Number of *Drosophila* flies collected at different times in a natural population near Madrid

Species	1969	1970					1971			1972				
	Dec.	March	April	June	July	October	March	June	July	April	June	July	August	Total
D. busckii	0	0	55	27	0	3	1	253	36	238	19	0	1	633
D. melanogaster	0	0	4	219	40	445	0	0	84	3	155	365	45	1.360
D. simulans	94	0	2	0	10	856	0	0	17	2	0	44	10	1.035
D. subobscura	322	217	115	2	0	2	35	0	54	153	17	3	0	920
Other*	47	17	16	1	1	9	1	2	9	14	–	–	2	119
Total	463	234	192	249	51	1135	37	255	200	410	191	412	58	4.067

* 7 other species were collected, with their total numbers given in parentheses: *D. phalerata* (67), *D. hydei* (14), *D. transversa* (13), *D. funebris* (12), *D. cameraria* (11), *D. confusa* (1) and *D. repleta* (1).

the relative abundance of *D. subobscura* is significantly negative ($r = -0.8239$, with 11 degrees of freedom, 95% confidence interval from -0.42 to -0.94). These correlations reflect the predominance of *D. melanogaster* in late spring and summer, and of *D. subobscura* in late autumn and winter. The other 2 species are associated with transition temperatures: *D. busckii* with ascending temperatures, *D. simulans* with descending ones.

Species abundance is often associated with temporal heterogeneity[3]. In the locality sampled in this study, it seems unlikely that migration plays a major role in determining species abundance, since the population is considerably removed from other habitats suitable for *Drosophila*. Changing local environmental conditions are the main factor responsible for the succession of relative abundance. It is suggested that seasonal changes may play a major role in making possible the existence of related species in the same habitat. The ability of different species to exploit common resources may change as the physical conditions of the habitat change.

1 I thank Dr F.J. Ayala for useful discussions and help in preparing the English text. I appreciate comments on the manuscript from Dr A. Prevosti and Dr A. Fontdevila.

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Chromatin circles in amphibian previtellogenic oocytes[1,2]

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Summary. Previtellogenic oocytes of *Odontophrynus americanus* display hundreds of chromatin circles. Electron microscopy of spread preparations of isolated nuclei shows that the circles originate from the chromatin. The circles change their morphology and form new copies. The length of the DNA packed in the nucleosomal circles is about 2.5–3.5 μm or multiples of this value. Assuming that histones need not be removed from chromatin before DNA replication[3] we suggest that the circles might belong to the process of rDNA amplification.

Eukaryotic chromatin consists of repeating units of DNA-histone complexes, the nucleosomes. The nucleosome core is formed by an octamer of histones (H_3, H_4, H_2a, H_2b) circumscribed by a DNA double helix containing 140 base pairs. This DNA segment describes $1\frac{3}{4}$ turns around the outside of the histone octamer, each turn consisting of 75–82 base pairs. The nucleosomes are interconnected by a DNA linker which is variable in length[4]. The H_1 histone is associated with the linker DNA and is implicated in the maintenance of higher-order chromatin structures[5–7].

The nucleosome is a dynamic structure as shown by crystallographical studies[4]. The findings that transcribing genes have altered nucleosomal configuration[8] clearly demonstrate the dynamism of the nucleosome. Regarding chromatin replication, it was found that histones do not need to be removed from chromatin segments before DNA replication, the nucleosomal pattern being rapidly restored afterwards[3]. In addition, some evidence has been found that the distribution of histones among daughter chromatins follows a conservative segregation pattern[9].

In this paper we describe a chromatin replication process in previtellogenic oocytes of *Odontophrynus* resulting in the formation of a high number of circles containing chromatin. The length of the DNA packed in the nucleosomal circles and the stage of meiosis where they occur, suggest that the mechanism could be related to the process of rDNA amplification.

Material and methods. The preparations were obtained from oocytes belonging to 10 females of the frog *Odontophrynus americanus* 4n = 44 (Ceratophrydidae). The previtellogenic oocytes have a diameter of 0.6 mm and a nuclear diameter of 0.2 mm (vitellogenic oocytes have a diameter of 1.3 mm and a nuclear diameter of 0.5 mm). Chromatin fibres were prepared through a modification of Miller's method[10]. The oocytes were isolated in 0.1 M KCl, then transferred to 5:1 of 0.1 M KCl + 10^{-3} M Ca $(NO_3)_2$ for 20 min. Each nucleus was put on to a drop of bidistilled water (pH 8.7, adjusted with sodium borate buffer) and allowed to swell for 15 min. The material collected on parlodium (2%) covered grids was fixed with 10% formalin in 0.1 M sucrose for 10 min. The grids, washed in water, were stained in ethanolic uranyl acetate (1:3 aqueous uranyl acetate plus 95% ethanol, 1 min). Some grids were treated with DNase I Sigma (50 μg/ml) 1 min before fixation. The electron micrographs were obtained in a Siemens Elmiskop I, 60 kV.

Results. The nucleoplasm of previtellogenic oocytes contains hundreds of chromatin circles. They measure ~ 100 Å

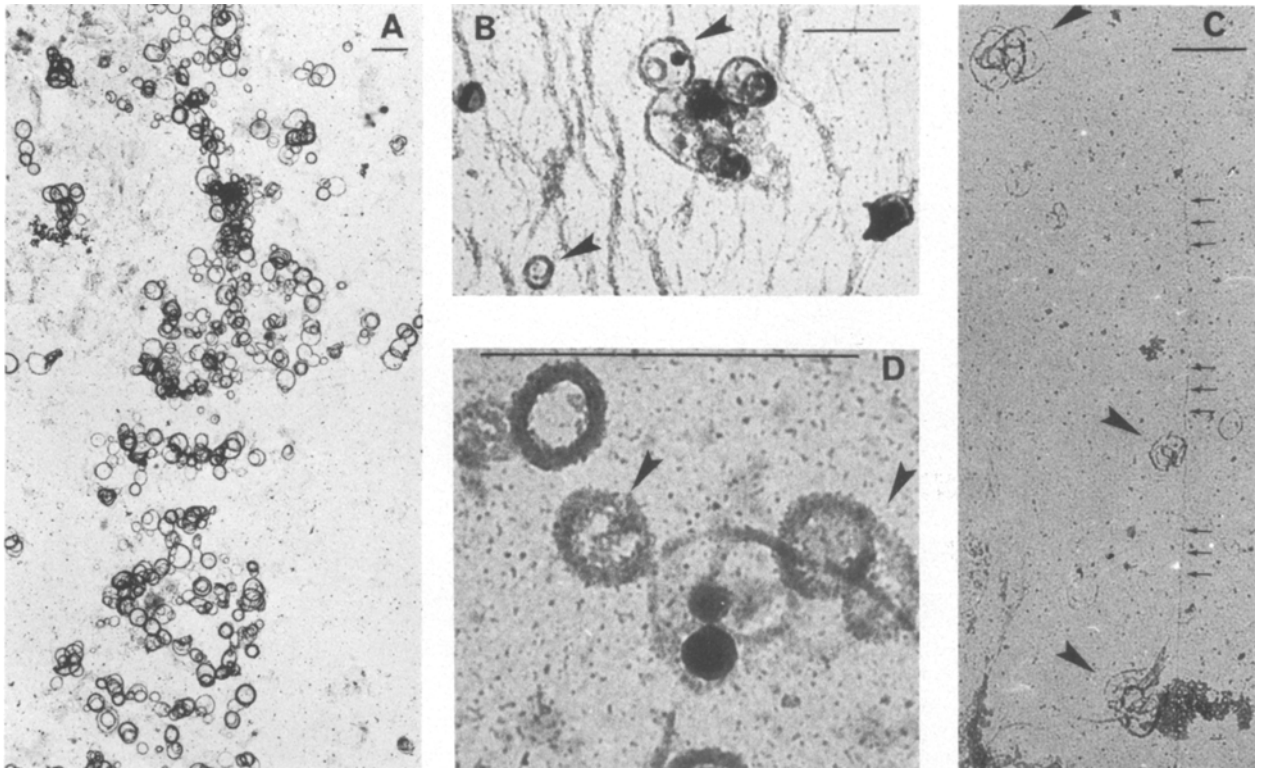

Fig. 1. Chromatin circles obtained from previtellogenic oocytes. *A* Free circles in the nucleoplasm; *B* circles (arrows) attached to chromatin fibres; *C* the upper circular structure (arrowhead) shows many turns of the chromatin which is beaded, 2 other interlocked circles (arrowheads) are attached at a chromatin fibre (arrows); *D* the bead aspect of the interlocked circles (arrows) revealed by DNase I treatment. Alcoholic uranyl acetate, bar = 1 µm.

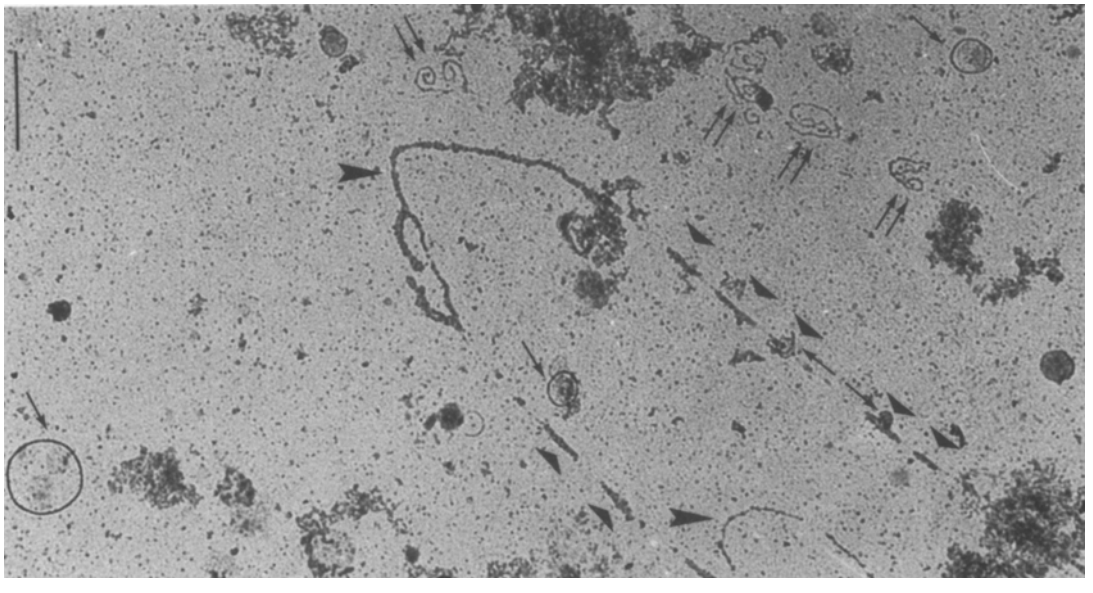

Fig. 2. 2 chromatin loops (arrowheads) the upper presenting double segments (triangles). Several circles (arrows) as well as coiled forms (double arrows) are present. Alcoholic uranyl/acetate, bar = 1 µm.

in width but can be larger in interlocked circles formed by several turns of the chromatin. There are also circles connected at their periphery to other circles (figure 1, a). The circular elements may occur attached to chromatin fibres (figure 1, b). The nucleosomal aspect of the circles is frequently obscured, especially in the interlocked forms (figure 1, a). When these structures are unravelled (figure 1, c) or treated by DNase I (figure 1, d) they show a globular aspect that we interpreted as being nucleosomes.

The circular structures emerge from double segments of the chromatin. When free in the nucleoplasm the circles are frequently seen as coiled filaments (figure 2). We observed that these coiled forms are sometimes formed by a long 'tail' attached at a circle. They change their morphology,

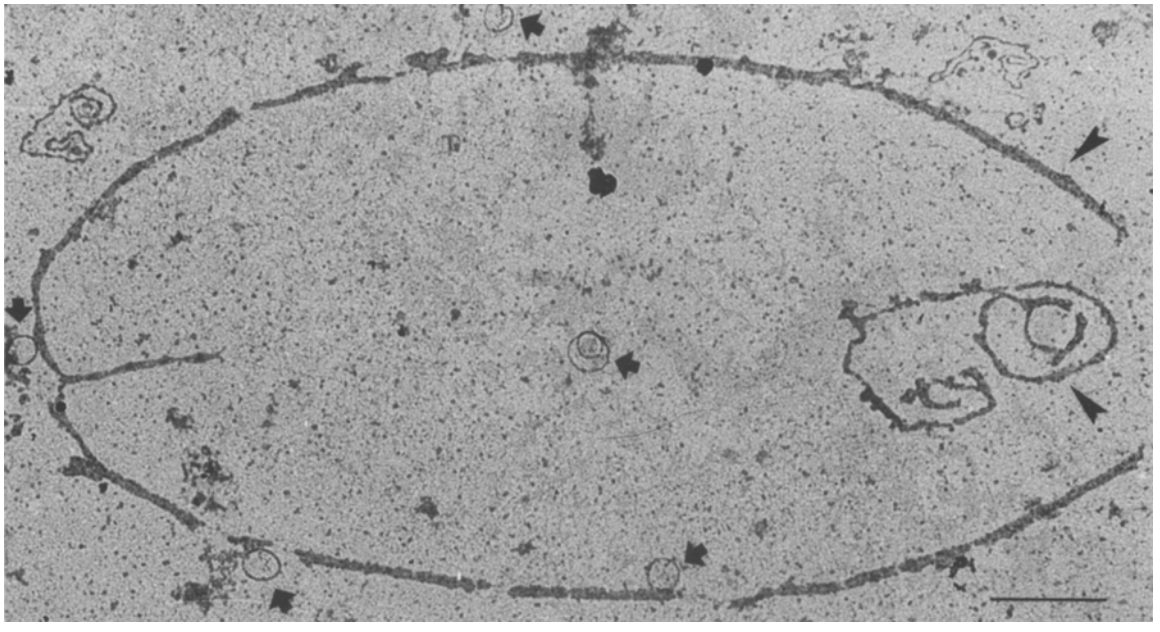

Fig. 3. 2 coiled filaments (arrowheads) the central presenting a long 'tail' joined to a circle. The filaments show altered morphology related with the presence of new circles (arrows). Alcoholic uranyl acetate, bar = 1 μm.

becoming wider and discontinuous, and forming the origin of new circles (figure 3). We ignore the question of whether these coiled structures are produced by the unfolding of interlocked forms or arise from changes in single circles.

The estimates based on DNA packing in a nucleosomal fibre[11] show that the circles contain DNA segments of ~ 2.5–3.5 μm in length, or multiples of this value. This data was obtained from 289 circles belonging to 3 nuclei.

The process described evolves synchronously during the pachytene, each oocyte presenting a distinct aspect of the same mechanism.

Discussion. The circular structures described were interpreted as chromatin-containing circles which can be single or interlocked. The presence of nucleosomes in these configurations is a question about which the evidence is conflicting. They were observed in some unravelled forms and also after DNase I treatment, but the lack of beads was also verified in many circles. The non-appearance of well-individualized nucleosomes can be explained by the difficulty of recognizing them in interlocked circles, or because they might be altered in structure. The interlocked rings were interpreted as being produced by continuous turns of the chromatin wrapped around the same circle. Similar doughnut-shaped configurations produced by in vitro DNA-H_1 associations have already been reported[12]. We used near-physiological salt solutions known to preserve H_1 histone[5,7]. It is therefore possible that H_1 histone plays a role in joining the turns of the chromatin in the interlocked circles.

The observations on the way the circles come directly out of the chromatin and on their paths in the nucleoplasm indicate that we are probably dealing with a replication process in DNA-histone fibrils. This idea clearly supports the findings on maintenance of histones in replicating chromatin fibre[3]. Obviously many interpretations can be formulated to explain the mechanism reported. The length of the DNA contained in the circles as well as the stage of meiosis in which they occur suggest that we are dealing with the beginning of rDNA amplification. Similar chromatin circles, although at a lower frequency (10–50 per nucleus), have also been described in spermatocytes of the same species[11]. They were assumed to contain rDNA. Nevertheless formation of new circles was not observed in the nucleoplasm of the spermatocytes. The presence of nucleosomes in extrachromosomal rDNA circles has already been reported in previtellogenic oocytes[13]. The structures described in this paper are different, having interlocked rings with obscured nucleosomes and being able to originate new circles. These differences may result from the methods used or might be considered as distinct structural aspects found in the initial stages of amplification.

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