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Genetic decay of balancer chromosomes in *Drosophila melanogaster*

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Theoretical considerations predict that balancer chromosomes in *Drosophila melanogaster* should accumulate numerous deleterious mutations with time. We counted the number of recessive lethal mutations on two balancer chromosomes from the *In(2LR)SM1/In(2LR)Pm* strain maintained in our lab, after making the balancers heterozygous with deficiencies from second-chromosome Kyoto Deficiency kit strains. We detected 10 recessive lethal mutations in the balancer *In(2LR)Pm*, which is consistent with the mutation rate estimated previously. However, we detected only three mutations, a significantly smaller number, in the balancer *In(2LR)SM1*, although this may be an artifact. In conclusion, we observed genetic decay over an estimable timescale by using balancers with historical records. Thus, balancers of any strain may have accumulated many unidentified recessive lethal mutations.

Introduction

Deleterious mutations accumulate in the genomes of asexually reproducing organisms, as their chromosomes do not undergo genetic recombination (Muller's ratchet).[1] Even in sexually reproducing organisms, deleterious mutations should accumulate in regions where recombination is suppressed. The degenerated Y chromosome is an extreme example of the evolutionary consequences of this phenomenon.[2] Chromosomal inversions suppress recombination in regions where homologous chromosomes are not collinear.[3] The multiple inversions present on the balancer chromosomes (balancers) of *Drosophila melanogaster* suppress recombination over most of their length.[4,5] Moreover, balancers generally carry at least one dominant visible marker and are lethal in homozygotes, causing them to be maintained in a heterozygous state. Given that there is no selection against the retention of recessive, deleterious mutations in heterozygotes (but see Results and Discussion), it seems likely that balancers will accumulate many such mutations. Therefore, balancers provide a useful evolutionary model system for understanding the accumulation of deleterious mutations. Furthermore, quality control of balancers is necessary because Drosophila geneticists frequently use balancers to screen for new mutations or to track particular chromosomes during crossing experiments in the absence of recombination. Accordingly, it is worth examining the extent of "genetic decay" of balancers.

Results and Discussion

We crossed *In(2LR)SM1*/*In(2LR)Pm* females to males from each strain included in the second-chromosome Deficiency kit to detect recessive lethal mutations in the balancers.

At least 10 recessive lethal mutations were detected in *In(2LR)Pm*, whereas only three were detected in *In(2LR)SM1* (**Table 1**; **Table S1** and **Fig. S1**). The incidence of recessive lethal mutations on the second chromosome is estimated to be 0.0060–0.0062 per generation,[6,7] which is equivalent to 0.156 mutations per year, assuming a 2-week generation time (transferred by a 2-week interval at 25°C). Considering each inversion on a balancer that suppresses recombination, extrapolation of this mutation rate suggests that 10.6 and 10.2 recessive lethal mutations should be detected on *In(2LR)Pm* and *In(2LR)SM1*, respectively (**Table S2**). Assuming a Poisson distribution, the probability that *In(2LR)Pm* has 10 or fewer recessive lethal mutations is 0.508. Conversely, the probability that *In(2LR)SM1* has three or fewer recessive lethal mutations is 0.009 (**Table 1**). If we assume that the *In(2L)Cy* + *In(2R)Cy* chromosome is not a good balancer and that recombination has been suppressed over the whole length of *In(2LR)SM1* only after the other inversion was induced, 7.3 recessive lethal mutations should have been accumulated since 1953 on *In(2LR)SM1*. Even if this assumption is correct, the probability that *In(2LR)SM1* has three or fewer recessive lethal mutations is 0.024. In summary, we detected a number of new recessive lethal mutations on *In(2LR)Pm* that is consistent with the previous estimate[6,7] but not for *In(2LR)SM1*.

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Table 1. Numbers of recessive lethal mutations

Balancer	Observed	Expected	Probability (≤ observed)
In(2LR)Pm	10	10.6	0.508
In(2LR)SM1	3	10.2	0.009

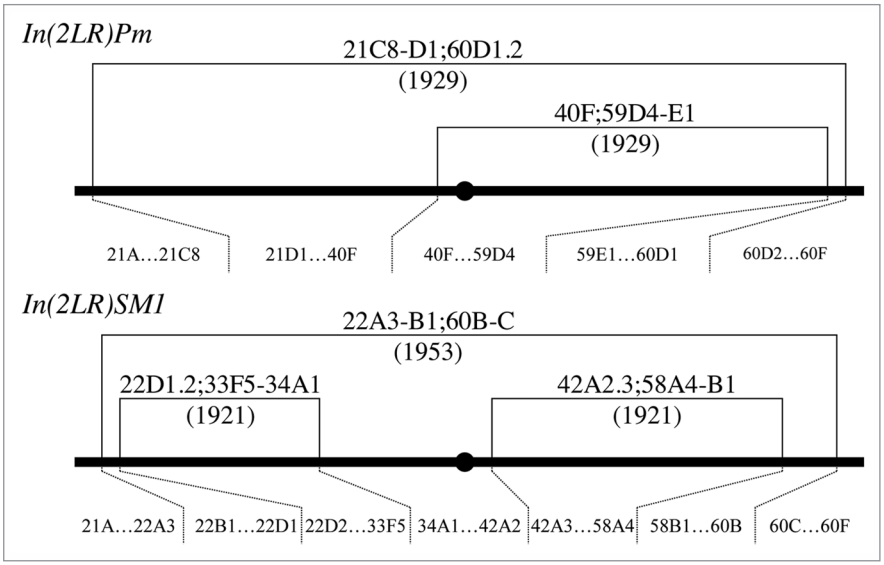

Figure 1. The second-chromosome balancers examined in this study, indicating the cytology and history of *In(2LR)Pm* and *In(2LR)SM1*. The new orders are 21A…21C8|60D1…59E1|40F…59D4|40F…21D1|60D2…60F and 21…22A3|60B…58B1|42A3…58A4|42A2…34A1|22D2…33F5|22D1–22B1|60C…60F, for *In(2LR)Pm* and *In(2LR)SM1*, respectively. The black bar represents the second chromosome, and the black circle represents the centromere.

Why are there so few recessive lethal mutations on *In(2LR)SM1*? The possibility remains that the value is an artifact. We may have not detected certain recessive lethal mutations because the deficiencies found in the second-chromosome Kyoto Deficiency kit strains do not completely cover the whole chromosome (ca. 94% coverage). Given that balancers derived from *In(2L)Cy* + *In(2R)Cy* [e.g., *In(2LR)SM1*, *In(2LR)SM5* and *In(2LR)O*], which have usually been used to isolate mutations and deficiencies on the second chromosome, may have common recessive lethal mutations, the deficiency kit may not contain strains with such deficiencies on their chromosomes in addition to chromosomal regions associated with haploinsufficiency.

It should be noted here that recessive lethal mutations are often also deleterious in heterozygotes.[8,9] If this is the case, recessive lethal mutations would accumulate more slowly than expected from the mutation rate when the population size is large enough. Here we did not observe such a slow rate of accumulation in *In(2LR)Pm*, which might be because the balancer strain has been kept as a very small population. Hence, balancers would decay at the upper bound rate with the usual methods of fly maintenance. It would be desirable to measure the dominance of accumulated lethal mutations on the balancers.

In summary, we observed genetic decay on an estimable timescale by using balancers with historical records. In keeping with our findings, balancers in any strain must, therefore, contain many unidentified, recessive lethal mutations. Furthermore, we predict that recessive lethal mutations accumulate to a greater extent on balancers than on other chromosomes. Because such recessive lethalities might be uncovered in certain genotypic classes that have inherited the balancers, we need to be aware that certain mutations or deficiencies may not be able to be isolated when using a particular balancer and that the segregation ratio may not be as expected for certain crossing experiments.

Materials and Methods

We employed the *Drosophila melanogaster* second-chromosome balancers *In(2LR)Pm* (FBab0004861) and *In(2LR)SM1* (FBba0000037). The standard second chromosome consists of 240 cytological compartments, with divisions 21–40 on the left arm (2L), divisions 41–60 on the right arm (2R) and subdivisions A–F in each division.[10] The balancer *In(2LR)Pm*, which carries the dominant visible marker *Plum* (= bw^{V1}) (FBal0001401), was discovered by H.J. Muller in 1929.[11,12] It has a nest of inversions extending over 236 compartments (40F;59D4-E1 in 21C8-D1;60D1.2), and we assume that *In(2LR)Pm* has been suppressing recombination over most of its length since it was discovered in 1929 (**Fig. 1**). The history of *In(2LR)SM1* begins with the discovery of *In(2L)Cy* + *In(2R)Cy* carrying the dominant visible marker *Curly* (FBal0002196) in 1921.[12,13] The chromosome contains two inversions: *In(2L)Cy* extends over 70 compartments (22D1.2;33F5–34A1), and *In(2R)Cy* extends over 97 compartments (42A2.3;58A4-B1) (**Fig. 1**). A large inversion extending over 229 compartments (22A3-B1;60B-C) was superimposed on *In(2L)Cy* + *In(2R)Cy* by Lewis and Mislove and reported in 1953,[14] and the resultant balancer is designated *In(2LR)SM1* (**Fig. 1**). We assume that *In(2LR)SM1* has been suppressing recombination over most of its length since 1953.

The balancers tested here are from the *In(2LR)SM1*/*In(2LR)Pm* strain (usually denoted *Cy*/*Pm*), which has been maintained in our laboratory for a long time and is believed to have been a gift of T. Mukai.[15] Because multiple recessive lethal mutations may have segregated in the balancers in our maintained strain, we isolated the balancers and established a new strain. To do so, we first mated a single *In(2LR)SM1*/*In(2LR)Pm* female to a wild-type Oregon-R male. For the second generation, we mated a +/*In(2LR)Pm* female and an *In(2LR)SM1*/+ male, and for the third generation, we mated a female and a male containing *In(2LR)SM1*/*In(2LR)Pm* to re-establish the strain.

To detect recessive lethal mutations in the balancers, we crossed the *In(2LR)SM1*/*In(2LR)Pm* females to males from each strain included in the second-chromosome Kyoto Deficiency kit (www.dgrc.kit.ac.jp). The strains in this collection are generally

heterozygous for both a deficiency (*Df*) and a balancer (*Bal*) (**Table S3**). Theoretically, four genotypic classes should appear in the crosses (**Fig. 2**). If *Df/In(2LR)Pm* or *In(2LR)SM1/Df* flies are not found, then a recessive lethal mutation should exist in the region rendered hemizygous by the deficiency.

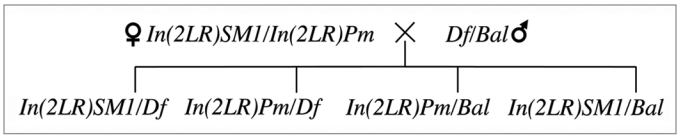

Figure 2. Test to detect recessive lethal mutations on *In(2LR)Pm* and *In(2LR)SM1*.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental material may be found here:
www.landesbioscience.com/journals/fly/article/24466

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