

Posch, N.A.* University of California at Los Angeles, California. Development time of *D. melanogaster*: dependence on yeast content, pH, and consistency of the medium.

Two types of media were used in this study. Banana agar medium, in ready to prepare form, was obtained from General Biological Supply House. This medium (pH 4.8) consisted of dried banana flakes, agar, brewer's (killed) yeast, white corn syrup, distilled water, and n-butyl

parahydroxybenzoate as a mold inhibitor. The concentration of killed yeast was 38 mg/ml medium. The second type of medium used (pH 3.4), contained cornmeal, agar, brewers yeast, unsulphured molasses, distilled water, and propionic acid as a mold inhibitor. The concentration of killed yeast was 10 mg/ml medium.

The natural food of *Drosophila* larvae is live yeast. The inclusion of killed yeast in a *Drosophila* medium is to provide the nutrition normally supplied by living yeast. We decided to determine the effect of live yeast on the development time of *Drosophila* raised in media containing killed yeast. Development time refers to the length of time between introduction of the adult flies into the culture, and the time the first progeny emerge. Of the two media we used, the cornmeal medium contained only one fourth the concentration of killed yeast contained by the banana medium. The first experiment was to determine the amount of live yeast necessary to add to the cornmeal medium to prevent delayed development, as results from inadequate nutritional value of the medium (described by Northrop, 1917, J. Biol. Chem. 30: 181.)

Six male-female pairs of Oregon-R flies, aged 4-6 days, and pre-fed on cornmeal medium with 500 mg of added live yeast (Fleischmann's active dry yeast), were placed in each culture, a 250 ml glass bottle with 30 ml of medium, and maintained at $23^{\circ}\text{C} \pm 1^{\circ}$. Varying amounts of live yeast had been sprinkled on the surface of the cultures a few hours before introduction of the flies. Cultures were checked four hours after the introduction of the adults to be sure egg laying had commenced. The results are shown in Table I and indicate that addition of 100 mg of active dry yeast to each culture is more than sufficient to provide adequate nutrition for normal rate of development. The length of the pupal period remained essentially constant, regardless of yeast content; only the time period of larval development varied.

Table I

	Active dry yeast added to medium (mg)					
	None	3.1	6.2	12.5	25.0	50.0
Average development time (days)	15.3	13.7	13.0	11.7	12.0	11.7
Number of cultures	3	3	3	3	3	3

From the previous experiment, it was apparent that the cornmeal medium without added live yeast was nutritionally deficient. We then determined the effect of raising successive generations on the cornmeal and banana media, and both media with added live yeast. Wild type, red eye flies, obtained from General Biological Supply House, were used. The parental generation of flies was pre-fed for three days on live yeast seeded media. Six pairs of flies were used to start each culture and left for five days. Flies were raised at $22^{\circ}\text{C} \pm 2^{\circ}$. The results are shown in Table II.

Table II

Medium	Generation				Number of Cultures	Average Development Time (days)
	F ₁	F ₂	F ₃	F ₄		
	Development time (days)					
Banana	12.3	14.3	13.4	13.8	48	13.5
Banana + 100mg live yeast	12.5	12.4	12.6	12.5	24	12.5
Cornmeal	14.1	15.2	17.3	18.3	68	16.2
Cornmeal + 100mg live yeast	11.5	11.3	11.7	11.3	24	11.5

From the results, several conclusions may be drawn: 1. The banana medium, with 38 mg killed yeast/ml, was nutritionally slightly sub-optimal, but sufficient to maintain continuous generations of healthy larvae with consistent development times.

2. The nutrition of the adult (female) affects the development time of its progeny. This is shown by the significantly shorter development time for F₁ than for subsequent generations on both cornmeal and banana medium due to the pre-feeding of the parental generation on yeasted media.

3. The cornmeal medium, with 10mg killed yeast/ml, was nutritionally quite deficient, with development time increasing with each successive generation (although the data are not presented, total progeny also decreased for successive generations).

4. Addition of live yeast to both media, in an amount previously determined to be sufficient for normal development, resulted in consistent development times for successive generations, but significantly different between the two media (i.e., compare the average development time on the two yeast supplemented media).

We performed a third experiment to determine if the difference in pH of the two live yeast supplemented media was responsible for the difference in average development times. Batches of banana medium were prepared in the usual manner, with the addition of varying amounts of hydrochloric acid. The acidity of each batch of medium was determined with a pH meter. Adult flies of the same stock as the previous experiment were pre-fed on yeast supplemented banana medium, and then transferred to the acidified cultures, to which no live yeast had been added. Six pairs of flies were placed in each culture and removed after four days. Temperature was $22^{\circ}\text{C} \pm 2^{\circ}$. The results of this experiment are shown in Table III.

Table III

	pH of acidified medium							
	<u>4.80</u>	<u>4.60</u>	<u>4.45</u>	<u>4.30</u>	<u>3.80</u>	<u>3.50</u>	<u>3.25</u>	<u>2.55</u>
Average development time (days)	12.5	12.6	12.6	11.8	12.6	12.4	12.6	12.8
Number of cultures	5	5	5	5	5	5	5	5
pH of banana medium: 4.8				pH of cornmeal medium: 3.4				

From these results, it is apparent that the difference in average development time on banana and cornmeal media is not due to pH difference. Indeed, the pH has no pronounced effect between 2.55 and 4.80. Sang (1956, J. Exp. Biol. 33: 45) reported that early growth of *D. melanogaster* is slightly retarded in a gel medium with 10% killed yeast, as compared to growth on live yeast alone. He suggested this was due to difficulty of the first and early second instar larvae in feeding on a non-particulate surface as opposed to the particulate nature of living yeasts and bacteria, the natural food. In our case, the living yeast is equally accessible to larvae in both types of media (on the surface). However, our cornmeal medium is much softer and more particulate than the banana medium, allowing the larvae to move through it more easily. This suggests that the larvae develop faster on the yeast supplemented cornmeal medium than on the yeast supplemented banana medium, either because they can move through the cornmeal medium faster, and therefore eat at a faster rate, or because they expend less energy in pushing through the medium, and therefore need to eat less volume in order to attain the necessary size for pupation, and therefore, pupate sooner.

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Studies on the ecology of
Drosophila in Korea.

Collections of *Drosophilid* flies were made during a period ranging from May to August in 1970 at Mt. Sok-ri (Chung book Province) and Mt. Kae ryong (Chung nam Province) in Korea. Most of these flies were collected by sweeping method. Six species of wild yeasts were isolated from the crops of *Drosophilid* flies. Among those yeasts *Saccharomyces florentinus* and *Saccharomyces cerevisiae* grew well on the medium for the Wagner Y-2 strain and also *D. auraria*

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Table 1. Wild yeasts isolated from the crops of *Drosophila*.

<u>Drosophila species</u>	<u>wild yeasts</u>
<i>Drosophila</i> (<i>Sophophora</i>) <i>rufa</i>	<i>Saccharomyces florentinus</i>
<i>Drosophila</i> (<i>Drosophila</i>) <i>brachynephros</i>	<i>Saccharomyces florentinus</i>
<i>D. (D.) nigromaculata</i>	<i>Saccharomyces cerevisiae</i>
<i>D. (D.) immigrans</i> (female)	<i>Trichosporon capitatum</i>
<i>D. (D.) immigrans</i> (male)	<i>Trichosporon fermentans</i>
<i>Leucophenga</i> (<i>Trichiasphiphenga</i>) <i>argentosa</i>	<i>Torulopsis salmanicensis</i>
<i>Leucophenga</i> (<i>Leucophenga</i>)	<i>Torulopsis dattila</i>

D. immigrans, *D. brachynephros*, and *D. busckii* bred well on cornmeal media with these yeasts (*Saccharomyces florentinus*, *Saccharomyces cerevisiae*).