

# Primordial germ cell proliferation in the salamander *Pleurodeles waltl*: genetic control before gonadal differentiation

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**ABSTRACT** PGC counts were carried out on larvae of *Pleurodeles waltl* (urodele amphibia) issued from standard, monosexual male and monosexual female offspring while the genital ridges were settling. During this period, which is characterized by a zero mitotic index (and is therefore called the Po period), and which lasts from stage 35 to stage 41, no PGC proliferation occurs. A statistical analysis indicated that PGC counts per larva are sex genotype independent and that offspring may be divided into three groups with average PGC counts of 96.9, 51.0 and 31.1, respectively. A fourth group with an average of 18.3 PGCs has been identified using experimental larvae reared at 30°C from stage 30. The PGC count of 96.9 would result from at least three mitotic cycles. Before the Po period, germ cells are not identifiable. A hypothesis concerning genetic control of PGC proliferation before Po was deduced from this analysis.

**KEY WORDS:** *amphibian, genital ridges, gonadal development, gonocyte counting, mitotic index*

## Introduction

At the beginning of the developmental period in amphibia, the question of the timing of germ cell proliferation and the understanding of control mechanisms have seldom been tackled. In Anura, germ lineage may be characterized from oocyte stages to colonization of genital ridges by electron dense bodies (also called nuage or chromatoid bodies) (Reed and Stanley, 1972; Coggins, 1973; Kalt, 1973) or by the germ plasm (Bounoure, 1929, 1931, 1935; Whittington and Dixon, 1975; Akita and Wakahara, 1985). Using these markers, the timing of germ cell proliferation was studied (Dixon, 1981). In Urodela, no markers are available to locate germ cells at embryonic stages and take us back to their presumed origin (Nieuwkoop, 1947, 1950; Sutasurya and Nieuwkoop, 1974; Maufroid and Capuron, 1973, 1977a, 1985). Dense bodies have, however, been noticed in primordial germ cells (PGCs) situated in the genital ridges of young *Triturus pyrrhogaster* larvae (Hamasima and Kotani, 1977) and of old *Ambystoma mexicanum* embryos (Ikenishi and Nieuwkoop, 1978). Germ plasm has only been indicated in the presumptive mesoderm area of asegmented eggs in *Ambystoma mexicanum* (Williams and Smith, 1971). In contrast to anurans, in urodeles there are

no germ cell markers continually present throughout development.

In the urodele *Pleurodeles waltl*, Maufroid and Capuron (1981) demonstrated that germ cells were induced by endodermal cells from the ectoderm within the presumptive mesoderm. The germ cells resulting from this induction, however, are only observable during and after late embryonic stages, when they become histologically identifiable, just before hatching stages. In *Pleurodeles*, the growth of the germ cell population was not studied. Houillon (1956) had, however, noticed on the basis of the age of animals that during and after the hatching stages, the PGC number per animal remained constant.

In the present study, a non-proliferation period is confirmed and precisely located during development, according to the stages defined in the development table of Gallien and Durocher (1957). Comparisons between offspring whose sexual genotype is known are carried out and a hypothesis concerning germ cell proliferation during the early embryonic period is inferred from PGC counts.

*Abbreviations used in this paper:* MI, mitotic index; PGC, primordial germ cell; Sd, standard offspring; St, stage; Sv, natural wild offspring

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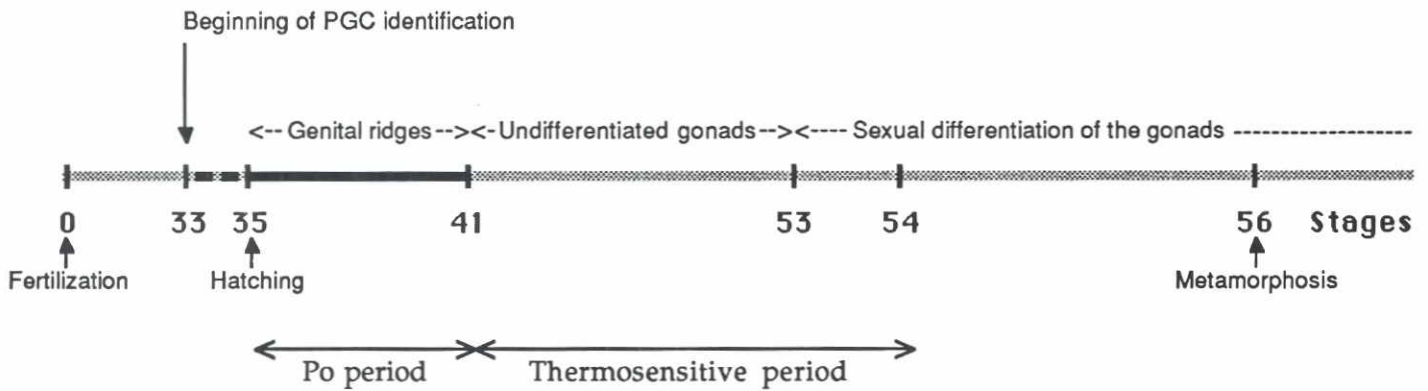


Fig. 1. Duration of the studied Po period in the development of *Pleurodeles waltl*.

**Results**

**Genetic control of the germinal population**

The results were derived from 15 different offspring: one wild, eight standard, three monosexual ZZ male and three monosexual ZW female, born between 1976 and 1987 and reared at room temperature.

*Identification and localization of PGCs*

When embryonic cells have substantial yolk platelets, PGCs cannot be distinguished from somatic cells. It becomes possible to identify PGCs at stage 33. At stage 35,

they are situated in the posterior region of the larvae near the primary ureters (= Wolffian ducts) and close to the future cloacal orifice. However, at this stage, an accurate count of PGCs is difficult, since PGC boundaries are indistinct due to germ cell accumulation and the persistence of yolk platelets. After stage 35, PGCs begin lining up along genital ridges parallel to the ureter and still contain yolk reserves. From stage 38 on, the PGCs are finishing consuming their yolk platelets and, at stage 41, they are in line in genital ridges and have no more yolk. During this last period, the cell count becomes more and more precise (Figs. 2, 3). After stage 41, the gonads again undergo mitosis.

TABLE 1

**MITOSIS, PGC COUNTS AND MITOTIC INDEX PER OFFSPRING AND PER DEVELOPMENTAL STAGE FOR 15 OFFSPRING STUDIED DURING THE Po PERIOD**

REFERENCE OF OFFSPRING	LARVAL STAGES							PER OFFSPRING	
	35	36	37	38	39	40	41	Total no of mitosis / Total no of PGCs	Mitotic index
ZZ 87							1/ 1278	1/ 1278	0.078
Sv 85							0/ 1156	0/ 1156	0
ZW 76				0/ 97	0/ 182	0/ 96	0/ 487	0/ 862	0
ZZ 85			1/ 204			0/ 322		1/ 526	0.190
ZW 82				0/ 697			0/ 415	0/ 1112	0
Sd5 77						0/ 183		0/ 183	0
Sd1 77		0/ 144	0/ 146	0/ 299	0/ 253	0/ 302	0/ 319	0/ 1463	0
Sd4 77			0/ 20	0/ 35	0/ 48	0/ 70	0/ 78	0/ 251	0
Sd3 77							0/ 451	0/ 451	0
Sd1 76			0/ 129	0/ 193	0/ 296	0/ 130	0/ 296	0/ 1044	0
Sd2 77	0/ 243	2/ 213	0/ 174	0/ 236	0/ 104	0/ 230	0/ 310	2/ 1510	0.132
Sd1 79							0/ 184	0/ 184	0
ZZ 86			0/ 103			0/ 172		0/ 274	0
Sd6 77						0/ 329		0/ 329	0
ZW 79				0/ 137			1/ 94	1/ 231	0.433
<b>P</b>	<b>0/ 243</b>	<b>2/ 357</b>	<b>1/ 776</b>	<b>0/ 1694</b>	<b>0/ 883</b>	<b>0/ 1834</b>	<b>2/ 5068</b>	<b>5/ 10854</b>	
<b>E</b>									
<b>R</b>									
<b>S</b>									
<b>T</b>									
<b>A</b>									
<b>G</b>									
<b>E</b>									
<b>Mitotic index</b>	0	0.560	0.129	0	0	0	0.039		0.046

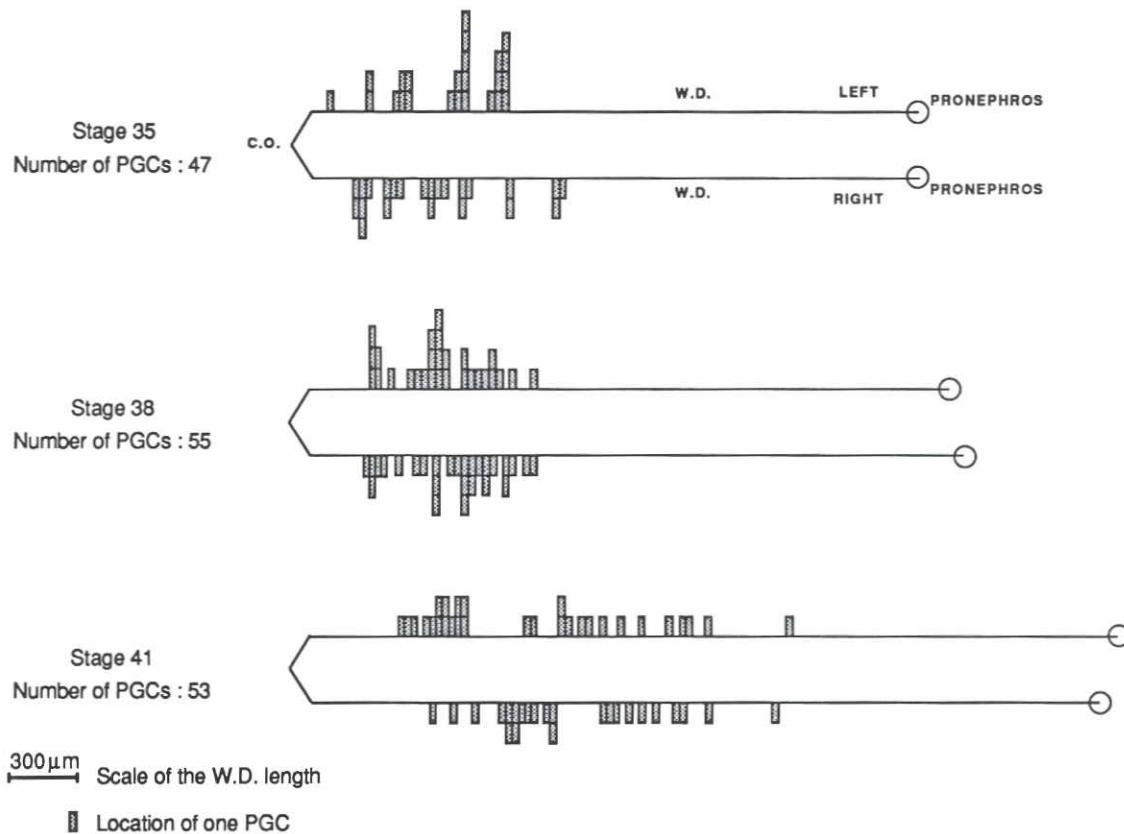


Fig. 2. Graphical reconstructions of characteristic PGC arrangement along ureters from three larvae with similar PGC numbers, at stages 35, 38 and 41 (C.O: cloacal orifice; P.G.C: primordial germ cell; W.D: Wolfian duct).

#### Mitotic index and *Po* period

From 185 young larvae, 10854 were counted; only 5 PGCs were observed undergoing mitosis. The mitotic index (M.I.) was 0.046. Whatever the offspring or whatever the larval stage, the M.I. can be considered nil (Table 1).

Thus, PGC proliferation is estimated nil over two weeks at  $20^{\circ} \pm 2^{\circ}\text{C}$ , and the period from stage 35 to stage 41 was thus called the *Po* period (*P* zero) (Fig. 1).

#### Genetic control of PGC proliferation

The 15 offspring studied are characterized by their average PGC count per larva which varies from 28.9 to 98.3.

It may be established, by calculation, that the average numbers (Table 2) are about the same as the median values

$$\frac{(\text{maximum number} + \text{minimum number})}{2}$$

We have also calculated that the differences between the minimum numbers and the values calculated for the 2.5 percentile, and between the maximum numbers and the 97.5 percentile, are slight. This indicates that, for each offspring, the extreme values are distributed symmetrically on either side of the average number and also of the median.

This symmetry, which persists while the average varies from 28.9 to 98.3, suggests some genetic control of germ cell proliferation.

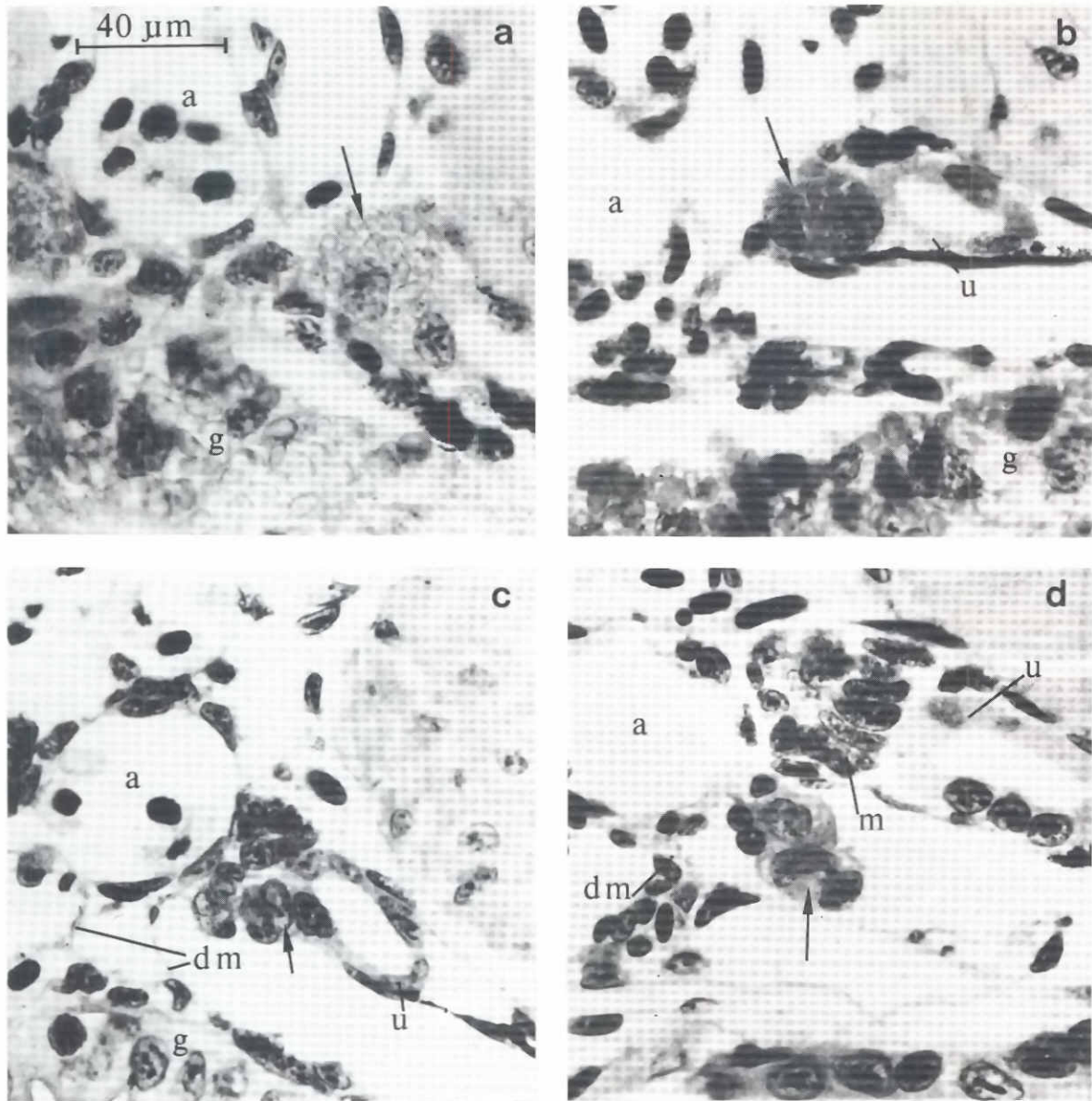
A graph was constructed from average, minimum and

maximum numbers of PGCs for each offspring (Fig. 4). It must be noted that the asymmetry of the figure is the result of its having been constructed from the different average counts of the 15 offspring.

From this graph, it is possible to deduce that, whatever the average number, the maximum is always about twice the minimum. Second, it is observed that the average PGC counts of the 15 offspring seem to be periodically distributed (Fig. 4). For this reason, a statistical analysis was carried out on the individual values. The results enabled us to classify the 15 offspring into groups with significant differences (Table 2). In group I, the offspring are characterized by an average number of 96.9 PGCs, in group II by an average number of 51.0 PGCs and in group III by an average number of 31.1 PGCs. That is, the PGC population doubles from one group to the next.

Whatever the approach, graphical or statistical, the results obviously indicate that PGC proliferation develops according to a regular pattern.

Two monosexual offspring, ZZ 85 and ZW 82, are respectively characterized by average counts of 71.3 and 65.4 PGCs, which fall between the average counts for groups I and II in which the differences are statistically significant. The parents of these monosexual offspring came from two different groups: groups I and II. The two offspring constitute a hybrid group. The PGC count of these hybrid offspring corroborates the existence of some genetic control of proliferation.



**Fig.3.** Transverse cross sections of *Pleurodeles waltl* larvae: PGC colonization of settling genital ridges. (a) stage 34; (b) stage 35; (c) stage 38; (d) stage 42 (arrow: primordial germ cell; a: aorta; d.m.: dorsal mesentery; g: gut; m: mesonephros; u: primary ureter).

**Sexual genotype independence**

From one larva to another, PGC number may be very different. In the nine normal offspring (one Sv + eight Sd), the minimum number of PGCs per larva is 14 and the maximum number is 158. In the three monosexual ZZ male and in the three monosexual ZW female offspring, the same scattering of the PGC counts is observed; the counts fall between 27 and 125 in the ZZ offspring and, between 22 and 149 PGCs in the ZW offspring. The same variability thus affects larvae issued from normal offspring (50% ♂ + 50% ♀ and larvae issued from monosexual offspring (100% ♂ or 100% ♀) (Table 2). Moreover, in each of the three groups, monosexual ZZ male offspring, monosexual ZW

female offspring, and standard offspring have the same average number of PGCs .

Therefore, in *Pleurodeles* it appears that the PGC count is independent of sexual genotype of larvae.

**PGC proliferation before the Po period**

The variation in average number of PGCs observed during the Po period would be considered as the expression of the modification in size of the germinal population during the embryonic period, prior to the Po period. In this hypothesis, the germ cell population of group I would result from at least two mitotic cycles involving an average PGC count increase from 31 (group III) to 51 (group II) and

TABLE 2

PGC COUNTS PER LARVA AND PER OFFSPRING DURING P<sub>0</sub> PERIOD AND STATISTICAL ANALYSIS. SNEDECOR'S F TEST PERMITS CLASSIFICATION OF THE 15 OFFSPRING STUDIED INTO THREE GROUPS AND ONE HYBRID GROUP

Reference of offspring	No of larvae examined	Total no of PGCs	Minimum no of PGCs observed in a larva	Maximum no of PGCs observed in a larva	Minl. no+ maxi. no 2 ( Median )	Average no of PGCs per larva in each offspring	F significance	Average no of PGCs per larva in the groups	F significance	Group
ZZ 87	13	1278	65	125	95.0	98.3 ± 5.2	N.S.	96.9 ± 3.8	S.	I
Sv 85	12	1156	61	158	109.5	96.3 ± 7.7				
ZW 76	9	862	67	149	103.0	95.8 ± 7.5				
ZZ 85	11	784	46	99	72.5	71.3 ± 5.1	N.S.	67.7 ± 0.2	S.	Hybrid
ZW 82	17	1112	32	86	59.0	65.4 ± 2.9				
Sd5 77	3	183	49	78	63.5	61.0 ± 8.7	N.S.	51.0 ± 1.4	S.	II
Sd1 77	26	1463	30	91	60.5	56.3 ± 2.5				
Sd4 77	5	251	20	78	49.0	50.2 ± 10.7				
Sd3 77	9	451	38	69	53.5	50.1 ± 3.7				
Sd1 76	21	1044	31	79	55.0	49.7 ± 2.5				
Sd2 77	31	1510	27	83	55.0	48.7 ± 2.4				
Sd1 79	4	184	36	57	46.5	46.0 ± 4.6				
ZZ 86	6	274	27	60	43.5	45.7 ± 5.1				
Sd6 77	10	329	14	51	32.5	32.9 ± 3.5	N.S.	31.1 ± 2.3	S.	III
ZW 79	8	231	22	45	29.5	28.9 ± 2.7				
Total	185	11112								

N.S.: no significant difference; S.: significant difference

then to 97 (group I). Two experiments have been carried out, the results of which were consistent with this hypothesis.

#### Effect of <sup>3</sup>H-labeled thymidine

Microinjections of labeled thymidine solution were administered in gastrulae issued from an offspring of group I. PGCs were counted at the end of the P<sub>0</sub> period (St 39 and 40). The average number of PGCs in control larvae (97.2 PGCs) is twice the PGC count in injected larvae (49.4 PGCs) which, consequently, could be classed in group II (Table 3). During this embryonic period, since the PGCs are unidentifiable, it may be considered that the low PGC count could be due to a reduction in PGC proliferation or even to cellular death subsequent to injection of labeled precursor. However, no dead cells were observed during the count period.

#### Effect of rearing temperature

Three batches issued from three standard offspring were reared at 30°C: from stage 30 for the first batch, from stage 33 for the second, *i.e.* before the P<sub>0</sub> period, and from stage 36 for the third batch, after the beginning of the P<sub>0</sub> period. Sample batches issued from the same three standard offspring were kept at constant room temperature (20° ± 2°C). For PGC counts, all control and experimental larvae were killed at the end of the P<sub>0</sub> period, at stage 41 (Table 4).

The difference between the PGC count in control larvae and PGC count in larvae reared at 30°C from stage 33 or

from stage 36 is not statistically significant. This shows that PGC proliferation is not modified during the P<sub>0</sub> period by heat treatment. However, the difference between the PGC count in control larvae and PGC count in larvae reared at 30°C from stage 30 is statistically significant. Thus, heat treatment restricts PGC proliferation occurring between stage 30 and the beginning of the P<sub>0</sub> period. Moreover, control larvae permit the classification of the offspring in group III (28.9 PGCs), but it is interesting to note that the average number of PGCs in experimental larvae (18.3 PGCs) is about half the average of the PGC count in group III and in fact, could constitute a fourth group. This experiment makes it possible to locate a mitotic cycle between stage 30 and the beginning of the P<sub>0</sub> period.

These two experimental observations, added to our previous observations, indicate that PGC divisions are probably cyclic and that the germinal population undergoes at least three mitotic cycles, the result of which is an average PGC count that increases from about 18, to 31, to 51 and then to 97.

## Discussion

Among our results, the wild Portuguese offspring Sv 85 presents an average PGC count (96.3) comparable with PGC count per larva (90 to 100) previously reported in *Pleurodeles* (Houillon, 1956; Capuron, 1963; Maufroid and Capuron, 1977b). This number is also roughly the PGC count for larvae of group I and therefore might be the PGC

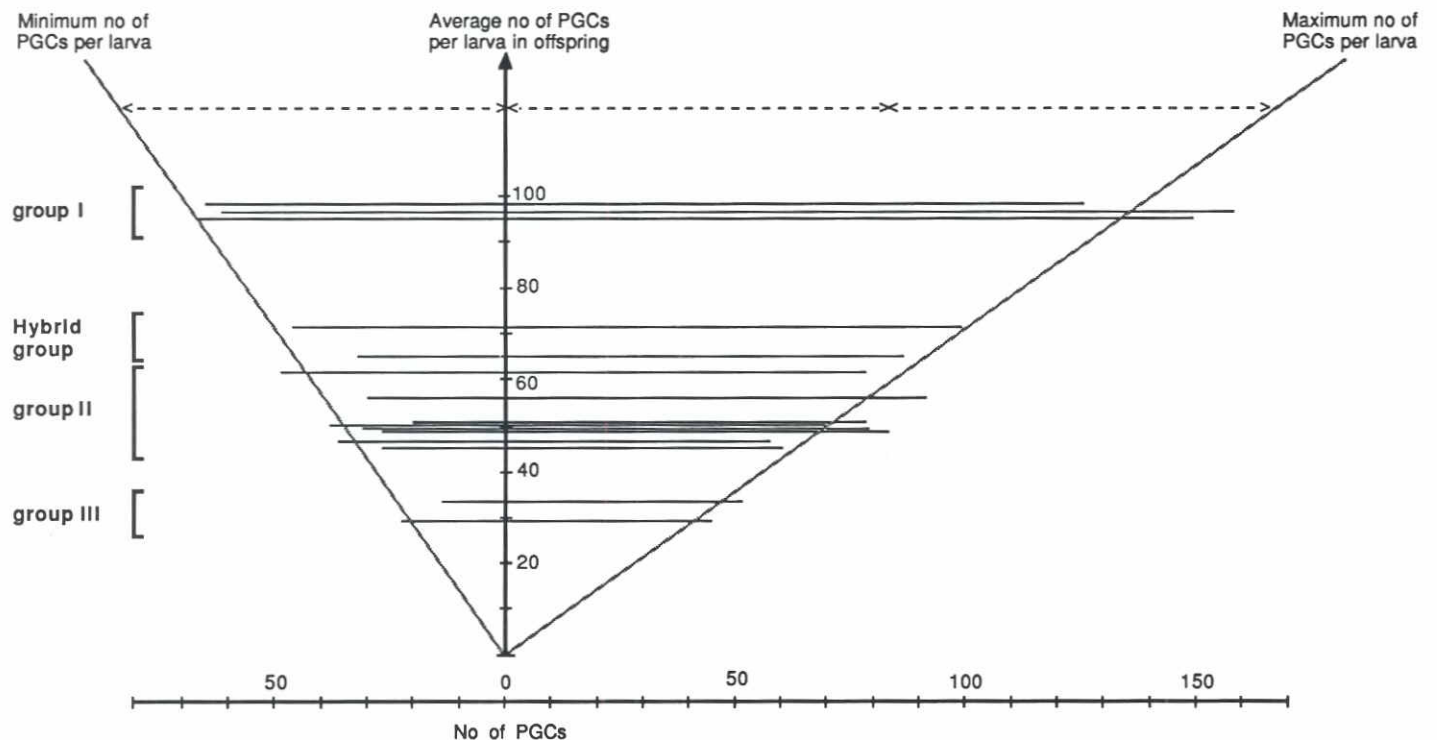


Fig. 4. Graph of the 15 offspring studied. Graph shows average numbers of PGCs as well as minimum and maximum numbers of PGCs per larva, in each offspring.

count characteristic of the species during the Po period. The PGC counts of groups II and III have never been found in previous work. These groups might result from consanguinity between some animals in our laboratory breeding. The problem of consanguinity as well as the characterization of hybrid group has been resolved from our genealogical knowledge of the lineages.

The results concerning PGC proliferation during the embryonic period suggest that, to some extent, after injection of [ $^3\text{H}$ ]thymidine at the gastrula stage, the size of germinal populations increases less in injected larvae than in control populations as if the precursor had suppressed a mitotic cycle. Previous reports indicate no modification in germ cell proliferation for some hours after injection of [ $^3\text{H}$ ]thymidine in *Xenopus laevis* (Dziadek and Dixon, 1977) and no modification in embryonic somatic cell proliferation in *Pleurodeles waltl* (Brugal, 1971). However, no investigations have been carried out concerning the effect of [ $^3\text{H}$ ]thymidine on further development of the germ line. Thus, the germ line could be more sensitive than the somatic line during precocious development.

Moreover, our results pointed to a reduction in PGC numbers following heat treatment at 30°C between stages 30 and 33, whereas proliferation is unaffected by that extreme temperature between stage 33 and stage 41. After a rise in rearing temperature, in poikilotherms it is generally observed that larval development quickens. In amphibians, the acceleration results from the decrease of cell cycle length while the dividing cell count is constant

(Brugal, 1971; Dournon and Chibon, 1974). Thus, it appears from our observations that germ cell and somatic cell proliferations have different sensibilities and that the epigenetic factor, *i.e.* the rearing temperature, can modify the expression of genetic control of germ cell proliferation.

In *Rana temporaria*, during the development of the paired genital ridges, Bounoure (1925) noticed an interruption in PGC multiplication together with a substantial individual variability in number. From PGC counts made by Bounoure on 15 embryos, Hardisty (1967), in a general review on germ cells, proposed the establishment of three different groups: a group with average counts of 22-23 PGCs, a group with 35-48 PGCs and a group with 76-106 PGCs. Such a regrouping of individual values (PGC count per larva) showed that two duplications of germ population occurred before the period considered. In *Xenopus laevis*, Kalt and Gall (1974), Ijiri and Egami (1975), Dziadek and Dixon (1977), Züst and Dixon (1977) evidenced a period, like the Po period, beginning with the development of genital ridges and lasting 12 days at a temperature of 18-20°C. In *Xenopus*, there is also individual variability in number of PGCs and a variation in average number depending on the offspring (Dixon, 1981). For instance, at stage 46 (Nieuwkoop and Faber, 1967), counts carried out per offspring indicate that average number of PGCs may be 20.8 (Züst and Dixon, 1977), 21.5 and 46.5 (Akita and Wakahara, 1985) or 48.8 and 59.6 (Ikenishi and Kotani, 1979). Before the Po period of *Xenopus*, Kamimura *et al.*, (1976) and Dixon (1981) demonstrated that the presump-

TABLE 3

**PGC COUNTS DURING THE Po PERIOD IN CONTROL LARVAE AND IN LARVAE INJECTED WITH [3H] THYMIDINE AT GASTRULA STAGE (REARED AT ROOM TEMPERATURE).**

No of larvae	Experimental larvae		Control larvae	
	Labeled PGCs	Unlabeled PGCs	Total	
1	20	16	36	90
2	37	12	49	94
3	44	6	50	102
4	33	20	53	103
5	59	0	59	-
<b>Average±S.E.</b>	<b>38.6±3.6</b>	<b>10.8±2.0</b>	<b>49.4±2.1</b>	<b>97.2±2.1</b>

tive germ cell number increased after segmentation. According to Dziadek and Dixon (1975, 1977) who studied nucleic acid synthesis, there should be three relatively synchronous mitotic cycles located between the end of segmentation and the beginning of genital ridge colonization. The first division should occur at gastrulation, at about stages 10-12, the second at about stages 22-24 and the third at about stages 37-39, the period of non-proliferation beginning at stage 43.

In the urodele, *Ambystoma mexicanum*, the halting of PGC proliferation has been observed at the beginning of larval life, over a period of time corresponding to stages 34 to 36 in *Pleurodeles waltl*. During this period, which lasts 8 days at room temperature, the mitotic index calculated from 10,554 PGCs is 0.028. The average number of PGCs is 182 per larva. The Po period of the axolotl may begin before stage 38 and may last after stage 39 (Dournon, unpublished).

Therefore, in anurans, the results published on *Xenopus laevis* and *Rana temporaria* agree with those we obtained in urodeles. In *Pleurodeles waltl*, the embryonic period involves at least three PGC mitotic cycles and is then followed by a period of non-proliferation of these PGCs. In *Ambystoma mexicanum*, a fourth cycle might occur before

Po, the average population increasing from 97 to 182 PGCs, on the average.

In *Rana pipiens* reared at 18°C, however, PGC proliferation spreads throughout genital ridge colonization. The average number of PGCs doubles in a week. No halt in PGC proliferation has been noted (Subtelny, 1980). In other poikilothermal vertebrates, such as the lamprey and the salmon, the period located very early in development, and during which there are no more gonial divisions, can be prolonged for several months (Hardisty, 1967). In homeothermal vertebrates, the numerical data concerning PGCs are rather scarce and sometimes contradictory. In chicken embryo, Swift (1914) and Goldsmith (1928) observed some rare gonial mitosis during germ cell migration into the blood vessels. Matsumoto (1932) noticed a slight increase of the total gonocyte number. On the other hand, in spite of the considerable natural fluctuation of 40 to 400 PGCs per embryo, Dubois and Cuminge (1978) indicated that the number of migrating germ cells, between somite stages 19 and 29, does not increase; the average number is about 180 PGCs. In mammals, the counts reported by Vanneman (1917) in the armadillo, Rubaschkin (1912) in the guinea pig, Chrétien (1966) in the rabbit and Witschi (1948, 1956) in the human, pointed to a progressive increase in germ cell number from the beginning of their migration. Thus, in mammals, there should be no interruption of PGC multiplication at the beginning of embryonic development.

Until gonadal differentiation in *Pleurodeles waltl*, PGC proliferation occurs in two phases. During the first period, which lasts two weeks at room temperature and which ends at stage 35, it is impossible to observe PGCs. However, we have been able to deduce from the results obtained between stages 35 and 41 that the average number of 96.9 PGCs per larva must be reached at stage 35, after a minimum of three mitotic cycles and starting from a maximum initial stock of about 18 PGCs.

The second period extends from stage 35 to stage 41. At 20 ± 2°C, this period lasts two weeks. It is characterized by non-proliferation of PGCs localized in genital ridges. At the end of this period, gonial mitosis starts again, and gonad differentiation begins.

Before the Po period, PGC proliferation is genetically

TABLE 4

**PGC COUNTS DURING THE Po PERIOD IN CONTROL LARVAE REARED AT ROOM TEMPERATURE AND IN THREE BATCHES OF EXPERIMENTAL LARVAE REARED FROM STAGES 30, 33 OR 36 TO STAGE 41 AT 30°C.**

	STAGE 30		STAGE 33		STAGE 36	
	Experimental larvae	Control larvae	Experimental larvae	Control larvae	Experimental larvae	Control larvae
No of larvae	14	8	9	9	6	10
Minimum no of PGCs	7	22	32	38	35	30
Maximum no of PGCs	36	45	59	69	48	57
<b>Average±S.E.</b>	<b>18.3±2.4</b>	<b>28.9±2.7</b>	<b>42.4±2.9</b>	<b>50.1±3.7</b>	<b>42.0±2.0</b>	<b>42.6±2.8</b>
F significance		S.		N.S.		N.S.

controlled although it could be modified by extreme temperature. Given the thermosensitivity of sex differentiation in *Pleurodeles waltli*, the question now is: how do germ cells proliferate, at room temperature and at sex reversing temperature, after the Po period during gonadal differentiation?

## Materials and Methods

### Origin of larvae and rearing conditions

Four types of offspring were used:

(i) natural wild offspring (Sv) from Portugal (ZZ ♂ x ZW ♀); (ii) standard offspring (Sd) issued from animals reared at room temperature by laboratory breeding (ZZ ♂ x ZW ♀); (iii) monosexual male offspring (ZZ) issued from crosses between ZZ standard males and ZZ neofemales obtained after estradiol treatment (Gallien, 1951); (iv) monosexual female offspring (ZW) issued from crosses between ZZ standard males and WW thelygenous females. These WW females were obtained as follows: standard ZW genotypic females were reared at 32°C from larval stages 42 to 54. Under these conditions, they became phenotypic males (Dournon and Houillon, 1984, 1985). These thermo-neomales were subsequently bred with ZW standard females. WW individuals were produced in the progeny and were selected by expression of peptidase-1 (Ferrier *et al.*, 1980, 1983; Dournon *et al.*, 1988).

The offspring of these four types were separately bred at room temperature (20° ± 2°C). At this temperature, the differentiation of sexual phenotype is in accordance with sexual genotype (Dournon *et al.*, 1988). The offspring were labeled by type (Sv, Sd, ZZ or ZW) and by birth year (Sv 85), preceded by a serial number (Sd6 77) when necessary.

### Stages of PGC observation and numbering

The PGCs were observed and counted in larvae of stages 35 to 41 (Fig. 1).

### PGC identification and PGC counting

Size allows discrimination between PGCs (Ø 30 µm) and somatic cells (Ø 10 µm). Moreover, the PGC nucleus is multilobed and the cytoplasm contains pigment granules (Lesimple *et al.*, 1987). With hemalum-eosin, PGCs are less colored than somatic cells.

PGC numbers were established by counting each PGC throughout histological serial sections of 7 µm thickness from the cloacal position up to place where the genital ridge disappears in the anterior region. Graphical reconstructions were also made of their arrangement along ureters (Fig. 2).

The margin of error was assessed as 10% between stages 35 and 38, and to 2% from stage 38 to stage 41.

### Microinjections of <sup>3</sup>H-labeled thymidine

Microinjections of 2.10<sup>-4</sup> ml of a <sup>3</sup>H-labeled thymidine solution dosed at 50 µCi/ml (specific activity 25 Ci/mM) were carried out in archenteron of gastrulae (stage 9). Both labeled and unlabeled PGCs were identified by histoautoradiography.

### Statistical significance test

Statistical analysis of the results is based on an analysis of variance: Snedecor's F-test (P = 0.05). This test makes it possible to compare each offspring with the rest and indicates whether the offspring may or may not be classified in a single group. The table of F values shows whether, in accordance with degrees of freedom, differences between the offspring are significant or not.

The dispersion of PGC count per larva on either side of the average number was estimated from the calculation of 2.5 and 97.5 percentiles (2.5 percentile = m - 1.960 σ, 97.5 percentile = m + 1.960 σ).

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