Table 2. Somal examinations of rat pups. The number of offspring examined and the frequency of skeletal (S) and visceral (V) abnormalities are shown. The incidence of major malformations in the Carworth CFE strain rat used in these experiments was less than 0.5 percent.

Griseofluvin (mg/kg)	Rats examined (No.)			Major abnormalities	
	Total	Skeletal	Visceral	Total	Type
None	264	183	81	0	
125	190	130	60	0	
250	231	161	70	1	S and V
750	302	209	93	1	V
1250	242	168	74	20	S
1500	90	65	25	9	S

While the incidence of resorptions increased by 50 percent when the dose was raised from 1250 to 1500 mg/kg, the incidence of malformations between the two treated groups increased only 2 percent. These changes suggest that 1500 mg/kg approaches the limit of fetal tolerance.

Since under specific conditions griseofulvin disrupts spermatogenesis in rats (*1, 2*), we performed other experiments to evaluate the effects of long-term administration on male and female fecundity.

In one study, we treated a group of males orally with 1500 mg/kg per day during spermatogenesis for 63 days and mated them with untreated females. The males were then killed, and the testes were removed and examined histologically. On day 14 after mating, the dams were killed and examined for a dominant lethal effect. Testicular histology, the number of nidation sites, the number of resorptions, and embryonic viability were normal.

The results show that, at high doses, griseofulvin affected embryonic development but not spermatogenesis. This difference is not surprising and has several possible explanations, of which three stand out. First, spermatogenesis and embryonic development both involve rapidly dividing cells, yet tissue differentiation characterizes only the latter. Second, enzyme induction may have occurred in males and not in females because the males were treated for a longer period. Last, the testes were not removed until 2 weeks after treatment, a delay which may have allowed them to return to normal if treatment had resulted in a transient change (*2*).

In another study, we segregated 100 male and 100 female rats into controls and 50-, 100-, and 250-mg/kg groups. Males were treated for 63 days and then mated with females that had been treated for 14 days. Males were treated until the end of the 3-week mating period, whereas females were treated until they were killed. About 40 of the females were killed on day 14 after mating; the remainder were killed when the offspring reached 21 days of age. Rates of conception, litter sizes, pre- and postnatal mortality, body weights of offspring, and somal development were normal in all groups.

From the results of these two studies, male and female fecundity appears to be unaffected.

From each group, 14 or 15 (F_1) male and female pups were reared to maturity and mated with members from the same group. On day 21, females were killed, and the number of resorptions and nidations, the conception rates, the litter sizes, and the numbers of viable F_2 offspring were all normal.

The results obtained by others giving griseofulvin intravenously cannot be correlated with those we obtained since we gave the drug orally. Our results can be correlated with those obtained in humans by MacLeod and Nelson (*11*). In their experiment, 14 men were given daily doses of 2000 mg of microsize griseofulvin for 3 months. After treatment, sperm counts and histological examination of testicular biopsies indicated that griseofulvin exerted no adverse effects on spermatogenesis.

From the results of our study we can conclude that (i) compared to controls, pregnant rats treated orally with high doses of griseofulvin have more malformed offspring, (ii) offspring from those dams which were treated with high doses have decreased pre- and postnatal survival rates, and (iii) griseofulvin given to male rats daily for 63 days in oral doses up to 1500 mg/kg does not adversely affect spermatogenesis.

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6. These doses were approximately 6, 12, 38, 63, and 75 times the human dose, based on a daily dose of 1 g of microsize (*7*) griseofulvin for a 50-kg person.
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Meiosis in Triploid All-Female Fish (Poeciliopsis, Poeciliidae)

Abstract. *Prior to meiosis in triploid gynogenetic all-female forms of* Poeciliopsis, *the chromosome number of the nucleus of the triploid oogonium is raised endomitotically to hexaploid. Recombination does not occur; instead, a triploid ovum with a genetic complement identical to that of the mother is produced by two conventional meiotic divisions. Sperm from a sympatric gonochoristic (bisexual) species stimulates the ovum to develop, but paternal genes are not incorporated into the zygote. This is the first cytologically verified case of natural endomitosis in the egg production in fish.*

The existence of natural populations of unisexual vertebrates raises questions about the evolutionary potential of these animals, both as individuals and as parts of unisexual-bisexual complexes with their gonochoristic (bisexual) relatives. To understand the evolutionary potential of unisexual forms, the sources of their genetic variability must be determined; this in turn requires an understanding of the meiotic mechanisms of these forms.

One mode of reproduction used by unisexual vertebrates is gynogenesis, that is, the development of an ovum after stimulation by a sperm but without fusion of the male and female pronuclei. This results in offspring genetically identical to the mother (matroclinous inheritance). Natural populations of gynogenetic vertebrates have been identified in teleost fishes (*1–4*) and salamanders (*5*). The viviparous genus *Poeciliopsis* (Poeciliidae: Cypri-

nodontiformes) comprises three morphologically distinct, gynogenetic, triploid, all-female fishes (*4*). Two of these, *P. monacha*-2 *lucida* and *P.* 2 *monacha-lucida* ($3n = 72$; where n is the haploid chromosome number), occur in the Rio del Fuerte of northwestern Mexico; and they utilize sperm from the sympatric diploid ($2n = 48$) gonochoristic species *P. lucida* and *P. monacha*, respectively (*4*).

Production of triploid offspring by gynogenesis requires a triploid ovum. This can be produced in one of three ways: (i) direct transformation from a triploid oogonium, by vitellogenesis without synapsis and reductional division, to form the "ameiotic ova" of Uzzell (*6*), a process that includes abortive tripolar spindles; (ii) suppression after synapsis of meiosis I, with concomitant suppression of crossing-over (*6*); or (iii) an endomitotic division that raises the chromosome number to hexaploid, followed by a complete meiosis and production of the triploid ovum. The last method is used by triploid *Poeciliopsis*.

Ovaries of immature specimens were sectioned (*7*); early prophase I stages were reconstructed by following individual chromosomes in all focal planes through a section, or through two adjacent sections if necessary. Chromosomes were usually sketched freehand or with the aid of a camera lucida, and the total number of chromosomes was counted from the sketch. In some cases reconstructions were not sketched; instead, the chromosome numbers were estimated visually (*8*).

Five reconstructions from ovaries of newborn and day-old *P. monacha*-2 *lucida* had hexaploid chromosome numbers. One contained 140 chromosomes, 4 less than hexaploid (Fig. 1A). In contrast, all 16 reconstructions made from gonochoristic diploids were diploid (Fig. 1B). A late pachytene cell of a newborn triploid fish contained at least 46 bivalents (Fig. 1C). Pachytene cells in air-dried preparations (*9*) of *P. mocha-2-lucida* were hexaploid (Fig. 1E).

Further evidence of hexaploidy was obtained from short-term tissue culture of the ovary of an adult *P. monacha*-2 *lucida* (*10*). Of 42 metaphase plates recovered, 10 (24 percent) had doubled chromosome complements (about 144 chromosomes). A common artifact of tissue culture is endoreduplication of chromosomes. In this process, each chromosome pairs with its sister replicate during c-metaphase, and a doubled chromosome number is produced (*11*). Because of endoreduplication, the occurrence of a hexaploid cell from an ovarian culture of a triploid fish is not remarkable. However, ten cells from ovarian cultures of two gonochoristic *Poeciliopsis* females showed no evidence of endoreduplication, and Chen (*10*) found only one tetraploid cell in 240 from ovarian cultures of diploid *Fundulus*. The short duration of these cultures (about 5 days) minimizes endoreduplication. The high proportion of hexaploidy in this triploid culture thus indicates that hexaploid cells normally are present in the ovary of the gynogenetic triploid and suggests that these hexaploid cells divide mitotically.

The meiotic mechanism of the other triploid gynogen, *P.* 2 *monacha-lucida*, is the same as that of *P. monacha*-2 *lucida*. Two early zygotene cells, one reconstructed from the ovary of a newborn fish and one from the ovary of a fish 2 days after birth, approximated hexaploidy (Fig. 1D).

Thus, *P. monacha*-2 *lucida* and *P.* 2 *monacha-lucida* reproduce by the formation of hexaploid primary oocytes through premeiotic doubling of the chromosome number by endomitosis. The primary oocyte then undergoes a conventional meiosis, during which triploidy is restored. Upon stimulation by a sperm, the triploid ovum develops gynogenetically into a triploid organism, with the mother as the sole source of the entire genetic complement of the progeny (Fig. 2).

Endomitosis followed by reduction is also used by the two triploid ($3n = 42$) gynogenetic salamanders of the *Ambystoma jeffersonianum* complex, *A. platineum* and *A. tremblayi* (*5*, *12*). Examination of lampbrush chromosomes from the primary oocytes of triploid females revealed 42 bivalents ($6n = 84$). Apparently, sister replicates of *Ambystoma* chromosomes remain associated as "pseudobivalents"; thus, synapsis may have occurred before the cell enters prophase I. In *Poeciliopsis*, the scattered chromosomes of early zygotene (Fig. 1, A and D) indicate that sister replicates dissociate from one another before

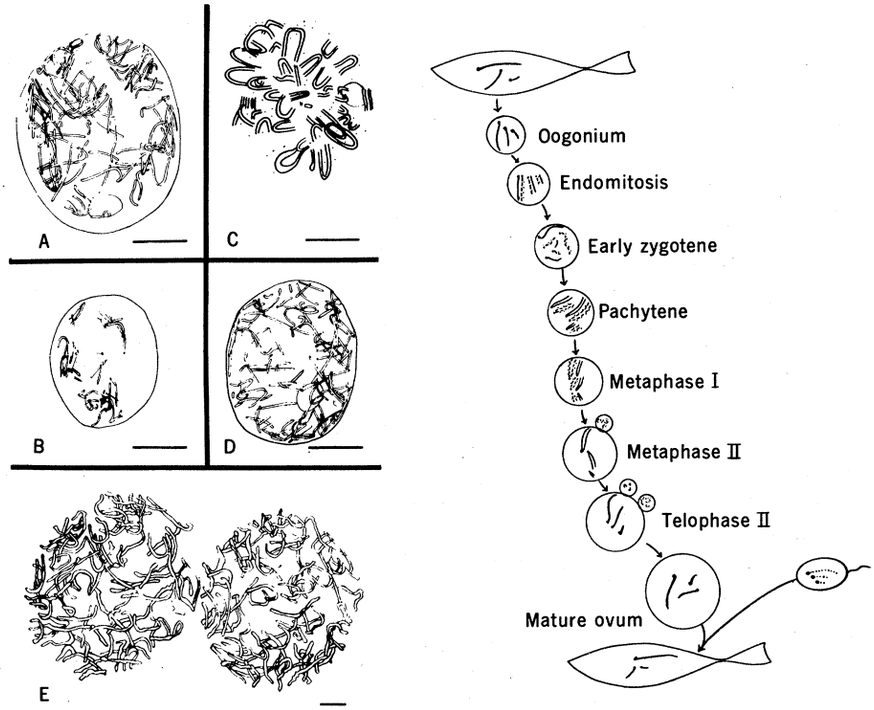

Fig. 1 (left). Meiosis in *Poeciliopsis*. All scales represent 5 μm; in A through D, magnifications are equal. (A) A reconstruction of an early zygotene cell from *P. monacha*-2 *lucida* is seen, with 140 chromosomes. (B) A reconstruction of an early zygotene cell from *P. lucida* is shown, with 48 chromosomes. (C) A reconstruction of a sectioned pachytene cell from *P. monacha*-2 *lucida* is seen, with 46 bivalents. (D) A reconstruction of an early zygotene cell from *P.* 2 *monacha-lucida* is shown, with 120 chromosomes. (E) Tracings of two early pachytene cells from an air-dried preparation of *P. monacha*-2 *lucida* are shown. That on the left has at least 87 chromosomes; that on the right, at least 91. Fig. 2 (right). Diagram of egg production and fertilization in gynogenetic triploid *Poeciliopsis*. For simplicity, only three chromosomes are shown. Inclusion of sister replicates instead of original homologs at telophase I would have no genetic significance.

meiosis. This dissociation is also suggested by the possibility of mitosis at the hexaploid level, as indicated by the tissue cultures.

The hexaploid oogonium that enters pachytene must therefore provide for two-by-two synapsis from sets of six homologous chromosomes. If a trio of homologs were represented by $I^AI^BI^C$, then the sister replicates added by endomitosis would be $I_r^AI_r^BI_r^C$. Random formation of bivalents from the homologous sextet $I^AI^BI^CI_r^AI_r^BI_r^C$ would result in combinations such as $I^AI_r^A$, I^AI^B, I^AI^C, and so forth. However, the gynogenetic inheritance pattern of triploid *Poeciliopsis* indicates that it is sister replicates that pair as bivalents. If univalents, trivalents, or higher associations occurred, or if bivalent pairing were random among homologs, then segregation would also be random, and the ovum would contain recombinations of chromosomes. I observed only bivalents (Fig. 1C), and 23 laboratory generations of triploids have shown no genetic evidence of random segregation (*4*). Thus, sister-replicate pairing ($I^AI_r^A$, $I^BI_r^B$, $I^CI_r^C$) conserves the genotype of the clone.

Endomitosis also occurs prior to the meiosis of the triploid ($3n = 69$) parthenogenetic lizard *Cnemidophorus uniparens*, which contains 69 bivalents during metaphase I (*13*).

Russian populations of triploid gynogenetic goldfish (*Carassius auratus gibelio*) possess a tripolar spindle during oogenesis (*1*). One chromosome set collects at each pole. The spindle aborts, and all three sets collect in the oocyte nucleus, a mechanism that is similar to that of the triploid parthenogenetic vegetable weevil, *Listroderes costirostris* (*14*). On the other hand, diploid gynogenetic goldfish from eastern Europe, studied by Lieder (*3*), apparently undergo both maturation divisions. Lieder concluded that diploidy is restored by suppression of the first cleavage division. However, this mechanism would lead to homozygosity of all alleles, including recessive lethals, and therefore must be viewed with some suspicion.

The first unisexual vertebrate discovered was the gynogenetic diploid poeciliid, *Poecilia formosa* (*2*). Although this species was known for almost 40 years, the mechanism by which it produces its eggs is still unknown. The techniques I used, if applied to *Poecilia*, might provide the answer. It is plausible that an endomitosis like that in triploid *Poeciliopsis* produces in *Poecilia* a tetraploid oogonium, which enters meiosis and is reduced to a diploid ovum. Three precedents for such a mechanism in vertebrates now exist.

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9. Air-dried preparations (*10*) were used for karyotype studies, to be reported elsewhere. Pachytene chromosomes were easily distinguishable from the chromosomes used for karyotyping, in that the latter are short, densely staining, and obviously composed of two chromatids connected by a centromere, whereas the former are thin, long, faintly staining, and apparently single.
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A Visual Pigment with Two Physiologically Active Stable States

Abstract. *Red illumination of a* Balanus amphitrite *photoreceptor that has been adapted to blue light leads to prolonged depolarization in the late receptor potential. This depolarization can be switched off by further exposure to a blue stimulus. The early receptor potential in this cell is purely depolarizing or largely hyperpolarizing; the former is true if the cell has been adapted to red light, and the latter, if blue light has been used. The color-adaptation "memories" for both early and late receptor potentials appear to be permanent. The existence of two stable states for the early receptor potential directly implies a pigment with two stable states, and these apparently contribute antagonistically to the late receptor potential.*

Correlation of the origin of photoreceptors' physiological response—the late receptor potential (LRP)—with the changes undergone by the visual pigment molecule after absorbing the photon has been hindered by the impossibility of substantially manipulating the pigment process in conditions where the LRP remains intact. We have now found a pigment that has two thermally stable and physiologically active states. These states have different absorption spectra and are interconvertible by light, so stimulation by different colors activates the two states to different degrees and leaves them in different relative proportions.

Figure 1 shows intracellular recordings in photoreceptors of the lateral ocellus of the barnacle *Balanus amphitrite* (*1*). The response to strong light (traces A and B) appears to be made up of two parts—a small, fast component, which is positive (depolarizing) in A and negative in B, and which we identify as the early receptor potential (ERP); followed by a large, slow, positive LRP. The slow response sometimes conveniently disappeared spontaneously, leaving the fast responses of traces D and E. The identification of these fast responses as ERP's (*2*) was confirmed by their short latency (less than 0.3 msec), their roughly linear dependence on intensity, their relative independence of ionic medium (for example, high concentration of K^+), and their survival after glutaraldehyde fixation, which destroyed the LRP (*3*).