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PRELIMINARY NOTE ON THE GENETIC ISOLATION OF *CIONA* SPECIES (ASCIDIACEA, UROCHORDATA)

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CIONA SPECIES
ASCIDIACEA
CROSSES
GENETICS

ABSTRACT – *Ciona intestinalis* is a solitary ascidian found in virtually all seas of the world. *C. savignyi*, originally described from Japanese seas is now also found in Long Beach and Los Angeles Harbors (California, USA). *C. edwardsi* and *C. roulei are* presently known from the Mediterranean Sea where they occur at deeper depths than *C. intestinalis*. We have removed sperm and eggs from *C. intestinalis* and *C. savignyi* collected from Long Beach Harbor and attempted heterologous and homologous fertilizations (homologous fertilizations used sperm and eggs from different individuals). Normal development was obtained from the homologous crosses but none of the eggs cleaved from the heterologous crosses in either direction. Similar crosses were attempted with *C. intestinalis*, *C. edwardsi* and *C. roulei* collected from the Mediterranean near Banyuls-sur-Mer (France). All of the homologous fertilizations resulted in normal cleavage but none of the six possible heterologous fertilizations resulted in cleavage except *C. intestinalis* sperm crossed with *C. roulei* eggs. The reciprocal cross failed to develop. From these studies we conclude that these species of *Ciona* are genetically isolated where they co-occur.

ESPÈCES DE *CIONA*
ASCIDIACEA
CROISEMENTS
GÉNÉTIQUE

RÉSUMÉ – *Ciona intestinalis* est une Ascidie solitaire qui vit dans pratiquement toutes les mers du monde. *C. savignyi*, originellement décrite des mers japonaises se rencontre actuellement aussi dans les ports de Long Beach et de Los Angeles (California, USA). *C. edwardsi* et *C. roulei* sont actuellement connues de la mer Méditerranée où elles apparaissent à des profondeurs supérieures à celles de *C. intestinalis*. Nous avons recueilli le sperme et les oeufs de *C. intestinalis* et *C. savignyi* récoltées dans le port de Long Beach, et tenté de réaliser des fécondations hétérologues et homologues (pour les fécondations homologues nous avons utilisé le sperme et les oeufs de différents individus). Les croisements homologues ont donné un développement normal chez les deux espèces dans l'une et l'autre direction. Des croisements similaires ont été tentés avec *C. intestinalis*, *C. edwardsi* et *C. roulei* récoltées en Méditerranée dans la région de Banyuls-sur-mer (France). Toutes les fécondations homologues ont donné des segmentations normales mais aucune des six fécondations hétérologues possibles n'a donné de divisions, excepté le sperme de *C. intestinalis* croisé avec les oeufs de *C. roulei*. Le croisement réciproque n'a pas été suivi de développement. Nous concluons de ces études que ces espèces de *Ciona* sont génétiquement isolées là où elles vivent conjointement.

INTRODUCTION

Ciona is a genus of large conspicuous solitary ascidians which while of global distribution contains only a few species. Opinions concerning speciation in this wide-spread genus have varied through time. *C. intestinalis* (Linné, 1767) is found in virtually all the seas of the world and has been widely used in physiological and developmental research. In the Mediterranean *C. intestinalis* is widely distributed. *C. roulei* Lahille, 1890 is rarer but clearly distinguishable on morphological grounds (Harant and Vernières, 1933). *C. edwardsi* (Roule, 1886), originally described from the Mediterranean, was synonymized with *C. intestinalis* (Harant and Vernières, 1933) but subsequently shown to be a distinct species based upon the structure of the branchial basket and very large egg size (Copello, Devos and Lafargue,

1981). All three species can be collected from the Mediterranean in the region around Banyuls-sur-Mer, France (Fiala-Médioni, 1974). *Ciona intestinalis* is generally found at shallower depths than *C. roulei* and *C. edwardsi*.

In addition to the ubiquitous *C. intestinalis*, Japanese waters also contain dense populations of *C. savignyi* Herdman, 1882 (Hoshino and Nishikawa, 1985). In their reconsideration of the genus Hoshino and Nishikawa (1985) concluded that *C. edwardsi* was indistinguishable from *C. intestinalis* and suggested that *C. intestinalis* and *C. savigny* were the only species present in temperate onshore waters of the world. We have now found that *C. savignyi* co-exists with *C. intestinalis* in Long Beach Harbor, California. Hybridization was attempted between the California species and also between the Mediterranean species. The preliminary evidence from these *in vitro* studies shows that all of the species are genetically isolated where they co-occur. Based on this evidence we suggest that there are actually a total of four *Ciona* species found in temperate near shore habitats.

MATERIALS AND METHODS

C. intestinalis and *C. savignyi* were collected from floating docks in Long Beach (Los Angeles County, California) and held at 15 °C until used. *C. intestinalis*, *C. edwardsi* and *C. roulei* were collected in the Mediterranean Sea by SCUBA near the Laboratoire Arago in Banyuls-sur-Mer, France. Eggs and sperm were removed from the gonoducts by dissection and the eggs washed several times in filtered sea water. Sperm were stored «dry» on ice until diluted just before use. Fertilizations were performed at 23 °C using heterologous and homologous inseminations using gametes from two different individuals. Development was assessed at 50 minutes when homologously fertilized controls had divided.

RESULTS

California Species

The eggs of the two species are similar in size and morphology. Neither the *C. intestinalis* sperm x *C. savignyi* eggs nor *C. savignyi* sperm x *C. intestinalis* eggs resulted in cleavage, although homologous fertilizations between two individuals yielded essentially 100 % normal development.

Mediterranean Species

The eggs of *C. intestinalis* and *C. roulei* are quite similar in appearance and about half the diameter of *C. edwardsi* eggs which are about 200 µm in diameter exclusive of the vitelline coat (chorion). Furthermore the follicle cells of *C. edwardsi* eggs have up to 6 birefringent bodies while the other 3 species each have only a single birefringent body. All of the homologous inseminations gave high proportions of normal two cell stages. All heterologous crosses yielded no hybrids except *C. intestinalis* sperm x *C. roulei* eggs, which developed into normal appearing tadpoles. The reciprocal cross failed to fertilize.

DISCUSSION

Most ascidian species have effective barriers to interspecific hybridization at the level of the vitelline coat (Minganti, 1959; 1974). However, *Corella inflata* and *C. willmeriana* can be hybridized easily in both directions in the laboratory with no morphological intermediates being found in nature where the two species co-occur (Lambert, Lambert and Abbott 1981). Thus the fact that two species can be hybridized in the laboratory does not necessarily place them in the same species. The situation is different in the converse; demonstrated interspecific sterility in the laboratory constitutes excellent evidence that one is dealing with distinct species. In the hybridization experiments reported here we support the conclusion that *C. intestinalis* and *C. savignyi* are distinct species as reported by Hoshino and Nishikawa based upon morphological grounds (1985). We disagree with their not distinguishing *C. edwardsi* from *C. intestinalis* on the basis of both possessing the endostylar appendage. The fact that this structure is missing in *C. savignyi* does not mean that all *Ciona* with it are *C. intestinalis*. Also, in addition to the hybridization data, these two species have been distinguished by branchial basket structure and egg size (Copello, Devos and Lafargue, 1981) as well as the differences in follicle cell morphology in this report.

The failure of *C. roulei* sperm to fertilize *C. intestinalis* and *C. edwardsi* eggs also argues for discrimination of this species from the other two. That *C. intestinalis* sperm could fertilize *C. roulei* eggs while not being able to fertilize those of *C. edwardsi* may indicate that *C. roulei* and *C. intestinalis* diverged more recently than the other species pair.

Although the experiments reported here are limited in scope and need many more replicates, they clearly show that genetic isolation is well developed between these species. This study

clearly supports the concept of four species of *Ciona* in temperate shallow seas and serves as a starting point for a more extensive study including morphology and behavior of the larvae as well as physiological differences in the adults.

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