MODIFICATIONS OF MEAN OVARIOLE NUMBER, FRESH WEIGHT OF ADULT FEMALES AND DEVELOPMENTAL TIME IN *DROSOPHILA MELANOGASTER* INDUCED BY DROSOPHILA C VIRUS

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ABSTRACT

Drosophila C virus, a picornavirus that has some influence on ovarian morphogenesis, was discovered in a French strain of *Drosophila melanogaster*. When the strain was infected by Drosophila C virus (DCV), the mean number of ovarian tubes and weights of the adult females increased, but the developmental time from egg to imago decreased. The maternal effects observed when DCV was present disappeared when the strain was DCV free but were restored by experimental contamination.

IN *Drosophila melanogaster* the mean number of ovarian tubes is a valuable biometrical character for differentiating geographical races (TEISSIER 1957; MELOU 1961) which is dependent on a polygenic system as shown by experimental selections (ROBERTSON 1957; TEISSIER 1957, 1958). Analysis of diallel tables with HAYMAN'S biometrical model (HAYMAN 1954a,b) showed that the concerned genes have additive effects and dominance deviation (THOMAS-ORILLARD 1967, 1982). Crosses between French or Japanese strains and stocks bearing genetic markers show that chromosome *2* and *3* have distinct influences on ovariole number and that the sex chromosomes are not involved (THOMAS-ORILLARD 1975a).

The main environmental factor influencing ovariole number is larval nutrition (SAVILIEV 1928; ROBERTSON 1957; TEISSIER 1958; DAVID 1960; THOMAS-ORILLARD 1972). For a given strain, the number of ovarioles and egg production may be positively (DAVID 1970) or negatively (ENGSTROM 1972) correlated, the productivity being limited by metabolic homeostasis. Reciprocal crosses between various strains give evidence of maternal effects on ovarian structure: the difference between the number of ovarioles per fly is more important with the French "Charolles" laboratory strain. To explain these results, the hypothesis of cytoplasmic inheritance was proposed (THOMAS-ORILLARD 1966, 1967, 1982). Different investigations led to the conclusion that viruses were present in the cells of the Charolles stock and that they were picornaviruses, RNA virions of 25–30 nm in diameter (JOUSSET, BERGOIN and

REVET 1977). Like the vertebrate picornavirus they are found in crystalline arrays in the cytoplasm of infected cells. These Charolles stock viruses, called Drosophila C virus (DCV), have been observed in the tracheal cells surrounding the cerebral ganglion, the muscles surrounding the intestinal tube, the Malpighian tubules and the ovarioles and in the follicular cells. The Charolles strain infected by the DCV seemed to be healthy; the virus did not produce any marked pathogenic effects. On the other hand, the flies injected with an extract of infected flies died within 3–4 days, and the number of virions in the dead flies was very high compared with that of other Drosophila virus isolates (JOUSSET *et al.* 1972). DCV is not transovarially transmitted but is horizontally transmitted by adult or larval contamination (JOUSSET and PLUS 1975). In 1982, N. PLUS (personal communication) observed that the Charolles stock was DCV free. The extract of the Charolles strain had lost its infectious lethal power toward the injected standard virus-free Drosophilae. Four successive blind passages (PLUS and DUTHOIT 1969) gave the same results: the injected flies remained healthy. Extracts observed by electron microscopy did not reveal any DCV. Infected flies are difficult to maintain in the laboratory; *e.g.*, 1 or 2 yr after being collected in nature, all of the fly strains infected by the picornavirus P virus have become virus free (JOUSSET and PLUS 1975). The present paper reports new observations and new experiments on the "safe" Charolles stock as well as on the same stock experimentally reinfected by DCV to restore the previous condition.

MATERIALS AND METHODS

Detection of DCV in a Drosophila stock: A sample of flies of the population to be tested was ground in 30 times its volume of Ringer's solution. This extract was clarified by low-speed centrifugation, and the supernatant was filtered through a 450-nm Millipore filter. This abacterian suspension was injected into about 50 *D. melanogaster* Champetières flies. It is known that the extract of DCV-infected flies kills the injected Drosophilae in about 3 days, even at a dilution 10^{-7}. The electron microscopic observation of the dead flies reveals a high concentration of virions typical of the Picornaviridae family (JOUSSET *et al.* 1972). In the present work, a Drosophila population was assumed to be DCV free when no mortality occurred after the injection.

D. melanogaster *strains*: The strains of *D. melanogaster* studied came from three French localities (Charolles, Banyuls E.B., Champetières), two Japanese localities (Tamashina, Otanu) and one Moroccan locality (Ouarzazate). Some of these stocks were DCV free; others provided to be infected by the DCV. The DCV-free Drosophila stocks are:

Champetières, classical standard wild-type strain, free of DCV and of all known viruses of Drosophila (PLUS *et al.* 1975).

Charolles-St, obtained by sterilization of the eggs from the Charolles stock naturally infected by DCV.

Ouarzazate-St, obtained in the same way from the Ouarzazate stock infected by DCV.

Five wild stocks, infected in 1972, which have lost spontaneously their DCV during the last 8 yr: Charolles-C^-, Tamashina-C^-, Banyuls-C^-, Otanu-C^- and Nagasaki-C^-. The infected Drosophila stocks are Charolles-C^+, from Charolles-C^- experimentally infected by the DCV isolated from the Charolles stock by JOUSSET *et al.* (1972); Ouarzazate-C^+, collected in Morocco, naturally DCV infected (JOUSSET and PLUS 1975).

Virus production: Champetières flies were anesthetized with ether, and each was injected with approximately 0.5 μl of virus suspension (L'HERITIER 1952). The DCV used for injections had been isolated from the Charolles strain (JOUSSET *et al.* 1972) and kept since 1972 at −20°. The injected flies, which died within 3–4 days, were stored at −20°.

Contamination of the DCV-free flies: The Charolles flies were infected at the larval stage by a contaminated medium. One fly was triturated in a glass microgrinder with 30 μl of Ringer's solution; 1 ml of this viral suspension per bottle was then applied to the surface of the food after eggs had been laid. The larvae were infected when they grew in the contaminated media, the most sensitive stage being the first instar (JOUSSET and PLUS 1975).

Rearing medium: All strains were maintained in rearing vials at 25° by mass culture on the usual cornmeal-molasses-yeast-agar medium seeded with live yeast and containing Nipagine (methyl parahydroxybenzoate) as a mold inhibitor. They were always raised in good feeding conditions, and great care was taken to keep the experimental conditions as constant as possible (THOMAS-ORILLARD 1972, 1975b). The infected and the uninfected strains were kept in separate rooms.

For the Charolles flies two samples were also reared on the axenic medium (DAVID 1959): Charolles-C^+ and Charolles-C^-.

Sterilization of the eggs: The eggs were dechorionated by placing them in a 4% sodium hypochlorite solution for 10 min; they were then washed extensively with Ringer's solution and put on an axenic medium.

Electron microscopy: Abacterian preparations extracted from the contaminated strains were observed with an electron microscope. The extracts were negatively stained with 2% sodium phosphotungstate (w/v) at pH 7.0, and the grids were examined with an Hitachi electron microscope at an accelerating voltage of 75 kV.

Crosses and measurements: Reciprocal crosses were made between the uninfected strain and the Japanese or French DCV-free strains. Each cross was made in five to ten bottles of cornmeal medium. All experiments were replicated two to three times. The parents were removed after 2 days. The next generation provided the data.

Quantitative characters: The following quantitative characteristics were studied: the mean number of ovarioles per fly, the mean fresh weights of young adults and the developmental time (from egg deposition to imago emergence). The techniques used for measuring the quantitative traits were extensively described in THOMAS-ORILLARD (1975b,c). Briefly, after emergence of adults, the flies in each culture were placed at 25° on fresh standard cornmeal-molasses-yeast-agar medium for 2 days. The ovaries were then dissected and immersed in a saturated potassium bichromate solution. Two days later, the ovarioles were counted after being separated one from another with a pair of microneedles. Observations were made with a dissecting microscope at ×30 magnification. It is usual for ovary biometrical traits to test about 25–30 females for each experiment: $13 < n < 122$ (ROBERTSON 1957), $2 < n < 30$ (DAVID, BOCQUET and PLA 1976), $n = 30$ (DAVID, BOCQUET and DE SCHEEMAEKER-LOUIS 1977), $19 < n < 34$ (SCHAEFFER, KIDWELL and FAUSTO-STERLING 1979). In this study, 70 females were initially dissected for each cross, but only 50 perfectly dissected females were retained. In the same way, each strain was regularly tested, the mean number of ovarian tubes did not change with time and the distribution of the samples was normal (THOMAS-ORILLARD 1975b). Thus, the biometrical characteristics of the strains cited in this paper were established at the same time as those of the crosses. On the other hand, 2-day-old imagoes were weighed individually on a Sartorius-Werke balance having an accuracy of 1/100 mg. Since GUILLAUMIN (1961) has shown that females are more variable than males, we weighed a larger sample of females than males (about 50 ♂ for 80–100 ♀).

The technique used to measure developmental time was described and discussed by DAVID (1955). At a specific time of day, females that had been with males for at least 2 days were put into a 3.5 × 2.4 × 2 inch plastic cage with axenic medium. At the same hour on the following day the females were removed, and eggs were collected and placed on a small piece of sterile paper in vials with rearing medium. After emergence, the flies were collected twice a day (morning and evening).

RESULTS

Experimental infection of the DCV Charolles strain: In the experiment involving larval infection of the DCV-free Charolles strain by DCV-contaminated food, some third instar larvae and some adults died rapidly.

TABLE 1

Mean numbers for ovarioles per fly for the stocks of Drosophila melanogaster

Medium	Strains	N^a	Mean ± SEM
Cornmeal-molasses	Charolles-St	35	33.11 ± 0.61
		50	34.52 ± 0.68
		37	33.43 ± 0.93
	Charolles-C$^-$	50	35.48 ± 0.48
	Charolles-C$^+$	50	39.27 ± 0.48
Axenic	Charolles-C$^-$	50	37.84 ± 0.53
	Charolles-C$^+$	50	39.44 ± 0.43
Cornmeal-molasses	Otanu-C$^-$	50	39.28 ± 0.49
	Tamashina-C$^-$	50	29.24 ± 0.45
	Banyuls E.B.-C$^-$	50	41.02 ± 0.50
	Champetières-C$^-$	50	40.48 ± 0.62
	Ouarzazate-C$^+$	50	36.12 ± 0.45

[a] *N*, number of females tested for each strain; for Charolles-St strain there are three replicates.

To verify that the experimental contamination was successful, Champetières flies were injected with an abacterian suspension of the sample that was believed to be contaminated. Nearly all of the flies died 3 or 4 days after injection (93% mortality). Abacterian preparations extracted from the contaminated strains were observed with an electron microscope. Both strains showed 30-nm particles identical with the DCV particles placed on the medium that had been reported for the first time in the Charolles strain (JOUSSET *et al.* 1972; JOUSSET, BERGOIN and REVET 1977). It is evident that a newly infected Charolles strain that we call "Charolles-C$^+$" has been established.

The mean number of ovarioles per fly: The mean numbers of ovarioles per fly for the different French, Japanese and Moroccan stocks are presented in Table 1. It appears that the five samples of the Charolles stocks reared on cornmeal-molasses medium are heterogeneous ($\chi^2_4 = 26.16$). This heterogeneity is probably correlated to the variances of the three replicates of the sterile stocks ($\chi^2_2 = 7.94$) and results from the sodium hypochlorite treatment possibly by selecting the eggs. Indeed, the comparison of two additional samples of eggs, one washed with a sodium hypochlorite solution and one with Ringer's solution, shows that embryonic mortality is greater in the first sample (24.5%) than in the second (11.32%). Therefore, it is not possible to pool all 122 females constituting the three replicated samples.

On the other hand, the variances of the DCV-free and the contaminated stock do not differ significantly ($F = 1.31$). In these conditions, the mean number of ovarian tubes of the infected flies is significantly higher than the mean number of the virus-free flies ($t = 5.37$, $P < 0.001$). It is the same result for the DCV-free and the contaminated stock reared on axenic medium ($t = 2.39$, $P = 0.02$).

It is known that a laboratory strain of sigma virus induces peculiar changes in the ovarioles of *D. melanogaster* females. The ovarioles present numerous

TABLE 2

Mean number and standard error of ovarian tubes per fly in reciprocal hybrids

Crosses	*N*	Mean ± SEM
Experiment 1		
♀ Charolles-C⁻ × ♂ Tamashina-C⁻	50	36.80 ± 0.52
	50	36.26 ± 0.34
♀ Tamashina-C⁻ × ♂ Charolles-C⁻	50	36.58 ± 0.40
	50	36.60 ± 0.55
♀ Charolles-C⁻ × ♂ Otanu-C⁻	50	41.28 ± 0.55
	50	40.88 ± 0.43
♀ Otanu-C⁻ × ♂ Charolles-C⁻	50	40.04 ± 0.34
	50	40.64 ± 0.49
♀ Charolles-C⁻ × ♂ Banyuls-C⁻	50	41.48 ± 0.47
	50	41.36 ± 0.60
♀ Banyuls-C⁻ × ♂ Charolles-C⁻	50	41.88 ± 0.47
	50	42.12 ± 0.51
♀ Charolles-C⁻ × ♂ Champetières-C⁻	50	42.93 ± 0.50
	50	42.62 ± 0.42
♀ Champetières-C⁻ × ♂ Charolles-C⁻	50	42.92 ± 0.45
	50	42.28 ± 0.50
Experiment 2		
♀ Charolles-C⁺ × ♂ Tamashina-C⁻	50	39.74 ± 0.36
	50	39.58 ± 0.43
	50	39.56 ± 0.41
♀ Tamashina-C⁻ × ♂ Charolles-C⁺	50	36.80 ± 0.34
	50	37.36 ± 0.45
	50	37.80 ± 0.51
Experiment 3		
♀ Charolles-C⁺ × ♂ Otanu-C⁻	50	42.96 ± 0.33
	50	42.56 ± 0.44
	50	43.40 ± 0.42
♀ Otanu-C⁻ × ♂ Charolles-C⁺	50	39.60 ± 0.54
	50	40.03 ± 0.60
	50	40.52 ± 0.39
Experiment 4		
♀ Charolles-C⁺ × ♂ Banyuls-C⁻	50	43.83 ± 0.57
	50	43.96 ± 0.46
	50	43.92 ± 0.59
♀ Banyuls-C⁻ × ♂ Charolles-C⁺	50	42.72 ± 0.67
	50	42.50 ± 0.49
	50	42.72 ± 0.62
Experiment 5		
♀ Charolles-C⁺ × ♂ Champetières-C⁻	50	44.32 ± 0.42
	50	44.00 ± 0.54
	50	44.64 ± 0.67
♀ Champetières-C⁻ × ♂ Charolles-C⁺	50	41.70 ± 0.70
	50	41.38 ± 0.61
	50	41.56 ± 0.57

In the first experiment (1), the Charolles strain is DCV free (C⁻); in the other experiments (2, 3, 4, 5) it is experimentally DCV infected (C⁺); for the different experiments, each Charolles-C⁺ stock was independently DCV infected.

TABLE 3

Analysis of variance for the mean number of ovarian tubes per fly in reciprocal hybrids when the Charolles stock is virus free (experiment 1) or DCV infected (experiments 2, 3, 4, 5)

Source	Degree of freedom	Mean squares	F	
Experiment 1				
Among reciprocal hybrids	1	0.15	2.058×10^{-4}	
Among strains (within reciprocal hybrids)	6	734.68	63.70	$P < 0.001$
Residual	792	11.53		
Experiment 2				
Among reciprocal hybrids	1	399.05	61.17	$0.001 < P < 0.005$
Among replicates (within reciprocal hybrids)	4	6.52	0.71	
Residual	294	9.16		
Experiment 3				
Among reciprocal hybrids	1	588.00	55.12	$0.001 < P < 0.005$
Among replicates (within reciprocal hybrids)	4	10.67	0.99	
Residual	294	10.67		
Experiment 4				
Among reciprocal hybrids	1	117.81	220.90	$P < 0.001$
Among replicates (within reciprocal hybrids)	4	0.53	0.03	
Residual	294	16.35		
Experiment 5				
Among reciprocal hybrids	1	576.85	180.07	$P < 0.001$
Among replicates (within reciprocal hybrids)	4	3.20	0.17	
Residual	294	18.35		

oocytes in stage 14 (JUPIN, PLUS and FLEURIET 1968). To determine whether DCV has a similar effect, four kinds of *D. melanogaster* strains were used: (1) experimentally infected at the first larval stage (Charolles-C^+), (2) naturally contaminated (Ouarzazate-C^+), (3) uninfected (Banyuls E.B., Champetières, Tamashina, (C^-), (4) experimentally virus free by sodium hypochlorite treatment (Charolles-St, Ouarzazate-St). All of the females had absolutely normal ovarian tubes except 11.5% of the Ouarzazate females in which numerous abnormal ovarioles were observed. In some cases the eggs had an inverse polarity.

In the first experiment, reciprocal crosses between the DCV-free Charolles stock and the Tamashina, Otanu, Banyuls E.B. and Champetières DCV-free stocks were made. The mean number of ovarioles of the reciprocal hybrids are presented in Table 2. From the analysis of variance performed on these data (Table 3), the following observations can be made: (1) There is no significant difference between direct and reciprocal crosses ($F < 1$), *i.e.*, no significant maternal effect. Consequently, the results can be grouped, giving 200 observations for each cross. (2) A specific strain effect for each strain crossed with the Charolles, within reciprocal crosses is very significant ($F = 63.70$, $F_{0.99} = 3.40$).

TABLE 4

The DCV effects on developmental time (from egg to imago) and on weights of 2-day-old adults of uninfected and DCV-infected strains or hybrids of D. melanogaster

Character measured	Rearing medium	Strains or F_1	N	Sex	Mean ± SEM	t-test	P
Developmental time (hr)	Cornmeal-molasses	Charolles-St	70	♀ + ♂	272.51 ± 2.14	2.77	0.01
		Charolles-C^+	164	♀ + ♂	265.46 ± 1.40		
	Axenic	Charolles-C^-	385	♀ + ♂	217.68 ± 0.45	20.68	0.001
		Charolles-C^+	250	♀ + ♂	203.00 ± 0.54		
		♀ Charolles-C^+ × ♂ Tamashina-C^-	130	♀ + ♂	212.53 ± 0.40	4.18	0.001
		♀ Tamashina-C^- × ♂ Charolles-C^+	389	♀ + ♂	216.68 ± 0.42		
	Cornmeal-molasses	Ouarzazate-St	441	♀ + ♂	296.79 ± 0.70	4.81	0.001
		Ouarzazate-C^+	1017	♀ + ♂	292.48 ± 0.50		
		Champetières-C^-	180	♀ + ♂	227.07 ± 0.78	5.45	0.001
		Champetières-C^+	79	♀ + ♂	220.48 ± 0.93		
Fresh weight (mg/100)	Cornmeal-molasses	Charolles-St	76	♀	158.48 ± 1.32	2.82	0.01
			46	♂	99.52 ± 1.44	0.05	
		Charolles-C^+	76	♀	163.89 ± 1.40		
			50	♂	99.62 ± 0.90		
		Ouarzazate-St	80	♀	162.88 ± 2.41	3.03	0.01
			101	♀	162.03 ± 1.42		
			100	♀	161.55 ± 1.34		
			50	♂	89.44 ± 0.90	0.07	
		Ouarzazate-C^+	102	♀	171.67 ± 1.64		
			73	♂	89.99 ± 0.73		

[a] From F. PERONNET (D. E. A. student).

In other experiments (Table 2), reciprocal crosses between four independent DCV-infected Charolles stocks and the Tamashina, Otanu, Banyuls E.B. and Champetières, DCV-free strains showed that (Table 3): (1) The difference between direct and reciprocal crosses is always very significant. (2) There is no replicated effect within reciprocal crosses ($F < 1$).

Other quantitative traits: The possible effects of C virus on developmental time and fresh weight of experimentally infected and uninfected flies of the same genotype were studied (Table 4). In all cases the infected flies performed better than the uninfected ones; the developmental time from egg to imago was shorter in cornmeal-molasses and in axenic medium, and the weights of the young females were higher. The differences are small but significant.

As can be seen, an acceleration of the developmental time and an increase in the weights of the young females appear for the infected flies; in other words, they produce more in less time. Since fresh weight does not increase in the males, it may be supposed that in females the increase in the fresh weight is correlated with the increase of the number of the ovarioles.

If we measure individually the number of ovarioles and the fresh weights of 56 infected Ouarzazate flies, it clearly appears that both characters are positively correlated ($r = +0.46$). Moreover, reciprocal crosses between Charolles-C^+ and Tamashina-C^- have been made. The difference between their devel-

opmental times are very significant ($t = 4.81$, $P = 0.001$). The developmental time of hybrids from Charolles-C^+ mothers was shorter than those of Tamashina-C^- mothers.

DISCUSSION AND CONCLUSIONS

The experiments reported in this paper clearly demonstrate that the picornavirus C does have some influence on quantitative characteristics of *D. melanogaster*: DCV infection decreases the developmental time (from egg to imago) and increases the fresh weights of females and their mean number of ovarioles. This is demonstrated by the comparison between (1) an uninfected strain and the same strain infected with DCV by larval feeding and (2) a normally infected strain and the same strain made virus free by dechorionation of the eggs. Maternal effects appear when the Charolles mothers are DCV infected, disappear when the virus is spontaneously lost and are restored by experimental contamination at the first larval stage. The maternal effects observed, therefore, appear to be associated with DCV infection.

In all cases infected flies have characteristics that could be advantageous in the proper environment. If this proves to be true, this would be the first time that viruses appeared to have a beneficial effect. If naturally infected populations have greater fitness because of the DCV influence on some of the metric characters, particularly on developmental time and mean number of ovarioles, it would be possible to understand why DCV was maintained in natural populations. Presently, five isolates of DCV are known in Drosophila from laboratory strains and wild populations of flies collected from geographically different regions (MOORE *et al.* 1982).

The DCVs have been detected by genetic experiments. Their presence was first revealed by their phenotypic manifestations in geographical races of Drosophila that seemed to be healthy (THOMAS-ORILLARD 1966, 1975b,c). Now we must determine what kind of effect DCV has on the quantitative characters and on the traits affecting the reproductive value of the population. Two hypotheses may be considered: (1) DCV acts on measurable parameters as an environmental factor; if this is the case, care should be taken with the results of quantitative experiments as well as with their mathematical treatment and interpretation. (2) DCV acts as an integrated virus, in which case the evolutionary interest for a natural population to possess such a virus would then require consideration. In fact, it is possible that the natural viruses of Drosophila are partially responsible for the rate of mutations occurring in the wild populations of this insect (BERG 1974; GOLUBOVSKY *et al.* 1974). It is now known that mutability level of the DCV-infected strain was three times higher than that of the virus-free strain for the *X* chromosome and was two to three times higher for the second chromosome (GOLUBOVSKY and PLUS 1982).

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