Strategies for Outcrossing and Genetic Manipulation of Drosophila Compound Autosome Stocks

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ABSTRACT Among all organisms, Drosophila melanogaster has the most extensive well-characterized collection of large-scale chromosome rearrangements. Compound chromosomes, rearrangements in which homologous chromosome arms share a centromere, have proven especially useful in genetic-based surveys of the entire genome. However, their potential has not been fully realized because compound autosome stocks are refractile to standard genetic manipulations: if outcrossed, they yield inviable aneuploid progeny. Here we describe two strategies, cold-shock and use of the bubR1 mutant alleles, to produce nullo gametes through nondisjunction. These gametes are complementary to the compound chromosome bearing gametes and thus produce viable progeny. Using these techniques, we created a compound chromosome two C(2)EN stock bearing a red fluorescent protein-histone transgene, facilitating live analysis of these unusually long chromosomes.

KEYWORDS C(2)EN long chromosome HisH2Av-mRFP1 neuroblast mitosis

Although much of genetic analysis has focused on the structure and function of individual genes, large-scale chromosome rearrangements also have played an important role in understanding higher levels of genome organization. In fact, the first functional genome-wide screen for regions of haploinsufficiency was achieved using a comprehensive collection of well-defined Y-autosome translocations (Lindsley et al. 1972). Chromosome rearrangements also have been essential for defining long-range interactions regulating gene expression and chromatin organization (Girton and Johansen 2008). One class of rearrangements, the compound chromosomes in which both homologs share a common centromere, have proven especially useful in functional genomic studies. For example, compound chromosomes have facilitated genome-wide screens for genes that must be zygotically expressed for completion of embryonic cellularization and gastrulation (Merrill et al. 1988; Wieschaus and Sweeton 1988).

Compound chromosomes are generated in a stepwise fashion using a series of complementary translocations, resulting in a doubling of the chromosome arm length (Ashburner 1989; Novitski et al. 1981). The arms are linked together with Y-heterochromatin, which cytologically appear as constrictions in the middle of the compounds arms (Figure 1, arrows). Compound chromosomes for the entire second and third chromosomes are referred to as C(2)EN and C(3)EN. For example, C(2)EN consists of both homologs of chromosome 2 sharing a single common centromere, creating a metacentric chromosome with arms twice the normal length (Figure 1). The structure of C(2)EN is 2R-Yhc-2L-C-2L-Yhc-2R. Despite these rearrangements, compound chromosome bearing flies are euploid, viable, and fertile. However, compound chromosome-bearing sperm are selectively lost after insemination (Dernburg et al. 1996). Because of their unusually long arm length, the compound chromosomes have been useful in examining the influence of chromosome arm length on chromosome segregation in different cell types. Studies analyzing C(2)EN syncytial embryos revealed that increased arm length resulted in an increased rate of errors in chromosome congression and segregation and loss of the damaged nuclei from the cortex (Sullivan et al. 1993). In contrast, a similar analysis in the slower dividing neuroblasts revealed that while the long C(2)EN chromosomes clearly lagged during anaphase, division failures did not occur (Gonzalez et al. 1991; Sullivan et al.
numerous C(2)EN strains, it is important to use C(2)EN bw1,sp1 during female meiosis. As soon as females are removed from the cold-depolymerizes microtubules, resulting in chromosome nondisjunction or mutation of interest, are kept at 10°C for example CyO, are collected. Virgin female bearing a second chromosome balancer with a dominant marker, as described by Ashburner (1989). Typically, ~300 virgin females protocol, we induce high rates of nondisjunction through cold-shock stably introduced into the compound chromosome stocks. In our approach, we introduced the white mutation (w1118) on the X chromosome into the C(2)EN stock (discussed in further detail in the sections to follow). We have also established C(2)EN stocks bearing the transgene, red fluorescent protein-tagged chromosome marker histone H2Av, or HisH2Av-mRFP1 [subsequently termed HisRFP (Schuh et al. 2007)], using an alternative method, as detailed in the sections to follow. We discuss the crosses necessary for establishing and maintaining stocks once transgenes and/or mutants have been introduced into C(2)EN-bearing flies.

Although cold-induced nondisjunction can be applied generally, this technique has some limitations. For instance, it would not work for some temperature sensitive mutations or dominant female sterile mutants. Thus, we developed an alternative method of inducing nondisjunction by using the heteroallelic combination of bubR1 alleles [bubR1[rev1]/bubR1[D1326N]] (Malmanche et al. 2007; Perez-Mongiovi et al. 2005). This allelic combination results in precocious sister-chromatid separation and high rates of nondisjunction during male meiosis (Malmanche et al. 2007). First, we constructed strains bearing the transgene HisRFP (Schuh et al. 2007) on the third.

1993; Kotadia et al. 2012). Thus, the rapid maternally driven embryonic divisions were much more sensitive to division errors than were the later zygotic divisions.

A major factor limiting the use of compound chromosomes is the fact that viable progeny are produced only when they are maintained as a stock. For example, the C(2)EN stock produces three progeny classes bearing no, four or two copies of chromosome 2. The first two classes are aneuploid and only the latter class produces viable fertile progeny (Figure 2A). If the C(2)EN stock is outcrossed, only inviable aneuploid progeny containing either one or three copies of chromosome 2 are produced (Figure 2B). Consequently, these stocks have been refractile to traditional genetic analysis such as introducing mutant alleles and transgenes into the stock.

Here we describe two strategies for producing viable progeny from outcrossed compound stocks. Both strategies are based on increased nondisjunction rates such that mutant alleles and transgenes can be stably introduced into the compound chromosome stocks. In our first protocol, we induce high rates of nondisjunction through cold-shock as described by Ashburner (1989). Typically, ~300 virgin females bearing a second chromosome balancer with a dominant marker, for example CyO, are collected. Virgin female flies, with the transgene or mutation of interest, are kept at 10°C for 7 d. The cold temperature depolymerizes microtubules, resulting in chromosome nondisjunction during female meiosis. As soon as females are removed from the cold-shock, they are mated to C(2)EN during female meiosis. As soon as females are removed from the cold-shock, they are mated to C(2)EN during female meiosis. As soon as females are removed from the cold-shock, they are mated to C(2)EN during female meiosis. As soon as females are removed from the cold-shock.
chromosome and the aforementioned bubR1 alleles on the second chromosome. We then crossed 80 C(2)EN bw1,sp1/+ virgin females to 60 bubR1<sup>mut</sup>/bubR1<sup>D1326N</sup>HisRFP males (Figure 3A). This cross produced large numbers of progeny bearing second chromosome markers bw<sup>+</sup> and sp<sup>+</sup>, indicating the presence of C(2)EN. Fluorescent analysis revealed these individuals also contained the HisRFP transgene. An advantage of this method over cold-shock is that it requires fewer flies to introduce genes into the compound chromosomes stocks. Disadvantages, however, are that this method is more time-consuming than the cold-shock method because the mutants and transgenes of interest must first be introduced into the bubR1 stock. In addition, many mutants and transgenes may produce synthetic lethal phenotypes when combined with bubR1.

Although the bubR1 mutant has proven useful for generating nondisjunction, other meiotic mutants such as mei-s332 or nod<sup>3TW</sup> can serve a similar purpose (Kerrebrock et al. 1995; Wright 1974). These mutants share in common with the bubR1 mutant, the property of high rates of chromosome nondisjunction. Therefore, one could potentially choose the mutant that causes the greatest level of chromosome nondisjunction during meiosis.

To easily identify and maintain a C(2)EN stock bearing a transgene, we took advantage of the mini-white gene associated with HisRFP. Using the cold-shock technique, we constructed a stock of C(2)EN bearing the X-linked mutant w<sup>1118</sup> and the third chromosome balancer, TM6B, Tb<sup>1</sup>, Hu<sup>1</sup>, e<sup>1</sup> (Figure 3B). These were generated by cold-shocking w<sup>1118</sup> mutant virgin flies bearing the double balancer Sp/CyO;Sb/TM6B,Tb<sup>1</sup>, Hu<sup>1</sup>, e<sup>1</sup>. After cold-shock treatment, these females were crossed to C(2)EN bw<sup>+</sup>sp<sup>+</sup> males. The resultant progeny were selected for non-Sternal pleura (Sp), non-Curly wings (CyO), non-Stubble (Sb), and Humeral (Hu). Because the w<sup>1118</sup> mutation is X-linked we can easily select white-eyed males due to their X/Y genotype. We then crossed these males to their brown-eyed sisters. The resultant progeny were crossed to C(2)EN bw<sup>+</sup>sp<sup>+</sup> males. The resultant progeny were selected for non-Sternal pleura (Sp), non-Curly wings (CyO), non-Stubble (Sb), and Humeral (Hu). Because the w<sup>1118</sup> mutation is X-linked we can easily select white-eyed males due to their X/Y genotype. We then crossed these males to their brown-eyed sisters.

![Figure 3](image-url) Introducing transgenes into the C(2)EN stock by promoting nondisjunction. (A) High rates of male nondisjunction were produced by a heteroallelic combination of bubR1 mutant alleles, a spindle assembly checkpoint gene. The generation of nullo-2 gametes allows the recovery of viable C(2)EN bearing progeny. (B) High rates of female nondisjunction were produced by cold shocking virgin females. Crossing scheme for generating C(2)EN flies in a mutant white-eyed background with a third chromosome balancer. In this case, nullo-2 gametes occurred due to a cold-shock of virgin females. Constructed strains: C(2)EN bw1,sp1; HisRFP/+; w<sup>1118</sup>; C(2)EN bw1,sp1; w<sup>1118</sup>; C(2)EN bw1,sp1; TM6B,Tb<sup>1</sup>, Hu<sup>1</sup>, e<sup>1</sup>; w<sup>1118</sup>; C(2)EN bw1,sp1; HisRFP/TM6B,Tb<sup>1</sup>, Hu<sup>1</sup>, e<sup>1</sup>.

should be chosen. Here we have focused on introducing mutant alleles and transgenes in C(2)EN but these techniques can be readily applied to other compound stocks as well. For example, the cold-shock technique can be used to create nondisjunction of the third chromosome to generate a C(3)EN stock bearing a mutant or transgene on the X, 2nd, or 4th chromosome.

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LITERATURE CITED

Ashburner, M., 1989 *Drosophila*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Dernburg, A. F., D. R. Daily, K. J. Yook, J. A. Corbin, J. W. Sedat *et al.*, 1996 Selective loss of sperm bearing a compound chromosome in the *Drosophila* female. Genetics 143: 1629–1642.

Girton, J. R., and K. M. Johansen, 2008 Chromatin structure and the regulation of gene expression: the lessons of PEV in *Drosophila*. Adv. Genet. 61: 1–43.

Gonzalez, C., J. Casal Jimenez, P. Ripoll, and C. E. Sunkel, 1991 The spindle is required for the process of sister chromatid separation in *Drosophila* neuroblasts. Exp. Cell Res. 192: 10–15.

Kerrebrock, A. W., D. P. Moore, J. S. Wu, and T. L. Orr-Weaver, 1995 Mei-S332, a *Drosophila* protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. Cell 83: 247–256.

Kotadia, S., E. Montembault, W. Sullivan, and A. Royou, 2012 Cell elongation is an adaptive response for clearing long chromatid arms from the cleavage plane. J. Cell Biol. 199(5): 745–757.

Lindsley, D. L., L. Sandler, B. S. Baker, A. T. Carpenter, R. E. Denell *et al.*, 1972 Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. Genetics 71: 157–184.

Malmanche, N., S. Owen, S. Gegick, S. Steffensen, J. E. Tomkiel *et al.*, 2007 *Drosophila* BubR1 is essential for meiotic sister-chromatid cohesion and maintenance of synaptonemal complex. Curr. Biol. 17: 1489–1497.

Merrill, P. T., D. Sweeton, and E. Wieschaus, 1988 Requirements for autosomal gene activity during precellular stages of *Drosophila* melanogaster. Development 104: 495–509.

Novitski, E., D. Grace, and C. Strommen, 1981 The entire compound autosomes of *Drosophila* melanogaster. Genetics 98: 257–273.

Perez-Mongiovi, D., N. Malmanche, H. Bousbaa, and C. Sunkel, 2005 Maternal expression of the checkpoint protein BubR1 is required for synchrony of syncytial nuclear divisions and polar body arrest in *Drosophila melanogaster*. Development 132: 4509–4520.

Schuh, M., C. F. Lehner, and S. Heidmann, 2007 Incorporation of *Drosophila* CENP-A and CENP-C into centromeres during early embryonic anaphase. Curr. Biol. 17: 237–243.

Sullivan, W., D. R. Daily, P. Fogarty, K. J. Yook, and S. Pimpinelli, 1993 Delays in anaphase initiation occur in individual nuclei of the syncytial *Drosophila* embryo. Mol. Biol. Cell 4: 885–896.

Wieschaus, E., and D. Sweeton, 1988 Requirements for X-linked zygotic gene activity during cellularization of early *Drosophila* embryos. Development 104: 483–493.

Wright, T. R., 1974 A cold-sensitive zygotic lethal causing high frequencies of nondisjunction during meiosis I in *Drosophila melanogaster* females. Genetics 76: 511–536.

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