

Species	2n
Hylidae	
Nyctimystinae	
<i>Litoria nasuta</i>	26
<i>Litoria chloris</i>	26
Leptodactylidae	
Cycloranae	
<i>Cyclorana alboguttatus</i>	26
<i>Mixophyes fasciolatus</i>	24
<i>Adelotus brevis</i>	24
<i>Limnodynastes dumerili</i>	22
<i>Limnodynastes terraereginae</i>	22
<i>Limnodynastes salmini</i>	22
<i>Limnodynastes ornatus</i>	22
Myobatrachinae	
<i>Ranidella parinsignifera</i>	24
<i>Taudactylus diurnus</i>	24
(incertae sedis)	
<i>Rheobatrachus silus</i>	24

terminalized chiasmata (usually 12 'ring' bivalents) as in the advanced families of the order⁸; since we have found a small number of spermatocyte metaphase plates showing a few dot-like univalents, it is possible that the microchromosomes of *Rheobatrachus*, like those of *L. hochstetteri*^{10,11}, are supernumerary chromosomes.

Karyological research on the Australian Leptodactylidae is still in a preliminary stage and, as we have seen, it is capable of providing cytotaxonomically interesting results. In particular, if new research confirms the peculiarity of the karyotype of *Rheobatrachus* within the Australian Anurans, then the presence of this primitive frog in an old Queensland refuge belt would be of relevance in the zoogeographical problems regarding the origin and radiation of the leptodactylid frogs, given that it could represent a relict from some stock karyologically intermediate between an ascaphoid and a leptodactylid (or pelobatoid) condition¹².

Summary. Fra i Leptodactylidi Australiani, *Cyclorana alboguttatus* ha $2n = 26$ come le forme più generalizzate della famiglia, mentre alcune specie di *Limnodynastes* hanno $2n = 22$; specie di altri 5 generi hanno $2n = 24$. Fra questi ultimi, il problematico *Rheobatrachus*, completamente acquatico, possiede alcuni microcromosomi come i membri più primitivi dell'ordine.

A. MORESCALCHI and G. J. INGRAM

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Istituto di Istologia ed Embryologia, Via Mezzocannone 8, I-80134 Napoli (Italy), and Queensland Museum, Brisbane (Australia), 3 April 1974.

Incorporation of Thymidine into the Chromosomes of Aphid (*Myzus persicae*) Embryos

In parthenogenetic female aphids, embryogenesis starts before the mother is born¹. Only a few days later, at the final moult, the maternal haemocoel contains 60–70 embryos, the oldest of which are usually fully-formed and ready for independent existence. Apart from the classical work on aphid embryology (reviewed by HAGAN²), and some more recent descriptive work^{3,4}, relatively little is known of this extremely rapid reproductive and developmental process which is a principal reason for the considerable importance of aphids as pests.

Regular ovulations take place from the germaria of the ovarioles, until each ovariole comes to contain a sequence of embryos at different development stages. The newly extruded oocyte is apparently supplied with a stream of nutrients during its initial growth phase by nurse cells in the anterior part of the germarium, but as the young embryo moves down the ovariole and further ovulations occur, this nutrient supply is presumably cut off and the later growth of the embryo, still continuing rapidly, must depend on provision of nutrients directly from the maternal haemocoel, through the follicular epithelium and the embryonic membranes. An indication of the probable nutritive function of the follicular epithelium is the fact that it persists until after the cuticula is formed in late embryonic development. However, the physiological mechanisms involved in what must be an extremely efficient provision of food materials to the developing embryo have not been investigated.

During the course of cytological studies on the chromosomes of *Myzus persicae* (Sulzer) attempts were made to label the chromosomes autoradiographically with tritiated thymidine so that they could be separately identified.

The principal method used provided information on the uptake of thymidine from the maternal haemocoel by young embryos.

Material and method. Preparturition apterous adults of *M. persicae* were anaesthetized by brief exposure to ether vapour and placed with the ventral surface uppermost in a small drop of water on the stage of a binocular microscope. About 0.5 μ Ci of tritiated thymidine (Amersham, specific activity 2000 mCi/mM) was injected with a fine glass needle through a thoracic intersegmental membrane into the haemocoel of each aphid. Care was taken to insert the tip of the needle only just below the cuticle to avoid damaging any embryos.

Aphids recovered from injection after 15–20 min and mortality was negligible. They were left on a potato leaf at 20°C for an interval ranging from 30 min to 3 h after injection. They were then dissected in 22% acetic acid and chromosome preparations made of the youngest embryos by a rapid Feulgen squash technique⁵. The embryos used were estimated to be between the 3rd and 6th day of their development. After freezing off the coverslip the cells on the slide were either covered with Kodak AR-10 stripping film or dipped in diluted Ilford K2

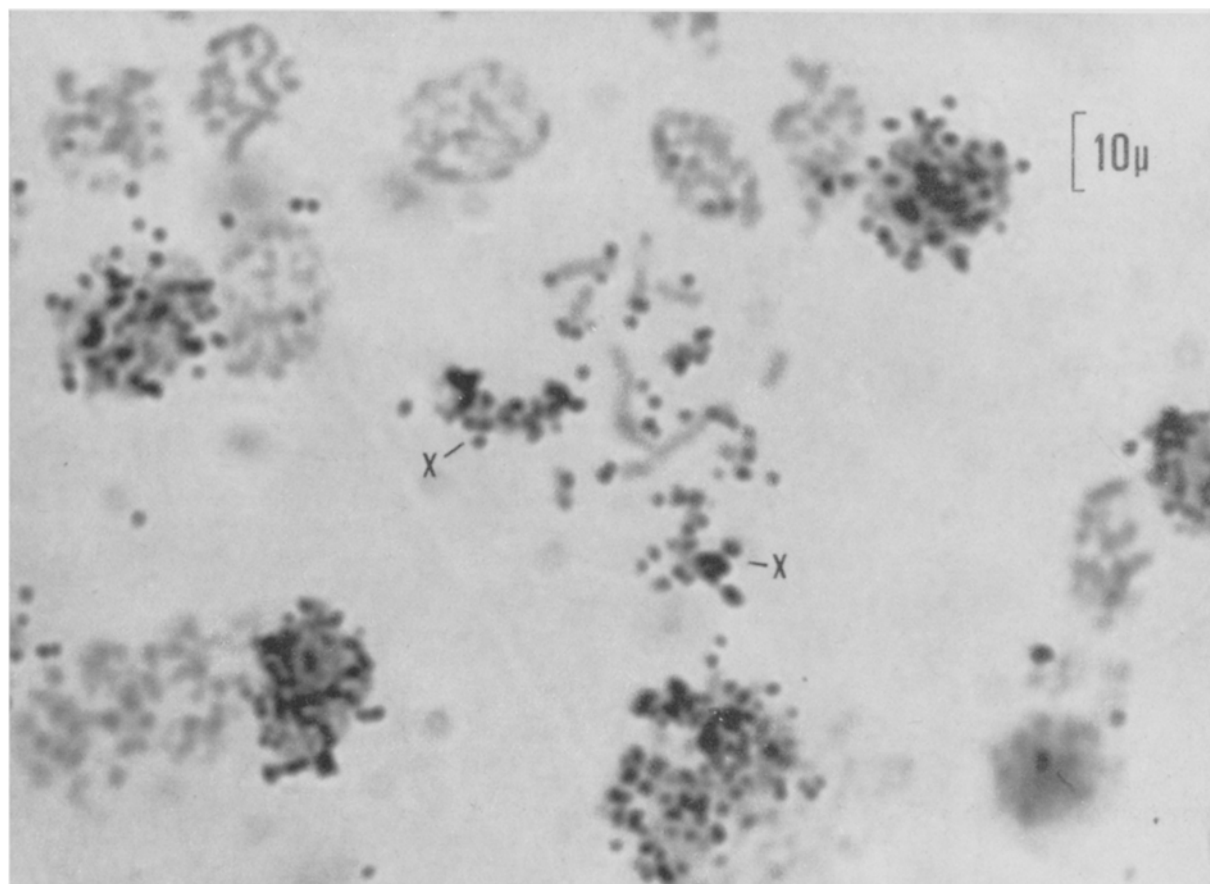
¹ L. B. UICHANCO, Philipp. J. Sci. 24, 143 (1924).

² H. R. HAGAN, *Embryology of the Viviparous Insects* (Ronald Press, New York 1951).

³ S. BRUSLÉ, Bull. Soc. zool. fr. 87, 396 (1963).

⁴ C. Y. OSETO and T. J. HELMS, Ann. ent. Soc. Am. 64, 603 (1971).

⁵ M. D. MACDONALD and A. M. HARPER, Can. J. genet. Cytol. 7, 18 (1965).



Autoradiograph of somatic metaphase of embryo of *M. persicae* fixed 90 min after injection of ^3H -thymidine into the maternal haemocoel. The exposure to Kodak AR-10 emulsion was for 3 days. The X-chromosomes are heavily labelled.

liquid emulsion. Only 1 to 3 day's exposure at 4°C was necessary before development in D19 for 3–5 min at 20°C .

Results and discussion. Many interphase nuclei were heavily labelled only 30 min after injection of thymidine into the maternal haemocoel. Chromosomes in metaphase were lightly labelled after $1\frac{1}{2}$ h, and heavily labelled metaphases were present 3 h after injection. Both X-chromosomes showed evidence of late replication. The labelling pattern appeared to be homozygous (Figure). Grain counts were not adequate for identification of late-labelling segments of autosomes, because of the small size of the chromosomes and the rather low percentage of clearly spread and labelled metaphases. Attempts to use colchicine to block mitosis were unsuccessful because even a low concentration caused excessive contraction of chromosomes.

The uptake of thymidine into the young embryos was almost immediate and probably took place directly through the follicular epithelium and embryonic membranes. Incorporation into the embryos by way of the ovarian nurse cells, which also take up thymidine⁶, appears to be ruled out by the very short period of time involved, even if a passage for nutrients to the developing embryos remained open within the ovariole. If thymidine can pass into the embryos so quickly then it seems likely that active uptake of amino-acids and other nutrients takes place from the maternal haemocoel. Further studies with other labelled compounds, either alone or in con-

junction with metabolic poisons to prevent active uptake, would be informative⁷.

Résumé. Les noyaux somatiques des embryons du puceron *Myzus persicae* prirent la marque de la thymidine H^3 30 min après son injection dans l'hémocoel maternelle. L'absorption des molécules de cette substance à travers l'épithélium folliculaire et les membranes de l'embryon fut donc très rapide. Le marquage des métaphases s'est produit après 90 min. Dans les chromosomes X, l'incorporation fut nettement tardive.

R. L. BLACKMAN

*Department of Entomology, British Museum
(Natural History), Cromwell Road, London SW7 5BD
(England), 17 May 1974.*

⁶ E. ORLANDO, *Caryologia* 20, 217 (1967).

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