# The circadian oviposition rhythm of *Drosophila melanogaster*

## II : Influence of biotic factors

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RESUME

**Le rythme circadien de ponte de *Drosophila melanogaster*.**

**II : Influence des facteurs biotiques.**

Des travaux précédents ont montré que le rythme circadien de ponte de *D. melanogaster*, analysé sous photopériode LD 12 : 12, est modulé par les conditions environnementales (Allemand, 1983). Outre le rôle de paramètres de nature abiotique (substrat, éclairement), des interactions entre les individus semblent exister. Une analyse de ces facteurs « biotiques » montre que l'augmentation de la densité de population provoque une synchronisation des femelles, diminue la ponte pendant les photophases et accroit l'amplitude du pic de ponte au début de la scotophase. Le comportement des mâles n'exerce pas d'effets marqués sur celui des femelles pendant les photophases. Les femelles vierges ont un rythme de ponte avec un pic élevé en début de scotophase ; elles sont insensibles à l'effet de groupement et gardent, une fois fécondées un rythme avec un pic de forte amplitude.

Les effets des facteurs biotiques s'ajoutent à ceux d'autres facteurs du milieu et tous agissent comme des déterminants exogènes sur le rythme de ponte en modulant l'expression du rythme circadien endogène de vitellogenèse. Cette sensibilité aux facteurs de l'environnement permet à l'espèce de réagir rapidement aux variations du milieu et de coloniser les sites de ponte favorables. D'après les données écologiques et comportementales, le rythme de ponte de *D. melanogaster* devrait être considéré plutôt au niveau de la population que de l'individu.

*Mots clés :* Rythme circadien. Comportement de ponte. *D. melanogaster*. Facteurs biotiques. Effet de groupe.

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SUMMARY

Previous paper (Allemand, 1983) has demonstrated that the circadian oviposition rhythm of *D. melanogaster*, analysed under LD 12 : 12 photoperiod, was highly dependent on environmental conditions. Beyond the role of some parameters (laying substrate, lighting), interactions between flies can occur. An analysis of these "biotic" factors shows that the increase of the population density causes a synchronization of the females, reduces the oviposition rate during photophases and increases the amplitude of the laying peak at the start of darkness. The activity of the males does not appear to exert influence on the oviposition during the photophases. The oviposition rhythm of virgin females shows a high peak at the beginning of the scotophase ; these females are insensitive to the grouping effect and maintain, once inseminated, a rhythm with a high night peak.

The biotic factors added to other environmental factors act as exogenous determinants on the circadian ovarian activity. The sensitivity to environment allows *D. melanogaster* to react rapidly to variations of the surroundings and to colonize the laying sites. According to ecological data and the behavioural observations, the oviposition rhythm should be considered on a population level, rather than on the individual level.

## INTRODUCTION

Numerous environmental factors act on the oviposition behaviour of *Drosophila melanogaster* (see Grossfield, 1978) and the action of certain of these factors appears in the expression of the circadian oviposition rhythm (Allemand, 1983). Under LD 12 : 12 photoperiod, the rhythm can show several patterns depending on the experimental conditions. The oviposition usually shows a peak at the beginning of the scotophase but sometimes the maximum laying takes place during photophase, and the peak at dusk is not observed. The rhythm pattern results from the more or less inhibiting action of the environment on ovulation of the oocytes which are numerous within the ovaries during photophase. The main environmental factor having an inhibiting effect is light, whereas on the contrary, the laying substrate favours rapid oocytes deposition (see Allemand, 1983). Some other factors can also have an action on the expression of the oviposition rhythm, notably some biotic factors. For example, results obtained in measuring the effect of substrate renewal would suggest that interactions between individuals may exist.

These interactions may occur in particular on natural sites between individuals of the same species or of different species because the egg laying sites are also feeding sites and are therefore the meeting place of many individuals (David, 1971 ; 1973 ; Lachaise, 1974).

The effect of the interactions between individuals upon reproductive physiology has been known for a long time, as the grouping modifies the females fecundity (Pearl, 1932 ; Merle 1970 ; David and Van Herrewege, 1971 ; Rockwell and Grossfield, 1978). Regarding the oviposition behaviour, certain authors have shown that the egg laying could be aggregative, i.e. that the females would prefer to lay on the sites already used by other females or marked by males (Del Solar and Palomino, 1966 ;

Mainardi, 1968). The most currently accepted hypothesis used to explain these phenomena is based upon the existence of pheromones as have been shown in the case of the egg laying of other Diptera (Hudson and Mc Lintock, 1967 ; Osgood, 1971) and also for the meeting and the recognition of the sexes (see Fletcher, 1977 ; Ehrman, 1978).

In these conditions, it appeared necessary to investigate the effects of grouping on the oviposition rhythm. The effects of individuals on each other, and in particular males on females will be also studied. Once these results are obtained, it will be possible to draw conclusions concerning the determinants of the oviposition rhythm in response to the environment.

## MATERIALS AND METHODS

The stock and the experimental methods are identical to those previously presented : Brazzaville stock, 25° C, LD 12 : 12 photoperiod with ligth intensity of 1100 lux (see Allemand, 1983). All the experiments took place with a medium and substrate containing corn flour and killed yeast. The oviposition rhythm has been measured with only two techniques using discs to provide the egg laying substrate (techniques A and B defined previously). These experimental methods differ essentially by the renewal mode of the substrate and by the accessible surface of substrate. In the case of condition B (discontinuous renewal), the cages can be equipped with removable partitions dividing their volume and the access surface to the substrate by 2 or 4.

For breeding and the conservation of the adults before the experiments, flies were kept in low density, in order to avoid the influence of grouping on the ovarian activity. All the experiments were carried out on groups of adults consisting of the equal number of males as females. These groups were formed at the moment of rhythm measurement.

## RESULTS

### Effect of grouping

The effect of the number of adults on the oviposition rhythm has been studied by varying the number of flies present in the cages. The experiments were carried out on pairs (the same number of males and females). The average oviposition rhytms observed for 4 densities, expressed by the number of females : 1, 2, 4 and 12 are shown in *figure 1* for the 2 measuring conditions A and B.

Firstly the results obtained with 4 females and 4 males, which are the number of flies regularly used in the experiments, are identical to those previously presented and confirm the effect of the measuring method (Allemand, 1983). The rhythm shows a strong egg laying during photophase with technique B, whereas, it is quite different with technique A, reaching a maximum at the beginning of the beginning of the scotophase.

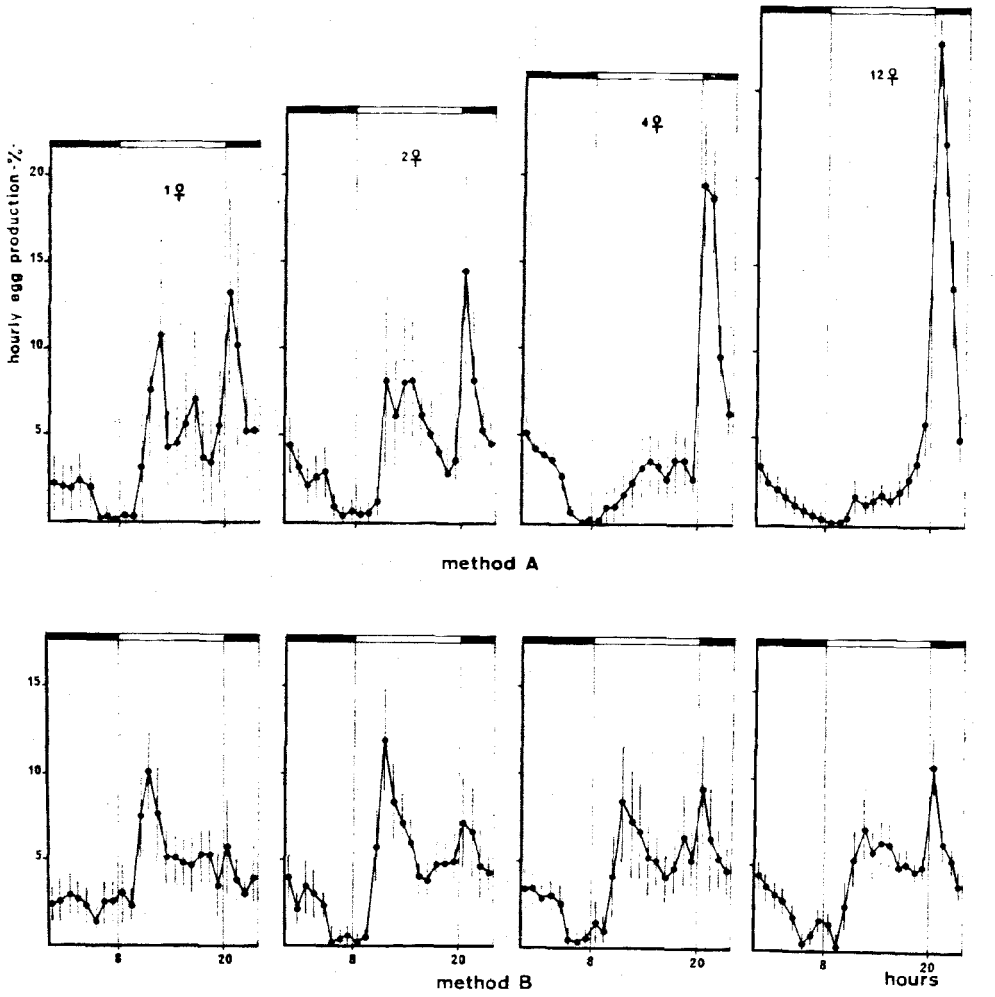


Fig. 1 : *Influence of grouping on the circadian oviposition rhythm of* D. melanogaster. *Rhythms measured under LD 12 : 12 photoperiod with the 2 techniques A and B, and with 4 densities : 1, 2, 4 and 12 females (sex ratio = 1). The mean values (hourly percentage of the total daily egg production) are plotted with their confidence interval (p = 0.05).*

Fig. 1 : *Influence du groupement sur le rythme journalier de ponte de* D. melanogaster *mesuré sous photopériode LD 12 : 12 avec 2 techniques de mesure et avec 4 densités (1, 2, 4 et 12 femelles ; sex ratio = 1). Les valeurs moyennes sont portées avec leur intervalle de confiance (p = 0.05).*

The increase in the number of flies causes an amplification of the oviposition rhythm. Technique A shows up this phenomenon more clearly, but in both cases, the variability of the average laying per hour decreases with the number of flies because of a mean effect due to the number of flies. The daily individual fecundity rate was sometimes affected by density. When the effect was significant, the fecundity

decreased with the increase of the number of flies, but the experiments length was too short for this effect to be noticeable (decrease of about 10 %).

In the case of technique A, the rhythm of isolated females is rather bimodal and shows a strong individual variability. Bimodality is observed for each fly and does not proceed from the average effect between flies which tend to lay during photophase or during scotophase. As the number of flies increases, the egg laying during photophase decreases and correlatively the oviposition peak at the start of darkness increases *(fig. 2)*. With 12 pairs or even 16 pairs, the egg laying during the first 4 hours of darkness represents about 67 % of the total laying, which corresponds to the physiological maximum (by taking into account the total daily fecundity), already observed in certain extreme rhythm conditions (Allemand, 1976).

The influence of the number of flies is much less clear in the case of technique B. However, a progressive decrease of the peak during photophase is observed when the number of females increases, compared with the peak at the start of the darkness. This can be graphically shown by plotting the values of the oviposition peaks against the number of females *(fig. 2)*. In the case of this technique which never shows high laying peak, a physiological constraint which results from the ovulation of numerous oocytes does not occur, and *figure 2* allows the definition of a density

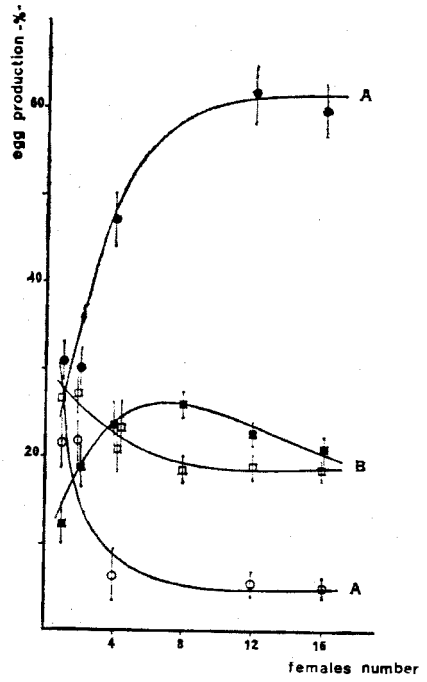


Fig. 2 : *Variations of photophase and scotophase egg laying " peaks " with grouping.*
● *condition A, scotophase peak ;*
■ *condition B, scotophase peak ;*
○ *condition A, photophase peak ;*
□ *condition B, photophase peak.*
*The peaks are estimated by the cumulated egg laying for 3 consecutives hours.*

Fig. 2 : *Variations en fonction du groupement des « pics » de ponte de la photophase et de la scotophase.*
● *condition A, pic de la scotophase ;*
■ *condition B, pic de la scotophase ;*
○ *condition A, pic de la photophase ;*
□ *condition B, pic de la photophase.*
*Ces pics sont estimés par la ponte cumulée pendant 3 heures consécutives.*

(8 females) with which the rhythm amplitude shows the highest increase. In these experimental conditions, this density corresponds to a volume of 20 ml per female.

The population density can be attributed to the volume of the cages or to the amount of accessible laying substrate. In this comparative study of techniques A and B, the cages are of equal volume but the acessible surface to the substrate is different. We have shown that the modification of the access surface has no effect on the rhythm (Allemand, 1983). Moreover, a decrease in the volume of the cage with a constant number of flies, does not affect oviposition rhythm pattern (technique B, 4 females and 4 males, results not presented). Therefore, to a certain extent, the density effect tends to be linked to the interactions between flies placed together. The main factor is the absolute number, rather than the relative number, with regard to the substrate surface or the volume. In applying this hypothesis, it seemed useful to observe the flies behaviour in the cages and, in particular the sexual behaviour of the males.

### Drosophila behaviour in the egg laying cages

The *Drosophila* behaviour was analysed during the measurement of the oviposition rhythm with the two conditions A and B. As for the previous experiments, the flies (4 females and 4 males) were young (5 to 6 days) and the females were always in the presence of males. The observations took place each hour during the second part of the photophase (15 to 19 h) for two consecutive days (at this time, the egg laying is always high with condition B and low with condition A). The presence of males and females on the cage floor, their presence on the laying substrate (access to the substrate in the centre of the floor) and the sexual activity of males (courtship or attempt) were recorded each time for 3 minutes. In the case of method B, the observations were taken 30 minutes after the substrate renewal.

Throughout all the observations, no mating has ever been noticed. A preliminary experiment has shown that matings only occur during the first hours of photophase ; this is consistent with the results published by Hardeland and Stange (1971).

The results obtained *(table I)* show no differences between the two methods, either for the frequentation of substrate, or for the male activity, because of the strong variability between cages. On the whole, the flies are very calm and do not move. From time to time, the males attempt to court the females which refuse them by running away. The substrate frequentation by the females is independent of the number of eggs laid. This result, which appears paradoxical, explains within certain limits why the decrease of the substrate does not modify the rhythm. It must also be remembered that the egg deposition is a very rapid event ( a few seconds).

Table I : *Substrate frequentation and sexual activity of males during photophase (LD 12 : 12 photoperiod). The behaviours have been studied during the oviposition rhythm measurement with techniques A and B. Every 3 minutes observation has been taken on groups of 4 females and 4 males (between 15 and 19 h). The values are the average of 10 observations for two consecutive days. * Mann and Whitney test, N.S. non significant comparison test (p > .05), S significant test.*

Tableau I : *Fréquentation du substrat et activité sexuelle des mâles pendant la photophase (photopériode LD 12 : 12). Les comportements ont été observés pendant la mesure du rythme de ponte avec les deux techniques A et B. Les observations de 3 minutes chacune ont porté sur des groupes de 4 femelles et 4 mâles par cage et ont été réalisées entre 15 et 19 heures. Les valeurs sont les moyennes de 10 observations faites pendant 2 jours consécutifs. * Test de Mann et Whitney, N.S. test de comparaison non significatif (p > 0,05), S test significatif.*

| | Condition A (high laying peak at "dusk") | | | | Condition B (strong laying during photophase) | | | | | | Comparison A - B | Variability between cages |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cage | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Average number of males on the substrate | 0.4 | 0.4 | 0.5 | 0.3 | 0.4 | 0.3 | 0.2 | 0.3 | 0.4 | 0.8 | N.S. | N.S. |
| Average number of females on the substrate | 0.2 | 0.9 | 1.0 | 0.7 | 0.6 | 1.5 | 0.2 | 0.2 | 0.9 | 0.5 | N.S. | S ($p < .005$) |
| Average number of flies on the floor | 4.6 | 5.4 | 7.8 | 6.3 | 5.2 | 4.8 | 5.9 | 6.4 | 6.5 | 6.3 | N.S. | S ($p < .001$) |
| Frequency of courtships | 0.5 | 0.1 | 0.2 | 0.4 | 0.8 | 0.3 | 0.2 | 0.4 | 0.1 | 0.2 | N.S.* | — |

INFLUENCE OF THE PRESENCE OF MALES

The influence of the presence of males on the females laying activity was also analysed by measuring the oviposition rhythm of females deprived of males for 24 hours. The results obtained with the two techniques are shown in *figure 3*. The absence of males does not modify the expression of the rhythm, which confirms the negligible role of males on the females' behaviour.

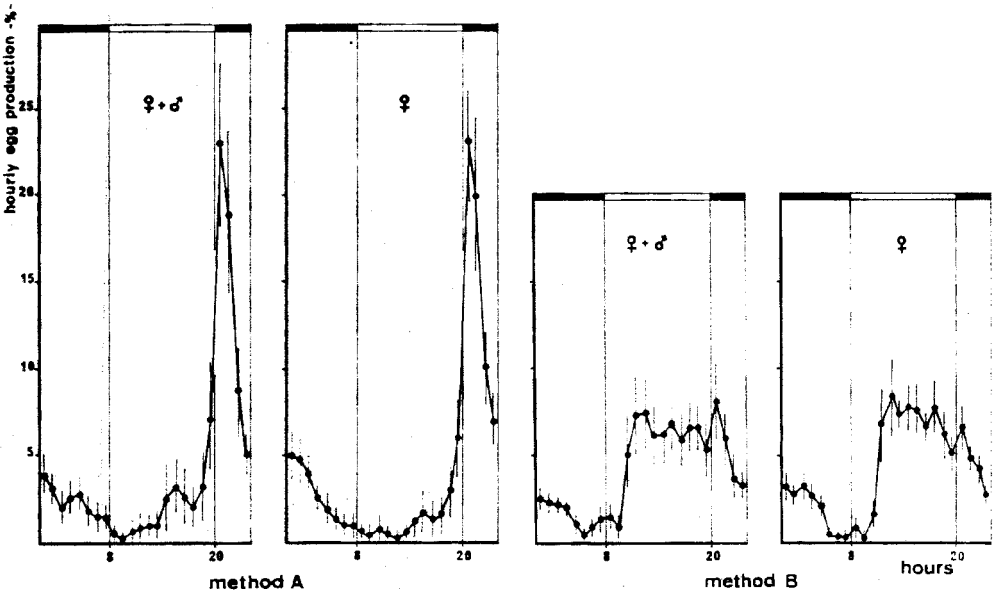


Fig. 3 : *Influence of the presence of males on the oviposition rhythm. Rhythms measured on 4 inseminated females with conditions A and B.*

Fig. 3 : *Influence de la présence de mâles sur le rythme de ponte. Rythmes mesurés sur des groupes de 4 femelles fécondées, dans les conditions A et B.*

VIRGIN FEMALES OVIPOSITION RHYTHM AND EFFECT OF INSEMINATION

The reproductive physiology of *Drosophila* females depends on the presence of males and on insemination. Virgin females are able to lay although their physiology is different to that of normally inseminated females (Boulétreau, 1974 ; Mahowald and Kambysellis, 1980). The oviposition rhythm of these females and the insemination effects on the rhythm have been studied with 3 densities (condition A) : 2, 6 and 12 females. These rather high numbers have been chosen because of the low virgin females fecundity rate which makes the rhythm measurement difficult, and because grouping regulates their fecundity (Merle, 1970). At the age of 8 days, the virgin females have a fecundity level which is sufficient and stable enough to be studied (about 20 eggs/female/day).

In the case of virgin females, the rhythm curves do not show the effect of population density *(fig. 4)*. In the three cases, the rhythm has

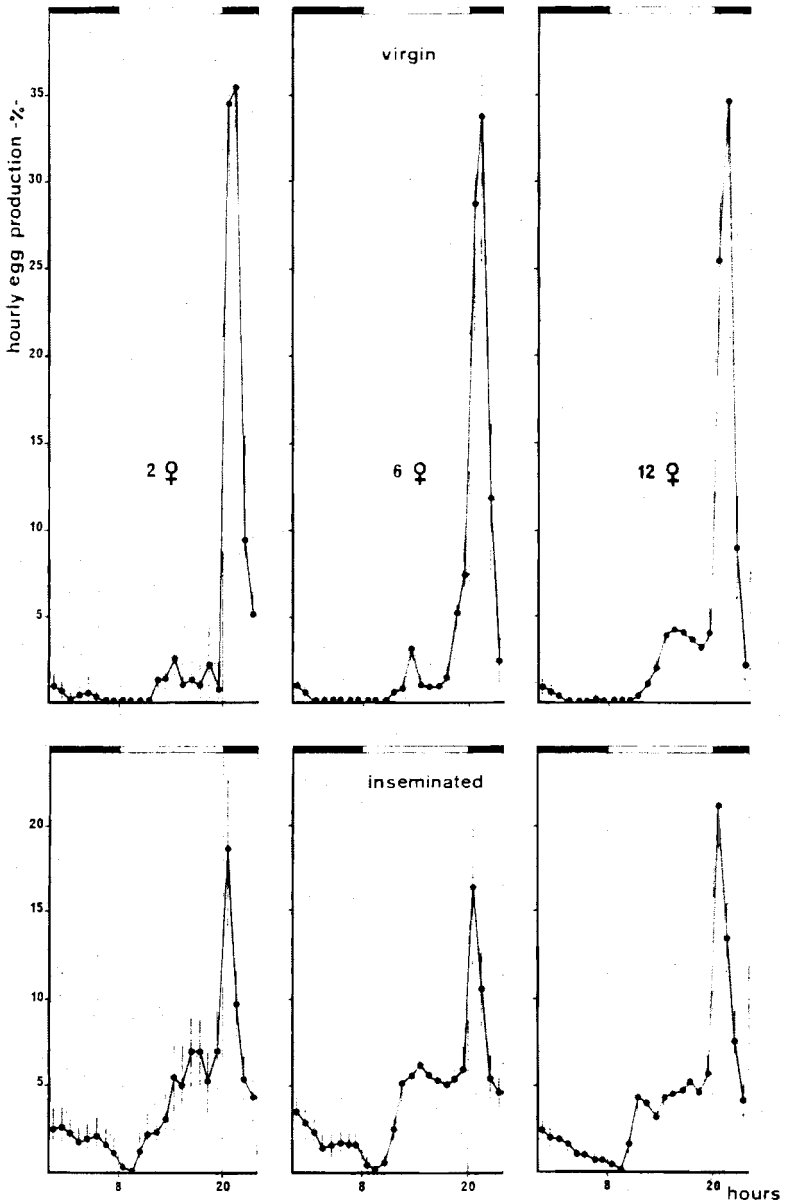


Fig. 4 : *Oviposition rhythm of virgin females. Effect of grouping before and after insemination (condition A).*

Fig. 4 : *Rythme de ponte de femelles vierges. Effets du groupement avant et après l'insémination (condition A).*

a high peak at the start of the scotophase exceeding 30 % of the daily egg laying.

Two days after insemination, the rhythm was measured on the same females (in the presence of males). The fecundity doubled after mating, compared with the previous days, but was lower, about 30 %, than that of females normally inseminated after emergence.

After fertilization, the rhythms remained similar *(fig. 4)*, but the peaks only reach 16 to 20 %. The density effect is much less noticeable than when the females have been in the presence of males throughout their life *(fig. 1)*.

## DISCUSSION

Several results presented in this paper deserve to be discussed : the grouping effect, the effects of the males behaviour on the females egg laying, and the oviposition rhythm of the virgin females. Then it will be possible to attempt a synthesis of these results with those obtained in response to some abiotic factors, or those published by other authors, and finally to discuss the actual nature of this rhythm.

In *Drosophila*, some effects caused by grouping are known, in particular the decrease of the fecundity (Pearl, 1932 ; Grossfield, 1978). However, in this work, we are only interested in the immediate effects on the oviposition behaviour, since the duration of the experiments was too short to ascertain important modifications of the daily fecundity.

The grouping of the flies causes an increase in the laying peak at the beginning of scotophase ; therefore this factor can be considered as momentary-inhibiting, bringing about oocytes retention (Allemand, 1983). This phenomenon leads to a paradoxical situation because the more numerous the flies are, the more their egg laying is synchronized and therefore the more the laying sites are congested. Obviously, this is only possible if the laying surface is sufficient for each fly to have access to, and consequently, the extent of this phenomenon is reduced in the case of overcrowding.

The grouping influence can also be considered as an environmental factor which synchronizes the flies. Such group effects or social stimulation have been shown in vertebrates (see Regal and Connolly, 1980), but relatively little is known on the effects of social interactions on the circadian rhythms of non-social insects (Rivault, 1981). In *Drosophila*, the grouping effect does not seem to depend on the available surface substrate or on the volume of the cage, since the decrease of these parameters does not cause a rhythm amplification (constant number of flies). The effects seem to be linked to the interactions between flies which, in the experimental conditions, are placed in a enclosed area. These interactions can be direct between the individuals and/or the consequence of marking by the flies of the laying substrate and the cage. We have pre-

viously suggested that the effect of the renewal mode (comparison of conditions ; Allemand, 1983) could be explained by the marking of the substrate by the flies. This marking which inhibits the egg laying during photophases must be of short duration since the already used substrate does not bring about significant effects on te oviposition rhythm. This hypothesis of the effects of marking, inhibiting the egg laying for a few moments, would thus explain the conflicting results of Mainardi (1968), Ayala and Ayala (1969) and Bartolozzi *et al.* (1975) concerning the possible influence of pheromones on *Drosophila* oviposition. According to some authors, females prefer to lav on intact substrates ; others believe the flies to be indifferent to the previous presence of males or females. In this work, the marking would only proceed from females (since males do not exert influence on the laying behaviour), but the direct interaction between individuals has not been observed. It could be derived at the moment of the laying peak from the females or from the eggs of stimulating substances which would act on the other females. Such pheromones are known to exist with some other Diptera (Osgood, 1971).

The males do not seem to act on the female oviposition rhythm since their sexual behaviour is identical during the laying phases of the two types of rhythms. However, considering the circadian flies organization, the general and sexual activities of males are out of phase with the females laying activity. General activity is nocturnal (Hardeland and Stange, 1971), matings occur at the end of scotophase and at the start of photophase when the females laying is always the lowest.

The virgin females have a quite different physiology from that of inseminated females, and in particular, their oogenesis is weaker (Boulétreau, 1974 ; Mahowald and Kambysellis, 1980). Their egg laying behaviour presents a strong variability and seems to be more sensitive to environmental agents (Boulétreau, 1974). An increase in density exercises a stimulating effect on their fecundity, contrary to what is observed with the inseminated females (Merle, 1970). Their oviposition rhythm is also different since the laying peak is very high. Such a phenomenon has already been shown with another Diptera for the activity rhythm (Jones and Gubbins, 1978), the activity of virgin females being more cyclical. With *Drosophila*, the more distinct oviposition rhythm can be explained by a greater sensitivity to the environmental factors and also by low fecundity, i.e. a greater capacity for oocytes retention.

The grouping effect observed with the females kept with males is not observed with the females which have remained virgin for a long time before insemination. These females are old (about 12 days) and their fecundity remains low, which can favour mature oocytes retention. This would explain the behavioural differences between these females and the normally inseminated females ; however, the hypothesis of a physiological tolerance to retention cannot be excluded.

The numerous environmental factors which have effects on the egg laying behaviour act as exogenous determinants on the circadian ovipo-

sition rhythm, and therefore, the rhythm characterization of the species *D. melanogaster* is difficult to define. The effects of biotic factors complete the model proposed to explain the oviposition rhythm expression. The females have numerous oocytes in their ovaries during photophase and the observed rhythm is the result of the more or less inhibiting effects produced by the environment which act on the ovulation *(table II)*. If the favourable factors (calm environment, convenient

Table II : *Main environmental factors acting on the circadian oviposition rhythm studied under LD 12 : 12 photoperiod. (1) see Allemand (1977), (2) see Allemand (1983).*

Tableau II : *Principaux facteurs de l'environnement agissant sur l'expression du rythme circadien de ponte étudié en photopériode LD 12 : 12. (1) d'après Allemand (1977), (2) d'après Allemand (1983).*

| Factors favourable to oviposition<br>Oviposition rhythm<br>with strong egg laying during photophase | Factors inhibiting oviposition<br>Oviposition rhythm<br>with a high amplitude peak at " dusk " |
|---|---|
| Low intensity light | Strong intensity light (1) |
| Fresh and attractive substrate | Dry, stale and repulsive substrate (2) |
| Calm environment | Disturbances, slight shakes at the time of substrate renewal (2) |
| Known, marked environment | Unknown environment (2) |
| Insemination | Virginity |
| Isolation | Grouping |

substrate, isolation...) prevail, the oviposition rate is high during the photophases ; and on the other hand, if the inhibitig effects (grouping, strong lighting...) are prevailing, the rhythm shows a high peak at the start of darkness. These environmental effects have been tested on flies showing a high fecundity, which was similar between experiments ; however, variations of the ovarian production can also modify the rhythm expression, in particular the low productions may facilitate both strong retentions and rhythms with high amplitude peaks. Owing to this model, the results obtained by other authors can be interpreted and discussed by studying their experimental conditions carefully. The rhythm shown by David and Fouillet (1973, condition A) would be difficult to interpret without putting forward the idea of a genetic variation ; this point will be the object of a further analysis (Allemand and David, in prep.). The effect of the discovery of an unknown environment explains the rhythm modifications observed by Gruwez *et al.* (1971) and by Fluegel (1978). The latter has observed bimodal rhythms which are explained by the study of isolated females placed under medium light intensity. On some oviposition curves, the effect of the daily substrate changing can be

also noticed. Ohnishi (1977) has studied isolated females whose egg laying was very low because of a non-nutritious substrate, and consequently the oviposition took place during the photophases.

The high sensitivity of *Drosophila* oviposition rhythm to environmental conditions implies that this rhythm has strong exogenous determinants, which is contrary to what is generally accepted for insects (Brady, 1974). However, under free-running conditions, an oviposition rhythm remains with a period of 25 hours (Allemand *et al.*, 1981). Only this result demonstrates that the oviposition rhythm has an endogenous circadian determinism. These conflicting conclusions are in fact compatible in the frame of the proposed model since the endogenous rhythm corresponds to the ovarian rhythm which also exists in free-running conditions (Allemand, 1976). In such constant conditions, the oocytes are not retained in the ovaries and are laid without delay. If the environmental conditions are cyclical, this fundamental rhythm can be conceled by variable, partial retentions, which explains the oviposition rhythm variability. The physiological controls of ovarian function and ovulation are very different, which thus reinforces the duality of the mechanisms involved. The vitellogenesis control seems to involve several regulation mechanisms (juvenile hormone ; see Mahowald and Kambysellis, 1980). On the other hand, the oviposition control seems rather to depend on the central nervous system, which allows the female to respond rapidly to external stimuli (Grossfield and Sakri, 1972).

The oviposition rhythm has been studied in laboratory conditions, on young, well-fed females with a constant supply of substrate. In the field, females are submitted to more difficult conditions than in the laboratory. The environmental factors, which have a prevailing role on the rhythm, present in natural conditions, more diversity and greater variations. Therefore, it appears necessary to discuss the repercussions of these results by placing *Drosophila* in its natural environment.

This species develops on decaying plants, vegetables and fruit. The ecological observations have essentially consisted in measuring the activity rhythm by means of traps. The collected individuals are searching for feeding or laying sites. In the tropical regions from where *D. melanogaster* originates, this species is attracted during photophase and remains on the fruit during the night (David, 1971, 1973 ; Lachaise, 1974). This data is compatible with observations obtained for the oviposition rhythm studied in laboratory (laying at the end of photophase and at the start of scotophase). The ovarian state of the collected females is quite different from that of the females confined in laboratory conditions ; these females differ greatly from each other, the difference being due in part to age, but also to their individual history and to their nutritional state. The ovarian activity is lower, the vitellogenic oocytes are less numerous, and most females have mature oocytes in retention (Boulétreau, 1978 ; Boulétreau *et al.*, 1982). A study carried out in Africa with a view to measurig the daily rhythm of ovarian activity has not

given significant results for two reasons : the variability between females and because of the numerous females showing a blocked oogenesis with retained oocytes (David and Cohet, pers. com.).

In natural conditions, the females' dependence on the environment is stronger than that observed in the laboratory, and the main difference between the two types of conditions is the food availability on which the ovarian activity depends. There are also the sudden changes in climatic factors (light, temperature, humidity), but their effects can be reduced by the behavioural response of the females which can move to escape them. Moreover, on the laying sites, the females are submitted to an inter and intraspecific competition which constitutes a limiting factor in the oviposition capacities. In such natural environments, retentions may constitue a normal phenomenon and an effective mechanism for species survival. The laying activity is in fact opportunist and very different from that of laboratory conditions where the laying substrate allows the oviposition without a lenghty retention. Therefore, the oviposition rhythm must be defined at the population level rather than at individual level, and because of this, it may well be that rhythm with high peaks at the beginning of the scotophase represents the actual *D. melanogaster* oviposition rhythm.

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