Calcium and magnesium antagonism with caffeine in cytokinesis inhibition

Treatments	Mononucleate cells			Binucleate cells			Binucleate (%)		
	0.04	0.06	0.10	0.04	0.06	0.10	0.04	0.06	0.10
Control	35.500	18.446	14.190	850	867	823	2.45 ± 0.08	4.71 ± 0.15	5.87 ± 0.19
10 mM Ca^{++}	15.833	8.028	4.385	190	289	228	1.31 ± 0.08	3.60 ± 0.20	5.21 ± 0.33
10 mM Mg^{++}	5.213	6.606	6.480	75	218	423	1.30 ± 0.15	3.21 ± 0.15	6.06 ± 0.29
Ca^{++} + Mg^{++} (10 mM)	20.500	4.454	3.379	106	98	196	0.52 ± 0.05	2.23 ± 0.21	5.86 ± 0.40

For cytological analysis, the roots were fixed in 3:1 ethanol-acetic mixture and the specimens prepared by staining the roots with acetic orcein according to the technique of Tjio and Levan[14]. Every treatment was studied in at least 2 bulbs and every root-tip was studied by scoring about 1000 meristem cells at random.

As binucleate cells were considered all cells with 2 nuclei sharing a common cytoplasm, in spite of the presence of partial cell plates.

Results and discussion. Caffeine efficiency in cytokinesis inhibition. With onion roots, a steady state of growth can be obtained under controlled conditions, characterized by a constant growth rate, a reliable cycle time and a mitotic index which remains steady over a long time period[9]. To estimate the caffeine efficiency, roots in such a steady state were incubated in different drug concentrations and the results are summarized in the figure. As a consequence, the concentration 0.04% (about 2 mM) of caffeine has been considered the 50%-inhibition-dose. Very low concentrations, below 0.02%, do not induced binucleate cells, while higher concentrations such as 0.08 or 0.1% show the maximum efficiency.

Caffeine-calcium antagonism. According to Paul and Goff's hypothesis, caffeine must inhibit cytokinesis by interfering with intracellular calcium, and we expected its competition to be detected easily by testing with 50%-inhibition-doses. Our results are recorded in the table, where the reduction in caffeine efficiency induced by calcium is remarkable at concentration 0.04% and negligible at 0.1% caffeine. It is logical to assume that the calcium concentration required to reverse the 0.1% caffeine solution must be too high to be compatible with in vivo assays.

Caffeine-magnesium antagonism. Similar results were obtained with magnesium nitrate, 10^{-2} M, and both cations appear to have a synergic effect (table), for the presence of both in the culture medium hardly depreses the caffeine efficiency at 0.04% to about 20% of control binucleate cell production.

On the other hand, the presence of any cation does not significantly modify the induction of binucleate cells when the roots are incubated in caffeine at 0.1%. This steady efficiency at this caffeine concentration strongly discounts the effects of cations as a general depression of the mitotic rate, for in this case the binucleate cell production at any caffeine concentration would also necessarily decline.

In short, we conclude that the effect of caffeine on cytokinesis may be modified by calcium and magnesium, especially at threshold concentrations of caffeine. We propose that both cations must play a role in plant cytokinesis, probably related with the fusion of Golgi vesicles to give rise to the new cell wall. The intracellular concentrations must be adequate under control conditions, but insufficient in the presence of caffeine at certain concentrations.

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An ammoniacal silver staining technique for mitotic chromosomes of *Triturus* (Urodela: Salamandridae)[1]

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Summary. An ammoniacal silver staining technique was applied to mitotic metaphase chromosomes of 2 species of Newts (Triturus). The method is useful for identifying nucleolar organizer regions. In addition, it reveals other sites of unknown significance.

Two procedures for a differential staining of certain chromosome regions have recently been proposed by Howell et al.[2] and Goodpasture and Bloom[3]. The first is an ammoniacal silver technique, designated AS-SAT, which stains the satellite regions of D and G group human chromosomes. The second one is a simpler ammoniacal silver staining reaction, designated Ag-AS, which stains differentially the nucleolus organizer regions (NORs) of some mammalian species. We tried both techniques on the mitotic chromosomes of Triturus to improve our knowledge of karyotypic events involved in evolution and speciation mechanisms within the genus[4].

Material and methods. 30 larvae and 5 males from Pisa and a couple from the River Sarno, near Pompeii, of Triturus vulgaris meridionalis (Boulenger, 1882) as well as 10 lar-

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vae and 5 males from Pisa of T. cristatus carnifex (Laurenti, 1768) were studied. The animals were previously treated with colchicine 0.3% for 24 h. Larvae, guts and testes excized from the adults were kept in distilled water for 10 min and then fixed in absolute ethanol: glacial acetic acid 3:1 for 30 min. The cytological preparations were made following the 'dry-ice method' and then stained according to either of the 2 above-mentioned techniques[2,3]. The best results were obtained by following the procedure proposed by Howell et al.[2] with a denaturation treatment of only 2 min. This technique does not, however, produce uniform results, since either too pale or overstained cells are always present in a given preparation beside properly stained mitotic metaphases.

Results and discussion. In T. vulgaris meridionalis, the chromosomes appear golden-yellow to light orange. Their pericentric regions, which are heterochromatic in larvae treated by cold[5] and which appear as C-bands in adults[6], are faintly stained. By contrast the NORs, previously identified on chromosome XI[7], are differentially marked by a subterminal black dot (figures 1 and 2,a, b and c). This localization seems to correspond to the sites of the genes for 18S + 28S rRNA determined by in situ molecular hybridization[8,9]. Besides the NOR, some AS-SAT additional sites are sometimes present: their number and localization tend to be constant in a given specimen, but they may vary between individuals. Figures 1 and 2,b show mitotic complements of a male from Pisa with both the NORs on the 2 homologues XI and 2 AS-SAT additional sites on only 1 homologue of one of the largest metacentrics, the site present on the shorter arm being more conspicuous. 2-min intercalary granules are present on the long arm of both homologues VII, but only in part of the cells (figure 1).

The karyological picture of a female collected in the River Sarno was peculiar: the mitotic complement has constantly shown a stained NOR on only 1 homologue XI, 2 intercalary additional sites on the long arm of the same chromosome but not on its homologue, and 1 site also on 1 homologue VIII (figure 2,c). In spite of the occurrence

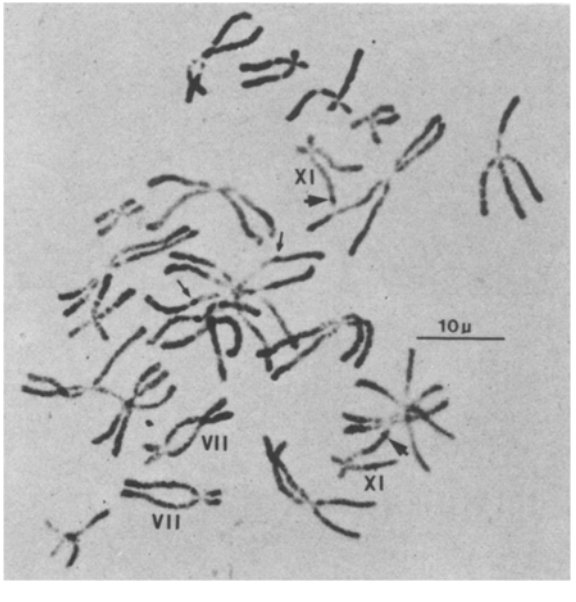

Fig. 1. The 24 mitotic chromosomes of T. v. meridionalis. The 2 subterminal NORs on chromosomes XI are indicated by →; the additional sites on one large metacentric are indicated by → (male from Pisa; gut; AS-SAT technique).

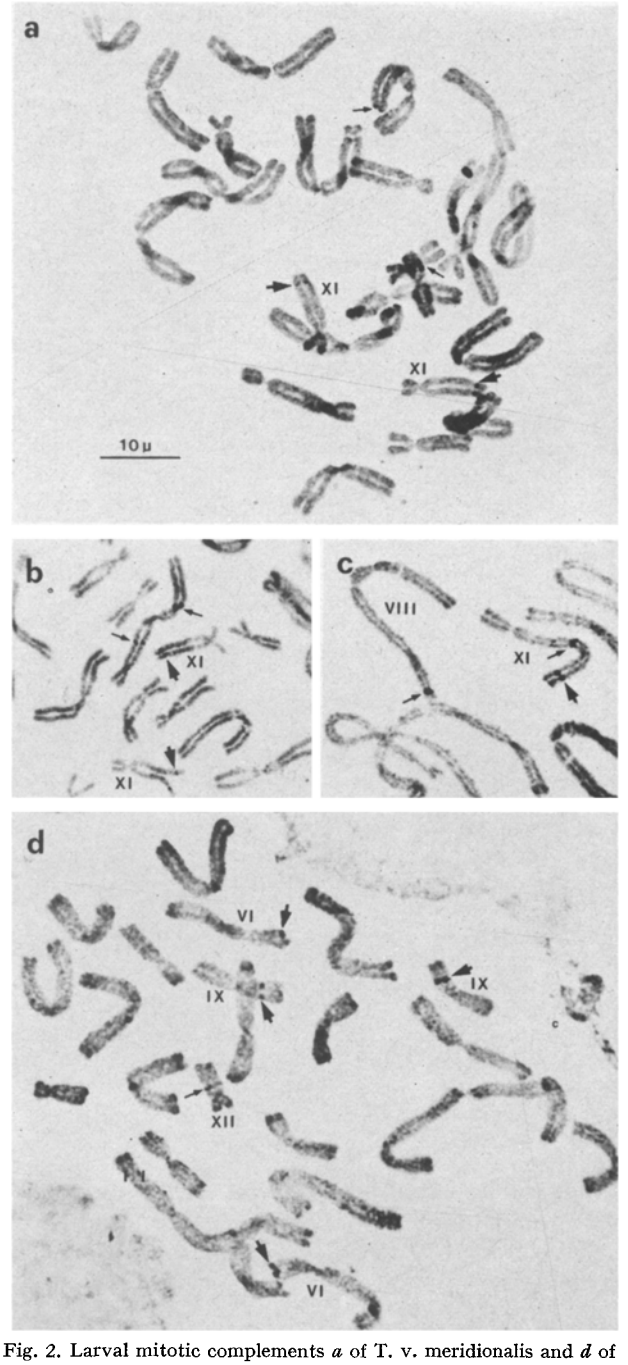

Fig. 2. Larval mitotic complements *a* of T. v. meridionalis and *d* of T. c. carnifex. *b* Part of the mitotic complements of the same specimen as in figure 1 and *c* of a female of T. v. meridionalis from the River Sarno. NORs are indicated by →; additional sites are indicated by → (AS-SAT technique).

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of only 1 NOR, this specimen cannot be regarded as heterozygous for this region, since the relationship between functionality of the nucleolus organizers and their visualization induced by the ammoniacal silver staining technique must be further verified.

T. cristatus carnifex seems to share the above-mentioned features. When its chromosomes are treated by the AS-SAT technique, the 2 NORs (subterminally on the short arm of chromosome VI, and, in intercalary position, on the short arm of chromosome IX[10-12]) clearly show a black coloration; some AS-SAT additional sites are also evident, for instance on chromosome XII (figure 2,d).

The procedure proposed by Howell et al.[2] for humans, thus appears to be of cytotaxonomic interest in Salamandrids, since the technique is particularly effective in revealing the number and localization of NORs, and of possible additional sites, in those species where they are still unknown.

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11 G. Barsacchi Pilone, I. Nardi, R. Batistoni, F. Andronico and E. Beccari, Boll. Zool. *41*, 456 (1974).
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An empirical relationship between the prey density and predatory efficiency of *Gambusia affinis*

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Summary. Larval density of Culex fatigans essentially influences the predatory rate of the mosquito-fish Gambusia affinis. The feeding rate of the fish gradually increases up to a maximum value at intermediate prey densities beyond which there is an asymptotic saturation in the feeding rate. The relationship is represented by an empirical equation which helps in the prediction of the ideal stocking rate of the fish in natural systems.

It is well known that predation by the fish Gambusia affinis alters the number and composition of populations of mosquito larvae[1]. In a predator-prey system, the functional responses of either species are considerably influenced by the properties of the other component[2]. Early theories of trophic ecology are based on the assumption that predatory rates are proportional to prey abundance[3]. Such assumptions have little significance in forecasting the success of predation as well as the stocking rate of the predator in natural systems. So far, workers in the field of larvivorous control of mosquitoes have taken the area of the habitat as an index for stocking predatory fishes[4]. More important than this, would be the study of interrelationship between the predator-prey properties, to arrive at the ideal stocking rate of the biological control agent. The present paper elucidates the influence of density of Culex fatigans larvae on the predatory rate of G. affinis.

Material and methods. Gambusia affinis collected from the Bellandur tank (near Bangalore) were grouped into males, non-gestating females and gestating females depending on their sex and physiological state. 5 individuals of similar body weights (male: 151.3 ± 12.94; non-gestating female: 160.3 ± 7.07 and gestating female: 249.2 ± 11.94 mg) were selected from each group and kept in separate aquaria (surface area: 625 cm^2) containing 1 l of aerated freshwater. The fish were starved for 3 days prior to the start of the experiment to elicit hunger in them[5]. The prey consisting of live 4th instar Culex fatigans larvae, were offered to these experimental individuals in different densities (5, 10, 25, 50, 75, 100, 125, 150 larvae/625 cm^2). The fish in each group/at each density level were allowed

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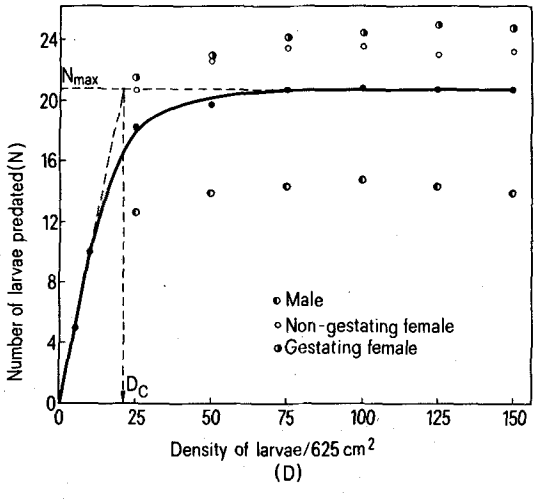

Fig. 1. Relationship between the density of 4th instar Culex fatigans larvae and the predatory efficiency of Gambusia affinis.

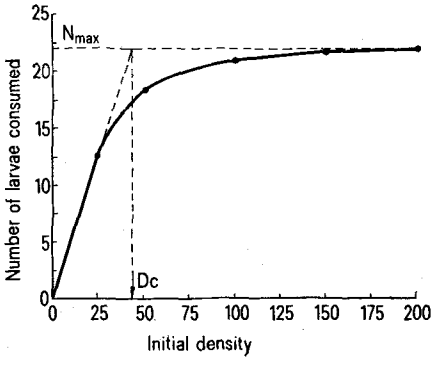

Fig. 2. Predatory efficiency of Mesogomphus lineatus at different larval densities[7].