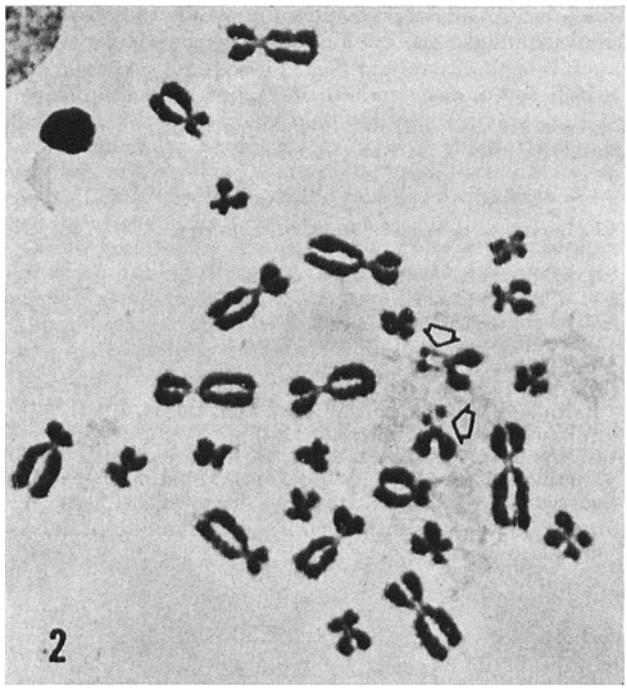

Fig. 2. Intestinal metaphase plate of a ♂ of *Pelobates cultripes*. The arrows indicate the chromosomes provided with heterochromatic areas. Mayer's acid hemalaun.

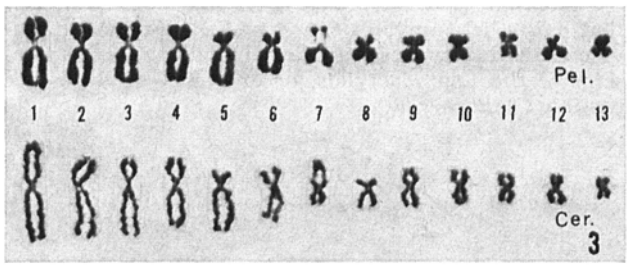

Fig. 3. Comparison between the genomes of the 2 species, reconstructed schematically by using a single homologue of each pair of the set shown in Figures 1 and 2.

The phyletic significance of the vast and perhaps heterogeneous grouping of the Leptodactylids is under discussion today; this grouping seems older than was previously thought and some authors consider that it is here that the differentiation arises in the forms that have produced the Anuran of the more highly evolved families (LAURENT[7]; REIG[4]; HECHT[6]).

Within the Leptodactylids, *Ceratophrys* appears as a primitive karyological form, the relationships of which with *Pelobates* seem extremely interesting, particularly in view of the fact that the derivation of the *Pelobatidae* from the stock of the *Discoglossidae* seems karyologically improbable, contrary to the commonly held view: according to the karyological data collected by myself[8], the *Discoglossidae*, together with the *Ascaphidae*, could constitute a branch of Anuran that had become differentiated from the remaining families at an early period and had evolved along lines of its own[9].

Riassunto. Il cariotipo di *Ceratophrys calcarata* (*Leptodactylidae* o *Ceratophrydae*) consta di 26 cromosomi, 12 grandi e 14 più piccoli; il settimo paio mostra zone eterocromatiche. Il genoma di *Ceratophrys* presenta una stretta somiglianza con quello di *Pelobates cultripes* (*Pelobatidae*); l'A ritiene che questo fatto possa fornire utili indicazioni sulle relazioni tassonomiche dei *Ceratophrydae*.

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Istituto di Istologia ed Embriologia dell'Università di Napoli (Italy), 26 July 1967.

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Antigenic Relationship between the Cysts and Trophozoites of *Entamoeba invadens*

The antigenic relationship of the trophozoites of various species of *Entamoeba* has been studied previously using the gel-diffusion technique[1], the fluorescent-antibody technique[2] and the immobilization reaction[3]. However, there is no information on the antigenic relationship between the cysts and trophozoites of the same species. This is an initial report of such a study in which the fluorescent-antibody technique has been used to study the surface antigens of the 2 main stages in the life cycle of *E. invadens*.

The strain of *E. invadens* (BC) used in this study encysts in large numbers, 5–6 days after subculture, when grown in Difco's Entamoeba medium[4]. To prepare the antigens, the cysts were washed 3 times in sterile distilled water by centrifugation and stored at 5 °C overnight. The process was repeated daily for 3 days until a pure suspension of cysts, completely free of trophozoites, was obtained. The cysts were transferred to physiological saline and injected into rabbits for immunization. The immunization schedule consisted of 4 injections given i.v. at 7-day intervals. In total, approximately 1 million cells were injected per animal.

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The sera, taken 7 days after the last injection, were pooled and used for conjugation. The globulin fraction of the serum was conjugated with fluorescein isothiocyanate on celite[5] according to the method described by RINDERKNECHT[6]. The unconjugated dye was removed by passing the serum through a column of Sephadex G25[7]. The serum was then absorped twice against rabbit liver powder before use.

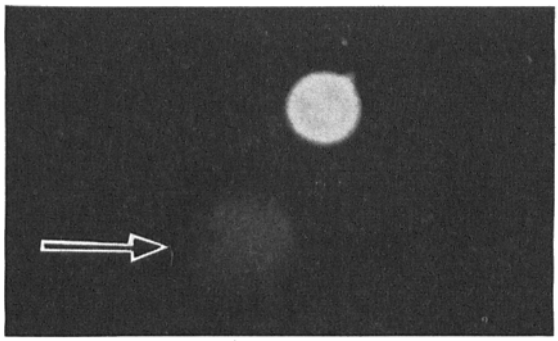

Fig. 1. The difference in the intensity of fluorescence between the cysts and the trophozoites after treatment with anti-cyst serum. The trophozoite is indicated by an arrow.

Fig. 2. Arrow marks the trophozoite emerging from a large aperture in the cyst which has been treated with anti-cyst serum. The remaining cyst wall is brightly fluorescent but the trophozoite shows negligible fluorescence.

The cysts fluoresced brightly after being treated with anti-cyst serum for 30 min in a serum dilution of 1:5 made with physiological saline. This reaction has been described previously[8] and is a true antigen-antibody reaction as it does not occur with normal serum and is inhibited by pre-treatment of cysts with unconjugated anti-cyst serum.

The trophozoites of *E. invadens* also showed some fluorescence when suspended in anti-cyst serum in a dilution of 1:5. However, it was possible to modify this reaction by absorping anti-cyst serum against a heavy suspension of trophozoites. The absorped sera then produced a negligible fluorescence of trophozoites but continued to produce an intense fluorescence of the cysts. Figure 1 shows the difference in the intensity of fluorescence between a cyst and a trophozoite after being treated with anti-cyst serum. In some cases excystation of the amoeba occurred during the treatment with anti-cyst serum and Figure 2 shows a trophozoite emerging from a large aperture in the cyst. The trophozoite shows negligible fluorescence but the remaining cyst wall is brightly fluorescent. This study indicates that the surface antigens of the cyst are different from those of the trophozoites and that this difference is recognizable by the fluorescent-antibody technique. Investigations are at present in progress to compare the internal antigens of the cysts with that of the trophozoites using the gel-diffusion technique.

Résumé. La technique des anticorps fluorescents a été appliquée à l'étude de la relation antigénique des cystes et des trophozoïtes d'*Entamoeba invadens*. Le sérum anticyste préalablement absorbé par les trophozoïtes a montré chez ces derniers une fluorescence négligeable mais il continua a produire une fluorescence intense chez les cystes, ce qui indique que les antigènes de surface des cystes sont différents de ceux des trophozoïtes.

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The Implications of the Temperature-Independent Binding and the Temperature-Dependent Action of Interferon

A number of vertebrate systems have been shown to respond to virus infection by producing interferon[1]. Interferon is the name given to a group of proteins which are capable of initiating the development of an antiviral state in cells of the appropriate species. Many studies have shown that interferon itself is not directly antiviral. Rather, interferon induces the formation of an antiviral state in treated cells. This induction is known to require both DNA dependent RNA synthesis and protein synthesis[2,3], but its details are not known. In fact, even the nature of initial interaction between cells and interferon is unclear. BARON and BUCKLER were unable to show that the induction of an antiviral state by interferon involved detectable loss of interferon from the medium[4]. This raised the question of whether interferon binding or

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