THE MAINTENANCE OF THE LETHAL GENE CURLY IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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SUMMARY

An analysis of the fitness components responsible for the maintenance of the lethal gene *Curly* in *Drosophila melanogaster* was made. The two competing strains, *Curly* and *vestigial* were the same except for chromosome 2. Three populations were studied for more than twenty generations and all attained similar equilibrium frequencies. The fitness components were measured. Equilibrium was maintained by a balance between *Cy* homozygote lethality, disadvantage of *Cy* heterozygotes in viability, heterosis in fecundity, and a frequent dependent heterosis for sexual selection. The role that each major component of fitness is expected to play in experimental populations, as the gene frequency changes, is discussed.

1. INTRODUCTION

Fitness is the result of complex and antagonistic forces. The first analysis, both in terms of a mathematical model and in terms of experimental measurement, was made in the early forties by Teissier (1942*a* and 1942*b*), in the special case of the lethal gene *Curly*. The problem was then nearly completely abandoned until Prout's articles (1965, 1969, 1971*a* and 1971*b*) followed by those of Anderson (1969) and Bundgaard and Christiansen (1972). To-day, some analyses of fitness components are available, which concern egg-to-adult viability and male sexual selection, whose relative importance depends upon the strains involved.

Maintenance of lethal genes being specially paradoxical, a new insight into the question might be of interest. Teissier's model was a good one and we decided to reconsider it. We thus undertook: (a) to analyse more precisely the fitness components, including sexual selection, considered today as an important component of fitness (for a review, see Petit and Ehrman, 1969; Spiess, 1970), (b) to determine the relative importance of heterosis, as demonstrated by Teissier, and also frequency-dependent selection, which was suspected by L'Heritier and Teissier (1934, 1937) in experimental populations, analysed by Petit (1951, 1954, 1958) and demonstrated in sexual selection and viability (for a review, see Ayala and Campbell, 1974, Petit, 1974).

To be sure that *Curly* would not be eliminated, *vestigial*, whose poor viability is known, was chosen as a competitor.

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2. MATERIAL AND METHODS

The balanced stock *Curly Oster* was used, being a very good suppressor of crossing over in chromosome 2. The *vestigial* stock had been kept for several years in the laboratory and was genetically homogeneous. The two strains were made isogenic for the rest of their genome, in order to prevent segregation effects for other loci; for this purpose, full-sib matings were performed for seven generations in fourteen lines as follows (female first):

$$\text{Generation 0: } \frac{Cy0}{33K_{Pml}} \times \frac{+vg}{+vg}$$

$$\text{Generation 1: } \frac{Cy0+}{+vg} \times \frac{Cy0+}{+vg}$$

$$G_2 \text{ to } G_7: \frac{Cy0+}{+vg} \times \frac{+vg}{+vg}.$$

Some lines were lost during inbreeding, but only one of each type was needed. The two strains $Cy(Cy0+ / +vg)$ and $vg(+vg / +vg)$ were highly inbred, except for chromosome II, which was protected from recombination by the pericentric inversion of $Cy0$.

(i) *The populations*

Four populations were maintained by serial transfer in half-pint milk bottles on S101 food medium enriched by fresh yeast. In populations H_1 and H_2 , the initial phenotypic frequency of Cy was 1; in populations L_3 and L_4 , the frequency was 0.10. They were kept at 25°C, 80 per cent RH and a photoperiod of 12 hours at 1000 lux. Competition for food occurred among the larvae. Evolution was followed for 23 discrete generations. For each generation, flies of the n th generation hatched during the three days following the first emergence, were placed for a 24 hour-period in a fresh medium bottle, their eggs constituting generation $n+1$. The parents were then counted to evaluate the composition of the n th generation.

(ii) *Fertility*

Five day-old mated females were permitted to lay eggs for two hours on histological plates covered by charcoal blackened S101 medium. Some 22–30 hours later, the number of unhatched eggs were counted and the proportion of hatched eggs estimated.

(iii) *Larvo-pupal viability*

Different ratios of Cy and vg flies were allowed to lay eggs. For each of them, one group of 150 first instar larvae were placed on a rich food medium to reduce larval competition (uncrowded series: UCS); they provided a sample of adults developed from the first stage larvae. Another group of 150 larvae were placed on a poor medium, so that larval competition was about the same as in the population bottles (crowded series: CS). The difference of frequencies between the two series was due to larval competition. Several runs were made at each frequency.

(iv) *Female fecundity*

The number of eggs laid from 1–12 days was measured for the different mating types. One female and two males, all newly hatched, were placed in a vial with maize medium and fresh yeast. They were transferred every two days into a new vial. Females that died were not replaced, but males were. Differences in means were tested by the Mann-Whitney *U* non-parametric test, since fecundity is not distributed normally.

(v) *Sexual selection*

The influence of female genotype was studied in 4 day-old virgin males and females. 12 males and 12 females of each genotype were put into an Elens-Wattiaux chamber (1964) and observed for $2\frac{1}{2}$ hours; the type and time of occurrence of every mating was recorded. To have a better evaluation of sexual selection in the bottles, the multiple choice technique was used: 100 3 day-old virgin *Cy* and *vg* males, in different phenotypic proportions, were put together with 100 virgin *vg* females. 24 hours later, the females were placed in individual vials. The male type they mated with was inferred from the progeny. *Cy* females were not used, since their progeny from the two mating types differs only in *Cy* frequency, making the analysis doubtful and time-consuming.

FIG. 1. Evolution of *Curly* phenotypic frequencies in the experimental populations.

3. RESULTS

(i) *Evolution of experimental populations*

Fig. 1 presents the evolution of phenotypic frequencies in the cages. The two H populations were stopped at the 7th and 20th generations respectively because of the occurrence of a recombination between *Cy* and *vg*. One of the H populations and the two L, each about 400 individuals, reached a genetic equilibrium about the 4th generation. This quick approach, explained by lethality in the H populations (Teissier, 1944), suggests frequency-dependent selection in one or several components of fitness in the low ones. The equilibrium frequencies, calculated from the 4th generation on, were very similar and averaged 0.75 ± 0.003 *Cy* phenotypes (table 1).

TABLE 1

Average phenotypic frequencies of Cy at equilibrium (calculated from the 4th generation to generation 19 or 23)

Line (to generation)	Phenotypic frequency of <i>Cy</i>	Theoretical phenotypic frequency
H ₂ ($\rightarrow$ G ₁₉)	0.767 ± 0.005	0.10
L ₃ ($\rightarrow$ G ₂₃)	0.755 ± 0.005	0.04
L ₄ ($\rightarrow$ G ₂₄)	0.737 ± 0.005	0.04
All	0.753 ± 0.003	—

These results might be compared to the theoretical evolution of a population in which *Cy* would be completely lethal when homozygous, and the adaptive values of *Cy* and *vg* phenotypes identical, that is $\sigma_1 = 0$, $\sigma_2 = \sigma_3$. Let n be the generation number, q_0 and p_0 the initial allelic frequencies of *Cy* and *vg*, $q_n = q_0 / (1 + nq_0)$. In H₂, $q_0 = 0.50$, then $q_n = 0.05$; in L₃ and L₄, $q_0 = 0.05$, then $q_n = 0.02$. The differences between observed and theoretical frequencies (table 1) demonstrate that the above hypothesis is false, a conclusion first obtained by Teissier.

TABLE 2

Egg fertility of Cy and vg phenotypes depending upon the mating type in Drosophila melanogaster

Mating number	Mating type*	Eggs laid	Eggs hatched	Eggs not hatched	χ^2	Hatched %
1	<i>Cy</i> $\times$ <i>Cy</i>	305	234	71		76.7 ± 1.7
2c	<i>Cy</i> $\times$ <i>vg</i>	365	324	41		88.8 ± 1.7
2v	<i>vg</i> $\times$ <i>Cy</i>	131	118	13		90.1 ± 2.6
2c+2v		496	442	54		89.1 ± 1.4
3	<i>vg</i> $\times$ <i>vg</i>	831	697	134		84.0 ± 1.3
Comparison						
2c v 2v					0.21	
1 v v(2c+2v)					22.27**	
3 v (2c+2v)					7.00**	

* Female first.

** significant at the 0.01 level.

(ii) *Egg fertility and embryonic mortality*

The results (table 2) demonstrate that, in mixed matings (mating types 2c and 2v), the mother's genotype had no influence on hatching percentage. The results for 2c and 2v could thus be combined, and compared with the $Cy \times Cy$ and the $vg \times vg$ matings. Both with Cy and vg the differences were highly significant, with eggs from the mixed matings having a higher fertility than that of homogametic matings: $89.1 > 84.0 > 76.7$.

It was difficult to give a direct evaluation of the egg viability for each genotype, as Cy 's lethality seems to depend on the strain used (Kuroda and Minato, 1976). An indirect estimate could be made by combining the results from different cross types, using the simple mendelian expectations (equations in table 3). Solving these equations gives $x_1 = 0.36$; $x_2 = 0.94$; $x_3 = 0.84$. These demonstrate: (a) heterosis in embryo fertility; (b) only a partial lethality of $Cy + / Cy +$ genotypes in the embryonic stage.

TABLE 3

Evaluation of the egg fertility coefficients. (x_1, x_2, x_3 represent the fertility of $Cy + / Cy +$, $Cy + / vg$ and $+vg / +vg$)

Experiment number	Mating types
1	$Cy \times Cy \rightarrow 0.25x_2 + 0.25x_3 = 0.77$
2c	$Cy \times vg \rightarrow 0.50x_2 + 0.50x_3 = 0.89$
2v	$vg \times Cy \rightarrow 0.50x_2 + 0.50x_3 = 0.90$
3	$vg \times vg \rightarrow x_3 = 0.84$
	Estimates
	$x_3 = 0.84$
	$x_2 = 0.94$
	$x_1 = 0.36$

(iii) *Larvo-pupal viability in competitive competition*

Larvo-pupal viability of Cy homozygote had first to be tested. To check on this, 150 Cy females were mated individually with vg males. If Cy were lethal in the larvo-pupal stage, all adults phenotypically Cy would be heterozygous $Cy + / +vg$; a female of this genotype mated with a vg male would produce about 50 per cent of *vestigial* flies. If it were only sublethal, some of the 150 Cy females would be $Cy + / Cy +$ and, mated with vg males, would produce $Cy + / +vg$ progeny. However, vg appeared in the progeny of the all females, which demonstrated that all phenotypically Cy females were $Cy + / +vg$ and $Cy + / Cy +$ was really lethal in our strain; its larvo-pupal viability was zero.

Larvo-pupal viability of heterozygous and *vestigial* genotypes was estimated by the ratio of the frequencies of adult genotypes emerging in the crowded (CS) and in the uncrowded (UCS) series. Let r and $1-r$ the observed frequency of the Cy and vg phenotypes among the UCS series, R and $1-R$, the corresponding frequencies among the CS series. Viabilities of the genotypes are as follows:

$$y_1(Cy + / Cy +) = 0$$

$$y_2(Cy + / + vg) = \frac{R}{r}$$

$$y_3(+ vg / + vg) = \frac{1 - R}{1 - r}.$$

Then, the relative viability of *Cy* heterozygotes versus *vg* homozygotes is:

$$Y = \frac{R}{r} \cdot \frac{1 - R}{1 - r}.$$

The standard error on this coefficient is $Y\sqrt{1/NR(1-R)}$ (Petit, 1958), *N* being the number of emerged adults in the crowded series.

Several frequencies of *Cy* were tested, viability being often frequency-dependent (Petit, 1966; Kojima and Yarbrough, 1967; Ayala and Campbell, 1974; Anxolabehere, 1976; Palabost, 1980). The results of the different runs of each frequency being statistically homogeneous, they have been pooled (table 4). Whatever the frequencies, *Cy* are at a disadvantage.

Occurrence of frequency-dependence has been tested by means of a ratio diagram, Fig. 2 (Ayala, 1972). This diagram uses the ratio between

FIG. 2. Larvo-pupal viability: Ratio diagram for the regression of the output frequencies (log. *Cy/vg* in crowded series); on the input frequencies (log. *Cy/vg* in uncrowded series).

the two genotypes and plots the logarithm of the output (number of flies emerged from the crowded series) in the ordinate versus the logarithm of the input (number of flies emerged from the uncrowded ones) ratios in abscissa. The equation of the regression line is: $y = 1.02 \times -0.30$. Its slope was tested against unity by a *t*-test. The standard-error is 0.10 and *t* equals 0.20. So the slope of the regression line is not significantly different from unity, which suggests no frequency-dependence. The location of the

TABLE 4
Larvo-pupal viability in competitive conditions

Phenotypic Cy frequency in eggs	Replicates	Uncrowded series			Crowded series			Relative viability of Cy versus vg: $\bar{Y} \pm \text{s.e.}$
		Cy	Total number	Phenotypic frequency of Cy (r)	Cy	Total number	Phenotypic frequency of Cy (R)	
0.15	8	129	766	0.17	45	346	0.13	0.73 ± 0.12
0.29	9	231	738	0.31	92	350	0.26	0.78 ± 0.09
0.40	9	316	738	0.43	84	294	0.29	0.54 ± 0.07
0.55	10	398	692	0.58	210	405	0.52	0.80 ± 0.08
0.66	9	620	906	0.68	264	412	0.64	0.82 ± 0.08

r and $1-r$: frequency of Cy and vg phenotypes in uncrowded series; R and $1-R$ the same in crowded series.
 Y : viability coefficient of Cy versus vg. $\bar{Y} = R/r : (1-R)/(1-r)$. $\text{S.e.}_{\bar{Y}} = Y\sqrt{1/NR(1-R)}$.

regression line under the diagonal demonstrates that *Cy* is at a disadvantage, which confirms results obtained by *Y* coefficient. The mean value of *Y* is 0.74.

Next, viability from egg to adult can be calculated combining fertility and larvo-pupal viability. Relative to *vg*, *Cy* fertility and larvo-pupal viability being 1.12 (0.94:0.84) and 0.73, *Cy* versus *vg* viability from egg to adult equals 0.82. This result differs from Teissier's, who demonstrated heterosis. The causes of this difference will be discussed in the general conclusion.

(iv) *Female fecundity*

Female fecundity has been evaluated: (a) during the first 4 days, to reproduce the population's conditions, where parents of every generation were killed on the 4th day following emergence, (b) during the 10 or 12 first days' to evaluate the potential fecundity (table 5). The evaluation during the first 4 days demonstrates that mixed crosses *Cy* × *vg*(2*c*) and *vg* × *Cy*(2*v*) have the same fecundity. Both homogamic genotypes have a lower fecundity, with a bigger disadvantage for *vg* than for *Cy*, in spite of *Cy* lethality; this probably ensues from facilitation phenomena (Lewontin 1955) which occur when *Cy* and *vg* develop in the same medium.

TABLE 5
Fecundity during the first 4 days and the first 10 days for four mating types

Mating type	Mating type phenotypes ♀ ♂	Number	Average fecundity (4 days)	Fecundity versus <i>vg</i> × <i>vg</i>	Average fecundity (10 days)	Mann-Whitney <i>U</i>	
						4 days	10 days
1	<i>Cy</i> × <i>Cy</i>	25	66	1.37	214		
2 <i>c</i>	<i>Cy</i> × <i>vg</i>	20	101	2.20	271		
2 <i>v</i>	<i>vg</i> × <i>Cy</i>	24	111	2.20	217		
3	<i>vg</i> × <i>vg</i>	12	48	1.00	141		
Comparison							
1 <i>v</i> 2 <i>c</i>						2.63**	2.44*
2 <i>c</i> <i>v</i> 2 <i>v</i>						0.83	2.03*
2 <i>v</i> <i>v</i> 3						3.96**	2.38**
1 <i>v</i> 3						1.30	2.89**

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Given the technique used, these results in fact include not only fecundity, but also premating time, developmental time and *Cy* homozygote viability.

To reduce the effect of premating and developmental time, fecundity has been evaluated during the first 10 days of adult life (table 5). Mixed crosses (2*c* and 2*v*) then have a different fecundity depending upon the mother's phenotype. Crosses between both males and females *vg* (3) have a much lower fecundity. This table does not seem to demonstrate any influence of female phenotype on fecundity. However two points have been neglected: (a) the *Cy* homozygotes disappear in the progeny of *Cy* × *Cy*. Thus the mean egg production of this type of cross (neglecting egg to adult

viability) would be $214 \times 4/3 = 285$ eggs; which is not statistically different from 271 eggs, obtained from $Cy \times vg$, (b) in the crosses $vg \times vg$, females begin to lay eggs on the third day instead of on the first for the other crosses (unpublished data). The fertility of the first 10 days of laying is then that of the first 12 days and equals 210, quite near the value of 217, for the $2v$ mating type. Thus, fecundity *sensu stricto* depends on female phenotype, and Cy females are heterotic. Nevertheless this heterosis is reduced in populations by $Cy+ / Cy+$ lethality and differences in premating time.

The actual fertility results (4th column in table 5) have to be taken into account in the evolution of the populations.

(v) Sexual selection

Comparisons with random mating and influences of male or female genotypes were tested by means of a chi-square for goodness-of-fit to a random mating distribution. The frequency-dependent mating success was measured by K , Petit's coefficient. S and $1-S$ being the ratio of females mated with Cy and vg males, s and $1-s$ the ratio of Cy and vg males, N , the number of mated females, $K = S/s : (1-S)/(1-s)$. The standard error on this coefficient is $e_K = K\sqrt{1/NS(1-S)}$ (Petit, 1958). Taking into account the number of matings ($N=274$), the bias on this coefficient (Goux and Anxolabehere, 1980, Anxolabehere *et al.*, 1982) is negligible.

The results obtained in Elens-Wattiaux chambers from intermating of 636 Cy and 636 vg pairs are given in table 6, and demonstrate a discrepancy

TABLE 6
Sexual selection between Cy and vg males studied in Elens-Wattiaux chambers (Cy phenotypic frequency: 0.50)

	Number of matings		Total
	$Cy \text{ } \varnothing\varnothing$	$vg \text{ } \varnothing\varnothing$	
$Cy \text{ } \sigma\sigma$	233	188	421
$vg \text{ } \sigma\sigma$	47	69	116
Total	280	257	537

$$\chi^2_{\text{panmixia}} = 182.57^{**}.$$

$$\chi^2_{\varnothing\varnothing} = 0.98.$$

$$\chi^2_{\sigma\sigma} = 173.24^{**}.$$

$$\chi^2_{\text{homogeneity}} = 8.02^{**}.$$

** Significant at the 0.01 level.

from a panmictic distribution ($\chi^2 = 182.57$). It is due to a male selection in favor of Cy males ($\chi^2_{\sigma\sigma} = 173.24$ for 1 df), and not to any female selection ($\chi^2_{\varnothing\varnothing} = 0.98$ for 1 df); in fact, the ratio of Cy and vg females mated either with Cy or with vg males are not homogeneous ($\chi^2 = 8.02$, significant at the 0.01 level), which demonstrates a tendency to homogamic mating.

The results of multiple choice experiments, performed with 3220 vg females, with 2879 mated, (table 7) demonstrate a very strong frequency-dependent advantage of the heterozygous males (fig. 3). The quadratic regression curve is: $K = 44.01s^2 - 31.59s + 6.84$.

TABLE 7

Sexual selection between Cy and vg males, measured by the technique of multiple choice, and evaluated by Petit's K coefficient

Phenotypic frequency of Cy(s)	Males		♀♀ mated with ♂♂		χ^2 panmixia	Sexual selection coefficient of ♂♂ followed by its S.e.
	Cy	vg	Cy	vg		
0.10	40	349	149	232	336.25**	5.6* ± 0.59
0.20	160	632	340	358	321.29**	3.75* ± 0.28
0.39	230	357	295	209	79.73**	2.19* ± 0.20
0.60	354	232	342	184	10.34*	1.22* ± 0.11
0.80	455	113	423	73	16.41**	1.44* ± 0.18
0.90	266	31	248	26	2.02	1.11 ± 0.23

$K = S/s : (1-S)/(1-s).$

$S.e._K = K \sqrt{1/NS(1-S)}.$

s and 1-s: phenotypic frequencies of Cy and vg males.

S and 1-S: phenotypic frequencies of females mated with Cy or vg males.

N: total number of mated females.

FIG. 3. Sexual selection: variation of Petit's K coefficient of sexual selection as a function of Cy phenotypic frequency. ● Experimental points.

Though not ambiguous, frequency-dependence has been tested by a ratio diagram where the output represents the logarithm of the ratio of females mated with *Cy* and *vg* males, and the input, the logarithm of the ratio of *Cy* and *vg* males in the population. The equation of the regression line (fig. 4) is: $y = 0.63x + 0.75$. The standard error on the slope is 0.05. The comparison with the diagonal ($t = 7.4$ for 5 fd) is highly significant.

FIG. 4. Sexual selection: Ratio-diagram for the regression of the output ratio (log matings with *Cy* males/matings with *vg* males) to the input ratio (log *Cy* males/*vg* males).

A comparison of the results obtained by the two techniques had to be made. With 0.50 *Cy* males, $K = 4.76 \pm 0.79$ for *Cy* females, 2.77 ± 0.38 for *vg* females, in Elens-Wattiaux chambers (table 7) and $K = 1.50 \pm 0.15$ for *vg* females in multiple matings (fig. 3).

Thus, the selection coefficient of *Cy* versus *vg* males measured in Elens-Wattiaux chambers is twice the coefficient obtained by the multiple matings technique available from the type of experimentation practised so far. It may be due to the different physical environment of the two procedures, or to the presence of one or two female genotypes resulting in complex interactions. New experiments will be necessary to answer the question.

In any case, a strong frequency-dependent sexual selection has been demonstrated in conditions very similar to the conditions of the experimental populations.

4. DISCUSSION AND CONCLUSION

The three populations, followed for at least 20 generations, attained a balanced polymorphism by the 4th generation. The level of equilibrium,

very similar in the three, was 0.75 ± 0.003 Cy phenotypes (instead of 0.66 in Teissier's experiments). The fluctuations around the equilibrium were usually parallel, which might be explained by the isogenicity of the competing strains which removes all recombinational variability. Their polymorphism seems to be maintained by a balance between: (1) Cy lethality and smaller egg-to-adult viability of Cy heterozygotes, (2) higher fecundity of crosses in which one (or both) parents are heterozygous, (3) a high frequency-dependent sexual selection in favor of Cy heterozygous males with advantage of the rare type. In Teissier's model, Cy's lethality was balanced by a higher viability and fecundity of Cy heterozygotes, and the equilibrium value was 0.66.

Some of these differences might be explained by the different genetic backgrounds and different intensities in the competition for food. More puzzling is the fact that his model, in which sexual selection was neglected, fits well to the equilibrium value obtained experimentally, while sexual selection, clearly demonstrated in our strains, had every chance of interfering. Frequency-dependent selection can be responsible for the discrepancy. Teissier's populations, initiated with 100 per cent Cy attained a level of equilibrium of 0.66. Now, for these frequencies, the coefficient of sexual selection is about 1 and might be neglected, the quick fall to equilibrium being mainly due to Cy lethality, an explanation that fits our H_2 population. On the contrary, in our populations initiated with 0.10 Cy, sexual selection is important during evolution to equilibrium; the sexual selection coefficient varies from 5 to 1 while Cy phenotypic frequency rises from 0.10 to 0.60. When the populations are in equilibrium, sexual selection is no longer efficient and the polymorphism is maintained essentially by viability and fecundity.

These conclusions might be tested by a model. But the model built with our sexual selection coefficient would be biased as sexual selection was under-estimated in our experiments and will need new evaluations.

This series of experiments and its comparison with Teissier's results demonstrate that the role which each major component of fitness is expected to play in experimental populations changes as the gene frequency changes, and is consequently different in stable or evolving populations. This result, demonstrated by Bundgaard and Christiansen (1972) in the dynamics of a fourth chromosome polymorphism in *Drosophila melanogaster*, and by Anxolabehere (1980) in the competition at the *sepia* locus, must be found every time that the evolution of one of the components of fitness is controlled by frequency-dependent selection.

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