Ferre, J.*and J.L. Mensua. University of Valencia, Spain. (*Currently: Oak Ridge Natl. Lab., Tennessee). Quinolines in Drosophila melanogaster and their application to the chromatographic characterization of eye-color mutants.

Table 1. "Quinolinic pattern" of wild type and the mutants that have only affected the biosynthesis of the brown pigment.

Phenotype	xanthurenic acid	kynurenic acid	cardinalic acid
wild type	+	0	0
cardinal	+	0	+
cinnabar	0	+	0
karmoisin	-	0	0
scarlet	-	-	-
vermilion	0	0	0

0 = lack, + = presence, - = trace.

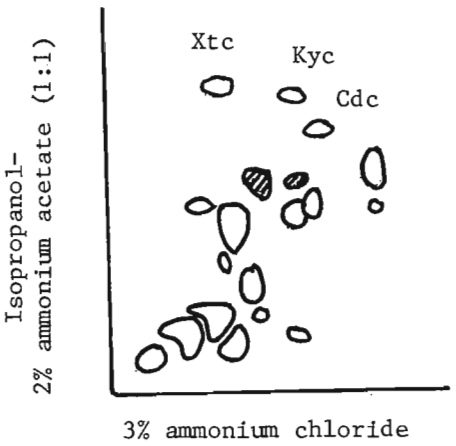
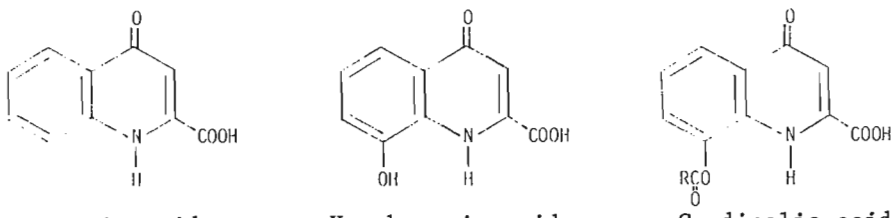


Fig. 1. Fluorescent pattern on thin-layer chromatography of a fly-head extract of D.m. (Shadowed spots are not fluorescent).



Kynurenic acid (Kyc) Xanthurenic acid (Xtc) Cardinalic acid (Cdc)

Fig. 2. Chemical structures of the three quinolines found in D.m.

In addition to the already known naturally occurring quinolines in Drosophila melanogaster, viz. xanthurenic acid (Umebachi and Tsuchitani 1955) and kynurenic acid (Danneel and Zimmermann 1954; Ferre 1983), a new quinoline derivative has been found on thin-layer chromatograms of some eye-color mutants. As it was formerly found in the cardinal mutant, it has been called "cardinalic acid."

Thin-layer chromatography in cellulose, as well as paper chromatography, have been extensively used to study the pteridines found in the eyes of D.m. (red pigments and related metabolites). We have found this technique to be very helpful in the study of the eye-color mutants that have affected only the brown pigment biosynthesis, viz. cinnabar, cardinal, karmoisin, scarlet and vermilion. These mutants show a wild type chromatographic pattern for the pteridines, but they can be characterized by their "quinolinic pattern" as shown in Table 1.

Two-dimensional thin-layer chromatography of the eye-color mutants was carried out as follows: Forty fly-heads (flies have to be at least 2 day old) were homogenized in 0.1 ml methanol-acetic acid-water (4:1:5 by vol) and 20 microliters spotted on a cellulose microcrystalline plate (20x20 cm, 0.1 mm thick, Merck). The first chromatographic solvent was isopropanol-2% ammonium acetate (1:1, v/v) and the second was 3% aqueous ammonium chloride (w/v). After cutting the heads off, all the steps were performed under dim red light to prevent photodecomposition. Figure 1 shows the fluorescent pattern seen under UV light. Xanthurenic acid, kynurenic acid and cardinalic acid have higher R_f values than the pteridines in the first chromatographic solvent and they have a sky blue fluorescence (pteridines have a darker blue fluorescence). Furthermore, the fluorescence of kynurenic acid appears gradually on the plate as it is irradiated with the UV-lamp (360 nm). This is the only spot that shows this behavior, so differentiating it unequivocally from cardinalic acid, the only compound with similar R_f values.

Column chromatography procedures were developed to purify cardinalic acid in milligram quantities. The infrared, ultraviolet and fluorescence spectra, together with its chemical properties (pK, color-reactions,

behavior in ion-exchange columns, etc.) indicate that cardinalic acid is an 8-ester of xanthurenic acid (Fig. 2).

Fifteen double mutants for eye-color, all of them carrying the mutation cardinal, were tested for the presence of cardinalic acid. This compound was only found in the cases where the other mutation allowed the accumulation of at least normal amounts of xanthurenic acid. This fact seems to indicate that xanthurenic acid is a precursor in the biosynthesis of cardinalic acid.

References: Danneel, R. & B. Zimmermann 1954, Z. Naturf. 9b:788-792; Ferre, J. 1983, "Accumulation of kynurenic acid in the "cinnabar" mutant of D. melanogaster as revealed by thin-layer chromatography, Insect Biochem. 13:289-294; Umebachi, Y. & K. Tsuchitani 1955, J. Biochem. (Tokyo) 42:817-824.

Table 1. Distribution of Drosophila fauna collected from Sampaje Ghats during August 1981.

Sites:	1	2	3	4	5	6	7	8	TOTAL
SUBGENUS SOPHOPHORA									
D.malerokotliana	28	-	6	1	80	122	20	11	268
D.bipectinata	-	-	-	-	91	311	7	-	409
D.nagarholensis	-	54	-	-	-	-	-	-	54
D.nigra	-	-	-	-	5	1	-	-	6
D.parabipectinata	-	-	-	-	-	2	-	-	2
D.jambulina	-	-	-	-	-	2	-	-	2
D.sahyadrii	-	-	-	-	-	1	-	-	1
SUBGENUS DROSOPHILA									
D.n.nasuta	3	20	8	3	74	151	55	34	348
D.s.neonasuta	-	3	1	3	12	53	1	-	73
*D.neoimmigrans	14	34	-	-	51	18	27	-	144
TOTAL	45	111	15	7	313	661	110	45	1307
# species per site	3	4	3	3	6	9	5	2	

*New species described by the authors.

Table 2. Distribution of Drosophila fauna collected from Shiradi Ghats during June 1982.

Sites:	1	2	3	4	5	6	7	8	9	TOTAL
SUBGENUS SOPHOPHORA										
D.malerkotliana	100	201	164	15	24	8	26	57	29	624
D.bipectinata	76	94	72	-	5	2	6	13	78	346
D.takahashii	3	2	-	1	-	-	-	-	-	6
D.eugracilis	-	1	5	-	-	-	-	-	-	6
D.nagarholensis	-	-	2	-	-	-	1	-	-	3
D.rajasekari	-	-	-	-	-	-	-	-	2	2
*D.barbarae	-	-	1	3	-	-	2	-	-	6
SUBGENUS DROSOPHILA										
D.n.nasuta	8	17	22	8	19	4	7	30	2	117
D.s.neonasuta	5	13	-	-	1	-	1	-	-	20
D.brindavani	-	-	-	-	-	-	-	-	12	12
*D.daruma	-	-	3	-	-	-	-	-	-	3
TOTAL	192	328	269	27	49	14	43	100	123	1145
#species per site	5	6	7	4	4	3	6	3	5	

*Species reported for the first time from INDIA.

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Studies on the Drosophila fauna from Sampaje and Shiradi Ghats, Karnataka, India.

The Western Ghats is known to harbour a number of Drosophila species because of its excellent ecogeographic conditions. It offers a rich abode for a variety of Drosophila species because of its luxuriant flora and varied climatic conditions. Though some parts of the Western Ghats of India have been investigated for Drosophila fauna (Sreerama Reddy & Krishnamurthy 1971; Hegde & Krishnamurthy 1980; Prakash & Sreerama Reddy 1978 & 1979), yet several areas of the ghats remain to be surveyed. Hence, the present collection trips were undertaken to Sampaje and Shiradi Ghats, which form part of the Western Ghats during August 1981 and June 1982, respectively. Collections were made both by fermenting banana bait and sweeping methods.

Table 1 shows the distribution of Drosophila species from Sampaje Ghats, whereas Table 2 shows the distribution from Shiradi Ghats.

Table 1 shows a total of 1307 flies trapped from eight spots. A total of 10 species were recorded, out of which 7 species represent the Subgenus Sophophora and the remaining 3 species represent