

**Anthony J Dornan** · University of Glasgow

Act-GAL4/CyO is a 'workhorse' driver used in Drosophila labs throughout the world, the CyO balancer effectively precludes any HR events, else problems with this line would have occurred and been reported ad nauseum previously.

So, while I hate to dispute Mark Mayhew, the most likely answer for your problems is simply contamination by another stock, presumably one carrying another GAL4 driver. Not much consolation to say 'it happens all the time'. That's why its always best to ensure a pristine set of your necessary/precious stocks are maintained at all times and if any question arises about the experimental stock arise you dump them and revert to the original stock (or get a replacement from the stock centres).

That said you should also be aware that the expression of Cy is suppressed in highly dense populations (reported as an effect of larval crowding) and also at lower temperatures. So if a CyO stock is maintained at 18° at a high population density and/or in lower amounts of food then you could 'lose' the Cy phenotype without losing the balancer. In this case increasing the temp the flies are raised at and reducing the population density should cause the Cy expression to become apparent again.