

100%), in the preparations which were excized from different regions of the renal pelvis, possibly indicates that the T-mechanism is particularly pronounced in special parts of the pelvic musculature. The adrenaline reactions were not significantly altered by application of tetrodotoxin ( $3 \cdot 10^{-6}$  moles/l) or propranolol ( $10^{-5}$  moles/l). Isolated strips of ureteral smooth muscle exhibited very slow spontaneous phasic activity, and responded to an increasing concentration of adrenaline with a progressive increase of contraction frequency, sometimes with a fusion of the single contractions giving the appearance of an incomplete tetanic contraction (figure 1). In contrast to pelvis preparations, the adrenaline reaction of ureter was virtually completely inhibited by nifedipine.

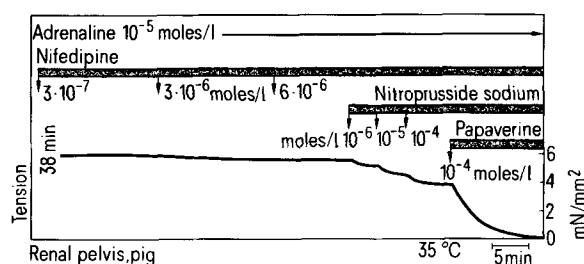


Fig. 2. Mechanical activity of an isolated muscle preparation of porcine renal pelvis, nifedipine-resistant part (T-component) of the adrenaline-induced activation. Inhibitory effects of nitroprusside sodium and papaverine. Preparation: 13 mm, 6.6 mg.

An increase of the nifedipine concentration from  $3 \cdot 10^{-7}$  moles/l to  $3 \cdot 10^{-6}$  or even  $6 \cdot 10^{-6}$  moles/l produced only a slight additional reduction of the T-component of the adrenaline reaction in pelvis preparations (figure 2), which indicates the high specificity of nifedipine in suppressing the P-activation in this type of smooth muscle.

Nitroprusside sodium, an effective antagonist of the T-activation in some tissues<sup>6,7</sup>, had only a partial inhibitory effect on the T-activation of porcine renal pelvis. Papaverine suppressed the T-activation completely, as also observed in other preparations<sup>6</sup>.

In the renal pelvis and ureter preparations of 3 rabbits, such a nifedipine-resistant adrenaline reaction was not observed. Furthermore, no indication for the existence of such a tonic component was found in earlier studies in pyeloureter preparations of guinea-pig and rat<sup>8</sup>. This suggests that the tonic component of renal pelvis musculature may have a special functional significance in larger multipapillary kidneys. First measurements in human preparations indicate that the results obtained with porcine preparations are also qualitatively valid for man. It appears likely that disturbances of the T-mechanism may contribute to special disorders of human urodynamics, and, consequently, that a differentiated pharmacological treatment of P- and T-components may be useful in therapeutics.

7 K. Boev, K. Golenhofen and J. Lukanow, in: Physiology of smooth muscle, p. 203. Ed. E. Büllbring and M. F. Shuba. Raven Press, New York 1976.

8 J. Hannappel and K. Golenhofen, Pflügers Arch. 350, 55 (1974).

## Oviposition rhythm of individual *Drosophila melanogaster*<sup>1</sup>

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**Summary.** Individually-housed *Drosophila melanogaster* show a gradual bimodal rise in egg production with a major crest shortly after dusk. The crest drifts toward noon after 2 to 3 days. The rhythm is hourglass.

Oviposition rhythm in *Drosophila melanogaster* is reported to occur at dark or dusk<sup>2-5</sup> as well as during midafternoon or before dusk<sup>6</sup>. Perhaps the method of collecting eggs influences results<sup>6</sup>. Several collections per day disturb the flies without providing the constant conditions necessary to study rhythms. A number of flies in one chamber may also disturb each other thus lowering egg production<sup>7</sup> and preventing constant conditions for each individual. An amorous male may disturb a female in her oviposition behavior when pairs are used. Consequently, it was desirable to test individual, mated females. My results are different from all other reports. I show the rhythm to be bimodal with the major crest initially at dusk. Individuals show a drifting or shifting of the peak towards noon.

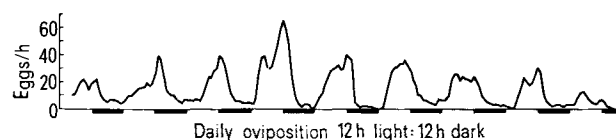


Fig. 1. A daily oviposition rhythm lasting 9 days on a 12L:12D regimen. Data from 18 surviving flies. Plot is a 3-point moving average. Note the minor peak during the dark phase.

**Methods.** A clock motor moves a conveyor belt at the rate of 72 cm in 24 h. A Plexiglas food tray on the belt has 25 lengthwise channels about 1 mm wide and 5 mm deep. The tray is about 15 cm wide and 84 cm long. Food is pressed into the channels. The food consists of 100 ml vinegar, 100 ml reconstituted frozen grape juice (Welch), 15 g viable dry yeast, 30+ g dried mashed potato (enough to make a paste).

In a stationary position above the tray are 25 vertical chambers made from glass tubing (ID 4 mm, OD 6 mm and 10 cm long) cemented to a metal plate. Each chamber is bisected by a channel below. There is just enough clearance to prevent escape of the individual fly and to allow protruding eggs to pass underneath the chambers. An aluminum rivet caps each tube. A ceiling of wet

1 I thank L. Cutkomp for the use of his facilities and material help during the initial stages of this study.

2 R. Allemand, J. Insect Physiol. 22, 1075 (1976).

3 P. Farb, in: Ecology: Life Nature Library, p. 74. Time, Inc., New York 1963.

4 G. Gruwez, C. Hoste, C. V. Lints and F. A. Lints, Experientia 27, 1414 (1971).

5 L. Rensing and R. Hardeland, J. Insect Physiol. 13, 1547 (1967).

6 J. David and P. Fauillet, Rev. Comp. Anim. 7, 197 (1973).

7 D. D. Sameoto and R. S. Miller, Ecology 47, 695 (1966).

sponges prevents the trays below from drying while humidity is kept high with a piece of polyethylene covering the complete conveyor system. Light is furnished by one 40 W white fluorescent bulb suspended 2 feet from the conveyor. For all studies reported here, except the hourglass experiment, lighting was on a 12L:12D regimen.

Mated female flies are 2 days old when placed in their separate chambers. They lay their eggs in the food, sometimes on the Plexiglas surface. If Norit or congo red is added to the food, the color contrasts with the eggs and facilitates recording. The exact location of each egg is marked on a strip of paper when the tray is viewed with an extension arm dissection microscope. The tray is moved on a sliding bar and every time an egg is seen, the location is recorded. Each channel is recorded in the same manner. The recording resembles an actograph of 25 flies.

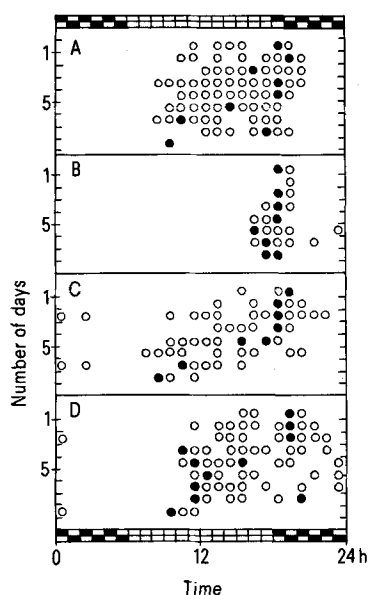


Fig. 2. Eggs per h are tabulated from the record. Daily peaks are marked (●) along with important numbers of eggs (○). Graph A is data from the same 18 flies used for figure 1; (○) 15 or more eggs per h. For flies B, C, D (○) is 2 or more eggs per h. Fly B was a very poor producer compared to C and D. Fly C shows gradual drift of peak, D shows abrupt shift. Note that in A, C and D the bulk of eggs are laid during the light phase. In B, C and D there are days when 2 peaks are equal.

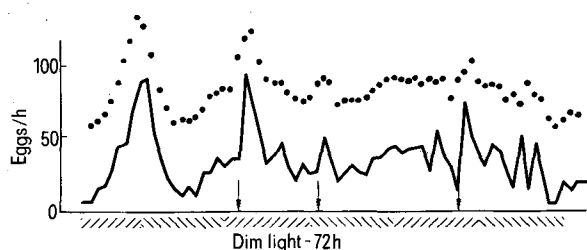


Fig. 3. Demonstration of the hourglass nature of oviposition. Dim light used for 72 h (shown as alternate 12-h-stripes; upward right stripes would have been normal light span, upward left the dark span). The line is a direct plot of the raw data from 21 flies surviving the experiment. Dots represent a 3-point moving average but are displaced upward by 50 eggs for comparison. Arrows represent time when food trays were changed. Similar results occur under constant light, i.e., a dampening out of the rhythm and tray change disturbance inducing oviposition.

**Results.** Variation in minute-by-minute, hourly, and daily production is seen in all flies. Some flies are better producers than others. Some are stimulated to oviposit as soon as a tray is replaced, while others will not lay eggs for several hours. This individual variability was noticeable during prototype experiments when trays were changed during random times and later under standardized procedures where trays were changed during the first hour of the light phase. The flies appear to be less sensitive at this time.

Individual production can be pooled with the results showing a bimodal rhythm. In figure 1, the record data was treated by using a 3-point moving average to smooth the curve. The bimodal nature of the curve survives the treatment on most days. The modes are generally not equal. The raw data can be plotted in a different manner to show when the major peak occurs. The peak drifts from dusk to noon (figure 2, A). Individual flies show this more dramatically (figure 2, C, D). The bulk of eggs are laid during the light phase and taper into the dark phase. The hourglass nature of the rhythm was found under continuous dim light (figure 3). Trays were changed at random times (arrows). In so doing, a surge of eggs were laid followed by a linear decline. The first peak with its earlier shoulder is comparable to the normal bimodal rhythm seen in other graphs. After what would be the first day, there is no discernable rhythm.

**Discussion.** The use of a rectangular tray has more advantages than using a disc<sup>8</sup>. All channels offer a uniform amount of food at a uniform rate. Recording the exact position of each egg for permanent records not only gives total eggs per h but allows other types of analyses. Disadvantages of the system are that a few eggs are laid on the chamber walls and the trays need to be changed at least once a day. Future use of a continuous moist food tape may solve the latter problem.

The individuality of each fly's oviposition is evident from examining the records directly. In total, the hint of a bimodal rhythm can be seen for most flies. However, the evidence is clear when information from individuals (not shown) and pooled data (figure 1) are graphed. Although the rhythm is bimodal, the modes are not equal. Apparently bimodal rhythms are quite common<sup>8</sup>. I find no other studies using a 12L:12D lighting regimen which show drifts or shifts after an initial set time for the peak.

When other investigators use groups of flies there may be a social effect which causes the oviposition crest to remain at dusk. Then, too, the investigators may not have run their experiments long enough to see the drift. An explanation for the drift may be that older females tend not to retain their eggs as long as younger ones<sup>9</sup>.

Evidence from light-dark studies of oviposition<sup>2</sup> suggests the hourglass nature of the rhythm. When I use constant conditions, the rhythm dampens immediately confirming the hourglass model.

8 J. Aschoff, *Ecology* 47, 657 (1966).

9 S. B. Yoon and A. S. Fox, *Nature* 206, 910 (1965).