PRELIMINARY EVIDENCE FOR A HOST EFFECT ON THE SIZE OF OFFSPRING FROM FOREIGN OVARY GRAFTS IN *DROSOPHILA MELANOGASTER*

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Introduction

It is possible in *Drosophila* to transplant a larval ovary to a host of the same stage, and, after this hatches as an adult and is mated, to obtain from the grafted ovary eggs fully capable of development. At the time of transplantation the ovary contains oogonia only, and its growth phase occurs in the environment provided by the body of the host; the first oocytes appear just before emergence of the adult, and the reduplication of their chromosomes at meiosis occurs therefore in the host.

In the experiments reported here, transplantation of ovaries was used in order to test the hypothesis that the host might exert some effect on a quantitative character of the offspring from ovarian grafts.

The same experimental procedure has been applied on mammals by Castle & Phillips (1909), Castle (1911, 1913), Russell & Hurst (1945), Russell & Gower (1949) and Russell & Russell (1948). However, an immunological reaction of the host is expected in mammals whenever the grafts come from unrelated donors. The possibility of avoiding this reaction has been studied by Harris & Eakin (1949) and Ferguson & Kirschbaum (1954). Other sources of complications in mammals are the effects of foetal nutrition, intra-uterine competition and other such secondary maternal effects. These difficulties make mammals rather unsuitable for inter-strain ovarian grafts, especially if a quantitative character is to be studied. Since in insects these difficulties do not arise, it would seem that experiments on *Drosophila* would supply useful and reliable information regarding maternal effects through the egg.

Materials and technique

The stocks used were several lines, mainly ES4, selected for small body size, and one line, LZ5, selected for large body size. These come from the collection of lines built up by Drs F. W. Robertson and E. C. R. Reeve and used by them for extensive studies on the inheritance of quantitative characters. I should like to express my gratitude to them for supplying me with these stocks.

The transplantation of ovaries was made by the operative technique of Ephrussi & Beadle (1935). Donors and hosts were always at the middle third instar larval stage.

The operated larvae were replaced in food vials and allowed to continue development at 25° C. The mortality was often about 50 %, but sometimes much less; there was a delay in hatching of about 1 day in comparison with unoperated larvae.

The imagos hatched from operated larvae were pair-mated with males of the donor line and were moved to new food vials every third day. No vial with more than sixty offspring was included in the measurements. Control matings of the donor line were simultaneously set up.

The measurements of thorax length and wing length were made, always by the same person, in the manner described by Robertson & Reeve (1952). The values obtained were listed in micrometer units without conversion to metric units.

Experiments

Experiment A

Ovaries from small lines were transplanted into large-line hosts. The operated females were mated to 'small' males and, where the graft was functioning, two types of offspring were obtained: homozygote small-line offspring from the graft, and large-small hybrids from the host's own ovaries. The two groups could be scored by means other than their size, since the host line LZ5 carries the sex-linked marker *w* (white eye colour) and the small lines are 'wild' for eye colour. The host's own offspring therefore were white-eyed males and red-eyed females who could produce white-eyed sons.

In practice this experiment met with an unforeseen difficulty: the 'small' line ovaries very rarely 'take' in a 'large' line or an unselected line host. The same trouble arose with all three small donor lines tested.

However, by persistent repetition of the experiment, the data summarized in Table 1 were obtained. They fall into three series representing the small lines IS4, ES4 and CS6 used as donors.

Table 1. *Numbers of offspring obtained from grafted ovaries and numbers of measurements made*

Exp. no.	No. of matings	No. of offspring	Measured ♂ Thorax	Measured ♂ Wing	Measured ♀ Thorax	Measured ♀ Wing
			(A) 'Small' ovaries in large hosts			
6 (ES4)	9	87	58	57	29	29
7 (CS6)	7	35	20	17	15	15
8 (IS4)	15	97	61	59	36	34
Total	31	219	139	133	80	78
			(B) 'Large' ovaries in small hosts			
1	4	63	29	29	34	32
2	16	189	65	76	122	113
3	15	125	49	19	76	64
4	15	210	91	89	107	121
Total	50	587	234	213	339	330
			(C) 'Large' ovaries in small hosts			
I	2	19	12		7	
II	3	18	6		12	
III	2	44	20		24	
IV	1	14	8		6	
V	1	25	14		11	
Total		120	60		60	

Experiment B

This is the reciprocal of A, ovaries from large-line donors being transplanted into small line hosts. In this case, both male and female offspring from the graft can be scored directly by their eye colour.

The numbers of offspring obtained are given in Table 1.

Experiment C

In the above experiments the operated females were mated to males removed at random from a mass culture of the donor line. In other words, the age of the males was not controlled, neither was the age of the control females.

Exp. C is a repetition of B with the refinement that males and females of both control and experimental vials are of the same age and the measurements from each age-group of their offspring are treated separately.

So far only a rather small test has been completed. The thorax only was measured, and some additional sensitiveness was imparted to individual measurements by using an objective lens of a higher magnification than in Exps. A and B.

RESULTS AND STATISTICAL ANALYSIS

Variance between vials in Exps. A and B. This was found in most cases to be significant, and therefore the individual measurements of vials harvested simultaneously could not be pooled. The vial means were therefore tabulated and used as the individual measurements for further analysis. These means and the means difference of control and experimental vials for each series are given in Tables 2 and 3. The figures appearing in these tables are the values of the means in excess of the following convenient levels:

For ♂ thorax: large line, 60; small line, 50
For ♀ thorax: large line, 70; small line, 57
For ♂ thorax: large line, 120; small line, 95
For ♀ thorax: large line, 140; small line, 105

The values are in micrometer units, each of which is equal to 0·0159 mm. The means difference of control and experiment vials is given a + sign when it is in the direction of a host effect and a − sign when it is in the opposite direction.

Table 2. *Offspring from small ovaries grafted into large hosts, Experiment A*

Exp.	Experimental vials		Control vials		Difference	$F = t^2$
	n	M	n	M		
			Thorax			
6 ♂	7	3·83	8	3·77	+0·06	0·007
6 ♀	4	5·6	8	5·0	+0·6	3·596
7 ♂	9	9·1	4	10·1	−1·0	0·107
7 ♀	6	6·3	4	2·7	+3·6	1·451
8 ♂	15	8·8	8	8·3	+0·5	2·255
8 ♀	9	8·8	8	9·2	−0·4	0·786
			Wing			
6 ♂	6	1·49	8	1·15	+0·237	0·306
6 ♀	4	2·39	8	2·25	+0·14	3·09
7 ♂	9	1·7	4	1·45	+0·25	0·14
7 ♀	6	5·33	4	3·6	+1·73	3·807
8 ♂	13	2·03	8	2·025	+0·005	0·005
8 ♀	8	2·58	8	2·6	−0·02	0·043

Table 3. *Offspring from large ovaries grafted into small hosts, Experiment B*

Exp.	Experimental vials		Control vials		Difference	$F = t^2$
	n	M	n	M		
			Thorax			
1 ♂	3	4·13	4	3·75	−0·38	0·55
1 ♀	4	3·45	4	3·85	+0·40	1·12
2 ♂	11	3·06	4	4·22	+1·16	6·10
2 ♀	16	2·11	4	2·50	+0·39	0·64
3 ♂	13	3·83	10	3·84	+0·01	0
3 ♀	15	2·40	13	3·03	+0·63	3·24
4 ♂	15	3·22	3	3·80	+0·58	1·39
4 ♀	15	2·15	4	2·50	+0·35	0·18
			Wing			
1 ♂	3	8·7	4	9·32	+6·2	0·083
1 ♀	4	7·07	4	8·9	+1·83	2·002
2 ♂	11	3·83	4	5·9	+2·07	3·515
2 ♀	15	2·9	4	4·4	+1·5	1·888
3 ♂	8	6·85	10	6·78	−0·7	0
3 ♀	13	5·52	9	5·94	+0·42	0·183
4 ♂	15	5·64	3	6·63	+0·99	1·130
4 ♀	17	4·70	4	5·72	+1·02	4·650

Analysis of data of Experiment A. The test applied to these data is a χ^2 test independent of any heterogeneity existing between the three series in this experiment, as each series represents a different inbred small-size donor line.

The value of F, which equals t^2, is calculated by analysis of variance for each series. The corresponding value of P, taking into account the sign of the means difference, is derived (*Biometrika Tables for Statisticians*, vol. 1, Table 9) and is used to calculate the value of $2 \log_e P$. The sum of these last values for all three series is the χ^2_6 for the group (Table 4).

Table 4. *Offspring from small ovaries grafted into large hosts*

Series	$E - C$	F	P	$-2 \log_e P$	χ^2_6	Probability
			Male thorax			
1	+0·06	0·007	0·469	1·514		
2	−1·00	0·107	0·625	0·940		
3	+0·50	2·255	0·074	5·207		
				——	7·761	Not significant
			Female thorax			
1	+0·60	3·596	0·043	6·293		
2	+3·60	1·451	0·132	0·050		
3	−0·40	0·786	0·806	0·431		
				——	6·774	Not significant
			Male wing			
1	+2·23	0·306	0·295	2·441		
2	+0·25	0·14	0·642	0·887		
3	+0·005	0·005	0·467	1·522		
				——	4·870	Not significant
			Female wing			
1	+0·14	3·09	0·055	5·800		
2	+1·71	3·807	0·043	6·293		
3	−0·02	0·043	0·580	1·089		
				——	13·182	Significant at 5 %

Analysis of data for Experiment B (*Table* 3). In view of the homogeneity between the $(\overline{C-E})$ values for the four series in this experiment, a more sensitive test, involving weighting of the $(\overline{C-E})$ values, was applied and will be illustrated by reference to the data on female thorax length.

The four series have $(C-E)$ values:

$$+0{\cdot}40, \quad +0{\cdot}39, \quad +0{\cdot}63, \quad \text{and} \quad +0{\cdot}35.$$

Weighting factors for each one are calculated by the formula

$$\text{Weighting factor } w = \frac{1}{\text{Variance of } (\overline{C-E})}.$$

The values obtained for these factors are:

$$7{\cdot}00, \quad 4{\cdot}2, \quad 7{\cdot}7 \quad \text{and} \quad 12{\cdot}5.$$

The mean means differences $(\overline{C-E})$ for each series are given by the formula

$$(\overline{C-E}) = \frac{\Sigma\,[w \times (C-E)]}{\Sigma w},$$

and its standard error by the formula

$$\text{S.E.} = \sqrt{\frac{1}{\Sigma w}}.$$

The $(\overline{C-E})$ was found to be $+0{\cdot}438 \pm 0{\cdot}173$. A value of t for $n+n-2$ degrees of freedom is thus obtained:

$$t = \frac{(\overline{C-E})}{\text{S.E.}}.$$

In this case the t value is equal to 2·53 for 73 degrees of freedom.

The probability P corresponding to this value is $P < 0{\cdot}02$, i.e. a highly significant value. In fact this P should be halved because it represents the probability of obtaining by chance a $(\overline{C-E})$ equal to $+0{\cdot}438$ or $-0{\cdot}438$, and not $+0{\cdot}438$ only. So that the P value is $P < 0{\cdot}01$.

The heterogeneity of the four series was tested by obtaining an heterogeneity χ^2 from the formula

$$\chi^2 = \Sigma[w \times (C-E)^2] - \frac{[\Sigma(w \times (C-E))]^2}{\Sigma w}.$$

In this case this $\chi^2_3 = 0{\cdot}39$. The significant value for the χ^2 for 3 degrees of freedom at the 5% level is 7·815, therefore our test is far from detecting any heterogeneity between the four series.

The same test on the wing-length measurements gives the following values

$$t = 2{\cdot}701 \text{ (highly significant)}$$

and

$$\chi^2_3 = 0{\cdot}947 \text{ for heterogeneity (non-significant).}$$

In the case of the male thorax and male wing measurements the tests give the following values:

$$t = 0{\cdot}08 \text{ (non-significant) for thorax,}$$

$$t = 1{\cdot}74 \text{ (significant at 5\%) for wing.}$$

Analysis of the data from Experiment C. In this small-scale test the variance between vials (1–3 in number) was shown not to be significant, and therefore measurements for each age-group were pooled. The values of F and other data for each age-group are given in Tables 5 and 6.

Table 5. *Offspring from 'large' ovaries in small hosts, thorax length*

	Experimental		Controls		Means		
Collection	Families	n	Families	n	Exp.	Controls	$C-E$
I ♂	2	12	4	42	2	1·9	0
I ♀	2	7	4	45	0·571	1·24	+0·7
II ♂	3	6	3	12	1·3	2·5	+1·2
II ♀	3	12	3	25	0·5	0·6	0
III ♂	2	20	5	46	−1·3	1·37	+2·7
III ♀	2	24	5	53	−1·37	0	+1·4
IV ♂	1	8	3	35	1·25	2·943	+0·7
IV ♀	1	6	3	34	−2·33	1·588	+3·9
V ♂	1	14	1	10	0·214	2·7	+2·5
V ♀	1	11	1	10	−2	2·8	+4·8
Totals	18	120	32	312			

Table 6. *Offspring from 'large' ovaries in small hosts, F tests, thorax length*

Collection	Means $C-E$	F values		F values at 0·1%	F values at 5%	Conclusion
I ♂	0	F^1_{52}	0		4·00	Not significant
I ♀	+	F^1_{55}	0·875		4·00	Not significant
II ♂	+	F^1_{16}	8·441	8·53	4·49	Significant nearly at 0·1%
II ♀	0	F^1_{35}	0·086		4·08	Not significant
III ♂	+	F^1_{64}	30·727	11·38	3·92	Significant at 0·1%
III ♀	+	F^1_{75}	12·758	11·38	3·92	Significant at 0·1%
IV ♂	+	F^1_{41}	74·045	12·61	4·08	Significant at 0·1%
IV ♀	+	F^1_{38}	17·578	12·61	4·08	Significant at 0·1%
V ♂	+	F^1_{22}	18·675	14·38	4·30	Significant at 0·1%
V ♀	+	F^1_{19}	55·132	15·08	4·38	Significant at 0·1%

Except for the first age-group and partly the second, the F values are generally highly significant (at the 0·01 level), in the direction of a host effect. Since the offspring of each age-group have the same parents the information from each age-group is not independent from the others.

Discussion

The largest sample is that of the females in Exp. B ('large' ovaries into 'small' hosts). Thorax and wing sizes are highly correlated so that the data for the one do not supply information independent of the other. But if one considers, say, the thorax, the data for the wing are a useful check. If the wing results contradict the thorax results, either the correlation does not exist or there is some grave mistake. If the wing data agree with the thorax data they add something to the evidence; although the correlation value cannot be calculated they can be considered as corroborative evidence.

The thorax can be measured with more accuracy than the wing; also the number of thorax measurements is in some vials larger than that of wing measurements, for sometimes the wings remain unfolded or are damaged.

For these reasons, the female thorax measurements are considered as the most reliable group of data. The statistical tests applied to them show (*a*) a highly significant difference

(at a level below 0·01) in the direction of a host effect, and (*b*) no heterogeneity between the four series. The results for the female wing simply confirm the above, with a difference significant at the 0·005 level and no heterogeneity. The male sample is smaller. It is also conceivable that any reaction of the one sex might not be accurately repeated by the other. In any case, the *t* test gives a non-significant value ($P < 0{\cdot}08$) for the thorax and a significant value ($P < 0{\cdot}05$) for the wing. In both cases the difference is in the same direction as in the females.

If the male tests were pointing in the opposite direction, they would introduce doubts about the validity of the results for the females, or they would indicate a (very improbable) reaction of the males to treatment in the opposite direction. As they do, in fact, point in the same direction they do not contradict the previous results. It is an unsolved question whether the difference of significance levels between the two sexes reflects a real difference or whether it is only due to the difference in the size of the samples. Further data may throw some light on this.

The above results may be viewed as constituting a *prima facie* case for the hypothesis under test. But statistical tests deal with probabilities and only the accumulation of more data can 'clinch the matter'. Actually, the results from Exp. C are the first, though small, instalment of such new data and they obviously support the above conclusions.

In Exp. A (small ovaries in large hosts) heterogeneity between the three series included would be probable, since each series represents a different donor stock. Such heterogeneity was in fact shown by the relevant χ^2 tests. The test applied in the data of Exp. B assumes lack of heterogeneity and is not therefore applicable to these data. The χ^2 which was used instead is independent of heterogeneity and gives the probability of getting by chance the means differences with the signs actually obtained in this experiment. It does not assume that each small line should react in the same way, whilst in Exp. B where the same line was used in all series such an assumption was justified. The results of Exp. A are only significant in the case of the female wing-length measurements. No conclusion about any host effect when small ovaries are grafted into large hosts can be based on these data. Since, however, the $(\overline{C-E})$ values have a positive sign, and in the single case of the female wing reach a significant level, the following conclusion may be justified: There is no evidence for, and there is in fact some evidence against, a shift in offspring size in a direction opposite to that of a host effect. There is no evidence, in other words, that the offspring from the grafted ovaries become smaller where the host is large. If there was, one might suspect that the shift towards smaller size in Exp. B is the result of some damage inflicted on the grafted ovaries during the operation, although this seems unlikely.

Following the conclusion that there is a *prima facie* case for the existence of a host effect, one has to consider: (*a*) whether there are any parallels from the work of other investigators with the same organism, (*b*) the probable mechanism of the effect, and (*c*) any useful modifications or additions to the experiments to follow.

The transfer from the haemolymph bathing the gonads, of some biological factor to the offspring of these gonads is not unknown in *Drosophila*. L'Héritier & de Scoeux (see L'Héritier, 1948) have found that the transfusion of haemolymph from a CO_2-sensitive stock to resistant flies transforms into sensitives not only the recipient individuals but also their offspring. The factor responsible must be able to 'infect' the gonad in the sense in which this term was used by Medawar (1947). Goldstein (1949) produced evidence

of the existence of a mutated form of the cytoplasmic factor associated with CO_2-sensitivity; therefore, this factor may have to be considered as particulate.

Brown & Hannah (1952) investigated the incidence of gynandromorphs arising by the loss in some tissues of the ring-X-chromosome carried by an experimental stock. The other X-chromosome (of the females) was marked with recessive genes which became phenotypically apparent in the tissues where the ring-X is lost. This permits the scoring of the gynandromorphs. The results showed that the frequency of gynandromorphs increases with the age of the mother.

A number of workers have transplanted larval gonads from sterile donors to 'normal' hosts in order to test whether any host influence might remedy the infertility of the implants. Dobzhansky & Beadle (1936) transferred to parent species hosts the infertile testes of hybrids of the *Drosophila* species *pseudoobscura* and *persimilis*; Clancy & Beadle (1937) transferred to 'normal' hosts infertile ovaries of donors homozygote for *singed, sn,* or *female sterile, fes,* and also of donors homozygote for *fused, fus,* which are fertile with some kinds of sperm and infertile with others; Suley (1953) transplanted infertile gonads of donors homozygote for *grandchildless, gs.* In all these cases, transplantation was made at the third-instar larval stage and the implant's infertility was not modified.

A similar experiment in mammals was the grafting of 'normal' mouse ovaries into sterile W^v/W^v hosts (Russell & Russell, 1948). The implants remained fertile.

Turning to quantitative characters, there is a claim (Durrant, 1955) that part of the variability in the number of the sternopleural bristles in certain pure lines is determined by the age of the mother. There also exist correlations positive or negative to the number of offspring. The above worker concluded that the factors involved segregate independently of each other.

It is conceivable then that the present effect also is mediated by some cytoplasmic factor (molecule or particle) transferred from the host haemolymph to the growing grafted ovary. One might assume that the large and small lines differ (correlated to their genotypic difference) in the relative amounts of two forms of a factor, the one form making for larger size and the other making for small size. This could come about by each genotype favouring the reduplication of the corresponding form and slowing down the reduplication of the opposite. It must be assumed, however, that the survival of any 'small' factors is not incompatible with the 'large' genotype. The reasons for assuming this are that small and large lines can be selected from a single 'wild' stock and that the hybrids are intermediate to the parents and should have a proportion of both factors.

Since the factors from the small hosts, which are assumed on this scheme to have entered the large ova, survive but are only allowed a small reduplication rate under the large genotype, they should become 'diluted' during growth to the adult stage. This provides an explanation for the fact that the size of the effect reflected in our data is extremely small, as can be seen in Table 7.

Table 7. *Average measurements in micrometer units*

(Experiment B)

	Large line	Small line	Difference approx.	'Effect'	'Effect' as % of difference
♀ Thorax	72·5	57·7	15·00	0·438	2·89 %
♀ Wing	146·2	111·2	35·00	1·064	2·97 %
♂ Thorax	63·9	51·3	12·6	0·307	2·43 %
♂ Wing	127·1	97·7	29·4	0·973	3·3 %

Should the effect be larger, then, at an earlier stage of development, say, at the first or second larval stages, when the infective factor would not yet be as diluted as in the adult? This is difficult to test, since the larvae derived from grafted ovaries cannot be distinguished from those derived from host ovaries. Furthermore, the lines used are isogenic for the size of the adult (as measured on the parts to be derived from the thorax-and-wing disk complex), and selection for this character may not necessarily coincide with selection for larval size.

If cytoplasmic factors can influence adult size, should this effect be detectable in the offspring of reciprocal crosses of the two lines used? No size difference between such reciprocal hybrids can be detected (Robertson & Reeve, unpublished). Strictly speaking, this applies to the females only, for a difference between the male reciprocal hybrids has been detected (Pantelouris, 1956), but as it seems to depend on the X-chromosome it does not affect the present argument. The absence of any size difference between the (female) reciprocal hybrids seems at first sight to make it improbable that there is a real effect of the host on transplantation. However, it should be taken into account that the hybrids are not comparable with the graft offspring in these experiments. The former contain a replica of each of the two genotypes, the latter are homozygous for the one only.

On the scheme outlined above, the hybrid genotype would allow the reduplication of both forms of the cytoplasmic factors and would bring the final ratio of the two to the point corresponding to the balance of the two genotypes, irrespective of whether there are in the ovum many small and few large ('small' mother) or vice versa ('large' mother) factors. Thus no difference in the reciprocal hybrids should be expected.

It would also follow that in further generations, derived from the graft offspring mated amongst themselves, the 'small' factors acquired would be diluted further and the effect would become smaller still and in practice undetectable in later generations. Such a phenomenon would fall under the definition of a Dauermodification, in contrast to the phenomena resulting from the transfer of *autonomously* reduplicating particles or plasmagenes.

It would obviously be interesting to test whether in fact the host effect persists with or without diminution in a second generation, but such a test is made difficult by the small size of the effect. It would be desirable therefore to find conditions under which the host effect can be 'enlarged' so as to be detectable with greater ease and confidence. Two ideas are under consideration. First, the possibility is being examined of using hosts which can be assumed to differ from the large donor line more than the small line does. Such hosts could be a stock of *Drosophila simulans*. The difficulty here is that the operated *simulans* females must be mated to *melanogaster* males and such matings rarely produce offspring. If this is due to mating difficulties these will persist also with the operated females; if it is due, however, to incompatibility of the gametes whilst copulation remains possible, offspring from the grafted (*melanogaster*) ovary might be obtainable. Secondly, it is being tested whether the host effect can be enlarged by environmental conditions such as changes in temperature or other factors. It would also be interesting to repeat the experiments with hosts and donors of an earlier developmental stage, for example, second-instar larvae. It can always be argued that a host effect would be more likely to become obvious then. Attempts in this direction have failed so far because operated second instar larvae fail to heal. It might be that at that stage the haemolymph is deficient in a factor necessary for healing, or that the wound from the operation interferes with moulting.

SUMMARY

Ovaries of an inbred line, L25, selected for large body size, were transplanted (at the third-instar larval stage) into hosts of a line, ES4, selected for small body size. The operated individuals were mated to males of the donor small line and produced two types of offspring: hybrids of the two lines and homozygotes of the 'large' line. Thorax and wing length measurements of the latter offspring group and of control 'large' line offspring were taken and compared. Statistical analysis of the data showed a significant reduction in the thorax and wing size of female and in the wing size of male offspring from transplanted ovaries. This reduction, however, amounted to only 3 % of the size difference of the two lines.

The reciprocal experiment ('small' ovaries transferred to 'large' hosts) proved more difficult to carry out due to failure of 'small' ovaries to grow in 'large' hosts in most cases. The data that could be collected show no effect of the host on the size of offspring from 'small' ovarian grafts, and also seem to exclude any size reduction as a result of the operation.

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