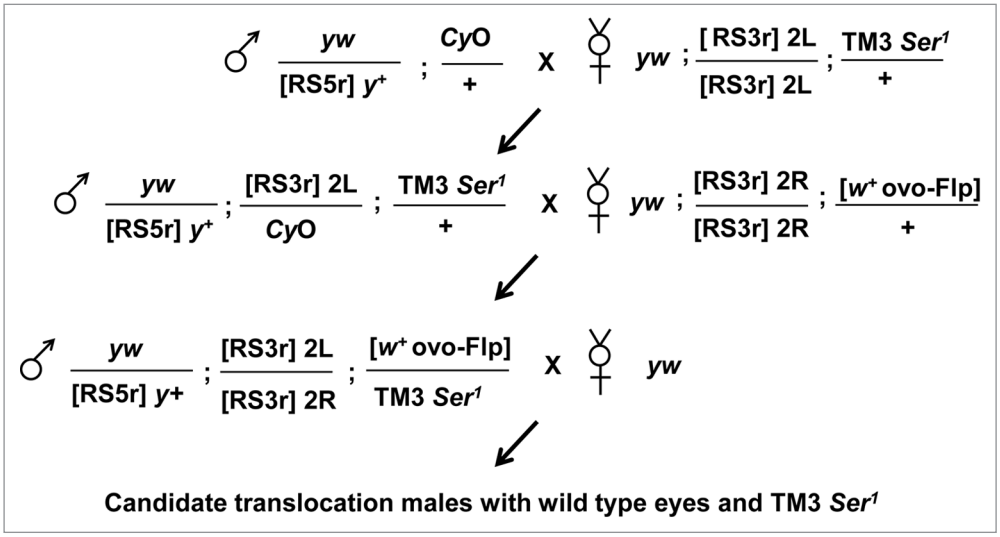

Figure 1. Mating scheme used to generate *T(2;Y)* translocations. The 3rd chromosome *FLP* source, [w^+ovo-*FLP*], was balanced over *TM3-Ser*^{1} to enable translocations to be positively identified. In practice, the eye color of translocations is sufficiently dark as to be readily distinguished in the [w^+ ovo-*FLP*] background.

one on *2L* and one on *2R*. Both *P{RS3r}* elements were oriented to enable production of *2;Y* translocations (**Fig. 1**). Cytological and molecular analysis was then used to identify the 2nd chromosome break point. The number of vials scored for each set of endpoints and the translocations recovered are presented in **Table 2**.

FLP-mediated recombination between *P{RS5r}* and *P{RS3r}* elements restores the mini-*white* marker, which segregates with the 2nd chromosome endpoint. Regeneration of w^+ made it possible to rapidly screen for recombinants, as only recombinant flies had deep red eyes. Our mating utilized a 3rd chromosome source of *ovo-FLP* in an element that itself produced a deep orange eye color. A *Serrate* balancer, *TM3-Ser*^{1}, was introduced to distinguish recombinants from flies carrying the *FLP* source. However, this proved unnecessary, as the reconstituted w^+ marker was distinctive, even in flies retaining the *FLP* chromosome. Use of an *X*-linked *FLP* source (BDSC # 8727) would obviate the need to distinguish male

offspring with light and dark eyes. The *Y*-linked half of the translocation can be followed by the y^+ marker, which continues to segregate with the *Y* chromosome. Both halves of the translocation are thus marked, making it possible to follow them independently in a yw background. Furthermore, each half of the translocation can be molecularly confirmed by amplification with genomic primers flanking the insertion sites and outward facing primers in the *P{RS5}* and *P{RS3}* elements (**Fig. 2**). Outward facing primers within each *P*-element and in flanking genomic sequence were used to confirm the second chromosome break point. A schematic of translocation between the *Y*-linked 1A1 insertion and the 22A2 insertion on *2L* is depicted in **Figure 2**. Flies with both ends of the translocation were w^+y^+, and, when used as template for PCR amplification, produced amplicons from proximal and distal *P*-element ends for the 1A1 and 22A2 insertions (**Fig. 2B**). Males with 2 wild type second chromosomes, and thus carrying half of the translocation as a duplication on the *Y* chromosome, were w y^+. As expected, these males lacked PCR products from the distal end of the insertion in *G9a* and the proximal end of the second chromosome insertion (**Fig. 2B**). No adult w^+y females were recovered from these stocks, indicating that these deficiencies are completely lethal.

We recovered 5 independent (*2;Y*) translocations with endpoints at 22A2 (3), 25B1 (1), and 57B1 (1). Translocations were named by the 2nd chromosome break point, for example, *T(2;Y) 25B1*. The rate of recovery of translocations was 0.85% (# independently recovered translocations/ # vials scored) × 100. This is comparable to translocation rates previously reported (0.33 to 0.97%).[4]

Both halves of translocations are clearly visible on polytene squashes (**Fig. 3A and B**). All 3 translocations can be separated to create viable (*Dp(2;Y)22A2*) or subviable (*Dp(2;Y)25B1* and *Dp(2;Y)57B1*) duplications. Duplications can sometimes be observed pairing with the wild type 2nd chromosomes (**Fig. 3C**). Translocation moves an *X* telomere from the *Y* chromosome to the tip of the truncated 2nd chromosome. This can be seen in a polytene preparation from the *T(2;Y)57B1* translocation (**Fig. 3D**). Importantly, both translocation and duplication males retain all fertility factors on the *Y* chromosome, and are thus fertile.

The w^+ and y^+ markers associated with each half of these translocations make it possible to maintain them by selection in a yw background. However, *Dp(2;Y)22A2* is viable, indicating that smaller translocations will be at a risk for loss without selection. Translocations were initially balanced by mating exceptional *XXY* females to a *CyO* (*In(2LR)O*) second chromosome. While possible to create stocks with both sexes carrying balanced translocations, these are plagued by low fertility. To enable easier maintenance, translocations were balanced over an *msl-1* mutation (**Fig. 4**). Females homozygous for *msl-1* are fully viable and fertile, however *msl-1* is male lethal.[6] The *msl-1* gene, situated at 36F on *2L*, is proximal to or on the opposite arm from translocation break points, and thus is present on the translocated second chromosome but absent from separated duplications. Stocks balanced by this method are vigorous and easily maintained. To control the risk of recombination posed by rare *XXY* females, duplicate stocks are maintained and periodically inspected. The location of other *male-specific lethal* genes, such as *mle* (42A6) and *msl-3* (65E4), suggests that all (*2;Y*) translocations and (*3;Y*) translocations on *3R*, or *3L* distal to 65E4, could be balanced by a similar strategy.

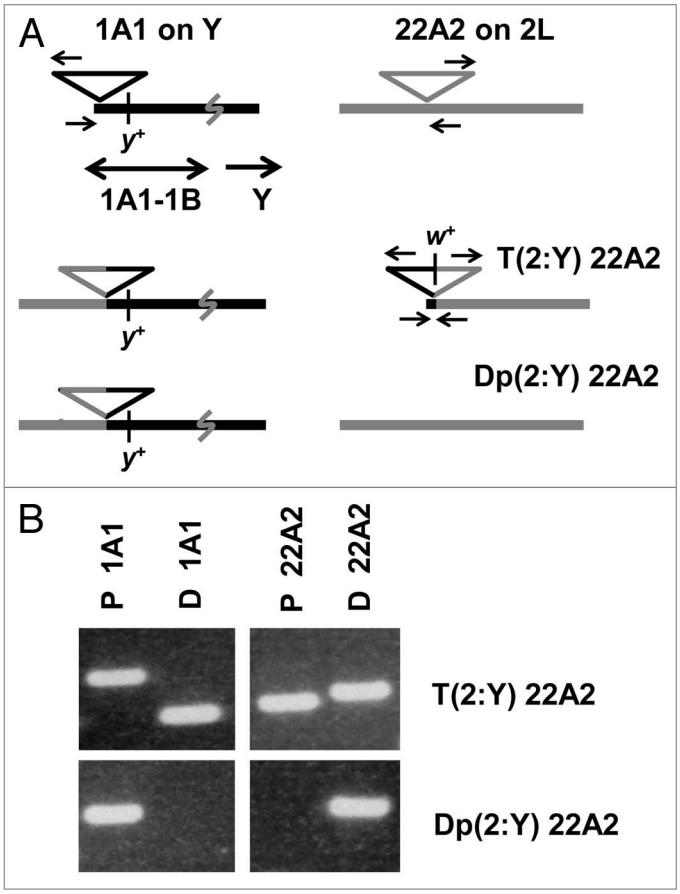

Figure 2. Molecular characterization of *T(2;Y)22A2*. (**A**) The insertion of *P{RS5r}* in *G9a* is in *Dp(X;Y)*y^+, containing 1A1–1B. The relative position of the y^+ marker is depicted with respect to *P{RS5r}* (triangle) and the junction between *X* and *Y* material (zigzag). Primers that amplify proximal and distal ends of all insertions were used; for simplicity, only those that amplify distal 1A1 (D 1A1) and proximal *P{RS3r}* at 22A2 (P 22A2) are depicted. Upon translocation, w^+ is reconstituted on the second chromosome. The template amplified by distal 1A1 primers is now at the tip of the second chromosome. Separation of the translocation halves to produce *Dp(2;Y) 22A2* removes the distal 1A1 and proximal 22A2 templates, but proximal 1A1 and distal 22A2 templates (primers not shown) are retained. (**B**) Amplicons produced by a fly carrying the complete *T(2;Y)22A2* translocation (top). All four *P*-element ends are detected. Amplicons from a *Dp(2;Y)22A2* fly identify only the proximal end of the *Y*-linked 1A1 insertion and the distal end of the 22A2 insertion (bottom).

Materials and Methods

Flies were maintained at 25 °C on standard cornmeal agar food. All fly strains used in this study are presented in **Table 1**.

Matings to generate translocations were set with 5 males and approximately 20 *yw* females per vial. A generalized overview of the mating scheme used to produce *T(2:Y)* is presented in **Figure 1**.

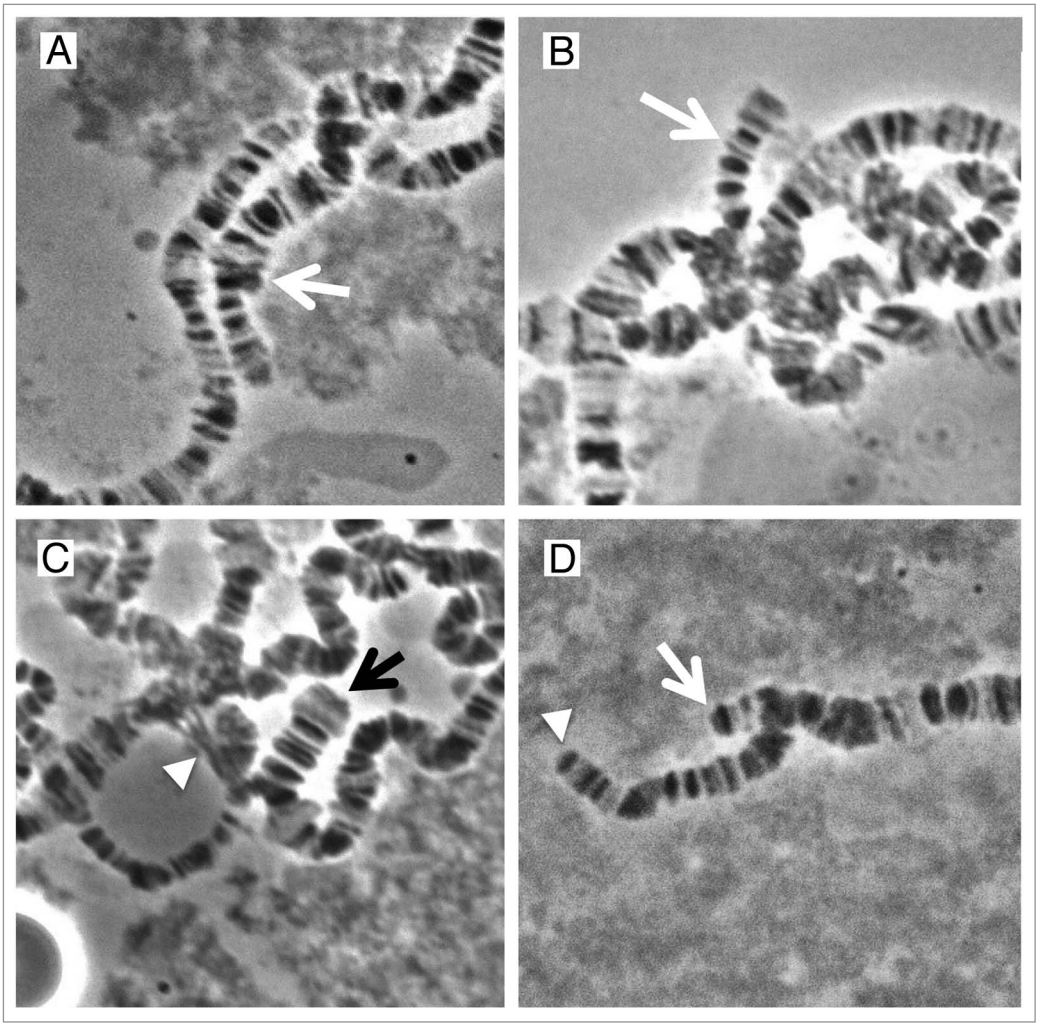

Figure 3. Cytological characterization of *2;Y* translocations. (**A**) Polytene preparations from a male larva with the *T(2;Y) 22A2* translocation display 1 second chromosome truncated at 22A2 (arrow). (**B**) The other half of the translocation is seen protruding from the chromocenter. (**C**) A male with *Dp(2;Y)22A2* shows pairing of the duplication with both wild type homologs (black arrow). A visible connection between the duplication and the chromocenter is marked by a white arrowhead. (**D**) Translocation moves the *X* telomere from the *Y* chromosome to the second. This is marked by a white arrow in a *T(2;Y)57B1* male. A white arrowhead marks the *2L* telomere on the normal homolog.

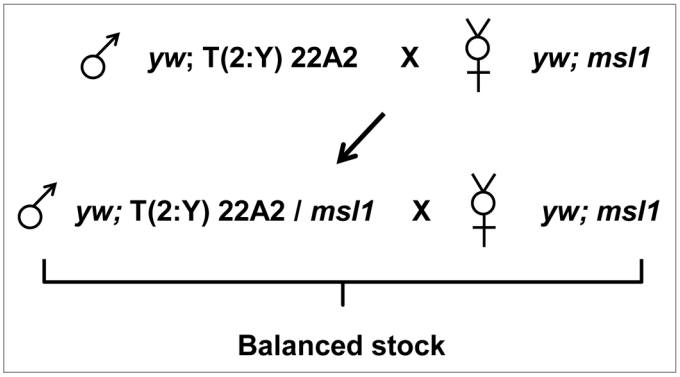

Figure 4. Balancing *T(2;Y)* over *msl-1*. Translocations were generated in a *yw* background to enable detection of y^+ and w^+ markers. *yw; msl-1* females are mated to males carrying a full translocation (y^+w^+). Sons carrying the full translocation are mated back to *yw; msl-1* females to establish a stock that can be maintained indefinitely without constant selection.

The primers used for the molecular characterization of translocations are: Proximal 1A1: Plac1(+)- CCAAGGCTGC ACCCAAGGCT CTGCTCCCAC and 1A1_R1- CGT TCTGCCGGCA GCGAAGTCGA CTG; Distal 1A1: Pry4(+) – TAATCAACAA TCATATCGCT GTCTCAC TCAG and 1A1_F1 - GAAGTGCGTG CGTGTGCGTG CGTC; Proximal *2L* at 22A2: Pry4(+) - TAATCAACAAT CATATCGCTG TCTCAC and 22A2_R1 – GTGCTGCCCA TCATCCTTAT GCC and Distal *2L* at 22A2: Plac1(+) – CCAAGGCTGC ACCCAAGGCT CTGCTCCCAC and 22A2 _F1 – CCGTCGCCTG CTGTGTACCGC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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