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INFLUENCE OF PARENTAL AGING ON THE REPRODUCTION OF THE F_1 GENERATION IN A HERMAPHRODITE NEMATODE *CAENORHABDITIS ELEGANS*

B. BEGUET and J. L. BRUN

Department of General and Applied Biology,
Associate Laboratory of the C.N.R.S.*, University of Claude Bernard,
Lyon I, 69 Villeurbanne, France

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1. INTRODUCTION

THE WORK of numerous authors, particularly that of Lansing (1947); Comfort (1953); David (1961); Lints (1963); Parsons (1964); Clark and Rockstein (1964) and Wattiaux (1968), has shown that in the higher organisms parental senescence has an effect on the F_1 generation, especially with regard to its reproductive functions. However, our knowledge of the causes determining the changes produced is still by no means complete. The variability seen in the behaviour of the F_1 generation is the basic reason for this.

It may be assumed that this variability stems to a large extent from the genotypic heterogeneity of the organisms used. It is, indeed, much smaller in the parthenogenetic rotifer *Philodina citrina* than it is in a gonochoric species like *Drosophila melanogaster*. And that being so, the study of an autogamous hermaphrodite animal, such as the nematode *Caenorhabditis elegans*, ought to prove fruitful since its constant autoreproduction should ensure the homogeneity of its genome. In addition, the brevity of the reproductive cycle of this animal ($4\frac{1}{2}$ days at 18°C) and its cultural requirements make it easy to repeat the experiments. Furthermore, its spermatogenesis and oogenesis can be studied relatively easily (Nigon, 1949; Nigon and Brun, 1955).

In this experiment, which is the direct outcome of work done by Brun and Lebre (1968), we used individual cultures to compare the fecundity of F_1 hermaphrodites that were obtained from parents having either a juvenile physiology or a senile physiology. This enabled us to get a clearer understanding of the way in which parental senescence acts on the sexual maturation of the F_1 generation as well as on its production of spermatozoa and ova.

2. MATERIALS AND METHODS

2.1 *Individual cultures of C. elegans*

The hermaphrodites used came from a strain that had been reared in the laboratory for about 2000 generations, mainly in individual cultures. These cultures were prepared by the method put forward by Nigon (1949). They were xenic cultures, for the bacterial

* C.N.R.S. Centre National de la Recherche Scientifique.

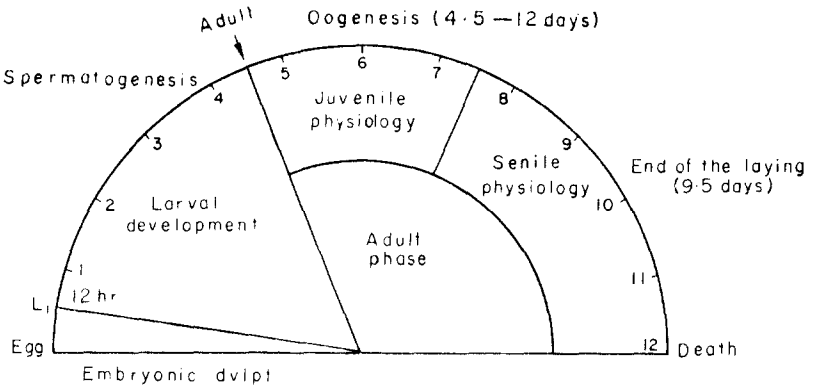

FIG. 1. Life cycle of the protandrous hermaphrodite *Caenorhabditis Elegans* at 18°C in individual xenic culture. The adult stage is characterized by the appearance of the 1st egg in the uterus.

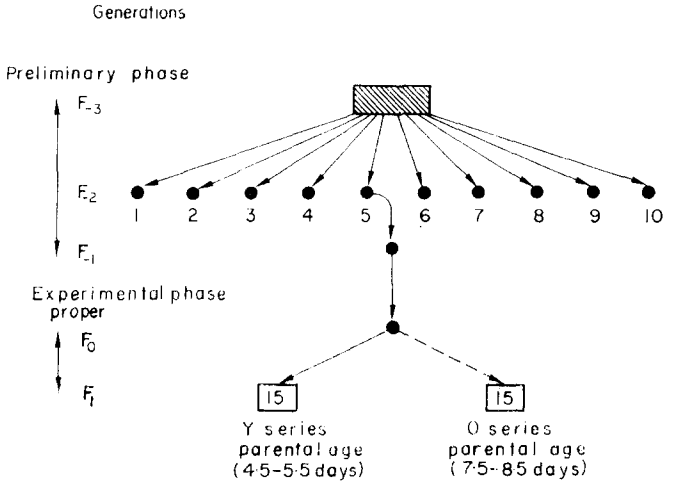

FIG. 2. Complete diagram showing the establishment in F_1 of two experimental series for comparison, citing the example of line no. 5, one of the ten sister lines used in one experiment.

▒ F-3 parent—the most fertile of the individual maintenance cultures—from which the ten lines were established.

F-2● Preliminary generations before establishing the two series.

F-1●

[15] Group of 15 parents for the line under consideration from which all the eggs laid were collected.

Arrows Culture of the 1st generation of 15 parents obtained from the O generation.
→ from young parents
⇢ from old parents

complex, constantly associated with the nematode (cf. Brun, 1966a) and developing on the surface of the nutrient agar, had not been removed.

Under these circumstances—and at 18°C—the life cycle of this protandrous hermaphrodite developed in the following way (Fig. 1).

2.2 *The experimental procedure* (*Fig.* 2)

This consisted of two successive phases: a preparatory phase in which 10 sister lines obtained from the same F_{-3} hermaphrodite parent were established, the cultures being ordered in such a way as to have the F_0 parents of very similar age ($\pm$ 3 hr);

then the experimental phase proper, in which the hermaphrodites of the two experimental series, Y and O, were cultured, each series being made up of F_1 progeny from the same F_0 parents, but derived when the latter were aged either 5 days $\pm$ 12 hr or 8 days $\pm$ 12 hr.

2.3 *Detection of the sexual maturity of the* F_1 *hermaphrodites*

Both in the Y and the O series the attainment of sexual maturity by the F_1 hermaphrodites was arbitrarily determined as taking place when the first egg was formed. This can readily be seen in the uterus of an adult animal on simple stereomicroscopic examination ($\times 25$).

2.4 *Measurement of the production of spermatozoa by the* F_1 *hermaphrodites*

This was done by counting the spermatozoa accumulated in the seminal receptacle of the F_1 hermaphrodites in the Y and O series, just at the time they reached sexual maturity. To do this these individuals were first identified by the method described above, then a smear was prepared from their reproductive apparatus, using the technique described by Nigon (1949), and stained by Feulgen's stain.

2.5 *Estimation of the production of eggs by the* F_1 *hermaphrodites*

This was carried out indirectly. In fact, it was not possible to count the eggs themselves, partly owing to the small size of the eggs (50 μ in length) and partly owing to their index of refraction, which is very close to that of the nutrient medium. The most easily measured parameter concerning the level of reproduction of a nematode is what is known as its fecundity. This is represented by *the number of adults obtained from the eggs laid by one parent.* An estimate of the actual number of eggs produced can be made from this parameter if the mortality rate of the eggs—determined in a significant sample—is taken into consideration.

(a) *Measurement of the fecundity of the* F_1 *hermaphrodites.* For each of the 10 lines (cf. Fig. 2) the fecundity of each hermaphrodite in the two series was determined with the help of a Leitz enlarger ($\times 20$), of the type used for reading. In this way a pair of readings for Y and O fecundity was obtained. The total results of an experiment consisted of the addition of all the Y readings and all the O readings. On average the fecundity of 5 parents for each line and for each series was determined.

In order to make the counting of the progeny of an F_1 hermaphrodit eeasier—they amounted to nearly 200 F_2 individuals—it was necessary to subdivide the laying phase into 24-hr periods by transferring it to a fresh nutrient medium each day. This operation led to considerable losses among the F_1 parents. Only those lines in which at least three F_1 parents remained in both series were used for our comparisons. This eliminated about 35 per cent of the lines studied.

(b) *The fertility of the* F_2 *eggs laid by the* F_1 *hermaphrodites.* As the method of individual culture is not suitable for collecting a large number of eggs (a hundred) over a short laying period (1 hr), the procedure was modified in the following way. For each of the Y and O

series, 20 F_1 nematodes reaching the adult state were cultured together instead of individually. Under these conditions it was possible to collect a large number of eggs from the surface of the agar and to follow their development (from 0 to 4·5 days) after they had been transferred to fresh nutrient medium.

3. EXPERIMENTAL RESULTS

3.1 *The modifications in fecundity seen in the F_1 hermaphrodites derived from old parents*

The study of the fecundity of the F_1 hermaphrodites was carried out in two ways: quantitatively, by counting the F_2 generation, and qualitatively, by studying the distribution of this F_2 progeny in relation to the F_1 laying days.

3.1.1 *The reduction in fecundity in the O series.* The results of 11 experiments (a–k) carried out under identical conditions were collected (cf. Fig. 2).

(a) *General study of the behaviour in the Y and O series.* The average fecundity readings for the two series were compared (Table 1). In the majority of cases (10 out of 11) the Y–O difference was positive. It was significant 8 times out of 10. Aging of the parental body thus generally led to a reduction in the fecundity of the next generation.

TABLE 1. AVERAGE FECUNDITY OF FIRST GENERATION PARENTS IN THE Y AND O SERIES IN EACH OF 11 EXPERIMENTS

Experiments	Y series	O series	Y–O comparison "t" test
a	160·67 ± 19·75	124·40 ± 16·33	2·77*
b	162·81 ± 17·41	136·89 ± 17·28	2·07*
c	163·16 ± 26·85	120·07 ± 19·95	2·53*
d	166·00 ± 22·05	133·13 ± 20·97	2·11*
e	169·75 ± 10·48	115·22 ± 15·14	5·80*
f	175·69 ± 18·22 ←→	185·75 ± 16·38	—
g	187·30 ± 16·22	124·26 ± 19·65	4·84*
h	204·10 ± 18·33	137·44 ± 29·28	3·15*
i	206·12 ± 20·33	173·94 ± 13·98	1·93
j	214·26 ± 17·54	187·50 ± 21·94	1·87
k	224·47 ± 19·99	177·45 ± 21·12	3·17*

The results on any one line relate to the same experiment in which the average fecundity of at least thirty F_1 hermaphrodites was assessed in parallel in the Y and O series. Each pair of readings is compared with the help of the "t" test. Where the Y–O difference is positive the threshold of significance is reached at 1·96 when $\alpha = 0{\cdot}05$. The sign * indicates a significant difference.
←→: This sign draws attention to the fact that the Y–O difference in fecundity is negative.

(b) *Study of the lines.* After eliminating the lines where fewer than 3 parents remained in one or other series (cf. technique used, 2. 5), we considered the Y–O pairs of readings relative to the 71 remaining suitable lines that were obtained from the 11 experiments carried out (Table 2). This grouping made it possible to study the effect of parental senescence on a large F_1 population.

TABLE 2. AVERAGE FECUNDITY OF THE LINES IN THE Y AND O SERIES OF F_1

Y series		O Series	Y series		O series
52·00	←→	80·75	187·00		59·60
99·33		55·80	190·00	←→	202·00
109·00		105·00	192·25		108·50
115·00	←→	164·16	193·20		121·00
118·50	←→	125·50	193·28		139·41
120·25	←→	134·83	193·75		137·66
124·66	←→	194·00	195·75		126·42
125·20	←→	127·70	196·00	←→	201·04
128·25	←→	144·33	197·33	←→	211·62
141·00	←→	167·83	199·44		144·50
150·66		133·57	199·50		117·20
152·00		141·33	201·33		175·00
152·57	←→	208·66	205·11		132·57
153·00		108·90	206·80		156·00
153·60	←→	168·25	208·75		208·33
154·66	←→	166·42	210·28		129·25
158·40		96·00	214·16	←→	218·00
159·00		94·66	214·60		117·75
159·50	←→	201·88	214·80		139·75
161·25		126·66	215·00		197·66
162·00		142·00	217·00		178·00
163·16		147·83	218·50		180·50
167·00		104·00	220·83		154·33
167·00		144·75	221·00		141·00
167·20	←→	182·14	222·40	←→	230·50
170·00	←→	218·33	230·37		132·66
172·28	←→	187·39	231·90		164·07
172·60		168·33	243·55		195·85
173·50		117·22	243·77		183·80
176·60		95·25	247·75		178·22
179·00		106·83	251·87		220·00
182·71		139·66	253·00		221·28
184·66		76·00	253·40		87·80
185·28		164·00	257·75		160·66
185·50	←→	186·83	258·00	←→	271·50
			275·00		271·50

←→Lines where the average fecundity is greater in the O series than in the Y series (Y–O < O).
Only 71 pairs of Y–O readings from the 11 experiments were used (cf. techniques used, 2. 5).

TABLE 3. THE DISTRIBUTION OF ADULT F_2 PROGENY IN THE Y AND O SERIES ACCORDING TO THE DAY THEY WERE LAID

TABLE 3a

Experiments (cf. Table 1)	Y series Laying days					O series Laying days				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
a	33·10	74·57	33·78	11·92	7·30	58·13	37·86	20·88	6·13	1·40
b	14·89	99·08	40·18	7·53	1·13	51·42	36·67	30·07	10·54	8·19
d	37·91	58·31	41·41	20·15	8·22	56·58	45·09	22·96	7·08	1·42
f	24·08	57·36	60·00	20·19	14·06	49·64	70·77	46·17	15·06	4·11
k	35·82	100·53	49·62	28·25	10·25	67·56	56·08	37·57	13·10	3·14
Average fecundity	29·16	77·97	44·99	17·60	8·19	56·66	49·29	31·53	10·38	3·65
Cumulative fecundity	29·16	107·13	152·12	169·72	177·91	56·66	105·95	137·48	147·86	151·51

TABLE 3b

Laying days	Y series	O series	t
1st day	29·16	56·66	4·81*
2nd day	77·97	49·29	3·38*
3rd day	44·99	31·53	1·72
4th day	17·60	10·38	1·15
5th day	8·19	3·65	3·60*

A group of five identical experiments in which the number of parents was always greater than 30.
*Significant difference—the threshold of significance being reached at $t = 1{\cdot}96$, when $\alpha = 0{\cdot}05$.

3.1.2 *Modification in the O series of the distribution of the F_2 generation in relation to the laying days of the F_1 parents.* Table 3a shows the results of the 5 experiments in which daily measurements of the fecundity of the F_1 hermaphrodites from the Y and O series, as nearly as possible of the same age ($\pm$ 3 hr), were carried out.

It should be noticed that, although there is a significant difference between the Y and O values if the first 2 days are taken separately (Table 3b), the number of F_2 descendants for the 2 periods considered together is the same: 107·13 and 105·95 respectively (Table 3a). It can also be observed that after this grouping the course of the laying is the same in both series and decreases steadily. Under these circumstances the differences in fecundity observed in the 2 series on the 1st and 2nd day cannot be attributed to a differential mortality rate and must be due to a different distribution in time of the F_1 laying. This is more intense on the 1st day in the O series.

3.2 *Modifications of the reproductive processes in the F_1O parents*

We have tried to link the reduction in the number of F_2 descendants in the O series to possible modifications in the reproductive mechanisms of the F_1 parents.

Indeed, the reproductive physiology of this protandrous hermaphrodite can be upset (Brun, 1955; 1966b):

either when the ovotestis starts to function, at the time the spermatozoa are formed;
or at the time of the maturation of the ovaries;
or during fertilization and the development of the eggs.

3.2.1 *Study of the production of spermatozoa by the F_1 hermaphrodites.* The counting of all the spermatozoa formed was carried out at the same time as the parallel experiment (e), on hermaphrodites coming from the same lines as were used for the determination of F_1 fecundity (Table 4).

TABLE 4. COMPARISON BETWEEN THE NUMBER OF SPERMATOZOA ACCUMULATED IN THE SEMINAL RECEPTACLES OF THE F_1 HERMAPHRODITES IN THE Y AND O SERIES

	Y series	O series	"t" test
Average number of spermatozoa for each individual	207·13 ± 9·27	180·30 ± 14·42	3·06*
Average fecundity of hermaphrodites belonging to the same lines	169·75 ± 10·48	115·22 ± 15·14	5·80*
Difference between the number of spermatozoa and fecundity	210–170 = 40	180–115 = 65	

The samples studied in this experiment (*e* in Table 1) always included more than 30 individuals.
(The threshold of significance was 1·96, when $\alpha = 0{\cdot}05$. The sign * indicates a significant difference).

The significant reduction in the number of spermatozoa formed by the F_1 hermaphrodites of the O series indicates the influence of parental aging on the spermatogenesis of the immediate F_1 generation. As the study of this spermatogenesis shows no cytological anomaly, it must be assumed that the reduction in the number of spermatozoa is due to a reduction in the duration of the male phase in the O series of hermaphrodites.

3.2.2 *Sexual maturity of the F_1 hermaphrodites.* The results of one experiment (1) are given in Table 5. It was observed that the arrival of the first formed eggs in the F_1 uterus occurred earlier in the progeny of aged parents. The F_1O subjects thus attained sexual maturity up to 6 hr earlier than the F_1Y subjects ($4\frac{1}{4}$ days instead of $4\frac{1}{2}$ days).

TABLE 5. APPEARANCE OF THE FIRST EGGS IN THE UTERUS OF F_1 HERMAPHRODITES OF THE SAME AGE IN THE Y AND O SERIES (EXPERIMENT 1)

	Y series	O series	Test
Number of F_1 individuals obtained from a laying period of 4 hr	141	96	
Number of F_1 hermaphrodites with at least one egg in the uterus	49	69	
Proportion of F_1 hermaphrodites with one egg in the uterus	0·347	0·718	4·00*

* A comparison of the percentages of hermaphrodites with at least one egg in the uterus by means of the "ε" test shows that they differ significantly at the threshold $\alpha = 0{\cdot}05$.

3.2.3 *Fertility of the F_2 eggs laid by the F_1 generation.* The average fertility of the F_2 eggs was defined as the percentage of adults obtained from eggs laid 4·5 days beforehand. It was measured for the first four days of laying in the experiment (*m*) in both the Y series and in the O series, a hundred eggs being used each time (Fig. 3).

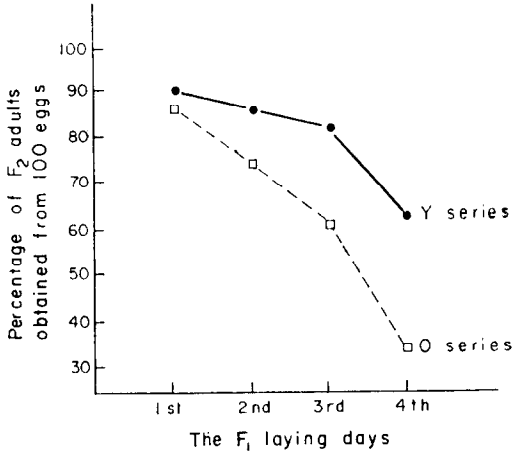

Fig. 3. Fertility of the F_2 eggs laid by the F_1 generation in the Y and O series. *The difference between the 2 readings is significant for the 4 consecutive laying days under consideration.

It can be seen that the fertility of the eggs laid by the F_1O subjects was always less than that of the eggs laid by the F_1Y subjects. Furthermore this reduction in the fertility of the

F_2 eggs laid by the O series of hermaphrodites increased as the laying days of the F_1 subjects proceeded. The gap rose from 5 per cent at the start of laying to 25 per cent at the end of laying.

These observations were completed by an investigation into the point at which the development was arrested (Table 6). To do this, in the experiment (*n*) the F_2 eggs obtained from a laying period of one hour only were watched until the 1st stage larvae (L_1) hatched, which occurred 10–12 hr after laying. It should be noted that the L_1 larvae derived from elderly parents hatched more rapidly than those from young parents. Then 5 days later we counted the number of adults that developed from these larvae.

TABLE 6. STUDY OF THE VIABILITY OF THE F_2 EGGS UP TO THE HATCHING OF THE FIRST STAGE LARVAE (L_1) AND STUDY OF THE POSTEMBRYONIC MORTALITY (EXPERIMENT *n*)

Laid by F_1	1st day			2nd day			3rd day			4th day		
	E	L_1	A	E	L_1	A	E	L_1	A	E	L_1	A
Y series	80	63	64	36	32	31	38	24	28	37	24	25
O series	36	21	25	35	26	22	35	24	20	35	25	19

E = Number of F_2 eggs studied.
L_1 = Number of L_1 counted.
A = Adults counted 5 days after deposition of the eggs.

Although our results were small in number we can state that there was no difference between the number of L_1 and the number of adults in relation to the period of laying. It may thus be concluded that the eggs became non-viable at an earlier stage, probably during the embryonic period, or even at the time of their formation.

4. GENERAL CONCLUSIONS AND DISCUSSION

In *Caenorhabditis elegans* parental aging generally leads to a significant reduction in the number of F_2 offspring produced by the F_1 parent. This observation thus confirms that the influence of parental senescence on the next generation of living beings is universal. This influence is seen both in organisms that reproduce asexually (as in the ciliates like *Paramecium aurelia, Tetrahymena rostrata, Euplotes crassus* (Siegel, 1967) and in those that reproduce by various sexual methods: gonochorism (*Musca,* Clark and Rockstein 1964), *Drosophila* (Wattiaux, 1968; Tsien and Wattiaux, 1971), *Mus* (Strong, 1968), parthenogenesis (*Philodina citrina,* Lansing, 1947), autogamous hermaphrodism (*C. elegans*).

The originality of this work lies mainly in the information it provides as to the modalities of this influence. In *C. elegans* the reduction in number of the F_2 progeny is correlated with changes in the reproductive mechanisms that are seen both in the functioning of the gonads of the F_1O parents and in the products of their ovotestes:

earlier sexual maturation of the F_1O hermaphrodites;
reduction in the number of spermatozoa produced by the F_1O;
increased mortality in the F_2O eggs.

Modification of oogenesis in the F_1O

Given that the reduction in the numbers of spermatozoa would appear likely to restrict the number of F_2 eggs formed, it might at first sight be thought that the last two processes

mentioned above could combine to produce the reduction in the numbers of the F_2O generation.

In reality, however, this is mainly due to the increased mortality rate of the F_2O eggs, as can be seen also in *D. melanogaster* (Butz and Hayden, 1962; O'Brian, 1961). Indeed:

1. The number of spermatozoa formed is always distinctly greater than the number of progeny observed (cf. Table 4).

2. In both the Y and O series the number of F_2 eggs formed is almost the same. Thus, for example, if we consider the results of the first 4 laying days of the F_1 parents in each series that are shown in Fig. 3, we can calculate the theoretical number of eggs needed to obtain the adult F_2 progeny shown in Table 3a: 169·72 in the Y series, 147·86 in the O series. We obtain a comparable number of about 200 F_2 eggs for both series. And this number does not differ significantly from the number of spermatozoa produced in the O series: 180·30 $\pm$ 14·42.

3. It does not seem that the spermatozoa produced by the F_1O hermaphrodites have lost their fertilizing ability. Indeed, crossing elderly F_1 subjects bred from old parents (O series) with young males of the original strain—which has the effect of making aging spermatozoa of the O series compete with young sperm for the fertilization of the oocyte —does not significantly increase the viability of the last F_2 eggs laid.

This last experiment is of interest also, because it makes it impossible to attribute the increase in the mortailty of the F_2O eggs to fertilization of the oocytes by abnormal spermatozoa, whose deterioration could not be seen on cytological examination. In these circumstances, therefore, it must be assumed that the increase in the mortality of the F_2O eggs is of oocytic origin. As in the parthenogenetic rotifer *Philodina citrina,* a disturbance of oogenesis would be produced in the F_1 offspring obtained from eggs laid by senescent parents. It is hardly possible to attribute this disturbance to a change in the genetic chromosome material for two reasons:

(a) no aberrant meiotic figures are seen before the 9th day (Brun, 1966b), which is the same as in the F_1Y;

(b) as is shown in another paper (Beguet, 1972) if the conditions producing the effects of parental senescence are suppressed in F_2 after 2 generations (F_0 and F_1) of O selection, the F_3 progeny thus obtained is quite as numerous as that of the Y series for the same generation.

Modification of the oocytic cytoplasm

This leads us to conclude that it is the oocytic cytoplasm that is modified, no doubt because of a disturbance in vitellogenesis. Clark and Rockstein (1964) reached the same point of view as a result of the differences observed in the gonochoric insects (*Drosophila melanogaster,* for example) following reciprocal crossing: elderly oocytes with young spermatozoa and young oocytes with elderly spermatozoa.

What then could be the basis of this maternal effect caused by parental senescence? To reply to this question two groups of findings must be considered.

1. The earlier sexual maturity of the F_1O (cf. Table 5) and the reduction in the number of spermatozoa that they produce (cf. Table 4) indicate an earlier transition from the spermatogenetic phase to the oogenetic phase.

2. The L_1 larvae bred from elderly parents hatched more rapidly than those bred from young parents.

This last observation implies that there is an intensification of metabolism in the embryos of the O series, shown particularly by an acceleration in cellular multiplication. There is every reason to suppose that this increase in mitotic activity extends to gametogenesis. But at this stage two processes occur that are considered by most authors (cf. Stern, 1970) to be mutually exclusive: gonial mitotic division on the one hand, and the cellular differentiation of these gonia into spermatocytes, and then into oocytes, on the other hand. If we accept, in agreement with the majority of authors, that the regulation of the cellular division forms the key to the phenomena of development and differentiation, it can be envisaged that the effect of parental senescence is to disturb the regulation of the gametogenetic cellular division in the F_1 generation, for numerous articles (Goldstein and Prescott, 1967; Rusch *et al.*, 1966; Gurdon, 1967; etc.) tend to show that the duplication of the chromosomes—which is the basic phenomenon of cell division—is under the control of the cytoplasm. Thus a modification in the oocytic cytoplasm occuring in the course of vitellogenesis in the gonads of senescent parents would be carried on into the F_1O egg that was formed, and then continued during the whole of its development. In this F_1O individual it would induce disturbances in gonial differentiation, shown by an early change-over to oocytosis and by an increasingly abnormal vitellogenesis in the formation of the F_2O oocytes.

Beyond a critical threshold of vitellogenetic disturbance the F_2O oocyte would no longer be viable, and this could explain how the mortality of the F_2O eggs increases with the age of the F_1O parent from whom they were derived (cf. Fig. 3).

Two different mechanisms can be put forward to explain the changes in the cytoplasm of the oocytes obtained from senescent parents, and their consequences. The sequel to this article may perhaps make it possible to choose between them.

1. These cytoplasmic changes could consist of an increase in the quantity of DNA in the cellular organelles, that are endowed with genetic continuity like the mitochondria. Indeed in *Drosophila melanogaster* Tsien and Wattiaux (1971) have shown that the eggs obtained from elderly parents are richer in mitochondrial and extramitochondrial DNA.

2. These cytoplasmic transformations might arise from changes in enzyme activity. Indeed, according to the work of Erlanger and Gershon (1970) on the free-living nematode *Turbatrix aceti,* the activity of various enzymes, such as acetylcholinesterase, α-amylase, malic dehydrogenase and isocitrate lyase, decreases significantly with the age of the nematode. In these circumstances we find that our conclusions on oogenetic differentiation are similar to those reached by Gurdon and Woodland (1968) on embryonic differentiation. "It must be assumed that there is a cyclical exchange of information between the nuclear genes and the cytoplasm, so that the cytoplasmic proteins, the end products of the genes, are able to determine the activity of the latter in the cells formed during the later stages of development".

REFERENCES

BEGUET, B. (1972) *Exp. Geront.* **7,** 209.
BRUN, J. L. (1955) *Bull. biol. Fr. Belg.* **89,** 326.
BRUN, J. L. (1966*a*) *Ann. Biol. Anim. Biochim. Biophys.* **6,** 127.
BRUN, J. L. (1966*b*) *Ann. Biol. Anim. Biochim. Biophys.* **6,** 267.
BRUN, J. L. and LEBRE, D. (1968) *C. R. Acad. Sci., Paris* **266,** 2149.
BUTZ, A. and HAYDEN, P. (1962) *Ann. Entomol. Soc. Amer.* **55,** 617.

CLARK, A. M. and ROCKSTEIN, M. (1964) In *Physiology of Insecta.* Chapter 6, Academic Press, New York.
COMFORT, A. (1953) *Nature, Lond.* **172,** 83.
ERLANGER, M. and GERSHON, D. (1970) *Exp. Geront.* **4,** 131.
DAVID, J. (1961) *Ann. Genet.* **3,** 1.
GOLDSTEIN, L. and PRESCOTT, D. M. (1967) In *The Control of Nuclear Activity,* p. 3, Prentice-Hall, Englewood Cliffs, New Jersey.
GURDON, J. B. (1967) *Proc. Natn. Acad. Sci. U.S.A.* **58,** 545.
GURDON, J. B. and WOODLAND, H. R. (1968) *Biol. Rev.* **43,** 233.
LANSING, A. I. (1947) *J. Gerontol.* **2,** 228.
LINTS, F. A. (1963) *Nature, Lond.* **197,** 1128.
NIGON, V. (1949) *Ann. Sci. Nat. Zool.* **11,** 1.
NIGON, V. and BRUN, J. L. (1955) *Chromosoma* **7,** 129.
O'BRIAN, D. M. (1961) *Ann. Entomol. Soc. Amer.* **54,** 412.
PARSONS, P. A. (1964) *Quart. Rev. Biol.* **39,** 258.
RUSCH, H. P., SACHSENMAIER, K., BEHRENS, K. and GRUTER, V. (1966) *J. Cell. Biol.* **31,** 204.
SIEGEL, R. W. (1967) *Symp. Soc. Exp. Biol. U.S.A.* **21,** 127.
STERN, H. (1970) In *The Cell Differentiation,* p. 141, Van Nostrand Reinhold, New York.
STRONG, L. C. (1968) In *Biological Aspects of Cancer and Ageing.* Pergamon Press, Oxford, London and New York.
TSIEN, H. C. and WATTIAUX, J. M. (1971) *Nature New Biol. G.B.* **230,** 147.
WATTIAUX, J. M. (1968) *Exp. Geront.* **3,** 55.

Summary—In the free-living hermaphrodite autogamous nematode *C. elegans,* parental senescence generally leads to a significant reduction in the number of F_2 adults derived from F_1 parents. Indeed, this reduction is seen in 70 per cent of lines. It is related to changes in the reproductive mechanisms of the F_1 generation, affecting both the physiology of the gonad and the products of the ovotestis:

Earlier sexual maturation of the F_1O hermaphrodite derived from elderly parents (4¼ days instead of 4½ days);

A 15 per cent reduction in the number of spermatozoa produced by the F_1O;

An increase in the mortality of F_2O eggs, rising to 50 per cent in eggs laid on the last day.

These findings suggest that the causes of the reduction in fecundity arise during oogenesis in the F_1O subjects. It is principally the cytoplasm of the oocyte that appears to be affected. Various hypotheses are put forward concerning the way in which this prolonged effect could be produced.

Résumé—Chez le Nématode libre, hermaphrodite autogame *C. elegans,* la sénescence parentale entraîne généralement une diminution significative du nombre de descendants adultes F_2 produits par la progéniture F_1. Cette diminution affecte, en effet, 70 pour cent des lignées. Elle est corrélative de modifications des mécanismes reproducteurs chez cette descendance F_1, observables tant au niveau de la physiologie de la gonade qu'en ce qui concerne les produits de l'ovotestis:

Maturation sexuelle plus précoce des hermaphrodites F_1 V, issus de parents âgés (4 j.¼ au lieu de 4 j. ½);

Diminution de 15 pour cent du nombre de spermatozoïdes produits par les F_1 V;

Mortalité accrue des oeufs F_2 V atteignánt 50 pour cent pour les oeufs pondus le dernier jour.

Les relations ainsi établies conduisent à situer les causes de la diminution de fécondité au niveau de l'ovogenèse des F_1 V. Il semble que ce soit essentiellement le cytoplasme de l'ovocyte qui soit perturbé. Diverses hypothèses relatives au déterminisme de cet effet prolongé sont présentées.

Zusammenfassung—In der freilebenden hermaphroditischen autogamen Nematode *C. elegans* führt parentale Seneszenz im allgemeinen zu einer signifikanten Reduktion der Zahl der erwachsenen F_2-Abkömmlinge von F_1-Eltern. Diese Reduktion wird bei 70% der Stämme beobachtet. Sie hängt mit Veränderungen im Fortpflanzungsmechanismus der F_1-Generation zusammen, welche die Physiologie der Gonade und der Produkte des Ovotestis beeinflussen:

Frühere sexuelle Reifung des F_1O-Hermaphroditen, der von älteren Eltern abstammt (4¼ Tage statt 4½ Tage);

eine 15%ige Reduktion der Spermatozoenzahl, die von den F_1O erzeugt wird;

eine Erhöhung der Mortalität von F_2O-Eiern, die bis auf 50% der Eier ansteigt, die am Tag zuvor gelegt wurden.

Diese Befunde legen nahe, dass sich die Ursachen der Reduktion der Fruchtbarkeit während der Oogenese in den F_1O-Tieren einstellen. Hauptsächlich scheint das Cytoplasma des Oocyten beeinflusst zu werden. Es werden verschiedene Hypothesen über die Art vorgetragen, in der dieser verlängerte Effekt hervorgerufen werden könnte.

Резюме—У свободно живущей нематоды, гермафродитной автогамной *C. elegans*, старение родителей обыкновенно влечет за собой значительное снижение в числе взрослых потомков F_2, произведенных поколеня F_1. Это снижение обнаруживается у 70% линий. Оно связано с изменениями в воспроизводительных механизмах этого поколения F_1 и выражается в физиологическом состоянии зонады и в продуктах, вырсбатываемых гермафродитной желе-ой:

—Более раннее половое развитие гермафродитов F_1 V, родившихся от престарелых родителей ($4\frac{1}{4}$ дней вместо $4\frac{1}{2}$ дней);

—Снижение на 15% числа сперматозоидов, выработанных у F_1 V;

—Повышенная смертность яиц F_2 V, доходящая до 50% у яиц, отложенных в последний день.

На основе установленных таким образом данных делается вывод, что причина снижения плодовитости лежит в овогенезе F_1 V. По-видимому, нарушеине касается в основном цитоплазмы яйцеклетки. Предгагаются различные гипотезы относительно факторов, обусловливающих этот длительный эффект.