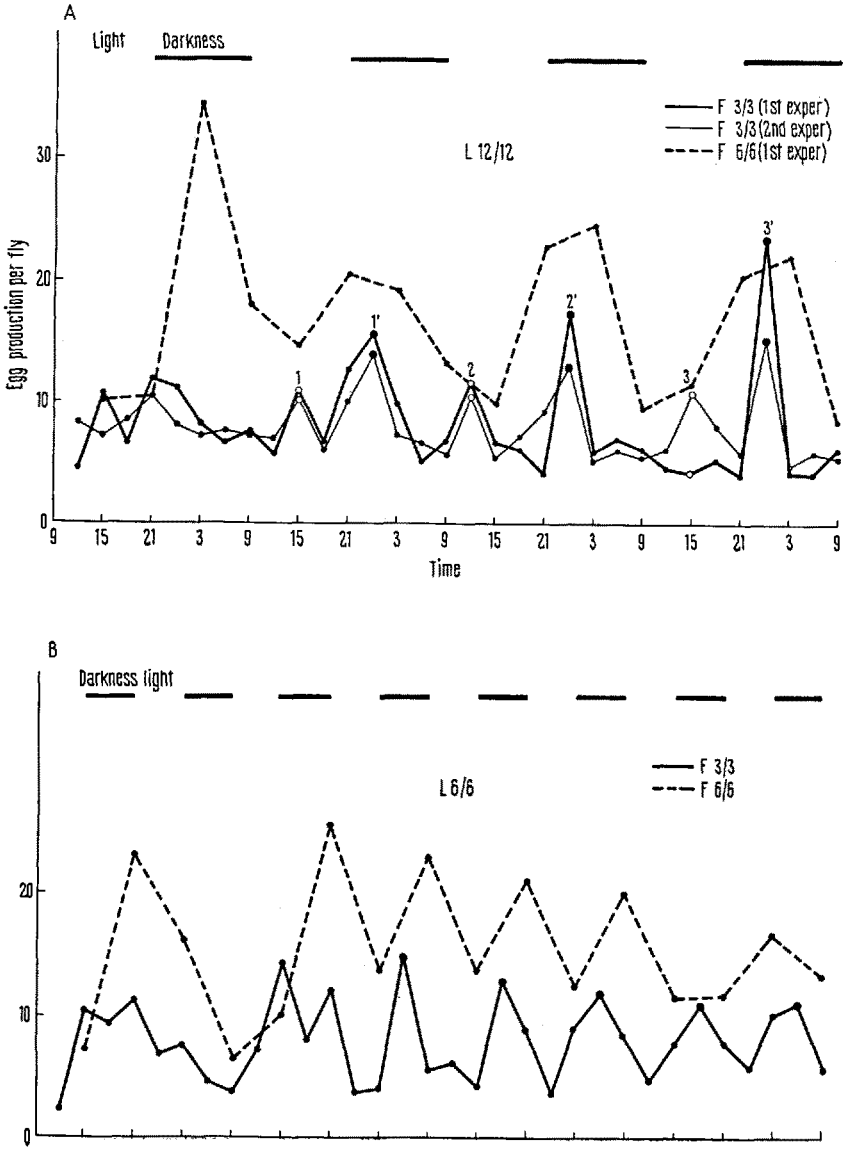
Oviposition Rhythm in *Drosophila melanogaster* and its Alteration by a Change in the Photoperiodicity

The egg-production of *Drosophila* is measured only once a day, occasionally twice a day[1]. It means that, implicitly or explicitly[1], the laying rhythm is considered to be constant throughout the day. In fact, no conclusive attempt to study the daily rhythm of oviposition and/or the influence of environment on such a rhythm by *Drosophila melanogaster* has ever been made. Yet the effect of photoperiodicity on oviposition appears plausible; different works have indeed shown that light influences *Drosophila* emergence rhythm[2] and behaviour[3–5].

Material and methods. A 'wild' *Drosophila melanogaster* strain was obtained by crossing 4 laboratory 'wild' stocks, namely *Chicago*, *Urbana 'S'*, *Gabarros* and *Oregon*, in such a way that each of these stocks contributed equally to the gene pool of the new 'wild' strain called CUGO. In a first experiment F_1 flies were used, whilst F_3 flies were studied in a second test. Flies were kept, at 25 °C, on the classical medium. Two photoperiodicities were used: 12 h light followed by 12 h darkness (L 12/12) and 6 h light followed by 6 h darkness (L 6/6). Under both these light conditions, the number of eggs laid by 4 series of 10 females, each female being isolated with a male, was measured every 3 (F 3/3), 6 (F 6/6), 12 (F 12/12) or 24 h (F 24/24)from the 3rd to the 6th day of imaginal life. In a second experiment F 3/3, F 6/6, F 24/24 measurements, made under a L 12/12 photoperiodicity only, were repeated with series of 22 females.

Results. The Figures A and B present the results of those experiments. The main points are as follows: 1. Under both photoperiodicities the total egg-production from the 3rd to the 6th day is equal (Table I) and a period of 24 to 36 h is necessary before a distinct rhythm of oviposition becomes visible (Figures A and B).

[1] H. P. DONALD and R. LAMY, Proc. R. Soc. Edinb. *57*, 78 (1937).
[2] C. S. PITTENDRIGH, Proc. Nat. Acad. Sci. *40*, 1018 (1954).
[3] F. W. CARPENTER, Am. Nat. *39*, 157 (1905).
[4] F. A. BROWN and B. V. HALL, J. exp. Zool. *74*, 205 (1936).
[5] H. B. WEISS, F. A. SCRACI and E. E. MCCOY JR., Jl. N.Y. ent. Soc. *51*, 117 (1943).



Mean egg-yield from the 3rd to the 6th day of life of *Drosophila melanogaster*. A) Photoperiodicity: L 12/12. Counting frequency: F 3/3 (1st and 2nd experiment), F 6/6 (1st experiment). B) Photoperiodicity: L 6/6. Counting frequency: F 3/3 and F 6/6.

2. With a photoperiodicity which fairly resembles normal light conditions (L 12/12), the manipulations of the laying females do not influence the total egg-production during the 4 days of observation. On the contrary, with a L 6/6 photoperiodicity frequent transfers influence the total egg-production: the more the flies are manipulated, the less they lay (Table I).

3. An analysis of variance (Table II) and the coefficient of correlation between the F 3/3, L 12/12 values ($r = 0.81$; $n = 32$; $P < 0.001$) show that the concordance between both experiments is excellent. At photoperiodicity L 12/12, the number of eggs laid in 3 h attains, twice a day, tops which occur 3 h, sometimes 6 h, after the light has been switched on or switched off. The number of eggs laid during the 3 hours following black out (solid dots 1′, 2′, 3′ – Figure A) is larger than the number laid during the 3h following lighting (open dots 1, 2, 3 Figure A and Table II).

4. The females whose ancestors had been, for generations, submitted to a L 12/12 photoperiodicity, have their own rhythm of oviposition. Indeed the correlation coefficient calculated between the values of L 12/12 F 3/3 and L 6/6 F 3/3 for the 12 first measurements, i.e. the first 36 h of the experiment, equals + 0.73 ($n = 12$; $0.001 < P < 0.01$). That rhythm, however, can be modified by a changed photoperiodicity; the correlation between L 12/12 F 3/3 and L 6/6 F 3/3 for the 20 last measurements, i.e. the last 60 h of the experiment, is equal to — 0.49 ($n = 20$; $0.01 < P < 0.05$). Under the abnormal L 6/6 photoperiodicity, the females now show a new rhythm of oviposition where the maximal number of eggs laid in a 3 hour period always occurs 3 hours after light is switched off (solid dots, Figure B).

Discussion. The rate of oviposition of *Drosophila melanogaster* may depend on 3 variables: the number of ovarioles and their production, the speed of growth of the successive stages of the egg-chambers and, according to

Table I. Analysis of variance of the mean total egg-production per fly from the 3rd to the 6th day of adult life for different photoperiodicities and counting frequencies

Photoperiodicity	Counting frequency	Egg-production
L 12/12	F 3/3	258.4 ± 13.9
	F 6/6	269.9 ± 9.5
	F 12/12	281.2 ± 17.4
	F 24/24	277.9 ± 12.1
L 6/6	F 3/3	254.6 ± 9.3
	F 6/6	252.8 ± 14.6
	F 12/12	300.4 ± 10.1
	F 24/24	305.4 ± 25.6

Analysis of variance

Sources of variation		S.S.	d.f.	M.S.	F
L 12/12	F 3/3 vs F 6/6	661.3	1	661.3	0.3
	F 12/12 vs F 24/24	54.5	1	54.5	0
	F 3/3+F 6/6 vs F 12/12+F 24/24	2,371.5	1	2,371.5	1.02
L 6/6	F 3/3 vs F 6/6	16.2	1	16.2	0
	F 12/12 vs F 24/24	125.0	1	125.0	0
	F 3/3+F 6/6 vs F 12/12+F 24/24	24,206.4	1	24,206.4	10.5
	L 12/12 vs L 6/6	832.5	1	832.5	0.36
	Error	166,577.0	72	2,313.7	
	Total	194,844.0	79		

[a] $P < 0.01$.

Table II. Comparison of the first and second experiment. Influence of light and darkness on oviposition rate. Mean egg-yield at the diurna and nocturnal tops and analysis of variance

Experiment I				Experiment II			
Tops	Diurnal tops	Tops	Nocturnal tops	Tops	Diurnal tops	Tops	Nocturnal tops
1	11.0 ± 1.5	1′	15.6 ± 3.0	1	10.6 ± 1.7	1′	13.9 ± 2.0
2	11.6 ± 1.3	2′	17.4 ± 2.1	2	10.5 ± 1.9	2′	12.9 ± 1.6
3	4.0 ± 1.1	3′	23.6 ± 2.0	3	11.0 ± 2.2	3′	15.9 ± 2.3
M	8.8 ± 1.5	M′	18.9 ± 2.4	M	10.7 ± 2.0	M′	14.2 ± 2.0

Analysis of variance

			S.S.	d.f.	M.S.	F
Experiment I	Light	top 1 vs 2	1.2	1	1.2	0
		tops 1 + 2 vs 3	247.7	1	247.7	2.8
	Darkness	top 1′ vs 2′	12.1	1	12.1	1
		tops 1′ + 2′ vs 3′	233.4	1	233.4	2.7
		light vs darkness	1,049.9	1	1,049.9	12.1[b]
Experiment II	Light	top 1 vs 2	0.2	1	0.2	1
		tops 1 + 2 vs 3	3.3	1	3.3	1
	Darkness	top 1′ vs 2′	10.9	1	10.9	1
		tops 1′ + 2′ vs 3′	85.1	1	85.1	1
		light vs darkness	411.3	1	411.3	4.7[a]
		exp. I vs exp. II	59.4	1	59.4	1
		error	13,985.0	162	86.4	
		Total	16,099.5	173		

[a] $P < 0.05$.
[b] $P < 0.01$.
Photoperiodicity: L 12/12; counting frequency: F 3/3,

our results, a photic signal regulating the ovariole activity and/or the oviposition. Flies which are compared must, however, be of the same age, the same genotype and grown in identical preimaginal environments. Indeed the activity and the number of ovarioles of a mature fly depend on age[6], genotype[7,8] and the conditions to which the larvae have been submitted[9].

In 'wild' *Drosophila melanogaster* imagos, aged 4 to 9 days, grown at 25 °C, under a L 12/12 photoperiodicity, there is under normal light conditions (L 12/12) a definite daily rhythm of oviposition. The rhythm is different when another photoperiodicity is tested (L 6/6). Under the L 12/12 photoperiodicity unequal tops happen 3 h after a passage from light to darkness and conversely, the nocturnal oviposition activity being, however, larger than the diurnal one. Under a L 6/6 photoperiodicity, equal tops always occur 3 h after switching off. Under both photoperiodicities, however, and for the period of time considered, the total egg-production is equal. The number of active ovarioles of our flies being, reasonably, supposed to be a constant, the production of the ovarioles therefore does not depend on the photoperiodicity.

Photoperiodicity, however, must influence the speed of growth of the successive stages of the egg-chambers. Indeed, under the L 12/12 photoperiodicity, the nocturnal oviposition activity is larger than the diurnal one; moreover under the L 6/6 photoperiodicity, tops only occur 3 h after switching off. The speed of growth of the egg-chambers stages may therefore be faster during the illuminated and slower during the dark period of the day. This is, however, not demonstrated. Indeed, while KING[10,11] and DAVID and CLAVEL[12,13] agree on the fact that 1 ovariole produces about 2 ovules a day, they disagree on the duration of vitellogenesis (stage 9 to 13 in KING's terminology) and of the mature egg stage (stage 14). DAVID and CLAVEL estimated the duration on counts made on F_1 mated hybrid females, aged 4 days, kept at 25 °C in total darkness. KING worked on a wild strain, kept at 25 °C and made his counts on females aged 0 to 7 days; no information as to the light conditions. Clearly, when dissecting females – at a not specified moment of the day! – the authors did not take into account the possible influence of a daily cycle; that lacuna may explain some of the discrepancies found.

The existence of the unequal tops under a L 12/12 photoperiodicity and of nocturnal tops only under a L 6/6 photoperiodicity may therefore be due to 2 different factors. The photoperiodicity may render the ovarioles activity, and more precisely the duration of growth of the egg-chambers, more or less synchronous, the activity being larger during the illuminated period than during darkness. Or if the mean ovariole production is constant throughout the day, light may provoke a retention of the mature ovules, and the passage of light to darkness may provoke oviposition properly so-called. It is probable that one of those hypotheses could be shown to be the right one by dissecting, at different moments of the day, females kept under different photoperiodicities.

Résumé. La mesure toutes les 3, 6, 12 ou 24 h, sous deux photopériodicités distinctes, de la ponte journalière de *Drosophila melanogaster* a montré que la ponte présente un cycle journalier bien marqué et que celui-ci peut-être modifié par un changement de la photopériodicité.

G. GRUWEZ, C. HOSTE, C.V. LINTS and F.A. LINTS

Laboratoire de Génétique,
Faculté des Sciences Agronomiques,
Université Catholique de Louvain,
92, Kardinaal Mercierlaan,
B–3030 Heverlee (Belgium), 24 May 1971.

[6] J. DAVID, Bull. Biol. Fr. Belg. *95*, 521 (1961).
[7] F. W. ROBERTSON, J. Genetics *55*, 410 (1957).
[8] G. TEISSIER, Suppl. Vol. of Cytologia (Proc. Int. Genetics Symp. 1956 (1957), p. 502.
[9] V. SAVELIEV, Trudy leningr. Obshch. Estest. *58*, 65 (1928).
[10] R. C. KING, Growth *20*, 121 (1956).
[11] R. C. KING, Growth *21*, 95 (1957).
[12] J. DAVID and M. F. CLAVEL, D.I.S. *42*, 101 (1967).
[13] J. DAVID and M. F. CLAVEL, D.I.S. *43*, 122 (1968).

Orientierende netzhautnahe pH-Messung im Glaskörper des Kaninchens bei intravenöser Glukoseinfusion

Neuerdings hat man tierexperimentell im Hirngewebe der Wistar-Ratte nach Glukoseinfusion einen Abfall des pH ähnlich wie im Karzinomgewebe beobachtet[1,2]. Als Erklärung wurde eine mit dem erhöhten Substratangebot einhergehende Zunahme der Glykolyse mit vermehrter Lactatbildung des schon bei normalem Glukose- und O_2-Angebot zu 8–9% glykosierenden Hirngewebes angenommen[2,3]. Auch die Retina ist seit WARBURG[4] als ein Gewebe mit der Fähigkeit zur Glykolyse bekannt, weshalb wir die nachfolgend skizzierten orientierenden netzhautnahen pH-Messungen im Glaskörper des Kaninchens bei Glukoseinfusion durchführten.

Methodik. Bei 12 Kaninchen von etwa 2 kg Gewicht wurde in Urethannarkose der Bulbus limbusparallel möglichst unter Vermeidung einer Blutung eröffnet. Die beiden Elektroden wurden durch die Skleraöffnung so in das Corpus geführt, dass die dünn ausgezogene Spitze der Glaselektrode (GA 70, Forschungsinstitut Meinsberg/Sa.) in ihrem empfindlichen Teil retinanahe im Glaskörper lag (Figur 1). Die Elektroden verblieben während des gesamten Versuches am gleichen Messort. Im Abstand von 5 min wurde der pH-Wert am Messinstrument der Firma Clamann & Grahnert, Dresden, abgelesen. Erst nachdem der pH etwa eine Stunde lang konstante Werte zeigte, begann die intravenöse Glukoseinfusion, wobei eine Infusionsrate von 12 g/kg^{-1}/100 min^{-1} durchschnittliche Blutzuckerwerte von 500–700 mg/100 ml erzielte. Die Dauer der einzelnen Versuche betrug jeweils 300 min. Bei 3 Tieren trat während des Experimentes eine massive Blutung in den Glaskörpern auf, so dass der Versuch abgebrochen werden musste.

Ergebnisse. Sofort nach dem Einführen der Glaselektrode misst man einen pH = 7,4, der sich nach einem relativ raschen Abfall auf einen Wert von pH = 7,12 $\pm$ 0,12 einstellt und mit geringen Schwankungen über mehrere Stunden beibehalten wird. Etwa 60 min nach Beginn der

[1] M. v. ARDENNE, *Grundlagen der Krebs-Mehrschritt-Therapie*, 2. Auflage (Verlag Volk und Gesundheit, Berlin 1970).
[2] M. v. ARDENNE und P. G. REITNAUER, Klin. Wschr. *48*, 658 (1970).
[3] U. GOTTSTEIN und A. BERNSMEIER, Klin. Wschr. *41*, 943 (1963).
[4] O. WARBURG, Biochem. Z. *184*, 484 (1927).