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Chromosomal effects in egg laying of *Drosophila melanogaster* under different conditions

J. Albornoz,
A. Domínguez,
E. Alcorta and
E. Santiago

Departamento de Genetica, Universidad de Oviedo, 33071 Oviedo, Spain.

Differences in the genetic determination of egg laying under three different conditions (in a suitable medium, in a non-suitable one and egg laying by virgin females) were studied through chromosome assay in two pairs of inbred lines. Egg laying of inseminated females on the suitable medium showed directional dominance and interchromosomal interactions, as expected for a trait related to fitness. Egg laying on the suitable and the non-suitable media are related, as suggested by the correspondence of some genetical effects. The difference between the egg laying in the two situations, which could be seen as a measure of retention, was affected by additive and heterotic effects. A chromosome mediating higher egg output than its holologue on the suitable medium can mediate a lower egg output on the non-suitable one. This shows that genotype × environment interaction cannot be due only to proportionate effects of all the relevant genes, but that different genes are acting in the two situations. The genetical effects on egg laying of virgin females were very different of those in the other two situations. This is an essentially additive trait, which suggests that it is not related to fitness.

INTRODUCTION

Dependence of *Drosophila melanogaster* egg laying on insemination and environmental variables has been reported (see Grossfield, 1978). However, interest in the effect of such conditions has largely been focused on non genetical aspects.

Oviposition is a complex trait which includes nutritional, hormonal and behavioural aspects that may be differentially involved when egg laying is measured under different environmental conditions (Grossfield and Sakri, 1972). While egg laying when females are inseminated, well fed and in a good oviposition medium could be seen as a measure of nutrient conversion, in the absence of insemination or of a suitable medium, females react by retaining mature eggs in the ovaries (Boulétreau-Merle and Terrier, 1986; Alcorta *et al.*, unpublished results). Therefore, other behavioural and physiological aspects must be implicated in the determination of the egg output in such situations.

Oviposition of inseminated females in a good environment was shown to present the features of a trait directly related to fitness (Chapco, 1977, 1980; Domínguez and Rubio, 1986). On the other hand, egg laying on a non-suitable medium and egg laying of virgins might be considered at first sight as having negative implications on fitness since egg retention in such situations would avoid waste of resources.

The objective of the present study was to survey, through chromosome assay, the genetical effects associated with egg laying under these three experimental conditions: inseminated females on a suitable substrate, inseminated females on a non-suitable substrate and virgin females.

MATERIALS AND METHODS

Four inbred strains of *D. melanogaster* were used: Teverga-5 (T), coming from our own laboratory, and Crkwenica (C), Israel (I) and Kreta-75 (K) lines which came from the Umeå *Drosophila* Stock Centre. Combinations of the three major chromosomes from both the K–T and C–I pairs of lines were synthesized following Kearsey and Komima's (1967) crossing scheme. The inversion chromosomes used were *Binscy*, *Cy O* and *TM*3, *Sb Ser.* Each substitution line is referred to by three capital letters denoting the source of the first, second and

third chromosome pairs. Letter X stands for a chromosomal heterozygote. These two sets of chromosomal combinations were tested for egg laying under three different conditions: inseminated females on a suitable oviposition medium (the whole culture medium), inseminated females on a non-suitable medium (a 0·8 per cent agar gel containing 2 per cent ethanol and 1 per cent acetic acid) and virgin females on the suitable medium. Henceforth we will refer to them as normal, agar and virgins respectively.

Culture conditions and the procedure to determine daily egg laying for virgin and inseminated females in the normal medium have been described elsewhere (Domínguez and Rubio, 1986), with the exception that the measurement period was from the 5th to 8th day inclusive of the females age. To determine egg laying in the non-suitable medium, a set of inseminated females kept in the normal medium until 4 days old were transferred to the agar medium on the 5th day. Egg laying on agar was measured only on the 5th day due to the lack of nutrients, which would lead to a great reduction in egg production later on. In these lines, total egg production (eggs laid + eggs stored) after a day on agar was, as a mean, 90 per cent of the eggs produced in the whole medium on the same day in a previous study (Alcorta *et al.*, unpublished results). Besides, even the females with a higher egg output on agar retained more mature eggs in the ovaries than control females placed in the normal medium. Therefore, the difference in egg output between normal and agar conditions is mainly due to egg retention.

Six egg laying vials (with three females each) per genotype and condition were tested simultaneously.

The statistical analysis of the genotypic variation has been conducted using the genetical model described by Mather and Jinks (1971). The phenotypic means are described by 26 parameters representing additions to or subtractions from a general mean. The parameters d_1, d_2 and d_3 refer to the additive effects of chromosomes I, II and III; h_1, h_2 and h_3 refer to their corresponding dominance effects and the other 20 parameters represent interchromosomal interactions. Parameters i refer to $d \times d$ or $d \times d \times d$ interactions, j are interactions $d \times h$ (*i.e.*, $j_{1\cdot2}$: interaction $d_1 \times h_2$), $d \times d \times h$ (*i.e.*, $j_{12\cdot3}$: interaction $d_1 \times d_2 \times h_3$) or $d \times h \times h$ (*i.e.*, $j_{1\cdot23}$: interaction $d_1 \times h_2 \times h_3$) and l are interactions $h \times h$ or $h \times h \times h$. Letters in parenthesis after each genetical component will denote the substitution set (K–T or C–I) concerned. These parameters were estimated by solving equations obtained by equating observed cell means to their corresponding sum of components.

Variances were heterogeneous between cells within each experimental situation. The log x, $\sqrt{x}$ and $1/x$ transformations did not remove the variance inequality; therefore, the analyses were conducted on untransformed data and errors of the genetical parameters were obtained from the variance-covariance matrix. Significance was tested by the approximate t-test proposed by Welch (t^*, see Brownlee, 1960, pp. 235–239, 265–268).

RESULTS

The mean daily egg production for every genotype in each of the three situations is presented in table 1. The components of variation were subdivided into the single degree-of-freedom contrasts of Mather and Jinks described earlier (table 2). Chromosomes II and III showed a significant homozygous effect (d_2 and d_3) on the K–T substitution set in agar and virgins, while only d_3 was significant in normal conditions. All significant d values were negative: chromosomes from line T were associated with higher egg output. The additive effect of chromosome I in the C–I substitution set was significant and of opposite sign for normal and virgins. Chromosome II had a homozygous effect only on virgins and the additive effect of chromosome III was significantly positive or negative depending on the situation.

Each chromosome, except chromosome I, in the set K–T, displayed dominance in the direction of higher egg output (significant h positive) or overdominance (h larger than its corresponding d absolute value) for egg laying on normal medium. The h_2 and h_3 in the K–T set were also significant on agar.

Out of 8 i interactions for each experimental situation, two were significant in normal medium, two in agar and four in virgins. The presence of i interactions implies that the homozygous effect of a chromosome depends on the homozygous constitution of the other chromosome pairs. Interactions among homozygous and heterozygous chromosome pairs (j) were significant in nine cases for normal medium, five for agar and one for virgins. Finally, heterozygous interactions (l) were negligible for virgins, while two of them were significant for agar and four for normal medium.

The genetical effects of chromosomes in normal medium and virgins were quite different. The d_3 (K–T) additive effect was significant and with the same sign in both cases; nevertheless, d_1 (C–I) and

Table 1 Daily egg laying per female for each genotype in each experimental situation

	K-T				C-I		
genotype	normal	agar	virgins	genotype	normal	agar	virgins
KKK	52·58±5·28	6·78±2·97	7·69±2·43	CCC	57·85±6·45	16·06±3·59	28·47±3·96
KKX	64·60±5·59	8·26±4·14	4·05±1·94	CCX	89·79±4·20	21·00±2·67	26·14±2·37
KKT	60·83±7·43	13·33±3·51	14·03±2·67	CCI	54·29±5·78	53·33±4·24	17·75±2·10
KXK	64·36±5·50	16·67±4·56	5·43±1·96	CXC	89·53±2·69	19·67±3·93	20·72±1·76
KXX	72·44±3·21	25·72±8·06	1·83±0·67	CXX	87·50±2·91	22·78±3·86	13·40±2·58
KXT	88·22±4·53	38·89±9·70	11·15±3·93	CXI	58·71±3·46	38·11±5·31	16·28±1·80
KTK	40·68±5·62	33·61±2·99	15·42±4·07	CIC	67·67±4·19	21·78±7·57	10·05±1·64
KTX	91·07±0·96	43·17±5·56	13·99±1·32	CIX	69·10±4·20	28·44±3·27	8·07±1·38
KTT	75·58±4·24	22·72±5·64	18·88±1·77	CII	51·13±1·48	36·50±2·49	5·33±1·12
XKK	46·40±4·16	3·61±1·01	4·99±2·35	XCC	82·43±5·64	10·56±3·63	17·65±1·44
XKX	71·06±3·24	1·72±0·35	8·27±3·04	XCX	79·89±3·59	29·03±2·19	12·57±2·44
XKT	57·53±3·09	10·83±3·78	12·69±5·28	XCI	73·55±3·67	53·38±4·19	16·10±1·21
XXK	70·25±2·47	13·89±5·51	5·22±2·99	XXC	101·00±1·95	26·67±2·00	10·13±2·11
XXX	78·76±5·55	25·89±5·94	3·82±1·08	XXX	92·58±5·57	50·33±7·46	8·44±1·46
XXT	65·64±4·79	17·56±5·76	8·06±1·07	XXI	55·17±3·41	38·83±2·74	12·54±1·97
XTK	50·08±8·19	35·06±5·01	15·29±2·84	XIC	71·71±3·16	20·39±3·99	11·71±2·66
XTX	90·24±2·70	54·94±10·54	17·81±5·88	XIX	81·07±2·87	37·56±4·56	7·10±1·09
XTT	82·83±1·91	33·47±5·50	19·31±4·07	XII	60·19±4·11	53·06±3·67	5·50±0·98
TKK	43·10±0·56	1·94±1·14	5·79±1·71	ICC	87·40±6·30	19·39±6·07	9·53±1·62
TKX	61·75±3·33	3·28±1·21	12·97±1·30	ICX	94·60±2·87	22·00±6·25	7·29±1·11
TKT	59·52±2·96	13·33±3·11	17·33±3·24	ICI	76·36±3·87	40·69±3·89	12·13±2·25
TXK	66·82±6·33	21·89±5·48	8·67±3·63	IXC	93·40±4·76	31·17±4·75	10·47±3·17
TXX	77·40±4·26	33·56±7·91	16·75±2·79	IXX	66·78±6·60	35·28±5·59	7·52±1·09
TXT	88·62±6·70	41·93±10·31	27·35±6·67	IXI	78·74±3·49	56·33±3·06	9·35±2·07
TTK	40·83±2·34	22·94±2·93	11·01±3·50	IIC	75·95±1·83	30·11±7·07	8·13±2·24
TTX	76·44±6·01	45·11±4·16	16·40±4·60	IIX	76·14±4·51	48·22±7·38	2·85±0·68
TTT	72·89±2·92	45·89±1·64	25·77±2·15	III	57·78±2·90	44·72±3·91	3·17±0·38

i_{12} (C-I) were significant and of opposite sign. Furthermore, the egg laying in the two situations showed rather different pictures in their dominance and interaction effects.

In the K-T substitution set there was some correspondence among the chromosomal effects on egg laying on normal or agar medium. The additive effect of chromosome III (d_3) was significant in both situations and in both cases the chromosome from line T is associated with higher egg laying. The dominance effects of chromosome II and III and two interaction effects were also significant and of the same sign. On the other hand, from the set C-I it was seen that the additive effect of chromosome III (d_3) changed the sign; that is, the chromosome III from line C mediates a higher egg output than its homologue from I in normal medium but a lower one in agar. There is also a significant change of sign in $j_{2\cdot3}$ (C-I). Nevertheless, $j_{3\cdot12}$ and l_{123} were significant and of the same sign in the two situations.

Even though the correspondence of some genetical effects could be due to the accumulation of different genes acting in the same direction for the two situations in the base lines, the fact that various chromosomal interactions coincide suggests that egg laying in normal and agar conditions are related.

Differences between the genetical effects on egg laying in normal and agar conditions are presented in table 3. Although the genetical parameters for egg laying under normal conditions over the 4-day period considered did not differ significantly from those estimated for the 5th day, differences between normal and agar were estimated from egg laying means on the 5th day to avoid any possible effect of the females' age in this difference. The d_2 (K-T), d_3 (K-T) and d_3 (C-I) additive effects were significantly different in the two experimental situations. The heterozygous effect of chromosomes III in the K-T set and of chromosomes II and III in the C-I set were higher in normal conditions than in agar ones. Also, differences in eight genetical interactions were significant.

DISCUSSION

Egg laying of inseminated females in normal medium showed directional dominance or overdominance and interchromosomal interactions as expected for a fitness trait (Breese and Mather,

Table 2 Estimates of the genetical parameters

	K-T			C-I		
	normal	agar	virgins	normal	agar	virgins
m	55·75***	20·07***	14·49	66·05***	32·82***	11·82***
d_1	1·67	−0·95	−0·49	−8·32***	−0·91	3·58***
d_2	−1·74	−11·22***	−3·28**	2·92	−0·45	5·15***
d_3	−11·45***	−3·75**	−4·51***	6·16***	−10·99***	2·22**
h_1	3·46	0·67	−1·42	5·91*	1·52	0·92
h_2	21·26***	9·77*	−1·34	14·04***	3·50	2·39
h_3	17·71***	4·89*	−2·64	16·36***	−2·91	−0·73
i_{12}	1·03	2·17	0·14	−4·59**	3·23	2·56**
i_{13}	0·67	4·83***	2·06*	−1·14	−2·01	1·63*
i_{23}	5·28**	−0·73	0·00	−2·52	−3·65	−0·19
$j_{1·2}$	−2·38	−1·11	−4·37	2·34	−6·52*	0·71
$j_{1·3}$	2·70	1·72	−2·34	5·36*	−4·29	2·44*
$j_{2·3}$	−8·55**	−7·96**	−0·06	6·87*	−7·96*	0·48
$j_{2·1}$	−5·50	−2·30	−0·95	3·10	−1·92	−1·02
$j_{3·1}$	0·48	2·34	1·58	−1·07	−7·89**	−0·28
$j_{3·2}$	0·00	−6·82	−1·59	5·21*	0·01	−0·84
l_{12}	−12·52*	−14·80*	−5·09	−7·93	−5·09	−3·79
l_{13}	3·72	2·70	2·61	−7·84	1·85	−2·17
l_{23}	−19·80***	−5·09	−1·22	−19·31***	−4·39	−3·01
i_{123}	1·38	−3·63**	−0·76	−0·73	−1·98	1·70*
$j_{12·3}$	−3·98	−0·43	−1·76	5·14	1·46	0·85
$j_{13·2}$	−1·18	−5·38	1·18	5·18*	3·69	−0·81
$j_{23·1}$	0·12	−1·47	−0·96.	1·86	1·12	−0·97
$j_{1·23}$	−4·47	−3·57	−0·25	10·98*	5·47	−3·79
$j_{2·13}$	6·21	−5·13	−0·47	−13·47**	6·07	−1·88
$j_{3·12}$	13·24*	6·39	3·10	12·61**	12·71**	−2·31
l_{123}	9·17	7·67	−1·57	25·30**	23·02*	3·02

* $p<0·05$; ** $p<0·01$; *** $p<0·001$.

1960; Kearsey and Kojima, 1967). It is of interest to examine interactions further. Two i interactions were significant, one positive and the other negative. The fact that new homozygous combinations were superior to the inbred parents (i negative) was also shown by Chapco (1980) in respect to chromosomal segments. The dominance effects were influenced by homozygous genetic background as evidenced by the significant j interactions. Homozygous × heterozygous interactions had also been revealed by Keller and Mitchell (1964) among entire chromosomes, and by Chapco (1977; 1980) and Chapco *et al.* (1978) among chromosome segments. Three of the double l interactions were significant and negative, while two were negative although not significant. Even though the triple l interaction in the C-I set was significantly positive, it was not larger than expected under additive combination of single heterozygotes. The existence of negative l interactions (of opposite sign to h's) means that the heterozygous effect of a chromosome is larger in an otherwise homozygous background than in a partially heterozygous one. This sort of interaction that mimics a duplicate gene action has also been found by Robertson and Reeve (1955), Dominguez and Rubio (1986) and it is noteworthy that in the studies of Chapco (1977, 1980) and Chapco *et al.* (1978) on chromosome segments and fecundity, six l interactions had been significantly negative and the other five were also negative although non-significant. The presence of interactions of the duplicate type, which would be expected with genes affecting fitness within a natural outbred population (Mather, 1973), seems to be common for fitness traits when combining chromosomes from widely different origins (Robertson and Reeve, 1955; Kearsey and Kojima, 1967; Chapco, 1977, 1980; Chapco *et al.*, 1978).

The difference between egg laying on normal medium and egg laying on agar could be seen as a measure of egg retention. Though line differences on egg production in a limited food resource would be included in such a measure, its contribution

Table 3 Estimates of the genetical parameters for the difference between agar and the 5th day of normals

	K-T	C-I
m	40·63***	44·99***
d_1	2·54	−3·52
d_2	11·69***	0·18
d_3	−10·64***	15·84***
h_1	−0·10	6·85
h_2	11·23	11·65**
h_3	11·55**	14·72**
i_{12}	−0·36	−6·82*
i_{13}	−4·08	−0·22
i_{23}	5·74*	−1·98
$j_{1\cdot2}$	−0·28	6·76
$j_{1\cdot3}$	−0·65	10·06*
$j_{2\cdot3}$	−6·83	11·81*
$j_{2\cdot1}$	−8·12	4·95
$j_{3\cdot1}$	1·33	3·06
$j_{3\cdot2}$	8·51	3·00
l_{12}	1·44	−9·36
l_{13}	4·17	−12·34
l_{23}	−15·05	−17·23*
i_{123}	4·49*	−1·73
$j_{12\cdot3}$	−3·77	3·18
$j_{13\cdot2}$	5·93	−0·85
$j_{23\cdot1}$	4·58	0·32
$j_{1\cdot23}$	1·93	8·84
$j_{2\cdot13}$	17·62*	−16·60*
$j_{3\cdot12}$	−0·62	4·07
l_{123}	4·99	7·32

* $p<0·05$; ** $p<0·01$; *** $p<0·001$.

must be small since the mean reduction in total egg production was only 10 per cent (8·89 eggs/female) in a previous study (Alcorta *et al.*, unpublished results). This measure of retention is affected mainly by chromosomes II and III in the two substitution sets and shows dominance in the direction of higher retention. Also, some interactions were significant. These features could be interpreted as denoting that the trait contributes to fitness. In both situations egg laying of inseminated females does not involve all the relevant genes proportionately, as was clearly seen from the genetical parameters (table 2). The significant changes of sign in genetical parameters suggest that different genes are acting in the two experimental situations. This result agrees with the suggestion of Schnee and Thompson (1984) that genotype × environmental interactions can perhaps best be thought of in terms of conditionally expressed polygenes.

The genetical effects on egg laying in virgin females were very different from those in the other two experimental situations. Egg laying of virgins was found to be essentially an additive trait. Although care must be taken in generalising from the effects of combining chromosomes from only two pairs of lines, this fact suggests that egg laying of virgins is not related to fitness (Breese and Mather, 1960; Kearsey and Kojima, 1967), contrary to what would be expected in view of the pointless loss of material which constitutes the deposition of unfertilized eggs. This result agrees with those of Lopez-Fanjul and Jódar (1977) in egg laying of virgin females of *Tribolium castaneum*, which they concluded to be a peripheral trait with respect to fitness.

It is clear that these three egg laying aspects have different genetic control. Egg laying of virgins may be considered a trait with little or no relation with egg laying of inseminated females. Hormonal and behavioural factors have a different influence on the three situations and generate large genotype × environment interaction effects that reveal egg laying to be a very complex set of traits.

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