ABSENCE OF rDNA AMPLIFICATION IN THE UNINUCLEOLATE OOCYTE OF THE COCKROACH *BLATTELLA GERMANICA* (ORTHOPTERA: BLATTIDAE)

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ABSTRACT

Amplification of the genes coding for ribosomal RNA occurs in the oocytes of a wide variety of organisms. In oocytes of various species of crickets (Orthoptera: Gryllidae) the amplified DNA is contained in a large extrachromosomal DNA body. Multiple nucleoli form about the periphery of the DNA body during the diplotene stage of meiosis I. In contrast to the general pattern of orthopteran oocytes, oocytes of the cockroach *Blattella germanica* demonstrate a single large nucleolus instead of many nucleoli. In order to determine whether the genes coding for rRNA are amplified in the oocyte of *B. germanica*, the relative amount of rDNA in oocytes was compared with the rDNA content of spermatocytes and somatic cells. An extrachromosomal DNA body similar to that present in crickets is not present in *B. germanica*. A satellite DNA band which contains nucleotide sequences complementary to rRNA accounts for approximately 3–5% of the total DNA in somatic and in male and female gametogenic tissues. Female cells contain approximately twice as much rDNA as do male cells. An XX-XO sex-determining mechanism is operative in *B. germanica*. *In situ* hybridization with rRNA indicates that the nucleolar organizer is located on one end of the X chromosome and that oocytes do not contain more than twice the amount of rDNA found in spermatocytes. The data indicate that rDNA is not amplified in the uninucleolate oocyte of *B. germanica*.

Amplification of the DNA coding for ribosomal RNA (rDNA) has been described in the oocytes of various species of mollusks (4), echiuroid worms (9), arthropods (15, 22), and chordates (4, 14, 28). In the oocytes of many orthopteran insects, rDNA amplification appears to be correlated with the presence of a large, extrachromosomal, DNA-containing body about which hundreds of nucleoli form during the diplotene stage of oogenesis (6, 7). In crickets (family: Gryllidae) such a DNA body has been identified in the pachytene-stage oocytes of representatives of the subfamilies Gryllinae, Nemobiinae, Brachytrupinae, Oecanthinae, and Gryllotalpinae (1, 8).

Previous studies suggest that amplification of rDNA occurs in oocytes with multiple nucleoli, but not in uninucleolate oocytes (28). This interpretation is not compatible with studies on the uninucleolate oocyte of the echiuroid worm *Urechis caupo*, in which amplification has been detected (9).

The oocytes of many cockroaches (family: Blat-

tidae) differ from those of other orthopterans in that they are uninucleolate (30) rather than multinucleolate. The number of genes coding for rRNA was determined in somatic and gametogenic tissues of the german cockroach *Blattella germanica*, in order to determine whether the genes coding for rRNA are amplified in this uninucleolate oocyte.

MATERIALS AND METHODS

Collection of Tissues

A colony of *B. germanica* was raised from five animals which were collected in Pulaski County, Arkansas. The colonies were reared in large glass tanks, the upper portions of which were coated with petroleum jelly to prevent escape. The animals were fed water, mouse pellets, apple, and potato. They received 14 h of light daily. The animals were dissected with the aid of a stereomicroscope. Gonads were collected from second to third instar nymphs which weighed less than 30 mg. Gonadal sex was determined at the time of dissection. The gonads were stored in 70% ethanol at −20°C. The femurs of several hundred adult animals, the sex of which had been identified prior to dissection, were frozen and stored at dry-ice temperature.

Preparation of DNA

Testes and ovaries (approximately 0.5 ml packed volume) were washed with several changes of 2× SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). They were extracted with 100 μg/ml beta-amylase (Sigma Chemical Co., St. Louis, Mo.) for 1 h at 37°C, and subsequently with 100 μg/ml ribonuclease-A (Worthington Biochemical Corp., Freehold, N. J.) for 2 h at 37°C. They were then washed with 2× SSC and suspended in 1 ml of lysing solution (0.05 M Tris; 0.1 M EDTA; 0.5% Sarkosyl NL-97; Geigy Chemical Corp., Ardsley, N. Y.). They remained in the lysing solution for 1 h at 37°C, and then were homogenized in a motor-driven glass Teflon homogenizer. The homogenate was centrifuged for 20 min at 1,500 *g*. The supernate was mixed with CsCl and brought to a density of 1.7 and a total vol of 5 ml. DNA was further purified by banding in gradients of CsCl. The DNA was centrifuged at 32,000 rpm, 20°C, for 60 h in the Spinco SW-50.1 rotor (Beckman Instruments, Spinco Div., Palo Alto, Calif.).

DNA was extracted from frozen femurs by a modification of the Pronase procedure. Tissues were homogenized in 5 ml of 0.1 M Tris, 0.05 M EDTA, and 0.5% sodium dodecyl sulfate. The homogenate was made 1 mg/ml Pronase (Calbiochem, San Diego, Calif.) and digested for 3 h at 37°C. The digest was phenol-extracted twice at 37°C for 30 min. Nucleate was precipitated from the aqueous phase by making it 0.2 M sodium acetate and 70% ethanol. After several ethanol washes, the precipitate was suspended in 2× SSC and extracted for 1 h each with 200 μg/ml beta-amylase, 100 μg/ml RNase-A, and 50 μg/ml Pronase. After two subsequent phenol extractions, DNA was precipitated with ethanol and wound onto a glass rod. The DNA was mixed with CsCl which was brought to a density of 1.70 and a vol of 5 ml. It was banded in CsCl as described above.

Preparation of Labeled RNA

Previous studies have demonstrated extensive homologies between the rRNA's of a wide variety of organisms (26). The rRNA of the South African clawed toad *Xenopus laevis* has been utilized extensively in such hybridization experiments. Labeled 18S and 28S rRNA were prepared by growing a cell line of *X. laevis* kidney cells (line CCL 102 A, obtained from the American Type Culture Collection, Rockville, Md.) in modified Leibowitz L-15 medium with 15% fetal calf serum. Cells were grown for 3–5 days in medium containing 25 μCi/ml [5-^{3}H]uridine (sp act 29.2 Ci/mmol, New England Nuclear Corp., Boston, Mass.) and then for an additional 24 h in two changes of fresh medium containing unlabeled uridine. Subsequently, RNA was extracted from the cells by a modification of the phenol procedure and 18S and 28S RNA were isolated as described elsewhere (6). Ribosomal RNA was reconstituted by combining one part 18S RNA with two parts 28S RNA (wt/wt).

Cytological Procedures

Ovaries were fixed for light microscopy in three parts ethanol:one part acetic acid. They were embedded in paraffin for sectioning or immersed in 45% acetic acid for squashing. Sections and squashes were stained with Azure B according to the procedure of Gabrusewycz-Garcia and Kleinfeld (13), according to the Feulgen procedure (12), and with Giemsa stain (16).

For electron microscopy, ovaries were fixed for 1 h at 4°C in freshly prepared 5.0% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Subsequently, the ovaries were washed overnight in several changes of 0.1 M buffer and then postfixed for 1 h at 4°C in 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.4. After dehydration, the ovaries were embedded in Epon-Araldite and sectioned with a diamond knife on a Porter-Blum MT-2 ultramicrotome. The sections were mounted on naked grids, stained with uranyl acetate and lead citrate, and viewed in a Siemens 101A electron microscope.

Nucleic Acid Hybridization

RNA-DNA hybridization on Millipore HAWP filters (Millipore Corp., Bedford, Mass.) was carried out as described previously (6).

RNA-DNA hybridization *in situ* was carried out according to a modification of the technique of Gall and Pardue (16). Treatment of the slides with 0.2 N HCl was omitted. Autoradiographs were prepared with full-

strength Kodak NTB2 emulsion (Eastman Kodak Co., Rochester, N. Y.). The autoradiographs of the hybrid structures were stained with Giemsa stain. In some cases, the hybridization was carried out on sectioned ovaries. The DNA in sections was denatured by placing the sections in 95% formamide in 0.1 × SSC for 2 h at 70°C.

RESULTS

In early diplotene-stage oocytes (Fig. 1), the nucleolus is seen as a single mass of material which stains blue-violet with Azure B (RNA). The nucleolus remains singular long after vitellogenesis has begun (Fig. 2). No Feulgen-positive material can be detected within it. Phase-contrast microscopy of whole, living oocytes confirms that there is but a single nucleolus in each germinal vesicle (Fig. 3). The nucleolus is visualized by transmission electron microscopy as an anastomosing network of ribbons or plates connecting larger masses of nucleolar material (Fig. 4). These larger masses of nucleolar material (Fig. 4) appear to have a fibrillar core and granular cortex. Areas that are primarily fibrillar and others that are granular are also associated with the ribbons (Fig. 5).

In neutral CsCl, the bulk DNA of *B. germanica* (main band DNA) has a density of 1.698 g/cm^3 (39% G+C), and DNA hybridizing with rRNA (satellite DNA) has a density of 1.714 g/cm^3 (55% G+C) (Fig. 6).

A satellite DNA of the same buoyant density as the DNA that hybridizes with rRNA (ρ = 1.714) is visualized by isopycnic banding in neutral CsCl in the analytical ultracentrifuge (Fig. 7). In gradients that were overloaded with DNA (0.5 OD_{260} U/gradient), the satellite is seen to account for 3–5% of the total DNA. No consistent differences were noted between male and female tissues or between somatic and gametogenic tissues in the relative proportions of satellite and main band DNA.

Approximately 0.02% of the DNA extracted from male tissues (testis and leg muscle) and 0.035% of the total DNA from female tissues (ovary and leg muscle) hybridize with labeled *Xenopus laevis* 18S and 28S RNA (Fig. 8). The results suggest that female cells have approximately twice as much rDNA as do male cells. An XX-XO sex-determining mechanism is operative in this species. If the genes coding for rRNA are localized on the X chromosomes, female cells with two X chromosomes would be expected to have approximately twice as many genes coding for rRNA as male cells. Chromosome counts made on the nuclei of spermatocytes confirm that n = 11 ($2n$ = 22 female, $2n$ = 21 male [29]) (Fig. 9).

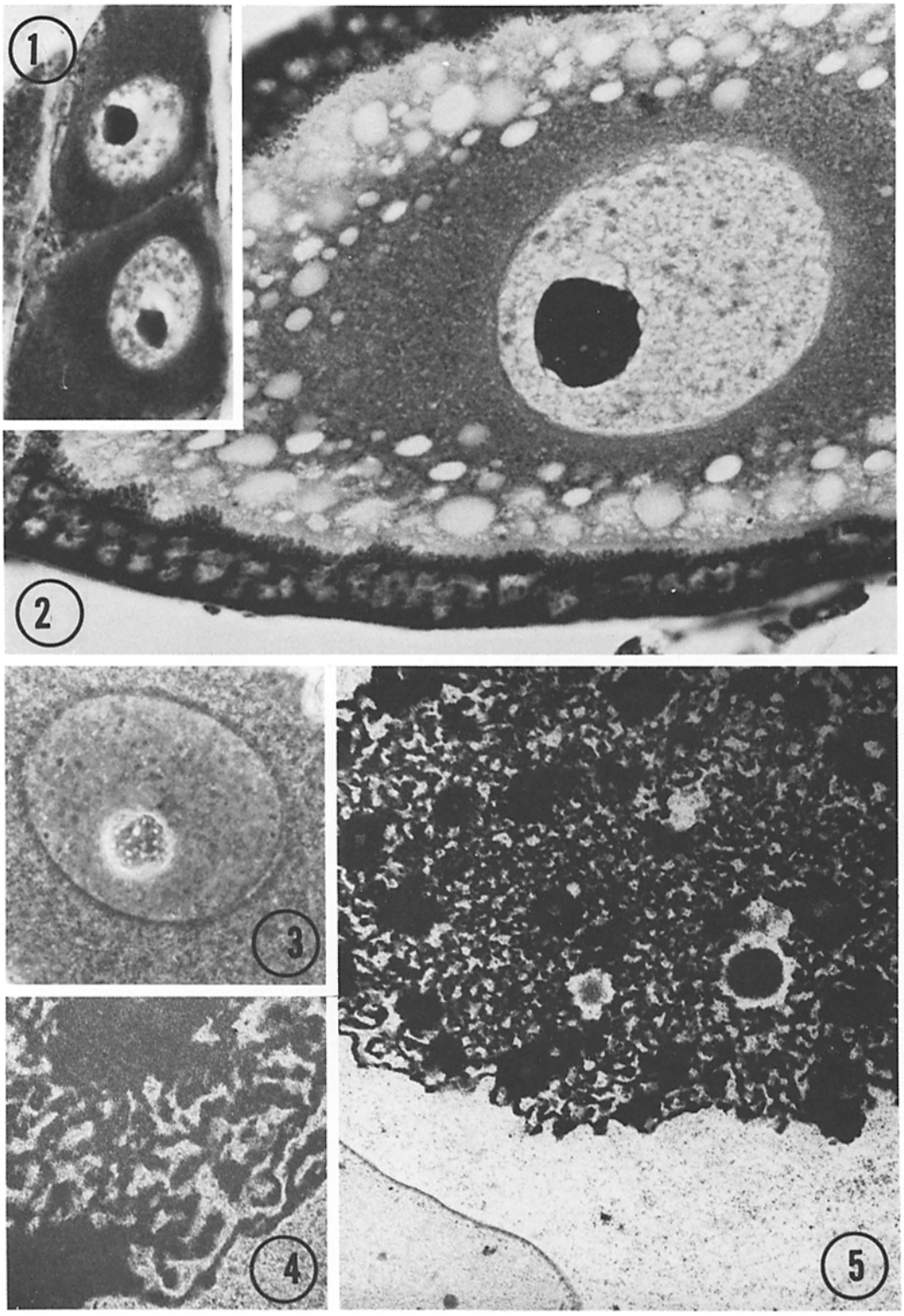
A Feulgen-positive extrachromosomal DNA body similar to that found in oocytes of gryllid crickets is not observed in either oocytes or spermatocytes of *B. germanica*. A small heteropycnotic knob is localized on the end of the presumptive X chromosome of spermatocytes (Fig. 11) and on its bivalent counterpart in oocytes (Fig. 10). The nucleolus persists well into the pachytene stage of meiosis in spermatocytes (Fig. 13). In autoradiographs of pachytene-stage spermatocytes hybridized with rRNA, most of the silver grains are localized over the nucleolus which is associated with the heteropycnotic knob on the presumptive X chromosome (Fig. 12).

Ovary DNA contains DNA derived from somatic cells as well as from oocytes. Perhaps the somatic cell DNA dilutes the oocyte DNA to such an extent that amplification cannot be detected in DNA extracted from whole ovaries. In order to determine whether rRNA is amplified in oocytes, autoradiographs of oocytes hybridized with rRNA were compared with autoradiographs of spermatocytes hybridized with rRNA. Since amplification of rDNA has been detected in premeiotic germ line cells in males of other species (23), it is necessary to determine whether the amount of rDNA increases during spermatogenesis. Grain counts made over nuclei of interphase somatic cells in the testis were compared with grain counts made over the chromosomes of pachytene-stage spermatocytes (Figs. 14 and 15; Table I). The data indicate that somatic cells and spermatocytes contain the same amount of rDNA (i.e., there is no amplification of rDNA during spermatogenesis). Next, comparisons were made between oocytes and spermatocytes which had been hybridized with labeled rRNA *in situ* (Figs. 16 and 17; Table I). Oocytes demonstrate less than two times the number of silver grains found over the nucleolar organizer regions of spermatocytes (i.e., there is no amplification of rDNA during oogenesis).

Finally, in sections of ovaries hybridized with *X. laevis* rRNA *in situ*, virtually no label was visualized over the nucleoli of oocytes. Labeling of oocyte nucleoli was the same as or less than that of the remainder of the nucleus, and less than that over the nucleolar area of interphase follicle cells.

DISCUSSION

The uninucleolate condition is common in the oocytes of invertebrates and in some vertebrates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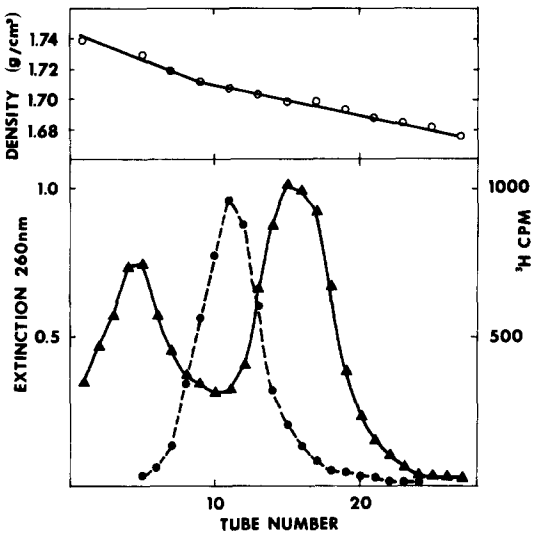


FIGURE 6 Buoyant density of DNA hybridizing with rRNA. DNA from leg muscle of male *B. germanica* was mixed with *M. lysodeikticus* marker DNA (ρ = 1.731 g/cm³) and sedimented in neutral CsCl for 60 h, 20°C, 32,000 rpm in the type-40 rotor of the Beckman L3-50 preparative ultracentrifuge. The gradients were fractionated and the density of each fraction was determined from the relationship between density and refractive index (18). The fractions were diluted to 1 ml with distilled water, and the optical density (260 nm) was determined. The DNA in each fraction was denatured and bound to filters. The filters were hybridized in a solution containing 1 μg/ml *X. laevis* rRNA (sp act 280,000 cpm/μg). (▲—▲), optical density 260 nm; (●—●), radioactivity on filters; (○—○), buoyant density.

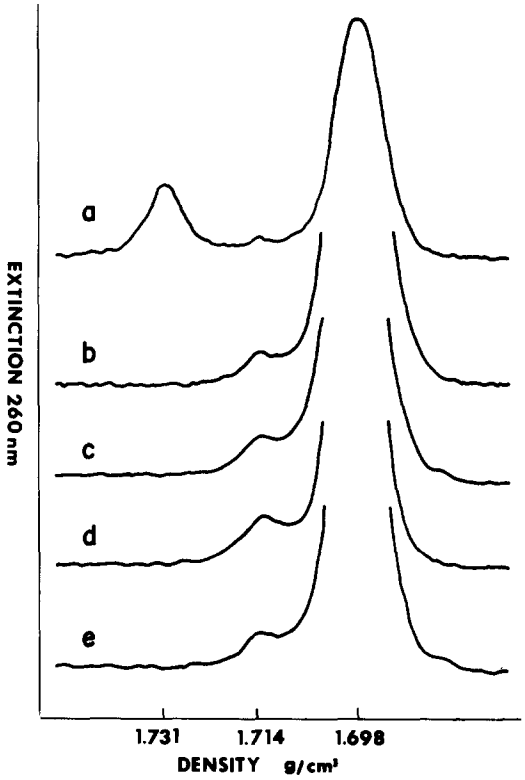


FIGURE 7 Buoyant density profiles of DNA from various tissues of *B. germanica* in neutral CsCl: (*a*) 0.2 A_{260} U of DNA from ovary with *M. lysodeikticus* DNA (0.05 A_{260} U, ρ = 1.731 gm/cm³) as an internal density marker; (*b–e*) 0.5 A_{260} of DNA from (*b*) ovaries of animals weighing less than 30 mg, (*c*, *e*) femur muscle from female (*c*) and male (*e*) adult animals, and (*d*) testis of animals weighing less than 30 mg. The minor peaks at density 1.714 in *b–e* represent 3–5% of the total DNA. Samples were centrifuged at 40,000 rpm for 18 h at 20°C with 2° double sector center pieces in the AnGTi rotor of the Beckman model E ultracentrifuge. Scans were made at a wavelength of 260 nm.

including the mammals. Multiple nucleoli are common in oocytes of fish, amphibians, birds, reptiles, and some arthropods (30). Exceptions to these general rules are common. The cockroaches

FIGURE 1 Light micrograph of an Azure B-stained 5-μm section through two early diplotene-stage oocytes in the ovary of *B. germanica*. Each contains a single nucleolus. × 1,140.

FIGURE 2 Light micrograph of an Azure B-stained 5-μm section through a late diplotene-stage *B. germanica* oocyte. Vitellogenesis has begun in the cytoplasm. The nucleolus remains a single prominent structure within the nucleus. × 1,140.

FIGURE 3 Phase-contrast micrograph of a living, late diplotene-stage *B. germanica* oocyte. A single large nucleolus is visualized within the oocyte nucleus. × 1,020.

FIGURE 4 Transmission electron micrograph through a portion of the nucleolus of a *B. germanica* oocyte. Areas which are primarily granular, the granules measuring 2.0–2.5 nm in diameter, and others which are primarily fibrillar are visualized. × 18,000.

FIGURE 5 Transmission electron micrograph through the nucleolus of *B. germanica*. Masses of nucleolar material connected by ribbon-like structures are visualized. × 10,000.

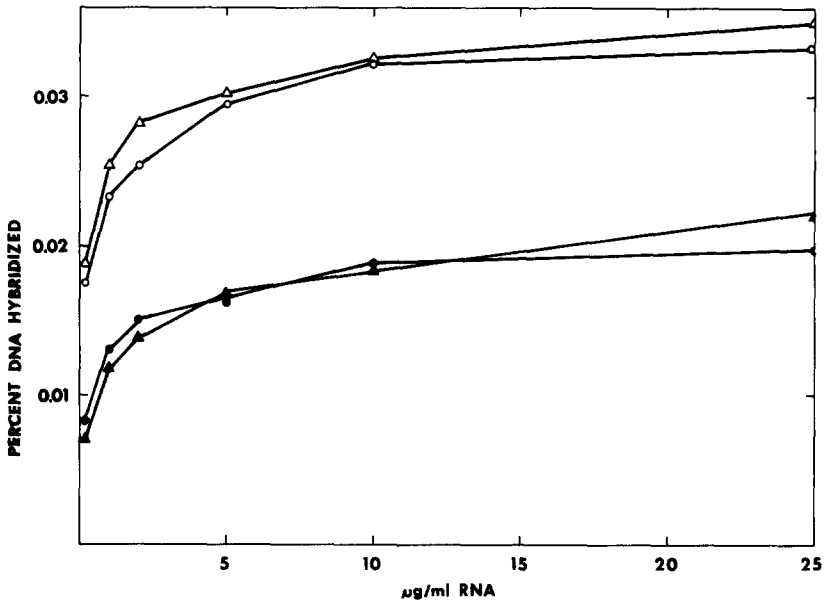


FIGURE 8 Filters containing DNA extracted from ovaries (△) and testis (▲) of animals weighing less than 30 mg, and femur muscle of male (●) and female (○) adult animals were hybridized with ^{3}H-labeled *X. laevis* rRNA (sp act 285,000 cpm/µg). Filters were hybridized for 18 h at 68°C in solutions containing 4× SSC and 0.4% SDS. The amount of DNA on each filter was determined after hybridization by hydrolyzing the filters in 5% perchloric acid for 30 min at 70°C and measuring the A_{260} of the hydrolysate.

TABLE I

*Grain Counts Made From Autoradiographs of B. germanica Testes and Ovaries Hybridized with ^{3}H-Labeled rRNA**

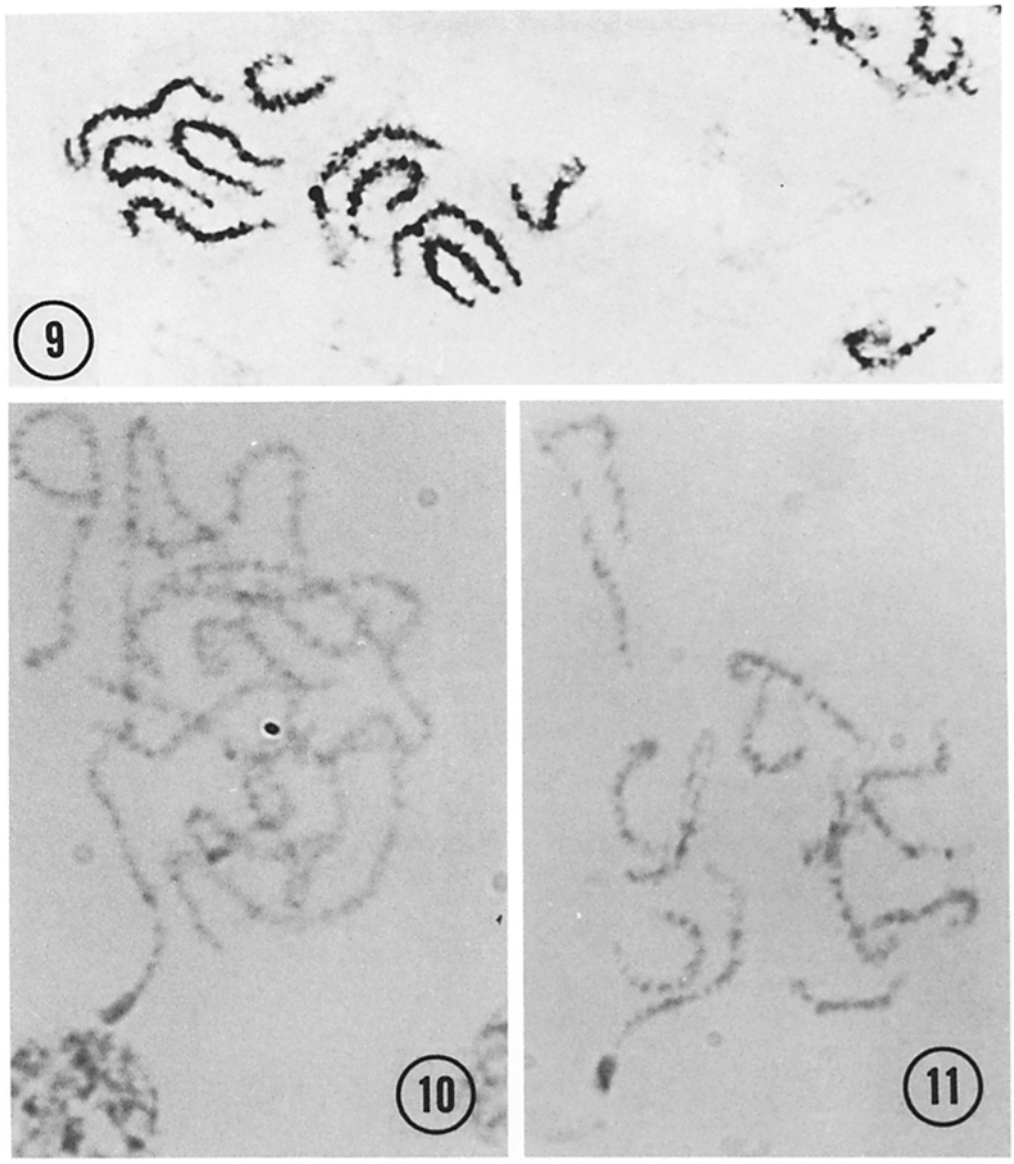
	Mean no. silver grains (±SE)	
	Male	Female
Meiotic prophase cells		
Grains over chromosomal nucleolus organizer	8.80 (±0.36)	9.95 (±0.41)
Grains over chromosomes (exclusive of nucleolus organizer)	6.57 (±0.54)	7.42 (±1.12)
Interphase somatic cells		
Grains over nucleolus and perinucleolar chromatin	9.38 (±0.31)	
Grains over nucleus (exclusive of nucleolus)	6.52 (±0.57)	

* Grain count comparisons were made on slides which were exposed for 120 days and developed under identical conditions.

represent such an exception. They belong to an order of insects, the orthopterans, most representatives of which demonstrate multinucleolate oocytes (30). Bier, Kunz, and Ribbert (2) have interpreted the nucleolar apparatus of *B. germanica* oocytes as being multinucleolate on the basis of its ultrastructural organization. In the present investigation, small masses of nucleolar material were occasionally visualized separate from the nucleolus. These probably represent material which is being transported to the cytoplasm rather than metabolically active nucleoli.

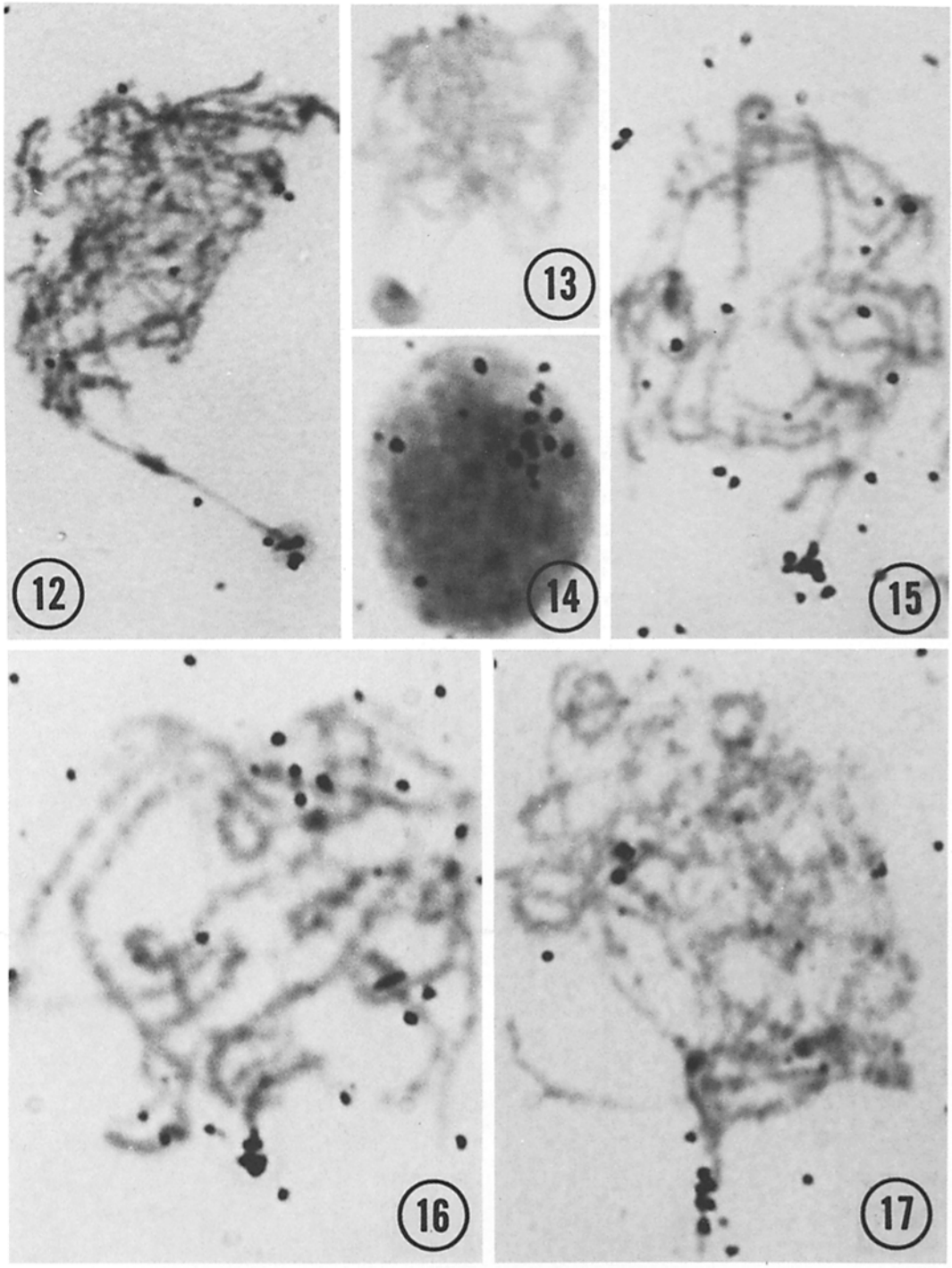
In *Drosophila melanogaster* the nucleolus organizer is localized on the X and on the Y chromosomes (25). Male *D. melanogaster* which are lacking a Y chromosome (X/O males) and females in which one of the X chromosomes has a deletion of the nucleolus organizer (X/X_{NO}- females) would be expected to have about 50% of the rDNA of their X/X mothers. Instead, they contain about 80% the amount of rDNA found in X/X females (27). This increase in rDNA which is somatic in nature (i.e., it is not inherited) is referred to as rDNA compensation. The percent DNA hybridizing with rRNA in male somatic and gametogenic tissues of *B. germanica* is 60% that of female somatic and gametogenic tissues. rDNA compensation does not appear to occur in *B. germanica* where the X/O condition is normal for males.

A large extrachromosomal DNA body is present in oocytes representing five subfamilies of crickets (Orthoptera: Gryllidae). RNA-DNA hybridization analysis indicates that the oocytes of



FIGURES 9–11 Giemsa- (Fig. 9) and Feulgen- (Figs. 10–11) stained squash preparations of *B. germanica* spermatocytes (Figs. 9 and 11) and an oocyte (Fig. 10) in the pachytene stage of meiotic prophase I. There are 10 bivalents and one univalent visualized in a spermatocyte (Fig. 9). The presumptive sex bivalent (Fig. 10) and univalent (Fig. 11) display a small heteropycnotic mass at one end. Fig. 9, × 1,000; Fig. 10, × 2,770; Fig. 11, × 2,250.

the house cricket *Acheta domesticus* contain about 100 times the amount of rDNA found in somatic cells. *In situ* hybridization localizes the amplified copies of rDNA in the oocytes (6). As the cells proceed through diplotene, DNA within the body becomes incorporated into the multiple nucleoli which form at the periphery of the DNA body. In one exceptional species of Gryllidae, the mole cricket *Scapteriscus acletis*, the diplotene-stage oocyte demonstrates a single nucleolus. A large extrachromosomal DNA body is visualized during pachytene. *In situ* hybridization



FIGURES 12–17 Light micrograph of an Azure B-stained spermatocyte (Fig. 13), and autoradiographs of an interphase male somatic cell (Fig. 14), pachytene-stage spermatocytes (Figs. 12, 15, and 17), and a pachytene-stage oocyte (Fig. 16) which were hybridized with ^{3}H-labeled rRNA *in situ*. Unstained squash preparations of *B. germanica* testis and ovary were hybridized *in situ* with 1 μg/ml ^{3}H-labeled *X. laevis* 18S and 28S RNA (sp act 950,000 cpm/μg) in 2× SSC for 18 h at 68°C. The autoradiographs were exposed for 60 days (Fig. 12), 100 days (Figs. 16 and 17), and 120 days (Figs. 14 and 15). The nucleolus persists in many pachytene-stage spermatocytes, staining green with Azure B, and being attached to the presumptive sex chromosome (Fig. 13). In pachytene-stage autoradiographs hybridized with rRNA, most of the silver grains are localized over the heteropycnotic mass which is embedded in the nucleolus (i.e., the nucleolus organizer) (Fig. 12). Approximately the same number of silver grains are found over the nucleolus organizer of pachytene-stage spermatocytes (Fig. 15) as are concentrated over the limited nucleolar area of the interphase somatic cell nucleus (Fig. 14). Approximately the same number of silver grains are localized over the nucleolar organizer regions of pachytene-stage oocytes (Fig. 16) and spermatocytes (Fig. 17). Fig. 12, × 2,170; Fig. 13, × 2,380; Figs. 14–17, × 2,850.

with rRNA demonstrates the presence of amplified rDNA in the DNA body as well as within the oocyte nucleolus (8).

Multinucleolate oocytes amplify rDNA hundreds of times. Amplification occurs at a very low level in uninucleolate oocytes (4, 9) or not at all (28, 21). Thus amplification of rDNA was readily detected in the multinucleolate oocyte of the striped bass, *Roccus saxatilis,* but not in the uninucleolate oocyte of the starfish *Asterias forbesii* (28).

Brown and Dawid (4) and Dawid and Brown (9) have observed approximately a five- to six-fold amplification of rDNA in the uninucleolate oocytes of the echiuroid worm, *Urechis caupo,* and in the surf clam, *Spisula solidissima.* The singular nucleolus in oocytes of the sea urchin *Strongylocentrotus purpuratus* binds [^{3}H]actinomycin D to a greater extent than the nucleoli of oogonia, indicating that a limited amount of rDNA amplification may occur during sea urchin oogenesis (11).

More recently, studies on oocytes of the coot clam, *Mulinia lateralis,* indicate that there is no greater than twice the somatic cell amount of rDNA in an oocyte (21). Studies by Vincent on the uninucleolate oocytes of several other species of marine organisms indicate that amplification does not occur (W. Vincent, personal communication).

In some organisms, ribosomes synthesized during oogenesis provide the protein-synthetic machinery of the embryo during the early stages of embryogenesis. When meiosis is completed in *X. laevis,* RNA synthesis ceases and is not initiated again until the onset of gastrulation (5). Synthesis of rRNA does not occur until the late blasteme stage (27 h after fertilization) during early embryogenesis in *A. domesticus* (17). A single *X. laevis* oocyte accumulates an amount of rRNA which is equivalent to the amount of rRNA in liver tissue containing about 200,000 cells (3). Assuming that a *X. laevis* oocyte contains the normal somatic rDNA complement and synthesizes rRNA at a rate equivalent to a rapidly growing cell in culture, it would take some 400 years to synthesize the amount of rRNA found in an oocyte (24). By increasing the number of genes coding for rRNA 1,000 times, this interval becomes 4–5 mo. Thus, amplification of rDNA is extremely advantageous to organisms in which the onset of rDNA synthesis is delayed until late in development.

In some organisms with uninucleolate oocytes, rRNA synthesis begins as early as the four-cell stage (10, 19, 20, 31). The early onset of rRNA synthesis does not appear to be correlated with the size of the egg since early rRNA synthesis has been detected in the relatively large eggs of the sea urchin *Strongylocentrotus purpuratus* (10) and the parasitic nematode *Ascaris lumbricoides* (19), as well as in the small alecithal egg of the mouse (31).

Amplification of rDNA enables the oocyte to synthesize large numbers of ribosomes which provide the protein synthetic machinery of early embryogenesis. Multiple nucleoli and extrachromosomal DNA bodies appear to be expressions of this synthetic activity. In the absence of rDNA amplification the nucleolus remains singular, the demands for ribosomes during early embryogenesis being met by the developing embryonic cells. Thus multiple nucleoli in the oocyte nucleus would appear to be representative of transcription of amplified rDNA.

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REFERENCES

1. Allen, E. R., and M. D. Cave. 1972. Nucleolar organization in oocytes of Gryllid crickets: subfamilies Gryllinae and Nemobiinae. *J. Morphol.* **137**:433–448.
2. Bier, K., W. Kunz, and D. Ribbert. 1967. Struktur und Funktion der Oocyten Chromosomen und Nukleolen sowie der extra DNS während der oogenese panoistischer und meroistischer Insekten. *Chromosoma (Berl.).* **23**:214–254.
3. Brown, D. D. 1966. The genes for ribosomal RNA and their transcription during amphibian development. *In* Current Topics in Developmental Biology, Vol. 2. A. Monroy and A. Moscona, editors. Academic Press, Inc., New York. 47–73.
4. Brown, D. D., and I. B. Dawid. 1968. Specific

gene amplification in oocytes. *Science (Wash. D. C.)*. **160:**272-280.

5. BROWN, D. D., and E. LITTNA. 1964. RNA synthesis during the development of *Xenopus laevis*, the South African clawed toad. *J. Mol. Biol.* **8:**669-687.
6. CAVE, M. D. 1972. Localization of ribosomal DNA within oocytes of the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). *J. Cell Biol.* **55:**310-321.
7. CAVE, M. D., and E. R. ALLEN. 1969. Extrachromosomal DNA in early stages of oogenesis in *Acheta domesticus*. *J. Cell Sci.* **4:**593-609.
8. CAVE, M. D., and E. R. ALLEN. 1974. Nucleolar DNA in oocytes of crickets: representatives of the subfamilies Oecanthinae and Gryllotalpinae (Orthoptera: Gryllidae). *J. Morphol.* **142:**379-394.
9. DAWID, I. B., and D. D. BROWN. 1970. The mitochondrial and ribosomal DNA components of oocytes of *Urechis caupo*. *Dev. Biol.* **22:**1-14.
10. EMERSON, C. P., and T. HUMPHREYS. 1971. Ribosomal RNA synthesis and multiple atypical nucleoli in cleaving embryos. *Science (Wash. D. C.)*. **171:**898-901.
11. ENESCO, H. E., and K. H. MAN. 1974. Nucleolar DNA in sea urchin oogenesis studied by ^{3}H-actinomycin-D binding. *Exp. Cell Res.* **86:**395-399.
12. FEULGEN, R., and H. ROSSENBECK. 1924. Microscopisch-chemischer Nachweis einer Nukleinsäure von Typus der Thymonucleinsäure. *Hoppe-Seyler's Z. Physiol. Chem.* **135:**203-248.
13. GABRUSEWYCZ-GARCIA, N., and R. G. KLEINFELD. 1966. The study of the nucleolar material in *Sciara coprophila*. *J. Cell Biol.* **29:**347-359.
14. GALL, J. G. 1968. Differential synthesis of the genes of ribosomal RNA during amphibian oogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **60:**553-560.
15. GALL, J. G., H. C. MACGREGOR, and M. E. KIDSTON. 1969. Gene amplification in the oocytes of Dytiscid water beetles. *Chromosoma (Berl.)*. **26:**169-187.
16. GALL, J. G., and M. L. PARDUE. 1971. Nucleic acid hybridization in cytological preparations. *Methods Enzymol.* **21D:**470-480.
17. HANSEN-DELKESKAMP, E., H. W. SAUER, and F. DUSPIVA. 1967. Ribonucleinsäuren in der Embryogenese von *Acheta domestica*, L. *Z. Naturforsch., Teil B. Anorg. Chem. Org. Chem. Biochem. Biophys. Biol.* **226:**540-545.
18. IFFT, J. B., D. H. VOET, and J. VINOGRAD. 1961. Determination of density distributions and density gradients in binary solutions at equilibrium in the ultracentrifuge. *J. Phys. Chem.* **65:**1138-1145.
19. KAULENAS, M. S., W. E. FOOR, and R. D. FAIRBAIRN. 1969. Ribosomal RNA synthesis during cleavage of *Ascaris lumbricoides* eggs. *Science (Wash. D. C.)*. **163:**1201-1264.
20. KARP, G. C., C. MANES, and E. W. HAHN. 1974. Ribosome production and protein synthesis in the preimplantation rabbit embryo. *Differentiation*. **2:**65-71.
21. KIDDER, G. M. 1975. The ribosomal DNA of clam gametes. *J. Cell Biol.* **67**(2, Part 2):208a (Abstr.).
22. LIMA-DE-FARIA, A., M. L. BIRNSTIEL, and H. JAWORSKA. 1969. Amplification of ribosomal cistrons in heterochromatin of *Acheta*. *Genetics (Suppl.)*. **61:**145-159.
23. PARDUE, M. L. 1969. Nucleic acid hybridization in cytological preparations. *J. Cell Biol.* **43**(2, Part 2): 101a (Abstr.).
24. PERKOWSKA, E., H. C. MACGREGOR, and M. L. BIRNSTIEL. 1968. Gene amplification in the oocyte nucleus of mutant and wild-type *Xenopus laevis*. *Nature (Lond.)*. **217:**649-650.
25. RITOSSA, F. M., and S. SPIEGELMAN. 1965. Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* **53:**737-745.
26. SINCLAIR, J. H., and D D. BROWN. 1971. Retention of common nucleotide sequences in the rDNA of eukaryotes and some of their physical characteristics. *Biochemistry*. **10:**2761-2769.
27. TARTOF, K. D. 1973. Regulation of ribosomal RNA gene multiplicity in *Drosophila melanogaster*. *Genetics*. **73:**57-71.
28. VINCENT, W. S., H. O. HALVORSON, H. R. CHEN, and D. SCHIN. 1969. A comparison of ribosomal gene amplification in uni- and multinucleolate oocytes. *Exp. Cell Res.* **57:**240-250.
29. WASSILIEFF, A. 1907. Die spermatogenese von *Blatta germanica*. *Arch. Microsk. Anat. Entwicklungsmech.* **70:**1-43.
30. WILSON, E. B. 1928. The Cell in Development and Heredity. Macmillan, Inc., New York.
31. WOODLAND, H. R. 1969. RNA synthesis during early development of the mouse. *Nature (Lond.)*. **221:**327-332.