PERSPECTIVES: GENETICS

Reversal of Fortune for *Drosophila* Geneticists?

William R. Engels

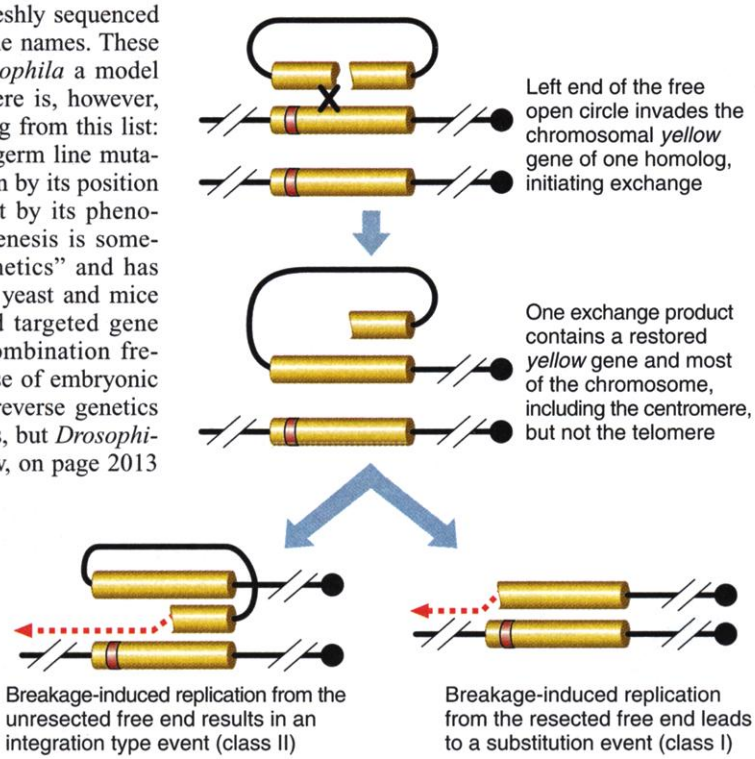
Drosophila geneticists love to talk about the advantages of their favorite research organism, and with good reason. After all, flies have giant polytene chromosomes, a wealth of chromosome rearrangements, P elements as multipurpose vectors, a freshly sequenced genome (*1*), and witty gene names. These characteristics make *Drosophila* a model of a model organism. There is, however, one important item missing from this list: a universal way to create germ line mutations when a gene is known by its position or DNA sequence but not by its phenotype. This kind of mutagenesis is sometimes called "reverse genetics" and has been used successfully in yeast and mice for gene replacement and targeted gene disruption. The high recombination frequency in yeast and the use of embryonic stem cells in mice make reverse genetics feasible in these organisms, but *Drosophila* has neither feature. Now, on page 2013 of this issue, Rong and Golic (*2*) describe a method for gene targeting in the fly. But some questions remain to be answered about the molecular mechanism that underlies their reverse genetics feat.

To date, there are two principal strategies for gene targeting in *Drosophila*, both using a family of transposable elements called P elements. The first is site-selected insertional mutagenesis: P elements are mobilized such that they insert randomly in the genome. Those lying in or near the gene of interest are selected in a polymerase chain reaction (PCR)–based screen. This can be accomplished by combining a PCR primer within the P element with one in the targeted gene (*3*). Thus, only P inserts lying within a few hundred base pairs of the second primer would permit amplification. By combining flies into pools and then testing them, large-scale screens are feasible. This technique has been subsequently refined but remains limited by the small size of the gene target. Furthermore, P elements exhibit a finicky preference in their selection of insertion sites. The rarity with which some sites are hit makes screening cumbersome even with the added benefits of pooling.

A significant improvement in the site-selected insertion approach involves turning the process around. Instead of using PCR to amplify only the insertions nearest the target, amplification of flanking DNA from all inserts is achieved by "inverse PCR." This amplified DNA is then hybridized with a large probe containing the targeted gene (*4, 5*). Thus, the effective target size is increased several hundred fold, eliminating the problem of insertional site specificity. Other limitations remain, however. The method is labor-intensive and is efficient only when several genes are screened simultaneously. In addition, inverse PCR—which requires cutting the genome and making the fragments form circles, thus allowing outwardly oriented primers to face each other—has a low yield when several P elements are mobilized at once. A potential improvement in this approach is to replace inverse PCR by a new technique, called TAIL-PCR (*6*). Here, a nonspecific primer is paired with two or more specific ones, thus eliminating the need to produce circular genomic fragments.

In the second strategy for *Drosophila* reverse genetics, a large collection of available P insertions (*7*) is analyzed to find one that happens to be close to the targeted gene. Then, one tries to coax that element to cause a mutation nearby. In some cases, P elements have been seen to jump "locally" such that targets near the insertion site have an increased frequency of new insertions (*8*). This method still requires a large screen, however, and has not always been successful.

Fortunately, there are other ways to make use of a conveniently situated P element. Gene replacement (*9*) is an option for cases in which a P element lies within about 2 kb of the target. Excision of the element makes a DNA double-strand break. Repair of the break results in replacement of flanking sequences with an in vitro modified version. This is the method of choice when its conditions are met. However, it is not always possible to find a P element close enough to the targeted site. In addition, a second copy of the gene on the homologous chromosome can compete with the in vitro modified sequence to make the process inefficient in many cases.



Breakage-induced replication. A single exchange between the free open circle and the *yellow* gene of one of the two homologous chromosomes of *Drosophila* may be initiated by invasion from one of the free ends of the open circle (**top**). After the exchange, the small telomere-containing fragment is lost, and the other fragment contains a broken end (**middle**). This free end can invade the homolog or sister chromatid (**lower left**) to initiate synthesis, which extends to the telomere (red dashed arrow). This results in an integration structure identical to the class II events described by Rong and Golic (*2*). Their class IV structure could also arise this way if the free open circle forms dimers. Note that class IV can retain an endonuclease cutting site, which would likely undergo additional rounds of breakage and repair. Such secondary breakage could be repaired by single-strand annealing or nonhomologous end joining to generate classes I, II, or III. Finally, class I structures can come about if the broken end is resected before invasion and synthesis (**lower right**).

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Less closely linked P elements can be mobilized to remove the targeted gene through flanking deletions. Such deletions have been recovered by selection for marker loss, but with sporadic success. A more systematic procedure to generate deletions is the "hybrid element insertion" process (*10*), which has been successful at several loci. Here, the left end of a P element joins with the right end of its twin copy on the sister chromatid. The resulting "hybrid element" can then insert into the homolog to yield a pair of recombinant chromosomes—one bearing a duplication and the other a deficiency of flanking sequences. The lack of meiotic recombination in *Drosophila* males makes screening for these recombinants easy. These deletion-based methods have two principal limitations: the variable frequencies for different P elements, and the potential for "collateral damage" or loss of other nearby or interstitial genes.

In Rong and Golic's new approach, most of the work is done by a pair of site-specific DNA-modifying enzymes from yeast. One is a recombinase whose action is to loop out a circular copy of the gene of interest. This copy comes from a transposon that was previously constructed and placed at a random site in the genome. The second enzyme is an endonuclease that opens the circle by cutting within the gene. This linear fragment can now find the targeted gene and recombine with it to produce a mutation.

There are some surprising aspects to Rong and Golic's results. The first is that their method works at all, given that another group (*11*) has recently tried a similar approach without success. The second is that two-thirds of the structures produced by Rong and Golic's method are not of the expected integration class, that is, they are not structures in which the linearized fragment is inserted into the targeted gene [see Fig. 4 in (*2*)]. A third surprise is that the frequency of mutations is much higher in females than in males.

The authors suggest possible reasons for these surprises, but there are alternative explanations that should be considered. A single exchange between the free DNA fragment and the chromosomal gene could lead to a double-strand break (see the figure). Repair of that break could make use of the homologous chromosome, especially if the event occurred before chromosome replication, when no sister chromatid is available for the job. This would explain the male-female difference, because the targeted gene (*yellow*) lies on the X chromosome and would have only one copy in males. Furthermore, *yellow* lies near the tip of the X chromosome, suggesting that the break could be repaired by "breakage-induced replication," as previously observed in yeast (*12*). In breakage-induced replication, a broken end is restored by synthesis from the homologous template all the way to the chromosome end. That would explain most of the structures observed by Rong and Golic (see the figure) as well as the lack of success in previous attempts where the targeted gene was not near a chromosomal tip (*11*).

The breakage-induced replication explanation can be readily tested. It implies that most mutational events are accompanied by recombination for outside markers, and that genes far from the telomere (end of the chromosome) would be refractory to mutation. If breakage-induced replication proves to be involved, Rong and Golic's findings will provide a valuable way to study this interesting repair pathway in *Drosophila*. Unfortunately, another implication is that the technique is unlikely to provide a broadly applicable approach to reverse genetics, because most *Drosophila* genes are not close to telomeres. On the other hand, if Rong and Golic's interpretation is correct and breakage-induced replication does not occur, then *Drosophila* geneticists will have a powerful and universal tool for reverse genetics at hand.

References and Notes

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13. I thank C. Flores for helpful discussions.

PERSPECTIVES: ECOLOGY

Weather Ruins Winter Vacations

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Our world is warming up (*1*). Climate models predict that this increase in mean annual temperature will continue for the rest of the 21st century (*2*). Whatever the reasons for the temperature increase, there is accumulating evidence that climate change may have a stronger impact on ecological processes than previously realized (*3*). Further evidence now comes from the report of Sillett *et al.* (*4*) on page 2040 of this issue. They show that large-scale regional variations in climate have a twofold effect on the demographics of a migratory bird species, affecting both its survival in the tropics as well as its reproductive performance in the north. One frightening consequence of these findings is that they illustrate how difficult it will be to reliably predict the effects of large-scale regional climate change on ecological systems.

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The El Niño Southern Oscillation (ENSO)—a quasi-periodic annual variation in global atmospheric and oceanic circulation patterns—influences rainfall worldwide (*5*). A quantitative measure of ENSO is provided by the Southern Oscillation Index (SOI), which is a standardized measure of the difference in atmospheric pressure between Tahiti in the South Pacific and Darwin in Australia. El Niños, assigned a low (negative) SOI value, generate milder and drier winters in the Southern Hemisphere. In contrast, high (positive) SOI values indicate La Niña conditions with more rainfall.

There has been an alarming decline in many migratory bird species that travel long distances to their tropical wintering grounds (*6*). It is well established that climate conditions at the wintering grounds may affect population fluctuations in long-distance migrant bird species. For instance, the size of the Dutch population of the purple heron, which winters in West Africa, is directly related to the annual variation in water discharges of the Senegal and Niger rivers (*7*). Small heron populations predominate after dry years when the rivers have little water. Another example is the British population of the sedge warbler. This increased after several winters of heavy rain in their West African wintering grounds, most likely because of higher survival rates among adult birds owing to an increase in the food supply (*8*). Such evidence suggests that unfavorable climate conditions at wintering grounds may explain the decline in many long-distance migratory bird species. The study of Sillett *et al.* (*4*) provides the first evidence that the demographics of a migrant bird, the black-throated blue warbler, may be strongly influenced by large-

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