is much simpler and faster than the RAGE method which requires restriction digestion and polyadenylation of uncloned genomic DNA and multiple rounds of PCR (Cormack and Somssich, 1997).

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Reichhart, J.M., and D. Ferrandon. UPR CNRS 9022, Institut de Biologie Moléculaire et Cellulaire, 15 rue René Descartes, 67084 Strasbourg Cedex, France. (email: reichhart@ibmc.u-strasbg.fr). Green balancers.

We have used the S65T green fluorescent protein (GFP; Chalfie *et al.*, 1994; Heim *et al.*, 1995) as a vital reporter to introduce a dominant innocuous marker onto the balancers of the three major chromosomes of *D. melanogaster*.

Construction: The drosomycin promoter contained in pJM802 (Ferrandon *et al.*, 1998) was replaced by the distal actin 5C promoter as an *Eco*RI-*Nhe*I fragment originating from pPac (Krasnow *et al.*, 1989) in which an *Nhe*I linker was inserted into the polylinker. The P element mediated transformation plasmid derived from pCaSpeR contained the actin 5C promoter, followed by the S65T version of the GFP and the drosomycin terminator. The nucleotide sequence of the transformation vector is available upon request. Transgenic fly lines were established as described (Driever *et al.*, 1990). One of the P element insertions obtained was remobilized using Delta(2-3) source of transposase. Insertions in FM7 (FM7i; Heitzler, 1997), CyO, and TM3 balancer chromosomes were selected. The following stocks were sent to the Bloomington stock center:

FM7i-pAct-GFP:

C(1)DX, f/FM7, y[93j],sc[8],w,oc,ptg,B,P[w^{+mC} act::GFP = pActGFP]

CyO-pAct-GFP:

w; ln(2LR)noc[4L],Sco[rv9R],b / In(2LR)O,Cy,dp[lvI],pr,cn[1],P[w^{+mC} act::GFP = pActGFP]

TM3-pAct-GFP:

w; Sb[1] / In(3LR)TM3,ri,p^{p},sep,l(3)89Aa,bx34e,Ser,P[w^{+mC} act::GFP = pActGFP]

Expression Pattern: Since their cuticle is transparent, third instar larvae carrying the marked balancers are easy to score under the fluorescent dissecting microscope. The main GFP expression pattern consists of a strong fluorescence in the salivary duct, the copper cells, the proventriculus and the visceral musculature of the midgut. A weaker signal can be detected in imaginal disks. In first instar larvae, the fluorescence appears to be restricted to the midgut (Burn *et al.*, 1989).

Adult flies carrying GFP balancers can be recognized by a deep pseudopupil type of expression in the eye, a mild fluorescence in the proboscis and a strong signal in the abdomen. Upon dissection, it appears that the abdominal fluorescence is due to:

– GFP expression in the reproductive tract of the male;

– GFP expression in ovaries (yolk of mature stages and musculature of the ovary sheath) and in the seminal receptacle in females.

In many animals, the visceral musculature of the midgut is also fluorescent.

In the embryo, there is a strong maternal contribution which masks the zygotic expression until about stage 15 of development, when a weak signal can be detected in the midgut, as in first instar larvae. In the absence of this maternal contribution, the expression of GFP can first be detected around 12 h after laying.

Selected pictures showing these expression patterns can be viewed at http://ibmc.u-strasbg.fr/upr9022/GreenBalancers.html

In conclusion, these "green balancers" constitute a highly useful tool to score living larvae, pupae, and adult flies, especially when working with mutations on the second chromosome.

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Som, Arundhati, and B.N. Singh. Department of Zoology, Banaras Hindu University, Varanasi, India.

No effect of marking flies either by nail polish on scutellum or by wing clipping on mating success in *Drosophila ananassae*.

In different species of *Drosophila*, males and females of different strains are marked for identification in mating preference tests. For marking the flies, different methods have been used by various investigators. These methods are:

a) wing-clipping -- margin of one wing is clipped in one of the strains (Ehrman, 1966, 1968)

b) placing a small drop of quickly drying enamel paint on mesonotum just anterior to the scutellum (Arita and Kaneshiro, 1979)

c) placing a small drop of nail polish on scutellum (Singh and Chatterjee, 1985)

d) placing a small mark of ink on both wings (Zouros and D'Entremont, 1980)

e) flies had been coloured with either pink or blue fluorescent dust (Markow, 1980) and

f) flies were fed red and green coloured food (Wu *et al.*, 1995).

In these studies, no effect of marking was found on the performance of flies or the outcome of mating preference test, because similar results have been found when the strains marked and unmarked are alternated in successive replicates.

Rare-male mating advantage which is an example of frequency-dependent selection, has so far been reported in nine species of *Drosophila* (Singh and Sisodia, 1997). *Drosophila ananassae* is a cosmopolitan and domestic species. This species occupies unique status in the whole of the genus *Drosophila* due to certain peculiarities in its genetic behaviour (Singh, 1985). Extensive work on population and behaviour genetics of *D. ananassae* has been carried out by Singh and others (for references see Singh, 1996). Rare-male mating advantage has also been reported in *D. ananassae* (Singh, and Chatterjee, 1989). It has been suggested by Bryant *et al.* (1980) that rare-male mating advantage is induced by wing-clipping in housefly and thus it is nearly an artifact resulting from alternately marking the rare and the common strains. On the other hand, Knoppien (1984) questioned the arguments given by Bryant *et al.* (1980) and proposed that any artificial rare-male mating advantage caused by wing-clipping is less important than suggested by Bryant *et al.* (1980). Further, Markow (1980) has clearly demonstrated that rare-male effect is not induced by marking with fluorescent dust in *D. melanogster*. In view of this, we have planned experiments to test the effect of marking on rare-male mating advantage in *D. ananassae*. Further, the phenomenon of rare-male mating advantage will be investigated in detail in *D. ananassae* by employing different wild type and mutant strains and inversion karyotypes as well as different experimental techniques. Before starting the detailed experiments, we have carried out preliminary experiments to test the effect of marking on mating success in *D. ananassae* and the results are reported in this note.

A wild type laboratory stock of *D. ananassae* (Bombay strain) established from a large number of flies collected from Bombay in 1985 was used. Virgin females and males were collected from this stock and aged for seven days.

Two marking procedures were used and for each procedure "male-choice" and "female-choice" techniques were employed:

A. Nail polish marking on scutellum

In "female-choice" experiments, males were marked by placing a small drop of quick drying nail polish on scutellum. Marking was done on lightly etherized flies 24 hr before the experiment. Twenty unmarked females with 10 marked and 10 unmarked males were introduced into an Elens-Wattiaux mating chamber and thus 20 pairs of flies were tested and the sex ratio was 1:1. Flies were observed for 60 minutes. When a pair commenced mating it was aspirated out and the type of male mated was recorded. In total five replicates were run.

In "male-choice" experiments, 20 unmarked males with 10 marked and 10 unmarked females were introduced into the mating chamber and were observed for 60 minutes. When a pair commenced mating it was aspirated out and the type of female mated was recorded. In total five replicates were run.

B. Marking by clipping the margin of wing of one side

Flies were lightly etherized and a small part of the distal tip of the right wing was clipped.

In "female-choice" experiments, 20 unmarked females with 10 marked and 10 unmarked males were introduced into the mating chamber. After commencement of mating, mated pair was aspirated out and the type of male mated was recorded. Observation continued for 60 minutes. In total five replicates were run.

In "male-choice" experiments, 20 unmarked males with 10 marked and 10 unmarked females were introduced into the mating chamber and were observed for 60 minutes. When a pair commenced mating it was aspirated out and the type of female mated was recorded. In total five replicates were run.