Chromosomal analysis of initial retention capacity in virgin *Drosophila melanogaster* females

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In natural temperate populations, virgin *Drosophila melanogaster* females present highly variable periods of preoviposition duration (from 2 to 25 days). Strains were selected for long and short initial retention capacity. Chromosome substitution between two of these lines produced, by appropriate mating procedures, every possible homozygous chromosomal combinations of the X, II and III chromosomes. Analyses of these lines demonstrate that both X and III chromosomes are involved in this egg-laying control, and have complementary effects.

INTRODUCTION

In temperate regions, the maintenance of an insect population not only depends on the egg production rate but also on the ability of females to manage egg laying in relation to the carrying capacity of the environment. *Drosophila melanogaster* populations originated in tropical Africa and adapted themselves during their geographical expansion, acquiring by natural selection a higher reproductive potential. However this potential is rarely expressed due to the scarcity and dispersion of food and egg-laying sites for a great part of the year, during which adults give priority to individual survival rather than reproductive effort. In European populations, *D. melanogaster* females avoid loss of material (eggs) by means of two egg-laying controls. The first control allows the female to stop egg deposition rapidly, in response to unfavorable change in environmental factors (food, temperature, humidity, light etc.). This nervous control acts on the oviposition process, at the uterus level, and occurs in both virgin and inseminated females.

The second control is specific to virgin flies and acts, at the ovariole level, on the ovulation process. In the absence of a sexual partner and insemination, some virgin females are able to postpone egg-laying for several days, keeping their first batch of eggs in the distal part of the ovarioles. The ovulation process is delayed by the lack of a myotropine brain hormone, the release of which

is normally induced by mating stimuli. Flies with this retention capacity are very rare in African populations, but the frequency of this physiological characteristic increases along a latitudinal cline. In French populations, this retention ranges from 2 to 25 days, but the frequency of individual capacities varies according to the season—high retention phenotypes being favoured by lower developmental temperatures (Boulétreau-Merle *et al.*, 1986). This physiological trait is genetically determined and opposite phenotypes for retention capacity can be rapidly separated by selection (Boulétreau-Merle, 1982). The opposite strains also differ in their ovarian activity and hormonal regulation (unpublished). The present results from chromosome transfers identify chromosomes carrying a gene (or a group of genes) involved in the determinism of this characteristic.

MATERIAL AND METHODS

The chromosome transfer experiment was carried out using two lines: line 18 and line 52, chosen from among various isofemale lines founded by wild flies from the natural population of Beynost near Lyon. They were brought to relative homozygosity by a few successive selections and then kept in laboratory conditions for five years.

Line 18 was characterised by a low retention capacity *i.e.*, the majority of the females which had

been kept virgin laid their first eggs within two or three days and presented relatively high and stable fecundity over the following days. In line 52, on the contrary, the flies presented a high retention capacity. They kept their first batch of mature eggs in the distal part of the ovarioles for a long preoviposition period—generally more than ten days.

Chromosome transfers were achieved using balancer stocks to test the role of the three major chromosomes. The second chromosome was marked with *Cy(In2LR)L/Pm* and the third chromosome with *In(3LR)TM3 Ser/Sb*. No markers were used for the X chromosome and wild chromosomes were manipulated, using their hemizygote state in males.

The technique of chromosome transfer (Robertson, 1954; Kearsey and Kojima, 1967; Pyle 1978) leads to the production of six strains, differing by the combination of homozygous chromosomes from the two original strains, and to two recreated strains, each with the three pairs of initial chromosomes. In this first experiment, no attempt was made to study the effects of the various chromosomes in heterozygous conditions.

The tested flies were reared at low density, 25°C and in total darkness on normal axenic medium (David and Clavel, 1965). The eight strains were tested at the same time. On emergence, over a short period (2 h), 50 virgin females for each strain, were taken randomly from ten rearing tubes, and immediately isolated in individual tubes. Nutritive

medium was renewed every day and the fecundity of each female determined daily. Retention capacity was measured in terms of the delay, in days, between emergence and first egg deposition.

Data analysis on the preoviposition durations are presented as frequency histograms of individual values and treated by usual statistical methods and by reciprocal averaging calculated on increasing class intervals (logarithmic classes). The aim of this correspondence analysis (Benzecri, 1973; Hill, 1973; Gauch, 1982) is to transform a classical contingency table row/column (duration/strain) in a single dispersion diagram by successive reciprocal ordinations of the averages in order to obtain a progressive optimization of the correlation between the two vectors. This eigen-vector method of ordination permits spatial representation and gives the relative position of the genetically different strains taking into account the values and the frequencies of individual phenotypes in each line.

RESULTS

Numerical results of chromosome transfers are presented in the first part of table 1. The frequency histograms of individual retention capacities in the initial strains and the eight genotypes obtained are given in fig. 1, with corresponding daily fecundities.

Table 1 Chromosome composition, mean retention duration (days) and global ten days fecundity of virgin females in the two initial lines and in the various lines obtained after chromosome substitution or in lines heterozygous for X chromosome

	Chromosome	Composition			N	Retention duration $m \pm \sigma m$	10 days fecundity $m \pm \sigma m$
Initial lines	line 18	①	②	③	32	1.34 $\pm$ 0.13	362.50 $\pm$ 19.33
	line 52	1	2	3	32	15.34 $\pm$ 0.64	1.87 $\pm$ 1.62
Lines after chromosome substitution	New 18	①	②	③	50	1.38 $\pm$ 0.09	413.02 $\pm$ 16.68
	line e	1	②	③	50	2.94 $\pm$ 0.50	238.04 $\pm$ 18.83
	line i	①	2	③	50	2.24 $\pm$ 0.19	337.48 $\pm$ 19.87
	line g	1	2	③	50	2.36 $\pm$ 0.21	190.12 $\pm$ 17.46
	line h	①	2	3	50	3.86 $\pm$ 0.31	160.90 $\pm$ 17.91
	line f	1	②	3	50	10.00 $\pm$ 0.88	19.98 $\pm$ 5.70
	line j	①	②	3	50	10.10 $\pm$ 0.89	27.46 $\pm$ 8.74
	New 52	1	2	3	50	15.54 $\pm$ 0.97	8.48 $\pm$ 3.59
Test for X chromosome influence	New 18	①	②	③	22	1.27 $\pm$ 0.11	447.50 $\pm$ 21.39
	♀e $\times$ ♂18	①	②	③	22	1.18 $\pm$ 0.08	346.77 $\pm$ 24.99
	♀18 $\times$ ♂e	①	②	③	22	1.59 $\pm$ 0.25	386.13 $\pm$ 23.91
	line e	1	②	③	22	3.50 $\pm$ 0.33	225.90 $\pm$ 29.00
	line h	①	2	3	22	4.09 $\pm$ 0.62	124.77 $\pm$ 26.82
	♀h $\times$ ♂52	①	2	3	22	7.45 $\pm$ 1.02	64.81 $\pm$ 18.71
	♀52 $\times$ ♂h	①	2	3	22	8.36 $\pm$ 0.92	52.50 $\pm$ 14.88
	New 52	1	2	3	22	12.04 $\pm$ 0.88	10.59 $\pm$ 5.72

Figure 1 Frequency distributions of individual retention durations and daily fecundity of virgin females in initial and homozygous chromosome substituted lines.

The distribution of individual values in the two reconstructed lines (New 18 and New 52) is similar to that of the original lines; the mean values are identical. A lower value was observed in the long retention line in a complementary experiment done one year later (second part of table 1).

It appears evident that the presence of a third chromosome from line 18 alone is sufficient to induce a low retention capacity (see g, fig. 1), while a third chromosome originating from line 52 is

responsible for the high retention capacity in lines f and j. The results are not so clear cut in line h and line e which have the inverse chromosome association. The shift of the distribution towards low retention in line h and towards long retention in line e might be due to a weaker effect of the sex chromosomes. The second chromosome is apparently not involved in determining the trait.

In spite of great differences in the distributions of the trait among the eight lines, an analysis of

variance of the three chromosome effects was carried out (table 2). This confirmed the predominant effect of chromosome III and the intervention, to a lesser extent, of the sex chromosomes. It also revealed the existence of interactions between these two chromosomes, and an unexpected interaction between chromosomes I and II.

Table 2 Analysis of variance of the three major chromosome influences on the initial retention capacity and the fecundity over 10 days, of virgin *D. melanogaster* females

Source	df	M.S.	F
Retention			
Chromosome I	1	1,098.92	59.99**
Chromosome II	1	1.10	0.05
Chromosome III	1	5,844.60	313.75**
Interaction I-II	1	668.22	35.87**
Interaction I-III	1	612.56	32.88**
Interaction II-III	1	6.00	0.32
Interaction I-II-III	1	1,092.30	58.63**
Error	392	18.62	
Fecundity			
Chromosome I	1	1,453,350.80	130.92**
Chromosome II	1	14.05	0.0
Chromosome III	1	5,781,860.70	520.86**
Interaction I-II	1	85,995.57	7.74*
Interaction I-III	1	164,957.83	14.86**
Interaction II-III	1	376,443.61	33.91**
Interaction I-II-III	1	186,062.82	16.76**
Error	392	11,100.50	

* $P < 0.01$; ** $P < 0.001$.

The influence of the sex chromosomes was tested one year later by reciprocal crosses between line h and New 52 and between line e and New 18, which differed only in sex chromosomes. Numerical data were presented in the second part of table 1. The distributions of individual retention capacities in three of these four strains were similar to those observed in the first experiment although the e line showed a peak for two day retention, instead of one day as in the previous year. The long retention line (New 52) showed a significantly lower value but remained statistically different from the other lines.

Heterozygote females, obtained by crosses between line 18 and line e, showed a distribution like that of New 18, especially in the case of females issued from line e mothers. The genetic determinant from the line 18 sex chromosome appeared dominant. This effect was also visible in the crosses between line h and New 52, in which virgin females of the two F1 strains showed very similar and intermediate retention capacities. Thus, this complementary experiment confirmed that the X

chromosome is also involved in control of retention capacity of virgin flies.

Reciprocal averaging (a more adequate correspondence analysis which is not limited by the distribution forms) gave a classification of the various strains (fig. 2). Among the eight strains obtained after chromosome transfer and the two initials lines (fig. 2(A)), the first axis discriminates between high and low retention phenotypes. It clearly opposes the strains, e.g., i and New 18, carrying the third chromosome from line 18 (low retention) from the strains f, j and New 52 carrying the third chromosome of the long retention line. On this axis, the h strain has an intermediate situation; moreover it is isolated by the second factor (vertical axis) which differentiates the strains composed of medium retention capacities, like strain h, from those constituted by a majority of extreme phenotypes.

Likewise, reciprocal averaging applied to reciprocal crosses between strains differing with respect to sex chromosomes (fig. 2(B)) shows the intermediate distribution of the F1 from 52 and h lines and the shift of the F1 from 18 and e lines towards very low retention capacities. Line h and above all line e, composed of more numerous intermediate phenotypes, were discriminated by the second vertical axis.

DISCUSSION

The present results confirm that the retention capacity of virgin *D. melanogaster* females is genetically determined and show that, as was previously suggested (Boulétreau-Merle, 1982; Boulétreau-Merle and Terrier, 1986), this determinism is relatively simple.

The major components are located on the third chromosome and are sufficient to induce the occurrence of extreme phenotypes but it is also shown that a second factor(s), located on the sex chromosome, enhances the expression of these phenotypes, leading to condensation of the distribution of short retention capacities and to a shift of the distribution towards very long retention in the opposite case.

The shift of the distributions for heterozygote females in the reciprocal crosses between line New 18 and e might be considered as a partial dominance of the X chromosome from the low retention line, if it were not for the fact that the effect is not apparent for the F1 females of the reciprocal cross between h and New 52 lines.

Figure 2 Representation of the composition of various lines for retention duration capacity in virgin females on the plane defined by first axes of reciprocal averaging. (A) initial and homozygous chromosome substituted lines; (B) homozygous and heterozygous lines for X chromosome.

This difference might be a consequence of chromosome interactions (see table 2). However, this shift, like the enlargement of the distribution in line f and New 52 towards the low retention capacities might also suggest the existence of a bias introduced by the possibly imperfect homozygosity of the initial line 52. The presence of a few individuals showing a retention duration less than ten days in the initial line 52, taken with the above results, might support this hypothesis. Nevertheless, this possible bias does not put the present localisation in question.

Although defined as egg laying control measured by preoviposition duration, this charac-

teristic is not only a behavioural trait like the oviposition blocking capacity which is a nervous control of uterus closing (Boulétreau-Merle and Terrier, 1986). The present control on the ovulation process is under hormonal regulation (Boulétreau-Merle, 1975) and thus is a physiological trait. Moreover, the extreme opposite phenotypes differ greatly in the kinetics of their egg production. Vitellogenesis starts in the same way in both phenotypes; but, whereas the process goes on in the low retention type, after a slight decrease on day three due to the lack of mating stimuli, vitellogenesis and germarium activity stop or become very low in the long retention type for more than

ten days (unpublished). It seems probable that hormonal activities also differ in the opposite genotypes. This problem is being studied by determination of juvenile hormones and ecdysone titers.

As the egg deposition rate is supported by active vitellogenesis in the low retention type, it is not surprising that global egg production for this type of virgin female was considerably higher than for the opposite one (see table 1). The chromosome transfer experiment shows that the origin of the X and third chromosomes are also of great importance for the level of this productivity (see h and i strains in fig. 1).

However, this higher production by virgin flies in low retention lines, does not prejudice the productivity of the same flies when inseminated. In the case of lines 18 and 52, the fecundity rate is effectively higher for mated females in line 18 than in line 52, but in several other selected lines we observed a significantly higher production in the long retention lines. Retention capacity and egg production in virgin females are independent of the ovarian productivity rate of inseminated flies. These traits do not have the same genetic determinism. This genetic independence has already been indicated by comparison of natural populations (Boulétreau-Merle *et al.*, 1982) and also by comparison of the first generation of isofemale lines in a temperate population (Boulétreau-Merle and Terrier, 1986). Albornoz *et al.* (1987) using chromosome assay, also came to the conclusion that egg laying by virgins may be considered a trait with little or no relation to egg laying by inseminated females.

Nevertheless, while Chapco (1977) showed the effect of various segments of the X chromosome on the egg production of inseminated females, Watanabe and Ohnishi (1975) studied the genes affecting productivity on the 2nd chromosome, and Dominguez and Rubio (1986) showed the importance of chromosome III for egg production by inseminated flies. These authors determined that genes influencing the trait are concentrated on the left arm of the chromosome and display complete dominance. After all, it is not surprising that the genetic determinants of such a very important and plastic function should be distributed among the three major chromosomes.

The genetic determinism of the trait in question here, i.e. initial retention in virgin flies, is also different from that involved in the oviposition blocking capacity, which is nervous control leading to a retention process at the uterus level, in reaction to unfavourable change in the environment: food and oviposition site (Boulétreau-Merle and Ter-

rier, 1986) or light regimen (Allemand and David, 1984).

The characteristic determined by these two groups of genes is important for the flies above all in temperate populations since it avoids a pointless loss of material constituted by unfertilized egg deposition. Moreover, this type of egg retention in the ovaries keeps the uterus empty and free for fertilization, whereas the oviposition blocking process stops egg laying but keeps an egg in the uterus when the substrate becomes unfavorable. So this retention capacity, specific to virgin females, seems particularly adapted when sexual partners are rare as in Spring.

Although it is not generally considered a major component of fitness, this characteristic has adaptive value and is actually subject to various types of natural selection. The frequency of the high retention genotype increases along a latitudinal cline from South to North according to a directional process (Boulétreau-Merle, submitted) while the trait is subject to balancing selection in European populations (Boulétreau-Merle, 1986) under the influence of temperature variations (Boulétreau-Merle *et al.*, 1986). This system leads to a variation in the relative frequency of the various phenotypes and so to a better-adapted composition of the population in the face of seasonal environmental fluctuations. The relative simplicity of the genetic determination permits this rapid modification of the genetic equilibrium.

Acknowledgements Balancer stocks were available by courtesy of Dr R. C. Woodruff (Bowling Green) and Dr T. R. F. Wright (Charlottesville). We are grateful to them and to Dr Allemand for his helpful advice in the choice of method.

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