Transpositions of P elements from chromosomes of the P strain,  $\pi_2$ , onto the balancer chromosomes was achieved through a series of "chromosome contamination" crosses in which the balancer was passed at least six times through dysgenic flies in the presence of  $\pi_2$  chromosomes. Figures 1 and 2 show the procedure for the M-5(P) and TM6B(P) stocks; the others are similar.

Following the "contamination" crosses, each balancer was tested by in situ hybridization to a P element probe to ensure that it had acquired numerous P elements. At least four sites were observed on each of the balancers. In most cases the "contamination" steps were carried out in multiple replicates so that the chromosome with the greatest number of acquired P hybridization sites could be selected.

The remainder of the genome was then replaced by chromosomes from the  $\pi_2$  stock through a series of backcrosses to  $\pi_2$  99. In the case of M-5(P), the balancer was also made homozygous.

Finally, the cytotype of each stock was determined using the sn<sup>W</sup> test (Engels 1984). Females from the stock to be tested were crossed to sn<sup>W</sup>(P) males, and the resulting sn<sup>W</sup>-bearing daughters (or sons in the case of C(1)DX/FM7(P)) were progeny-tested to measure the rate of mutations to sn<sup>e</sup> and sn<sup>+</sup>. All stocks were confirmed to have the P cytotype, as indicated by the lack of sn<sup>W</sup> mutability.

References: Craymer, L. 1980, DIS 55:197-200; Engels, W.R. & C.R. Preston 1979, Genetics 92:161-175; Engels, W.R. 1984, Science 226:1194-1196.

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USNA. Characterization of a novel quinoline
in Drosophila melanogaster: xanthurenic acid
8-0-β-D-glucoside.

0 0 1 7 8 N 1 COOH OH H

kynurenic acid

xanthurenic acid

cardinalic acid (xanthurenic acid 8-0-ß-p-glucoside)

The biosynthesis of xanthommatin, the brown eye pigment of **D.melanogaster**, can be represented by a series of reactions: L-tryptophan  $\rightarrow$  kynurenine  $\rightarrow$  3-hydroxykynurenine  $\rightarrow$  xanthommatin: Two branch points give rise to other metabolites: xanthurenic acid is derived from 3-hydroxykynurenine and kynurenic acid from kynurenine. The cardinal mutant is deficient in xanthommatin and extracts of the head contain an abnormal fluorescent component that is shown by two dimensional thin layer chroma-

tography. The cardinal mutant shows a fluorescent pattern like that of the wild type, except that the former has an additional blue fluorescent spot with  $R_{\rm f}$  values of 0.65 in isopropanol/2% ammonium acetate (1:1) and 0.53 in 3% ammonium chloride. This unknown compound has been called "cardinalic acid".

To obtain pure "cardinalic acid" for structural studies, the following purification procedure was set up: A Drosophila head extract (from the mutant pr cd which accumulates more cardinalic acid than the cd mutant) at pH 7 was loaded onto a column of Dowex AG 50W (H+ form) equilibrated with water.

Table 1.  $pK_a$  values of the quinolines found in Drosophila, as detected by changes in the UV absorbance.

	Cardinalic acid	Kynurenic acid	Xanthuren <sub>ic</sub> acid
pK <sub>a</sub> (N)	1.8	2.2	1.8
$pK_a(0_4)$	11.0	11.2	7.3
$pK_a(0_8)$	-	-	12.3

Figure 1. Structure of the quinolines from Drosophila.

Most of the fluorescent components of the extract were retained and cardinalic acid was eluted with water in the first fractions. Fractions containing cardinalic acid were loaded onto ECTEOLA-cellulose column (Cl<sup>-</sup> form) equilibrated with water. Cardinalic acid was retained as a thin blue fluorescent band on the top, and eluted with 0.01M HCl. Cardinalic acid obtained by this procedure was shown to be free of any contaminant that could interfere with the subsequent assays.

The ultraviolet spectra from cardinalic acid were very similar to those of xanthurenic and kynurenic acid. Cardinalic acid and xanthurenic acid had similar fluorescent color (emission maximum wavelength around 480 nm). Studies of their acid-base properties gave similar  $pK_a$  values for cardinalic acid and kynurenic acid (Table 1), suggesting the same free substituents for both molecules (Figure 1). These results pointed at the possibility that cardinalic acid was an 8-derivative of xanthurenic acid. The infrared spectrum gave strong peaks in the region around 100 cm<sup>-1</sup>, suggesting the presence of a sugar in the molecule. The chemical hydrolysis gave xanthurenic acid and glucose. This was further confirmed by hydrolysis of cardinalic acid with  $\beta$ -glucoside; since the products were xanthurenic acid and glucose, the linkage to the glucose was established. This is the first time that a glucoside of xanthurenic acid has been characterized from natural sources.

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Ford, S. and L. Tompkins. Temple University, Philadelphia, Pennsylvania USNA. An assay to measure the consumption of attractants in solution.

Falk and Atidia (1975) described an assay that measured the responses of **Drosophila melanogaster** males to repellents in solution. We have developed a similar assay in which the consumption of different attractants can be measured. 25-35 adult male flies, 3-5 days old, are starved for 18 hours in a 95 x 25 mm

shell vial that contains water-saturated filter paper. After starvation, the vial is inverted onto a  $35 \times 10$  mm plastic culture dish that contains 2 ml of a medium consisting of 1.5% agar, 1 M sucrose, glucose or fructose, water and red food coloring (3 drops/10ml). The flies are allowed to feed on the medium for 1 hour, after which they are anesthetized with carbon dioxide and the number of flies that have red-colored abdomens is determined.

We have observed that  $99\pm0$ ,  $97\pm1$ , and  $98\pm1$  % of the Oregon R males had red abdomens after being tested with sucrose, glucose and fructose, respectively (15 groups of flies were tested with each attractant). However, Oregon R females are somewhat less attracted to the stimuli;  $62\pm3$ ,  $58\pm2$ , and  $64\pm4$  % have responded to sucrose, glucose and fructose, respectively (15 groups were tested with each attractant).

We have also used this assay to screen populations mutagenized with EMS and have recovered two X-linked mutations that affect the responses of males to 1 M sucrose; specifically,  $64\pm2$  and  $57\pm3$  % of the males have red-colored abdomens (15 groups of males from each mutant stock were tested).

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Reference: Falk, R. & J. Atidia 1975, Nature 254:325-326.

