Crossing-over and inbreeding depression in attached-X chromosomes of *Drosophila hydei*[1]

P. Hutter[2] and H. Gloor

Genetics Department, University of Geneva, 154bis route de Malagnou, CH-1224 Geneva (Switzerland)
Received 27 April 1989; accepted 4 July 1989

Summary. In a white-eyed attached-X chromosome of *Drosophila hydei*, C(1)RM, $w^{iv} \cdot w^{iv}$ (homozygous for *white-ivory*), a spontaneous mutation occurred of w^{iv} to w^{a83}. Dominance order is $w^{+} > w^{a83} > w^{iv}$, where $w^{a83} \cdot w^{a83}$ homozygotes are indistinguishable from $w^{a83} \cdot w^{iv}$ heterozygotes. The frequency at which in heterozygotes these alleles become homozygous by recombination (homozygosis frequency) was investigated by a progeny test. For any locus situated at least 50 map units away from the centromere, the theoretical homozygosis frequency should be 16.6%. This value was approximated by our results. However, by selecting heterozygous females over eight generations, we observed an unexpected decline in homozygosis frequency as selection progressed. In parallel, the proportion of males substantially increased throughout the experiment.
Key words. Homozygosis frequency; inbreeding depression; attached-X chromosomes; *Drosophila hydei.*

Anomalous segregation of homologous chromosomes at meiosis has provided a powerful tool in genetic analysis. 'Half-tetrad analysis' in *Drosophila melanogaster* is especially noteworthy because of the important conclusions it has led to in classical genetics, but has also been useful more recently in intragenic recombination studies. In parallel, the introduction of extra chromosomes into a genome led to a number of studies on changes in genic balance. In *Drosophila*, chromosome non-disjunction (resulting from pairing or segregation failure) is tolerated only in the case of the sex chromosomes or the very small 'dot' autosomes.

The term 'half-tetrad analysis' refers to a situation where two normally independent homologous chromosomes become attached to a single centromere. As a consequence, the four homologous chromatids resulting from meiosis will be recovered two by two conjointly (attached) instead of randomly. When two X chromosomes in normal sequence become attached proximally to the same centromere (*c*) by breakage and reunion in different heterochromatic parts, as shown in figure 1, the resulting attached-X compound will have the sequence d – p – *c* – p – d, thus when it is considered as one chromosome, one arm is in reversed order with respect to the other. Hence it is termed Compound (1 = X) Reversed Metacentric, or C(1)RM. Such a mutant was first discovered in 1921 by L. V. Morgan[3]. Pioneer investigations of the behaviour of attached-X chromosomes during meiosis[4–7] shed considerable light on the rules governing recombination since two, instead of only one, of the four meiotic products can be recovered from females. Stocks carrying compound-X chromosomes attached in various heterochromatin regions have been widely used; they offer the advantage that they produce non-crisscross inheritance of X-linked genes. In *D. melanogaster*, investigation of recombination in attached-X chromosomes brought one of the best proofs that crossing-over occurs between chromatids rather than between whole chromosomes[4,8,9].

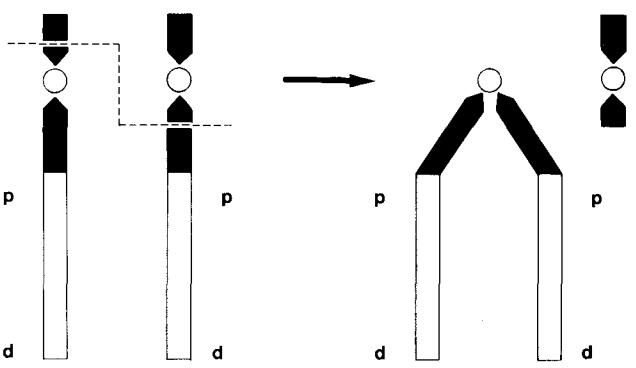

Figure 1. Diagram showing the origin of a compound chromosome by exchange in heterochromatin. Open circles = centromeres; black = heterochromatin; white = euchromatin; p, d = proximal, distal. The second, entirely heterochromatic chromosome produced in the exchange is lost.

In attached-X chromosomes the frequency with which one allele of a heterozygous gene-pair becomes homozygous in the following generation (referred to as the homozygosis frequency) has been shown to be a function of the amount of crossing-over. Anderson[4] first pointed out that if the four strands of attached-X chromosomes were associated at random, each recessive character should appear in one-sixth of the daughters. Sturtevant and Beadle[8] later showed that the homozygosis frequency for an allele close to the centromere is equal to one half of the crossover frequency between the locus and the centromere, or is equal to one fourth of the corresponding exchange frequency.

The purpose of the present study was to investigate homozygosis frequency in attached-X chromosomes of *Drosophila hydei*, since the X chromosomes in this species are known to differ from those of *D. melanogaster* in several aspects. For example, *D. hydei* attached-X chromosomes have no nucleolus organizer[10,11]. As the X-chromosomal nucleolus organizer region of *D. hydei* is located distally in the very large heterochromatic arm, it will generally be excluded in the formation of C(1)RM chromosomes[12]. Besides, both the chromosome length and the gene order differ between the two species.

Spencer[13] pointed out that gene exchanges for all loci examined are more frequent in *D. hydei* relative to *D. melanogaster*. Thus for X-chromosomal crossover frequencies, the relative map lengths as expressed in map units or 'centimorgans' are in the order of 2:1. The choice of the *white* locus was particularly indicated, as this gene appears to present a high degree of homology between the two species on genetic grounds[14] although a detailed comparison on the molecular level is not yet available. It is worth noting that evolutionary rearrangements have moved analogous genes into different relative positions in the two species. In *D. melanogaster*, the *white* locus is situated 64.5 map units away from the centromere near the distal end of the X chromosome, whereas in *D. hydei*, it is located at a distance of 97.0 map units away from the centromere[15]. In *D. hydei* most of the attached-X chromosomes that have been constructed are of the type 'reversed metacentric'[12,16–18].

Materials and methods

The *D. hydei* females used in this experiment stemmed from the original attached-X stock of Gregg[16], C(1)RM, $w^{iv}y^{Lt} \cdot w^{iw}y^{Lt}/Y$, where w^{iv} = *white-ivory* and y^{Lt} = *yellow-Light*. The accessory Y chromosome is indispensable in *D. hydei* because of its nucleolar organizer (see above). Since this stock is remarkably stable, that is, practically without any spontaneous detachments of the X chromosomes, it was used in various combinations to perpetuate sex-linked mutants in the male line. In one such stock a mutation in a female at the *white* locus spontaneously arose in 1983 with apricot eye colour (w^{a83}), adding to the already long list of alleles at the *white* locus[19].

Females carrying heterozygous attached-X chromosomes ($w^{a83} \cdot w^{iv}$) were selected at every generation. Each female, when aged 3–4 days, was mated in a vial to three 5-day-old wild type males. Fresh males were substituted in case of apparent sterility; otherwise the parents were removed when their progeny began to pupate. Breeding temperature was 25 °C ± 0.5 °C. Phenotypes of the F_1 progeny were scored daily for up to 7 days. From each generation a few females were retained for selection, each from a few distinct progenies that were thought to contain both w^{iv} and w^{a83} alleles. These selected females were individually mated to 2 or 3 wild type males. This procedure was repeated for 8 generations. All females which survived more than 14 days without producing any progeny were designated as 'attested sterile', whereas those females that died earlier were designated as 'putative sterile', except when a female died within the first week after mating; those cases were presumed to be accidental deaths and were not taken into consideration.

The new allele, w^{a83}, being completely dominant over w^{iv}, heterozygous $w^{a83} \cdot w^{iv}$ females are indistinguishable phenotypically from homozygous $w^{a83} \cdot w^{a83}$ ones. In order to distinguish between the heterozygous $w^{a83} \cdot w^{iv}$ and the homozygous $w^{a83} \cdot w^{a83}$ genotypic constitutions of female parents as judged from offspring phenotypes, the following method of statistics was used, with an arbitrarily selected probability level at 0.05. Mothers of progenies which yielded at least 17 w^{a83} females and no w^{iv} females were considered homozygous w^{a83}/w^{a83}, as computed from the binominal distribution assuming a theoretical ratio of phenotypes $w^{a83}:w^{iv}$ = 5:1 for genes situated at a distance of 50 or more map units from the centromere[4]. On this basis, the frequency of homozygosis, as the frequency with which homozygous females appeared among the progeny of heterozygous attached-X mothers, could then be estimated in every generation.

Since there was a possibility that egg-to-adult viability differed between $w^{iv} \cdot w^{iv}$ and $w^{a83} \cdot w^{a83}$ genotypes, a control experiment was conducted separately in order to test for possible differential survival rates. Newly hatched larvae of the same age (less than 30-min-old) were deposited with a thin paintbrush in fresh vials at two larval densities of 80 and 160 animals per vial. Both the above homozygotes as well as wild type controls (stocks W4-Madeira and W5-Zürich) were assessed both separately and in competition conditions and the emerging flies were scored daily. Experiments were carried out at 25 °C ± 0.5 °C on yeast-glucose medium (yeast 3%, glucose 6%, agar 0.5%).

Results

Figure 2 shows that the homozygosis frequency tended to decrease as selection progressed. At generations 7 and 8 the surprisingly high estimates remain difficult to explain. The linear correlation coefficient between homozygosis frequency and the progress of selection ($r = -0.693$) is significant at the 0.05 probability level, and at the 0.025 level ($r = -0.790$) if the exceptional value of generation 7 is left out.

Because from every generation of selection a single female was retained to contribute gametes to the next generation, a substantial inbreeding depression was expected to occur. This effect could be estimated by recording the females' fecundity in all successive generations, for both

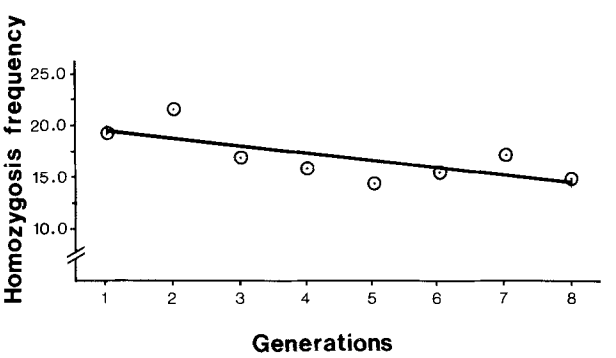

Figure 2. Homozygosis frequency as the number of $w^{a83} \cdot w^{a83}$ females/total number of females over 8 generations of selection.

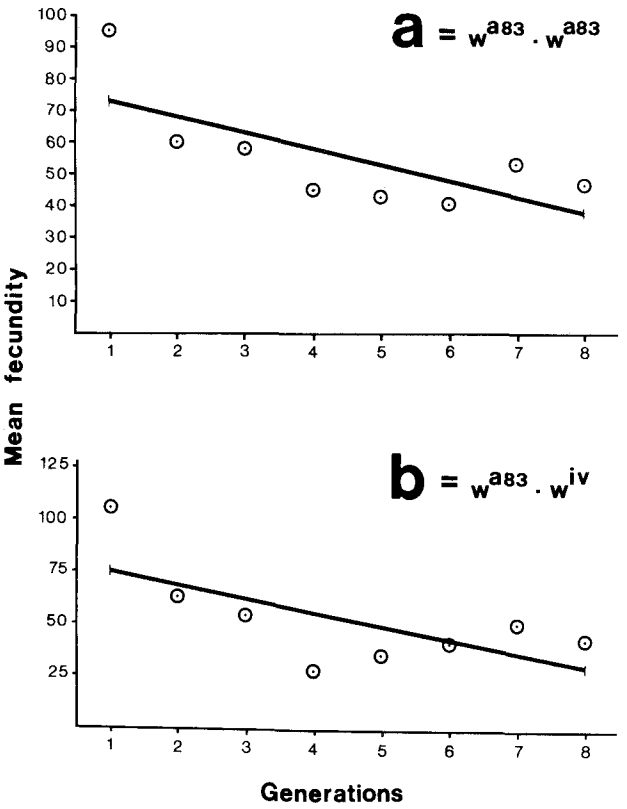

Figure 3. Mean fecundity as measured by the mean number of progeny of $w^{a83} \cdot w^{a83}$ females (*a*) and of $w^{a83} \cdot w^{iv}$ females (*b*).

homozygotes $w^{a83} \cdot w^{a83}$ and heterozygotes $w^{a83} \cdot w^{iv}$ separately. Figure 3 shows a strong effect of inbreeding on female fecundity. For both homozygotes and heterozygotes the correlation coefficients between mean female fecundity and selection progress are significant at the 0.05 level ($r = -0.701$ and $r = -0.649$ respectively). Besides, the mean fecundity averaged over the 8 generations of selection does not differ between homozygotes and heterozygotes ($|t| = 0.210$, $df = 14$, $p > 0.5$). Similarly to what was observed with homozygosis frequency (fig. 2), one can see in figure 3 that the mean fecundities of both genotypes tended to be surprisingly high at generation 7, which might suggest that some degree of recombination had occurred between loci whose favourable alleles were originally in repulsion linkage.

Tests on larva-to-adult viability were carried out in order to compare the survival rates of the two different genotypes (homozygous at the *white* locus) as well as to control for the possibility of larval competition between the two genotypes. Results presented in table 1 rule out the possibility of either differential survival rates or competition effect between the two genotypic constitutions. At both larval densities approximately 50–57% (one exception!) of the young larvae included in the experiments reached the adult stage, but the calculated standard deviations were relatively high. Still, it must be borne in mind that in theory these scores should not exceed 66.6%,

Table 1. Comparison of viability of $w^a \cdot w^a$ and $w^{iv} \cdot w^{iv}$

Larval density	Genotype	% viability (SD)
80	$w^{iv} \cdot w^{iv}$	57.5 (± 6.4)
80	$w^a \cdot w^a$	54.8 (± 8.9)
80	$w^{iv} \cdot w^{iv} + w^a \cdot w^a$	26,4; 26,4 Tot.: 52.8 (± 9.4) (± 9.0)
80	wildtype*	96.5 (± 4.0)
160	$w^{iv} \cdot w^{iv}$	54.3 (± 7.8)
160	$w^a \cdot w^a$	62.1 (± 12.1)
160	$w^{iv} \cdot w^{iv} + w^a \cdot w^a$	23,9; 26,8 Tot.: 50.7 (± 4.8) (± 9.9)
160	wildtype*	85.4 (± 9.1)

Percentage of viability (number of flies reaching adulthood) with standard deviations. Larval densities are given in number of larvae deposited per tube. *Stocks W4-Madeira and W5-Zürich.

since approximately one third of the larvae deposited were expected to be metafemales (XXX) which normally fail to survive to the adult stage.

Interestingly, the sex ratios of progenies indicated a consistent trend towards a male bias. This effect was noticeable at least in generations 1–3 and 8, where the numbers of emerged flies per vial were reasonably close to those found in the viability tests (see below). For the generations considered, the sex ratio was 1.55 for progenies with less than 25 adults ($n = 1115$) and 1.36 for progenies with at least 70 adults ($n = 10922$). Both differ significantly from 1 ($p < 0.001$ using a chi-square test); therefore the preponderance of males does not seem to be density-dependent. Nevertheless, this bias in sex ratio was not observed in the viability tests and table 2 shows that in none of the tests performed did the percentage of adult males substantially depart from an expected 50%. No control experiment was performed to determine at which life stage(s) some sex-limited lethality might have occurred (primary or secondary sex ratios).

As the genotypic constitution of the selected females was inferred from the phenotypes of their sisters, it was not possible to relate the presence of sterile females to either genotype. Table 3 nonetheless suggests that the percentages of 'attested' sterile (and 'putative' sterile) females slightly increased as selection progressed. Such an observation would be in line with the decline in fecundity simultaneously observed.

Once again the exceptional scores at generation 8 (and 7?) show up the salient situation at the end of the exper-

Table 2. Proportions of males present in the viability tests

Larval density	Genotype	% males	SD
80	$w^{iv} \cdot w^{iv}$	46.47	± 4.99
80	$w^a \cdot w^a$	50.74	± 5.81
80	$w^{iv} \cdot w^{iv} + w^a \cdot w^a$	53.16	± 10.44
80	wildtype*	49.95	± 3.11
160	$w^{iv} \cdot w^{iv}$	50.16	± 6.32
160	$w^a \cdot w^a$	54.58	± 5.25
160	$w^{iv} \cdot w^{iv} + w^a \cdot w^a$	46.67	± 6.95
160	wildtype*	53.74	± 5.20

Percentage of males/total number of flies and standard deviations. *Stocks W4-Madeira and W5-Zürich.

Table 3. Proportions of sterile females in generations 1–8 of selection

Generation	No. of ♀ scored	'Putative' sterile	% (*)	'Attested' sterile	% (*)
1	93	–	0	1	1.08
2	202	17	8.42	25	12.38
3	201	48	23.88	13	6.47
4	209	33	15.79	77	36.84
5	213	11	5.16	60	28.17
6	210	28	13.33	92	43.81
7	186	–	0	60	32.26
8	163	–	0	13	7.98

(*) Percentage of total number of flies.

iment, pointing to a sudden recovery of the overall fitness of the flies which will be discussed later.

Discussion

Testing for homozygosis frequency in a series of successive generations was not possible, in our experiment, without inbreeding. Hence some degree of inbreeding depression was to be expected. The unexpected result was a progressive decrease in homozygosis frequency observed during the selection experiment, for which there is no obvious explanation. In *D. melanogaster*, w^a mutants are known to be connected with the insertion of transposable elements. A like situation in *D. hydei* is not to be excluded. But instability due to the presence of a transposon could not account for the results, because transposon-mediated mutations are far too infrequent compared to regular crossover events.

A possible explanation may be sought in the genetic control of crossover frequency. A number of mutations are known to reduce recombination rates although there seems to be no a priori reason to expect that our selection procedure should have consistently favoured one type of allele affecting recombination. Nevertheless, we shall argue here that the effect observed most probably resulted from the artificial selection applied. Since chromosome rearrangements or centromere effects are known to affect crossing-over frequencies, one can reason that increased overall homozygosis (through the strong inbreeding programme we used) may incidentally have impinged on the frequency of recombination. Indeed this may have resulted from a disruption of the balance of the forces which tend to increase and decrease the actual recombination frequency. There is strong evidence that within-population genetic variance for the frequency of recombination exists in the wild[20]. Results from artificial selection on the recombination rate have indicated for a long time that genetic variation controlling recombination is important in *Drosophila*[21–27]. As Ebinuma[28] summarizes, the above studies show that recombination is under the control of a balanced polygenic system as first described by Mather[29].

It is worth noting that if some genetic factor(s) affecting recombination did not act so as to lower crossing-over rate but so as to enhance it instead, this could equally have resulted in a fall of homozygosis frequency because of a higher occurrence of double crossovers. Similarly, extranuclear factors influencing crossing-over might theoretically also have been responsible for an altered recombination rate. For example Thoday and Boam[30], Lawrence[31] and others have found crossing-over factors carried in the cytoplasm; it is therefore possible that again our selection procedure resulted in a substantial drift for such cytoplasmic factors. Some evidence that the selection procedure we used may have resulted in an increased level of homozygosity coupled with the fixation of alleles affecting the measured traits was provided by the abnormalities observed at generations 7 and 8. The sudden fluctuations in both homozygosis frequency and mean female fecundity might indeed have reflected a higher sensitivity of the flies to slight variations of environmental variables such as the age of food, temperature and humidity. It is well known that in so far as the enzyme activity of a gene is reflected in a measured character, homozygotes are expected to maintain an adequate level of enzyme activity over a narrower range of environmental variation than heterozygotes. Thus the phenotypic change observed at the end of the experiment was probably a direct consequence of the strong inbreeding exerted.

Another possible interpretation of the observed decline in homozygosis frequency would take into account the age at which parents contributed their gametes to the next generation, considering that, as maternal age increases in *Drosophila*, crossing-over sometimes tends to decrease[32]. With respect to this, one could argue that the strong inbreeding depression imposed by our selection regime resulted in females becoming fertilized increasingly later in life, as a consequence of a reduced mating success, or, alternatively, becoming fertile late because of slow maturation. This might then have resulted in the production of zygotes from oogonia formed later and later in life as selection progressed, therefore exhibiting a lower frequency of crossing-over.

Finally, the male-biased sex ratio observed in the selection experiment and not observed in the control experiment (table 2) can be interpreted by considering the relative population sizes of the X chromosomes in females as compared to that of the X chromosomes in males. In the latter case, at every generation new X chromosomes were introduced into the lines of selection while this was not the case for the female X chromosomes. Therefore one can expect a stronger effect of inbreeding depression in females than in males.

As Berg[33] has pointed out, the X chromosome appears to be the chromosome which in *D. melanogaster* at least carries the largest proportion of loci affecting male fertility as determined by X-ray induction of fertility mutants. In relation to that, Geer et al.[34] emphasize that the distribution of male sterility loci along the *Drosophila melanogaster* X chromosome is similar to the distribution of lethal loci, which suggests that there could be many

genes that may mutate to forms altering both viability and fertility (e.g. events common to mitosis and meiosis).

1 Work supported by the Swiss National Science Foundation, grant 3602079.
2 Correspondence address: P. Hutter, Genetics Department, Universitiy of Geneva, 154bis route de Malagnou, CH-1224 Geneva, Switzerland.
3 Morgan, L. V., Biol. Bull. *42* (1921) 267.
4 Anderson, E. G., Genetics *10* (1925) 403.
5 Emerson, S., and Beadle, G. W., Z. Indukt. Abstamm. Vererb. Lehre *65* (1935) 129.
6 Beadle, G. W., and Emerson, S., Genetics *20* (1935) 192.
7 Welshons, W. J., Genetics *40* (1955) 918.
8 Sturtevant, A. H., and Beadle, G. W., An Introduction to Genetics. W. B. Saunders Company, Philadelphia and London 1940.
9 Serra, J. A., Modern Genetics, vol. 1. Academic Press, London 1965.
10 Hennig, W., J. molec. Biol. *38* (1968) 227.
11 Beck, H., Drosoph. Inf. Serv. *50* (1973) 94.
12 Beck, H., Genet. Res. Camb. *26* (1976) 313.
13 Spencer, W. P., in: Genetics, Paleontology and Evolution, p. 23. Eds G. L. Iepsen, E. Mayr and G. G. Simpson. Princeton University Press, Princeton, N.J. 1949.
14 Frei, H., Arch. Genet. *49/50* (1976/1977) 42.
15 Clausen, R. E., Am. Nat. *57* (1923) 52.
16 Gregg, T. G., Univ. Texas Publ. *5721* (1957) 238.
17 Hess, O., Chromosoma *16* (1965) 222.
18 Breugel, F. M. A., Genetica *41* (1970) 589.
19 Frei, H., Molec. gen. Genet. *142* (1975) 105.
20 Maynard Smith, J., The Evolution of Sex. Cambridge University Press, London 1978.
21 Gowen, J. W., Genetics *4* (1919) 205.
22 Detlefson, J. A., and Roberts, E., J. exp. Zool. *32* (1921) 333.
23 Parsons, P. A., Am. Nat. *92* (1958) 255.
24 Mukherjee, A. S., Am. Nat. *95* (1961) 57.
25 Chinnici, J. P., Genetics *69* (1971) 71.
26 Kidwell, M. G., Genetics *70* (1972) 419.
27 Charlesworth, B., Mori, I., and Charlesworth, D., Heredity *55* (1985) 209.
28 Ebinuma, H., Genetics *117* (1987) 521.
29 Mather, K., Biol. Rev. *18* (1943) 32.
30 Thoday, J. M., and Boam, T. B., J. Genet. *54* (1956) 456.
31 Lawrence, M. J., Nature *182* (1958) 889.
32 Stern, C., Proc. natl Acad. Sci. *12* (1926) 530.
33 Berg, R. L., Genetics *22* (1937) 241.
34 Geer, B. W., Lischwe, T. D., and Murphy, K. G., J. exp. Zool. *225* (1983) 107.

0014-4754/89/11-12/1136-05$1.50 + 0.20/0

Proliferation of primordial germ cells of frogs stimulated by a fraction of granules derived from *Rana nigromaculata* embryos at the tail-bud stage

T. Shirane

Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730 (Japan)
Received 7 February 1989; accepted 1 June 1989

Summary. A fraction of heavy granules, obtained from embryos of the frog (*Rana nigromaculata*) at the tail-bud stage by centrifugation at 1700 × g, were shown to stimulate the formation or proliferation of the primordial germ cells not only of the same species but also of a closely related species.
Key words. Primordial germ cells; granule fraction; Anura.

Bounoure [1] proposed that there exists in *Rana temporaria* (Anura, Amphibia) a specifically stained cytoplasm (germ plasm) which is closely associated with the determination of primordial germ cells (PGCs), and which can be traced back from the PGCs in the genital ridges to the subcortical layer at the vegetal pole of eggs at the 2-cell stage. Since then, similar observations have been reported in several species of Anura by other investigators [2, 3]. In addition, Balinsky [4] found, by electron microscopy, that germ plasm contains clusters of particularly dense bodies. This observation has also been confirmed by other investigators [2, 3]. About ten years ago, Wakahara [5, 6] attempted to isolate the germinal determinant in *Rana chensinensis* and *Xenopus laevis*, and he confirmed that a certain fraction obtained by centrifugation was able to stimulate proliferation of the PGCs. However, he did not perform further experiments with respect to this fraction and its effects. Up to now, few researchers have succeeded in isolating the actual germinal determinant(s), even though this seems to be a necessary step for an understanding of their function, and of the mechanism of formation of PGCs.

The germ plasm cannot be detected in all species of Anura during all stages of embryogenesis. In *R. nigromaculata* and *R. brevipoda*, the cells containing the germ plasm (presumptive PGCs or pPGCs) are scarcely detectable after the neurula stage [7], as has also been reported in the case of *R. esculenta* [8]. In the above mentioned two species, it may be relevant that there were PAS-positive granules which were not restricted to a certain type of cell and which showed similar behavior to the pPGCs in terms of distribution in embryos [7]. Although such PAS-positive granules were present in the migrating PGCs, it remains to be determined whether these granules are true organelles, or artifacts formed during the preparation of samples. If they are actual organelles and are related to the formation of PGCs, it is possible that they may be concentrated in a certain fraction of granules prepared by centrifugation and that they may cause the PGCs in larvae into which they are injected to multiply. The pres-