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Genetic analysis of growth pattern in cell populations in vitro

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Genetica. — *Genetic analysis of growth pattern in cell populations in vitro* (*). Nota di ARIELLA OTTAVIANO GOTTARDI, presentata (**) dal Corrisp. C. BARIGOZZI.

RIASSUNTO. — È stato studiato l'accrescimento di culture *in vitro* di cellule embrionali dei ceppi inincrociati Aspra e Varese di *Drosophila melanogaster*, e dei loro incroci reciproci.

Questa analisi ha indicato l'esistenza di un chiaro controllo genetico delle modalità di accrescimento. L'accrescimento durante le prime 88 ore di cultura risultò assai differente nei due ceppi; le popolazioni cellulari ibride mostrarono modalità di accrescimento intermedie, parzialmente influenzate dal citoplasma dell'uovo, assai più omogenee e stabili alle condizioni di cultura che non le linee originali. Si è tentato di spiegare questi risultati come conseguenza di una differente modalità di accrescimento tipica degli embrioni di ciascun ceppo; ciò fu analizzato elaborando statisticamente il minimo tempo di schiusa di ciascun ceppo, ma questa ipotesi non ha potuto essere dimostrata.

INTRODUCTION.

Since previous data (Rezzonico Raimondi and Gottardi, 1967) on the growth of isolated embryonic cell cultures *in vitro* had proved the existence of statistically significant differences between wild inbred stocks of *Drosophila melanogaster*, the same line of work was pursued, studying the effect of interstock hybridization with the aim of securing the most convenient material for further attempts at maintaining long term cultures and establishing cell lines. The data presented here refer therefore to the following points.

1) how the character "growth pattern *in vitro*", peculiar to every inbred stock, behaves in cell populations derived from F_1 hybrid embryos;

2) whether different proliferation rates of cell cultures from genetically differentiated stocks may be connected with different developmental rates of the donor embryos, as suggested by previous results.

MATERIALS AND METHODS.

1) *Growth rate of cell populations* in vitro.

Inbred stocks Aspra (AS) and Varese (VA), which exhibit, as previously reported (Rezzonico Raimondi and Gottardi, 1967), the greatest difference in their response to *in vitro* culture conditions, and their reciprocal hybrids (♀♀ AS × ♂♂ VA—H_1 ; ♀♀ VA × ♂♂ AS—H_2) were used for the present research. Inbred and hybrid eggs were collected on coal-agar coated vessels, following the usual method. Cell suspensions were prepared by mechanical dissociation of the embryos and incubated in H–5 medium (Horikawa and Fox, 1964).

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(**) Nella seduta del 15 novembre 1969.

Sixteen cell populations, four for each type of donor embryo (AS, VA, H_1 and H_2), were analysed during a three month period.

All cultures were randomly distributed in time. Cell concentration controls were set up for all cultures at the same incubation period. The intervals chosen were: 0, 16, 40, 88, 136 and 184 hours of incubation.

The mean cell concentration values at each interval were estimated on comparable samples of the 16 populations, using the method previously described (Rezzonico Raimondi and Gottardi, 1967).

The data concerning the first 88 hours of incubation were analysed statistically using the following tests:

1) overall analysis of variance, including all considered types of cell populations, in order to appreciate, whether there was any significant difference in the growth rate of the four types during the whole increase period. The variance between times was subdivided into a linear component and a deviation from the linearity. The overall analysis allowed us to test the interaction between the four types and these items.

2) regression test, applied to the mean values of each type of cell population at each incubation interval, in order to analyse which kind of growth might characterize each type.

3) coefficient of variation, in order to compare the stability of the four considered genotypes as regards uncontrolled environmental variations (Table I).

2) *Minimal hatching time of the inbred stocks.*

This character was chosen as an index of the embryonic developmental rate. For this part of the research only the inbred stocks Aspra and Varese were considered. Young adult flies of both stocks, kept in glass bottles, were allowed to oviposit on coal-agar coated vessels for I hour. The vessels were then replaced by fresh ones, which were removed from the bottles after half an hour and kept finally in a thermostatic chamber at $25 \pm 0.5^{\circ}$C for 9 hours.

During the first hour of incubations the females are supposed to oviposit the oldest eggs they already have in the genital tract; during the following half an hour only more recently fertilized eggs are laid, so that all eggs utilized were about of the same age (Sonnenblick, 1950).

This experiment, including 6 repetitions on 6 succeeding days, was planned as follows. Each day the adult flies of five mass cultures for each stock were used. During the 9 hour incubation the vessels were searched for hatched larvae at half an hour intervals. For each vessel the minimal hatching time was expressed as the time period that elapsed between the introduction of the second batch of vessels into the culture bottles and the observation of the first hatched larva.

If up to the end of the incubation period no larva was noticed, a hatching time of 10 hours was arbitrarily attributed to the mass culture in which the first larva was about to hatch, even if not completely hatched.

The data collected following this scheme were submitted to analysis of variance, in order to test the existence of significant differences between the hatching times of the two parental stocks.

Results.

All 16 cultures have nearly the same initial cell concentration. A very high value was chosen (about 13×10^6 cells/ml), in order to ensure rather limiting environmental conditions. All potential differences between geno-

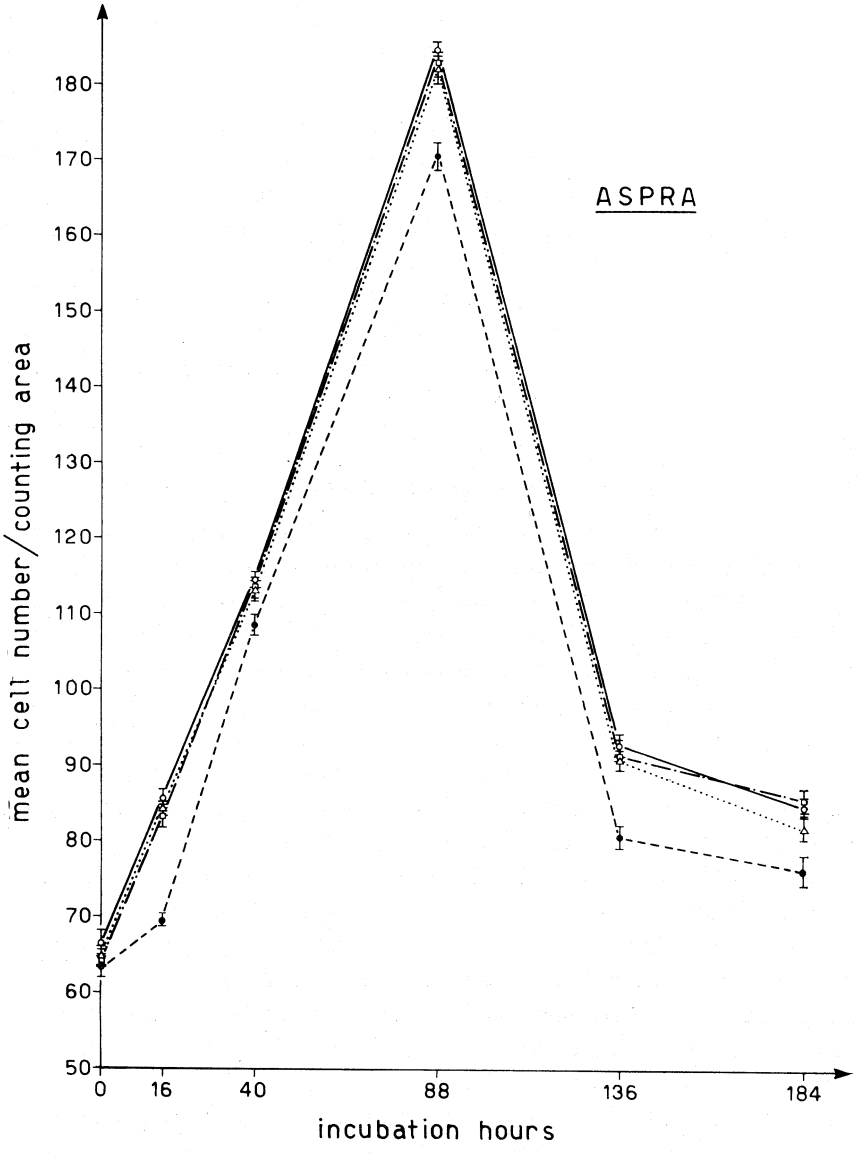

Fig. 1. – Growth graph of Aspra inbred cell populations *in vitro*.

types are in fact more clearly revealed in a crowded population resulting in an unfavourable milieu (Lints, 1962). After 88 hours of incubation, each culture reaches the highest cell concentration, the pattern shown by each stock being typical in all repetitions. After this point cell concentration decreases till 136 hours, and remains unchanged during the last incubation interval.

Let us now consider each stock with its characteristics.

AS. This inbred stock is characterized, as previously described (Rezzonico Raimondi and Gottardi, 1967), by a high proliferation rate, which leads to a triplication of the initial cell concentration within 88 hours.

Fig. 1 shows the roughly linear growth peculiar to this stock (Table I).

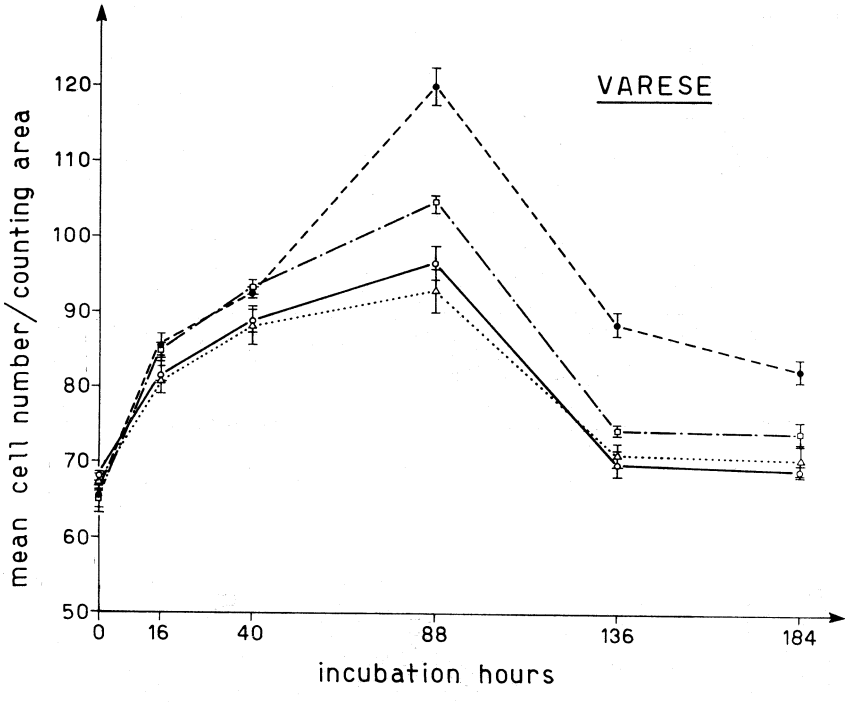

Fig. 2. – Growth graph of Varese inbred cell populations *in vitro*.

VA. The second inbred stock shows a lower growth rate. The multiplication activity slows down between 16 and 40 hours of incubation. Therefore the growth curve of this stock can be defined by a quadratic function (fig. 2; statistical test in Table I).

H_1. All hybrid cell populations of this type exhibit a peculiar pattern from 88 to 184 hours of incubation; cell concentration decreases only very little, when compared to the cultures of the other types of cell populations (fig. 3).

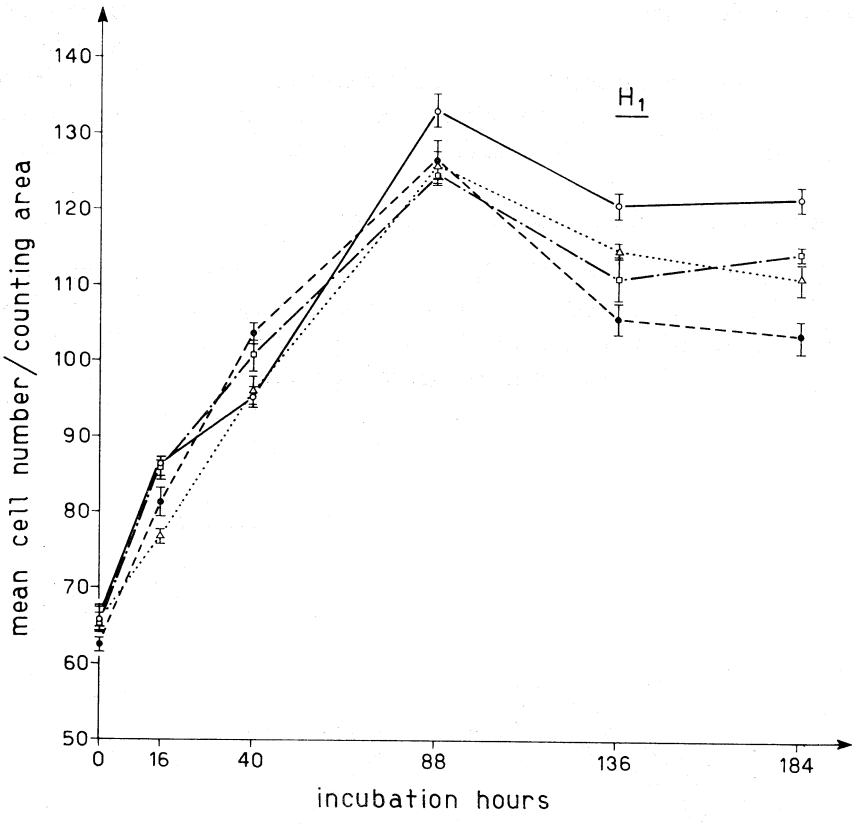

Fig. 3. – Growth graph of H_1 (♀♀ AS × ♂♂ VA) cell populations *in vitro*.

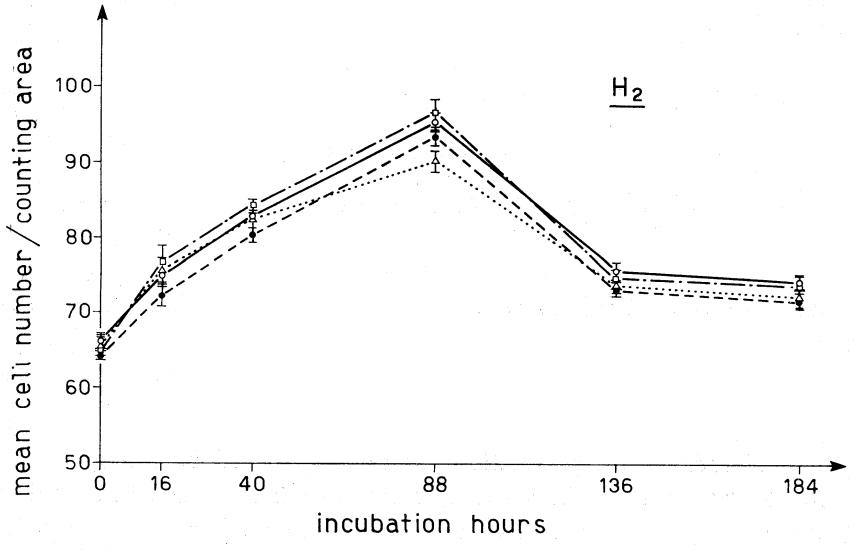

Fig. 4. – Growth graph of H_2 (♀♀ VA × ♂♂ AS) cell populations *in vitro*.

The values of cell concentration at 88 hours fall between those of the parental stocks. The scatter about line of the growth curve, even if lower than in Varese, is also significant ($0.01 < P < 0.05$).

H_2. The reciprocal hybrid cultures are characterized by the lowest growth rate among the four types of cell populations, being only 1/3 of the initial cell concentration.

TABLE I.

Statistical analysis of growth pattern of inbred and crossbred cell population in vitro. *Coefficients of variation (c.v.) are also shown.*

(* = $P < 0.05$; ** = $P < 0.01$)

Type of cell populations	Variances			c.v.
	linear trend	scatter about line	error	
Aspra	30925.1360**	80.0270	38.8069	0.0143
Varese	2793.9488**	134.8406*	32.4876	0.0165
H_1	8378.6162**	69.0569*	13.3347	0.0097
H_2	1671.3488**	34.5456**	3.6518	0.0060

Also in this case (Table I), the curve which best fits such a growth pattern is a quadratic one (fig. 4).

DISCUSSION.

The overall analysis of variance demonstrates a highly significant difference between the two inbred stocks, between the two hybrids and between inbred stocks and hybrids ($P < 0.01$); thus one may assume that the four types of cell populations react in remarkably different ways to the conditions offered by culturing *in vitro* (fig. 5). Four aspects of the behaviour *in vitro* of the different cell populations have now to be discussed.

1. *Individual growth pattern of the inbred stocks and of the inter-stock hybrids.*

The data concerning the inbred stocks confirm the previous results obtained by Rezzonico Raimondi and Gottardi (1967), proving furthermore the good repeatability of this kind of experiment and the stability of the stock reaction throughout many generations of flies.

Compared with inbred cell populations H_1 cultures exhibit intermediate behaviour during the increase period. The mean cell concentrations at each

incubation interval are in fact comprised between the corresponding values of the parental stocks. The growth pattern too shares both characteristics of parental stocks. In fact, the growth curve is characterized by an intermediate value of the linear regression coefficient ($b_{AS} = 1.3231$; $b_{HI} = 0.6887$; $b_{VA} = 0.3977$) and also of the variance of the scatter about line (Table 1).

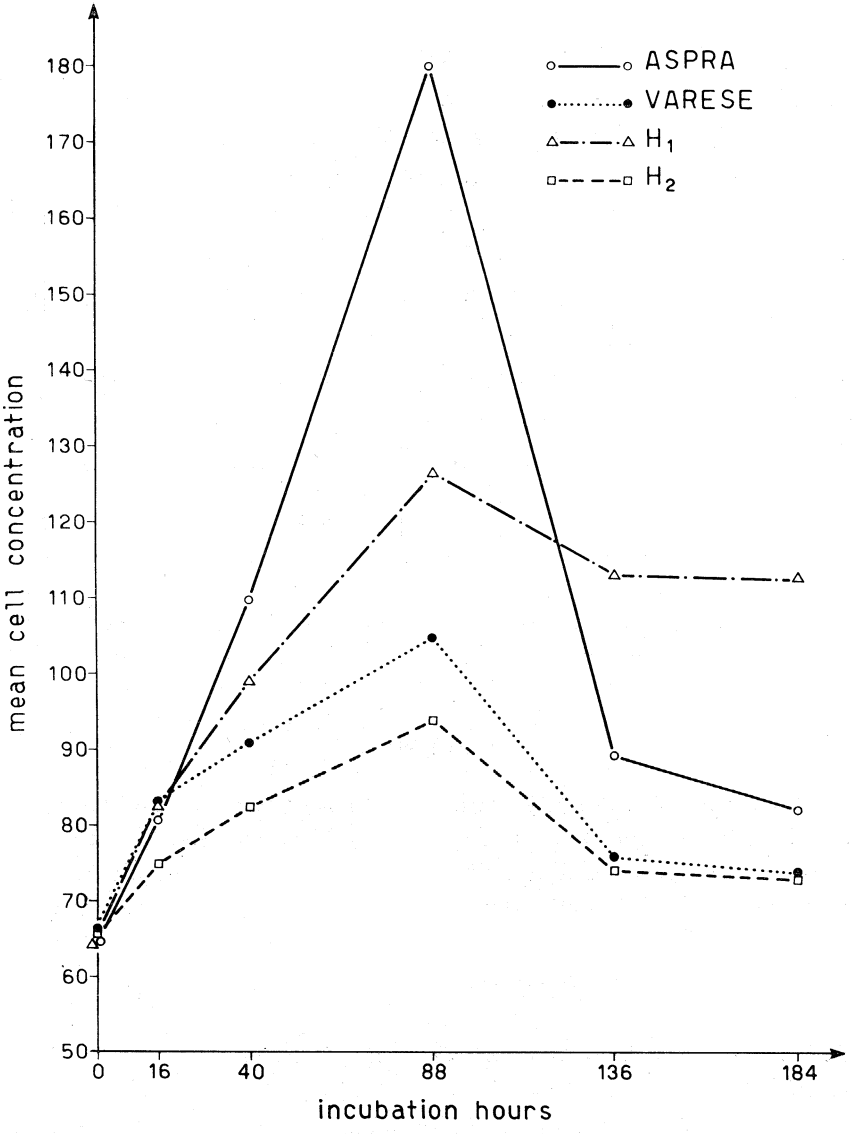

Fig. 5. – Mean growth curves of the four different types of cell populations.

The small cell concentration decrease after 88 hours of incubation could be accounted for by the hybrid advantage as regards increase of cell population resistance towards disadvantageous environmental conditions.

Two possible hypothetical explanations may be quoted to justify the results reported above (Rezzonico Raimondi and Gottardi, 1967).

The first one assumes that there is an optimal embryonic stage (Horikawa and Fox, 1964), at which the mechanically dissociated cells show the best adaptation to culture conditions. Therefore if each stock has its typical development rate, the embryo populations that reach the optimal stage in the highest percentage in the same time period will ensure the best cultures for growing *in vitro*. The second one refers to differential activities of several enzymes involved in the metabolic pathways of cell cycle.

To test the first hypothesis, the minimal hatching time for each inbred stock was determined, choosing as parameter of this character the hatching time of the first larva of a sample (Table II). Since the analysis of variance on the data collected fails to show any statistically significant difference ($P > 0.05$), the first explanation has to be discarded.

TABLE II.

Mean hatching time of the first larva in five mass cultures for each of the two inbred stocks Aspra and Varese. Overall mean and standard error (s.e.) are also shown.

Stock	Culture mean					Overall mean	s.e.
	1	2	3	4	5		
Aspra	8.67	7.57	8.35	7.47	8.64	8.14	± 0.36
Varese	10.00	7.12	8.58	8.14	8.90	8.55	± 0.36

The second one, although not based on any experimental evidence, and not considered during the present investigation, can be used as working hypothesis for future researches.

2. *Exceptionally low growth rate of* H_2 *cultures.*

To explain this peculiar pattern, one might invoke a negative interaction between cell characters derived from the females (i.e. from the egg cytoplasm) and the nuclear genotype.

3. *Significance of the difference between reciprocal hybrid cell populations.*

This is a clear example of maternal effect. Little more can be said, although it seems justified to attribute this phenomenon back to some properties of the egg. The initial trend of the growth curve of both crossbred populations up to 88 hours of culture depends on the growth pattern of the maternal inbred stock. In fact, the growth curve shows a less appreciable bend in H_1 than in H_2 (fig. 5).

4. *Higher stability of hybrid cell populations.*

This fact is well documented by the particularly low coefficient of variation of both hybrids (Table I).

This finding is interesting, because it shows which genetical conditions give the highest stability in response to uncontrolled environmental variations, and it is to be expected because of the higher buffering power of the heterozygotes in homeostatic phenomena, as demonstrated by Lerner (1954).

It is therefore of great interest from the point of view of investigations like biochemical assays which require and absolutely homogeneous behaviour of the material used.

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