Correlated responses in lines of *Drosophila melanogaster* selected for different oviposition behaviours

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Summary. D. melanogaster was subjected to selection for two different traits of oviposition behaviour in relation to oocyte retention in ovaries, i.e. the aptitude to stop laying in response to an unsuitable substrate, and the occurrence of a dusk peak in the circadian oviposition rhythm. Selection for high and low lines was rapidly successful for both characters. Each selected line was also tested for the non-selected trait. Results showed a genetic correlation between the two behaviours, suggesting a common mechanism for the control of oviposition, independently of the origin and the duration of oocyte retention.
Key words. Oviposition behavior; circadian rhythm; selection; *D. melanogaster.*

As in other insects, egg-laying in *Drosophila* results from complex physiological and behavioural processes, involving oocyte production, ovulation and oviposition [1]. Egg-laying must be considered to be dependent on environmental conditions, and regulation of oviposition includes intrinsic and extrinsic components [2]. Among experimental factors that have been shown to affect the egg-laying rate, most are related to the laying substrate (humidity, components, appearence...[3-6]). Moreover, the egg-laying pattern, in response to a photoperiodic cycle, shows circadian variations with a peak at dusk [7]. In all these cases, the behavioural response is expressed by an egg retention, as mature oocytes can be retained in the ovaries for several hours before deposition, independently of vitellogenesis. The ability to stop egg-laying (oviposition blocking) is under control of the nervous system and can occur in response to various environmental stimuli [2]. The retention allows *Drosophila* females to find suitable oviposition sites and to deposit several eggs in a few minutes. The adaptative value of such a trait might play a major role in patchy environments.

In natural populations, the existence of genetic variation for oviposition preference and oviposition time have been demonstrated [8-10], and artificial selection has been successful [5, 11]. However, only a few studies have investigated the genetic control of the egg-laying behaviour that relates to the ability to block oviposition. Artificial selection upon circadian oviposition rhythm and laying on an unsuitable site was therefore performed to induce retention for different times, i.e. a few hours for the first trait and more than 24 h for the second one. Selected lines were analysed to determine whether selection for one oviposition trait produced any correlated changes in the other. It appeared that a common genetic system controls both short retention (circadian rhythm) and long retention (unsuitable substrate).

Materials and methods

Experiments were performed with a natural population located in Beynost, near Lyon (France). About 200 flies were caught, and from these a mass-reared population and 50 isofemale lines were founded. Strains were selected either for their characteristics of daily oviposition rhythm or for their oviposition blocking capacity.

– Selection for oviposition blocking capacity: The term 'oviposition blocking capacity' was used instead of egg retention to differentiate this ability to stop egg-laying (in reaction to unfavourable conditions) from the initial egg retention occurring in virgin females. The latter is due to the delay of the ovulation process at the ovariole level, whereas the former is initiated at the uterus level [9]. From a pool of 50 mixed isofemale lines, selection for high and low ability to stop oviposition was carried on for six generations. Females unable to stop egg-laying and females capable of long delay were mass selected in the presence of unsuitable substrate. The main trait used for selecting parents was the oviposition rate on the first day after switching the substrate. In each generation, the 6–10 most extreme females were selected out of 50 to found the next generation.

– Selection for oviposition rhythm: Oviposition rhythm usually shows a broad mode during photophase and a sharp peak at dusk. Artificial selection attempted to decrease the dusk peak for the low line and to increase the dusk peak for the high line. From the mass-reared population, selection was made for 9 generations. In each generation, 10 pairs were isolated and the rhythm of their offspring was recorded. The two families showing the best scores for each line were isolated. They were mixed (40 flies) and used for the next generation out of which 10 pairs were isolated and tested.

– Experiments: The four selected lines were studied for 3 or 4 generations after selection took place. Flies were reared in low density, on a killed yeast medium, under LD 12:12 photoperiod at 25 °C. After emergence, groups of 4 males and 4 females were transferrred into plastic boxes. Both the traits were measured on these groups between the 7th and the 12th day, when egg production is maximum and stable. Therefore, the two behaviours could not be studied on the same individuals.

The substrate effect was studied by changing the complete, dead yeast medium for an agar + sugar medium

(without flour or yeast[9]) on day 10. Daily laying was recorded 3 days before and 3 days after the substrate had been changed. The circadian rhythm was recorded with an apparatus providing substrate to the flies continuously[7], with the egg position indicating the laying time.

Results

1) *Laying selection*: The killed yeast medium is very suitable for *D. melanogaster* oviposition. Under the experimental conditions used, flies from the (initial) mass-reared population laid about 60 eggs per day (table). When placed upon unsuitable agar medium, females kept the eggs in retention and oogenesis was rapidly stopped. The first day after the substrate had been changed, the laying rate was low (about 27 eggs) and no more eggs were laid after three days (total oviposition of 47 eggs, table and figure).

The oviposition-blocking capacity was modified by selection. The ability to retain eggs on deficient medium had shifted, producing two opposite, significantly different lines (table and figure). The realised heritabilities estimated from the 6 generations were 0.18 ± 0.08 for the high line and 0.31 ± 0.07 for the low line. For the former, the daily fecundity was also significantly reduced (table). Rates of egg deposition on agar medium during the first day were significantly different between the two selected lines: 60% and 20% of the normal daily laying. Both selected lines differed also from the initial population (table and figure). The total number of eggs that were laid for 3 days on agar substrate confirms this difference despite a progressive decrease of the blocking capacity (table).

2) *Rhythm selection*: The oviposition rhythm of the initial population, which showed a high laying rate during photophase and a peak after light-off (figure), was quite typical for populations from temperate Europe. Selection for an increased laying rate during photophase for the high line and during scotophase for the low line gave rise to modifications in the initial pattern but did not affect the daily fecundity. After 9 generations, two opposite tendencies were observed; a rhythm with a high peak at the beginning of the scotophase and a rhythm with a low peak at dusk and with a maximum laying rate during photophase (figure, see also Allemand et al.[10]). The effects of the selection are highly significant when expressed in number of eggs (table) since the night peak is enhanced by a coefficient of 4 between the two lines. The estimation of the realised heritabilities from the first 7 generations are 0.27 ± 0.09 for the high line and 0.47 ± 0.06 for the low line.

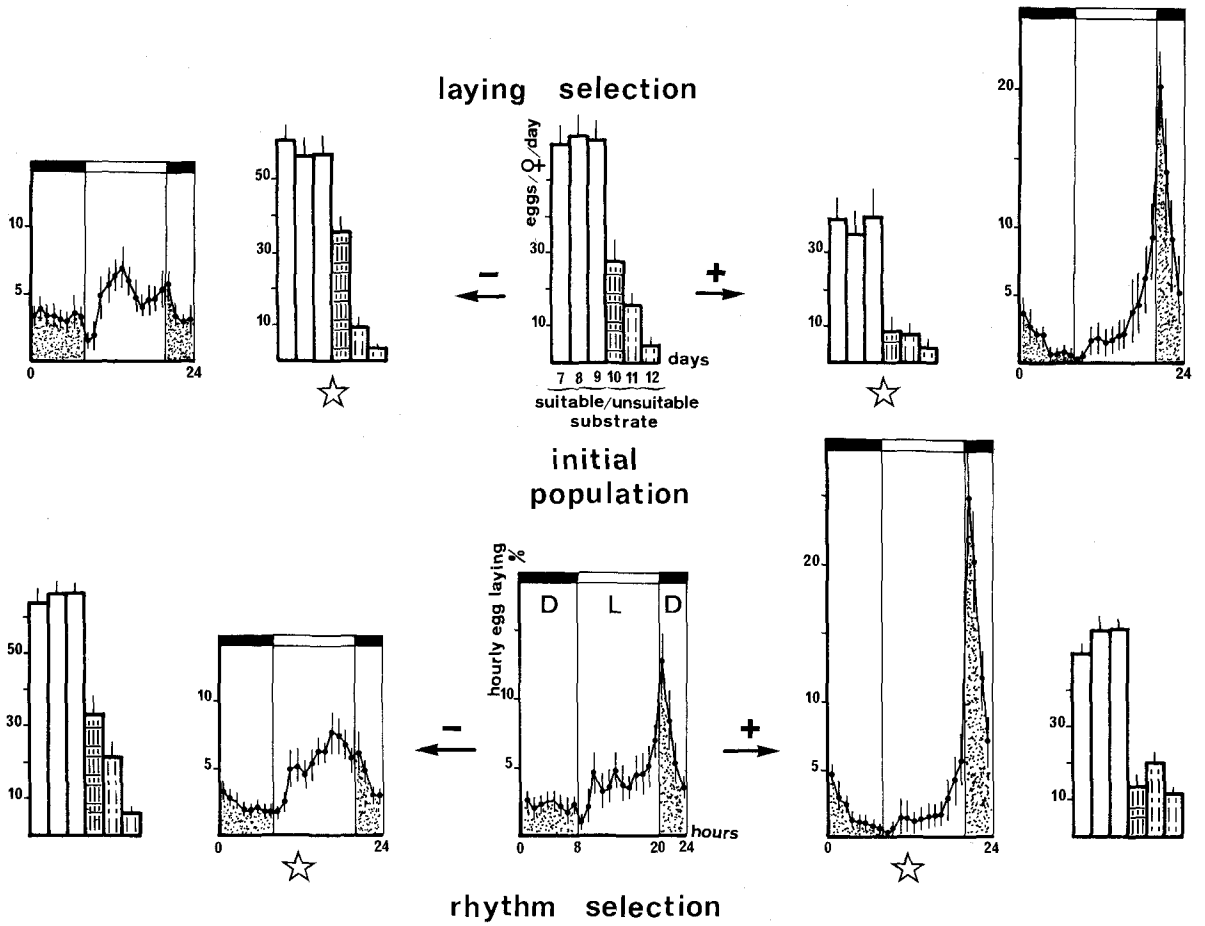
3) *Correlations between selections*: The lines selected for one behaviour were tested for the other one. Results are presented with the previous ones on the figure and in the table. The daily fecundities estimated in the two kinds of experiments are similar ($r = 0.94$, $p < 0.05$). In both cases, the trait that had not been directly selected was significantly modified and one can observe a good similarity between high laying after substrate change and a rhythm with a low dusk peak, and also between low laying after substrate change and a high peak. However, the correlation between the two traits, calculated on the lines and on the initial population, is not significant ($r = 0.85$, $p < 0.10$). The modification of the unselected trait was always of less importance than modifications in lines that were specifically selected for this character (and was sometimes insignificant).

Discussion

Artificial selection for oviposition behaviours can give rise to genetic divergences in *D. melanogaster*. Response to selection for the two behavioural traits, blocking capacity and daily rhythm, confirms the genetic plasticity of the oviposition behaviour which has been selected in the past for some aspects of the oviposition site preference (OSP), the choice between two media[11] or the tendency to insert eggs in the substrate[5].

Selection for egg-laying on an unsuitable substrate and for the circadian rhythm of oviposition. Fecundity and laying are expressed as the number of eggs per female. The laying rate (%), that is recorded the first day after the change of medium is expressed as a proportion of the mean fecundity before change. n = number of groups of 4 females. Mean ± standard deviation. Significance of the t-tests (comparisons to the initial population and between lines): * < 0.05, ** < 0.01, ns = no significant.

		Egg-laying Daily fecundity before change		1st day laying after change		3 days laying after change	Oviposition rhythm Daily fecundity		Peak laying 20.00–22.00 h	
Initial population		60.0 ± 3.3 n = 10		27.0 ± 2.9 (% 45.1 ± 5.3)		47.2 ± 3.4	68.3 ± 4.6 n = 15		13.7 ± 1.3	
Laying selection	Retention (high line)	40.1 ± 4.5** n = 10	**	8.3 ± 2.1** (% 21.2 ± 4.0)	**	20.0 ± 3.0	45.9 ± 4.2** n = 15	**	16.6 ± 1.4ns	**
	No retention (low line)	57.0 ± 2.7ns n = 12		34.0 ± 2.3* (% 59.7 ± 3.1)		46.5 ± 2.8	67.7 ± 4.0ns n = 14		6.5 ± 2.7*	
Rhythm selection	Laying peak (high line)	54.3 ± 7.6ns n = 11	ns	13.6 ± 1.6** (% 25.3 ± 3.0)	**	45.4 ± 1.9	57.8 ± 3.9ns n = 14	ns	24.4 ± 1.8**	**
	No laying peak (low line)	65.7 ± 7.8ns n = 11		33.2 ± 3.1ns (% 50.2 ± 4.5)		59.8 ± 2.1	68.2 ± 4.1ns n = 15		7.3 ± 1.4**	



Selections for two oviposition behaviours: egg-laying on an unsuitable substrate and circadian rhythm of oviposition.
The two behaviours are presented for the initial population and the selected lines. The trait that is subjected to selection is indicated with a star. (+): high line, (−): low line.

In our experimental conditions, females are young and well fed, with a constant supply of food and of laying substrate. When they are placed on an unsuitable substrate, the oviposition is partially blocked and the oogenesis is rapidly stopped, as shown by dissections. Only the few eggs that were already engaged in vitellogenesis achieved maturation and were laid during the three days after the substrates were switched. In such conditions, the laying substrate induces a strong retention which goes on for a few days [9]. By selection, the intensity of the blocking capacity was modified. In the high line, the daily egg production was decreased, which should make the retention easier.

In the case of the circadian oviposition rhythm, the photoperiod induces a cyclical activity of the ovarian physiology. Every day, numerous synchronous oocytes complete vitellogenesis during the photophase and are partially retained in the ovarian tubes for the end of the photophase, till the 'light off' signal results in the laying peak. The time of retention varies between oocytes and usually does not exceed a few hours. It depends on environmental conditions such as light intensity, interactions between individuals, etc.[7,13]. By selection, the rhythm patterns are strongly modified without significant change in the daily egg production. For both behaviours, the response to selection estimated by the heritability values was more rapid for the low lines, in which the retention time was lowered. This difference in the response can be related to the modification of the egg production (only significant for blocking capacity) which appeared when selection attempted to increase egg retention.

The two selected traits are quite different because they relate to distinctive physiological states and durations of ovarian retention. They are also different with respect to the environmental clues to which the selected flies responded. The short, normal and non-obligatory retention occurring in the daily rhythm can be opposed to a long, strong but obligatory one occurring in the presence of unsuitable substrate. Comparison between the two selection experiments showed that they led to similar phenotypes even for the non-selected trait. The correlation between traits could be due to pleiotropy of some major genes influencing the two traits. However, this phenomenon can be explained more simply by a common mechanism which becomes active in response to cues as different as unsuitable substrate or lighting. The selection may affect even the reactivity to environment or, more likely, the physiological mechanism controlling oviposi-

tion. The genetic response to very unfavourable environments (extreme stresses) has been investigated and most of the results can be explained by relatively few genes having broad and mainly additive effects [16, 17]. However, the egg retention affects the ovarian activity, and limits to selection would presumably be more severely canalized than those for specific actions of chemicals like ethanol or insecticides. In our conditions, the poor substrate or the photophase cannot be considered as stressful factors since flies are normally submitted to photoperiod and are often deprived of oviposition sites as shown by their ovarian state in the field [18].

The capacity to stop oviposition must be a muscular, voluntary closing of the genital duct, under the control of the nervous system, since most of the females in our experiments presented one egg in the uterus. The genetic bases of this egg-laying control need further investigation. Nevertheless, genetic variability between isofemale lines for both the oviposition rhythm and the response to an unsuitable substrate has been demonstrated already [9, 10]. Under natural conditions, the blocking capacity shows seasonal variations giving rise to an increasing frequency of flies with strong retention in the spring and in the autumn [14, 15]. Such observations express the great genetic plasticity of this behaviour, which seems to be selected by natural conditions (temperature...) in a few generations [14].

The ability to control the oviposition process in response to various environmental factors, which can be as different as photoperiod or laying substrate, must allow *Drosophila* females to adapt their response to environmental events, in particular by rapidly laying numerous eggs when conditions become more favourable. This trait might be particularly important for the population when the developmental sites are scarce and scattered, as is likely to be the case in temperate regions [18].

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