

Adhesiveness of the eggs of *Ecdyonurus venosus* to siliceous and calcareous substrate

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Abstract

The eggs of *Ecdyonurus venosus* are characterised by attachment structures represented by knob-terminated coiled threads (KCTs), which are frequently found on Ephemeroptera egg shells. KCTs represent adhesive devices. Scanning electron microscopy (SEM) investigation showed the pathways of KCTs extension in laid eggs. Transmission electron microscopy (TEM) observations gave evidence that the synthesis of KCTs occurs in the ovarioles enveloped by the follicle cells, which are presumably involved in this process. These chorionic devices have a complex structure. Each thread consists of an electron-dense core wrapped with thin coiled filaments. Threads and coiled filaments support the terminal knob. The thread joins the knob in its central part whereas the filaments slightly diverge and connect to thin relieves located on the inner side of the knob. Contact with water triggers uncoiling of the spring-like KCT so that its knob adheres to the substratum. Thread tension makes the terminal knob to slightly lift up, thus forming a sucker-like device. Chorionic tubercles dispersed among KCTs also perform an adhesive function. The different adhesiveness of the egg KCTs of *E. venosus* was tested in experimental conditions by allowing eggs to settle on the bottom of culture vessels containing glass cover-slips, quartz and travertine blocks. Quartz and travertine constitute the basic component of the natural substrates. Glass cover-slips give the best opportunity for adhesion, followed by quartz and travertine. The wider is the knob adhesive surface the stronger is the egg anchorage. Our data suggest that the mineral components, whose texture affects the smoothness of the surface to which the terminal knob adheres, promote a firmer egg anchorage.

Keywords: mayfly, egg chorionic devices, knob-terminated coiled threads, TEM, SEM.

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Introduction

Mayfly egg chorionic pattern has been the object of numerous investigation owing to its relevance in understanding the phylogeny of this insect group and in exploring the adhesive function performed by some eggshell decorations (Degrange, 1960; Kosova, 1967; Koss, 1968; Koss and Edmunds, 1974).

Morphological details of the chorion, revealed by ultrastructural investigation, coupled with experimental observations of eggs after their immersion in water, gave compelling evidence that chorionic devices and fibrous layers enveloping eggs are involved in enhancing egg adhesion to the substrate (Gaino and Mazzini, 1988; Provonsha, 1990; Gaino and Bongiovanni, 1993; Gaino and Flannagan, 1995; Gaino and Rebora, 2001; Gaino *et al.*, 2001). Whatever organised, mayfly attachment chorionic devices and coats seem to be adapted to accomplish the same adhesive function that is to prevent eggs from being dragged away into an environment unsuitable for their further development. Nevertheless, no investigation has been performed to test whether the lithology of the substratum can affect egg adhesiveness.

The effect of the mineral composition of the substrate on distribution and structure of benthic communities has been recently invoked to explain the lack of animal colonisation in the presence of quartz grains owing to the oxidant properties of the quartz crystal surface (Cerrano *et al.*, 1998). Consequently, the crystallographic characteristics of the substrate may play an important role in regulating the benthic communities, a feature so far neglected or poorly investigated.

The aim of the present work was to test egg/substrate interaction in experimental conditions by verifying the effect that the mineral composition may have on the egg adhesiveness, a crucial event for the survival of many Ephemeropteran species.

Material and Methods

Nymphs of *Ecdyonurus venosus* (FABRICIUS, 1775) were collected in the Ierna brook (a left tributary of the Nestore River), close to Tavernelle (Perugia, Umbria Region, Italy), at approximately 236 m a.s.l. in June 2000, and reared in laboratory. Specimens were differently processed for transmission and scanning electron microscopy investigation (TEM, SEM).

For TEM analysis, the ovaries dissected from nymphs were fixed in Karnovsky's medium

(1965), rinsed in 0.1 M cacodylate buffer (pH 7.2) at 4 °C, and postfixed in 1% osmium tetroxide in cacodylate buffer. Selected material was rinsed, dehydrated in a graded ethanol series, and embedded in Epon-Araldite. Sections were cut with a Reichert ultramicrotome, mounted on Formvar-coated copper grids, and stained with conventional uranyl acetate and lead citrate. Specimens were observed with a Philips EM 400 transmission electron microscope, belonging to the Electron Microscopy Centre of the University of Perugia.

Abdomen of 20 adults (emerged from reared nymphs) was slightly pressed on water-filled culture dishes. Eggs were allowed to settle on the bottom of three kinds of culture vessels: a first kind covered with glass cover-slips (used as control); a second kind covered with small and flat travertine blocks; a third kind covered with small and flat quartz blocks. Blocks represented two models differing in chemical and physical characteristics. For SEM observations, selected material (eggs adherent both to the glass cover-slips and blocks), dehydrated following the above described procedures for TEM study, was then critical-point dried using liquid CO₂ in a Bomar apparatus, attached to specimen holders by silver conducting paint, and coated with gold-palladium in a Balzer Union evaporator.

Two hours after settlement, the adhesiveness of about 1000 eggs on both cover-slips and blocks was tested (320 on cover-slips; 300 on quartzic blocks, 380 on travertine blocks), by gently shaking the experimental substrates, thus allowing the non-adhering eggs to slip away. Afterwards, the eggs attached to the bottom were counted under a stereomicroscope. Specimens were observed with a Philips EM XL30 scanning electron microscope (Electron Microscopy Centre of the University of Perugia). The basic descriptive terminology used by Koss and Edmunds (1974) is followed in the present paper.

Results

The eggs of *Ecdyonurus venosus* have peculiar chorionic attachment structures, as shown by scanning electron microscopy (SEM) (Fig. 1). According to the classification proposed by Koss and Edmunds (1974) they are considered to be of the type having individually knob-terminated coiled threads (KCTs). These attachment structures cover the egg chorion and are interspersed among tubercles (Fig. 2). KCTs tend

to gather at one of the egg poles, where they are larger than on the rest of the egg surface. In newly laid eggs, the knob loses its uniform covering and partially folds along its peripheral border (Fig. 3), thereby resulting in a gradual uncovering of the coiled threads beneath. Some TEM images reveal that the knob is connected to the coiled thread by regularly arranged filaments keeping the thread in its coiled configuration (Fig. 4). After contact with the water, KCTs gradually uncoil and project from the chorion, thereby assuming an extended configuration (Fig. 5). The inner side of the knob is regularly crossed by uplifted relieves (Fig. 6) converging towards the insertion of the thread (Fig. 7). The knob of each uncoiled thread firmly adheres to the substrate in such a way that, in their final configuration, KCTs act as cables stretched from the egg surface to the bottom (Fig. 8).

The ovarioles of *E. venosus* consist of a sequence of follicles of increasing size (Fig. 9). Analysis at transmission electron microscopy level (TEM), carried out on the ovarioles, revealed that the synthesis of KCTs ends in the ovarioles, as shown by sections of the egg chorion where KCTs are arranged between tubercles (Fig. 10). The terminal knob of these threads is held firm by the follicle cell epithelium (Fig. 11). Each thread supporting the terminal knob (Fig. 11) consists of an electron-dense core enveloped with coiled filaments (Fig. 12), which are connected to the terminal knob. In the ovulated eggs gathered in the oviduct, the KCTs keep their coiled pattern whereas the peripheral border of the knob tend to turn outward (Fig. 13). The knob consists of two superimposed electron-dense layers separated by a slightly less electron-dense region (Fig. 14). The thin peripheral border results from the extension of the innermost layer (Fig. 14). On the whole, the terminal knob consists of a thicker central region, to which the thread and coiled filaments adhere, and a thinner peripheral one. This organisation explains the slight folding of the knob border in the eggs entering the oviduct (Fig. 14).

Our assay, carried out in experimental conditions (see material and methods section), tested egg adhesiveness, resulting from the interaction of the terminal knob with the substrate (Figs. 15, 16), and proved that the response of the eggs to the siliceous substrata was higher than to the calcareous ones. Indeed, the percentage of adhesiveness on the glass cover-slips showed the maximum values (75%), followed by that on quartz (70 %) and on travertine (20%) (Fig. 17).

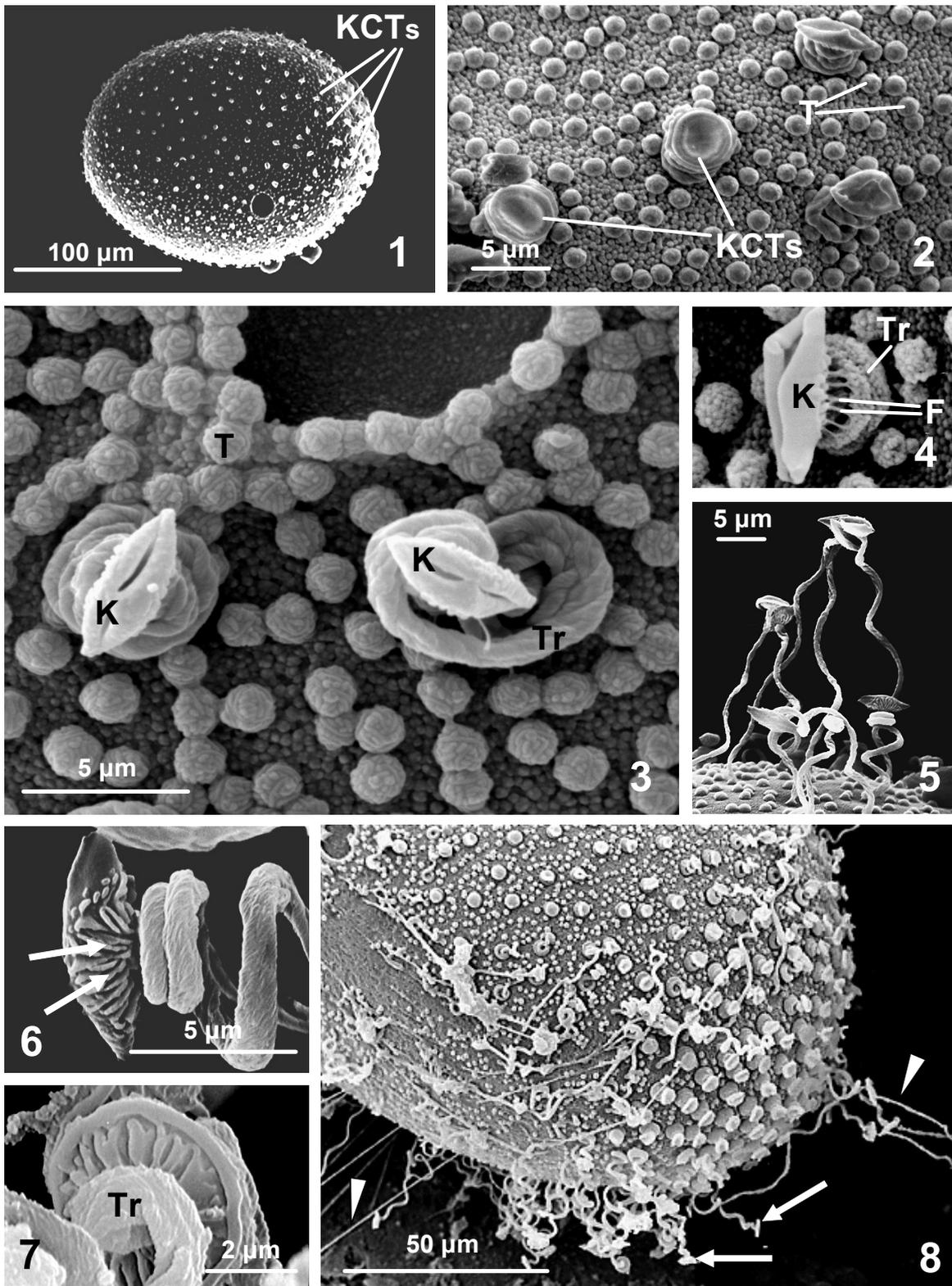


Fig. 1 – SEM view of the egg of *E. venosus* showing chorionic pattern of knob terminated coiled threads (KCTs); Fig. 2 – A region of the chorion showing KCTs among tubercles (T); Fig. 3 – Knobs (K) of the KCTs in their folded configuration. Note the coiled shape of the threads (Tr) underneath. T, tubercles; Fig. 4 – Enlarged view of a KCT showing the filaments (F) connecting the terminal knob (K) and its coiled thread (Tr) beneath; Fig. 5 – Several KCTs in their extended configuration; Fig. 6 – Detail of a folded knob the inner side of which is crossed by uplifted relieves (arrow); Fig. 7 - Zoomed view of the inner side of a knob showing the insertion of the thread (Tr) in its central region; Fig. 8 - Egg pole showing the partial (arrow) and complete (arrowheads) uncoiling of KCTs. Note the extended KCTs acting as cables anchoring egg to the substrate.

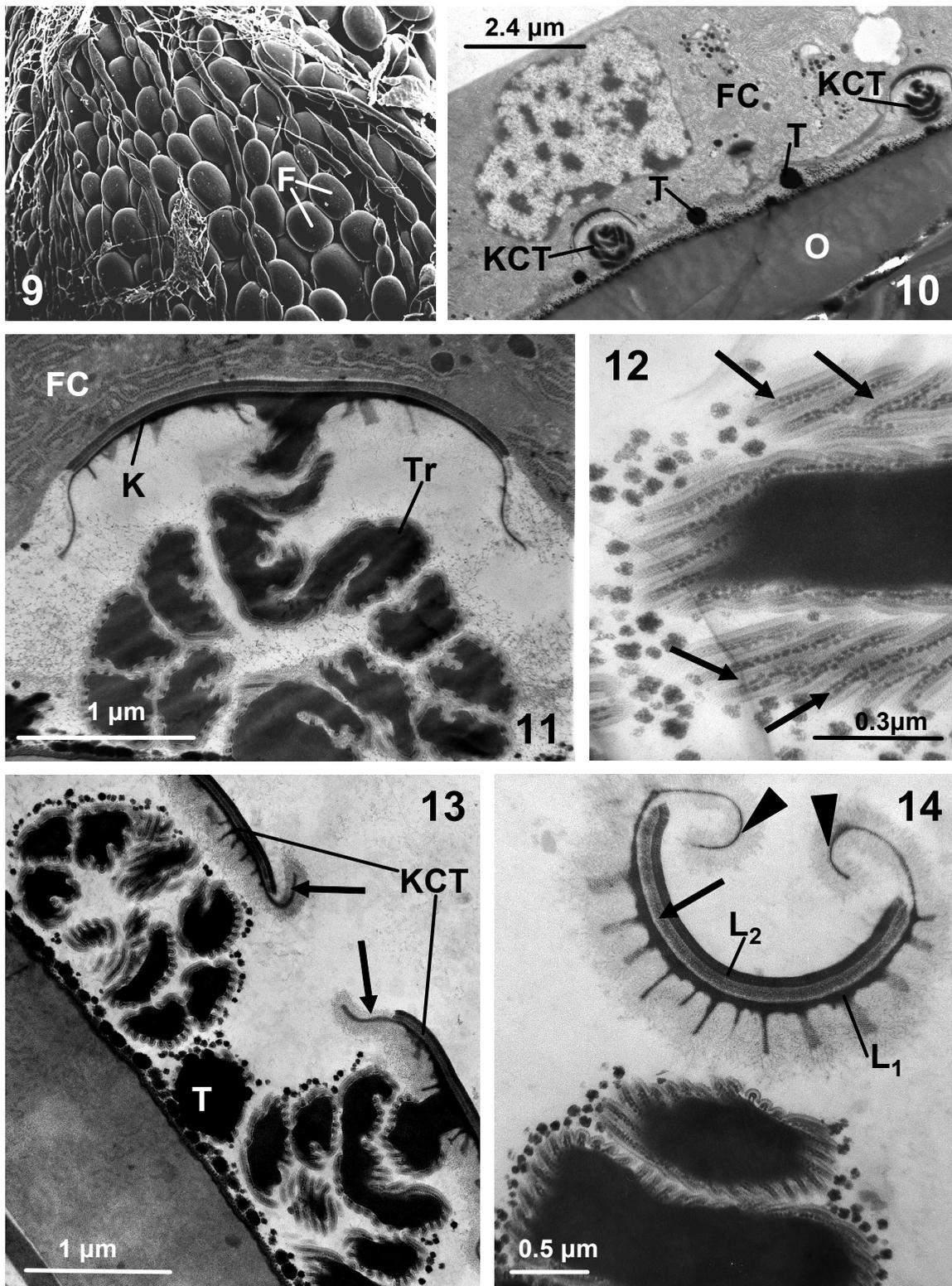


Fig. 9 – SEM view of the ovarioles showing the constitutive follicles (F) arranged in sequence of increasing size; Fig. 10 – Section of a follicle under TEM. Follicle cells (FC) envelope the oocyte (O). Note the arrangement of the KCTs among chorionic tubercles (T); Fig. 11 – Fine organisation of a KCT, the knob (K) of which is held firm by the enveloping follicle cell (FC). Note the electron-dense coiled thread (Tr) supporting the terminal knob; Fig. 12 – High magnification of a thread enveloped by coiled filaments (arrows); Fig. 13 – Detail of an ovulated egg showing 2 KCTs and an interposed tubercle (T). Note the slight turning outward (arrows) of the terminal knob; Fig. 14 – Detail of a terminal knob showing its organisation in two superimposed electron-dense layers (L₁-L₂) separated by a less electron-dense region (arrow). Note that the innermost layer extends to form a thin peripheral border turning outward (arrowheads).

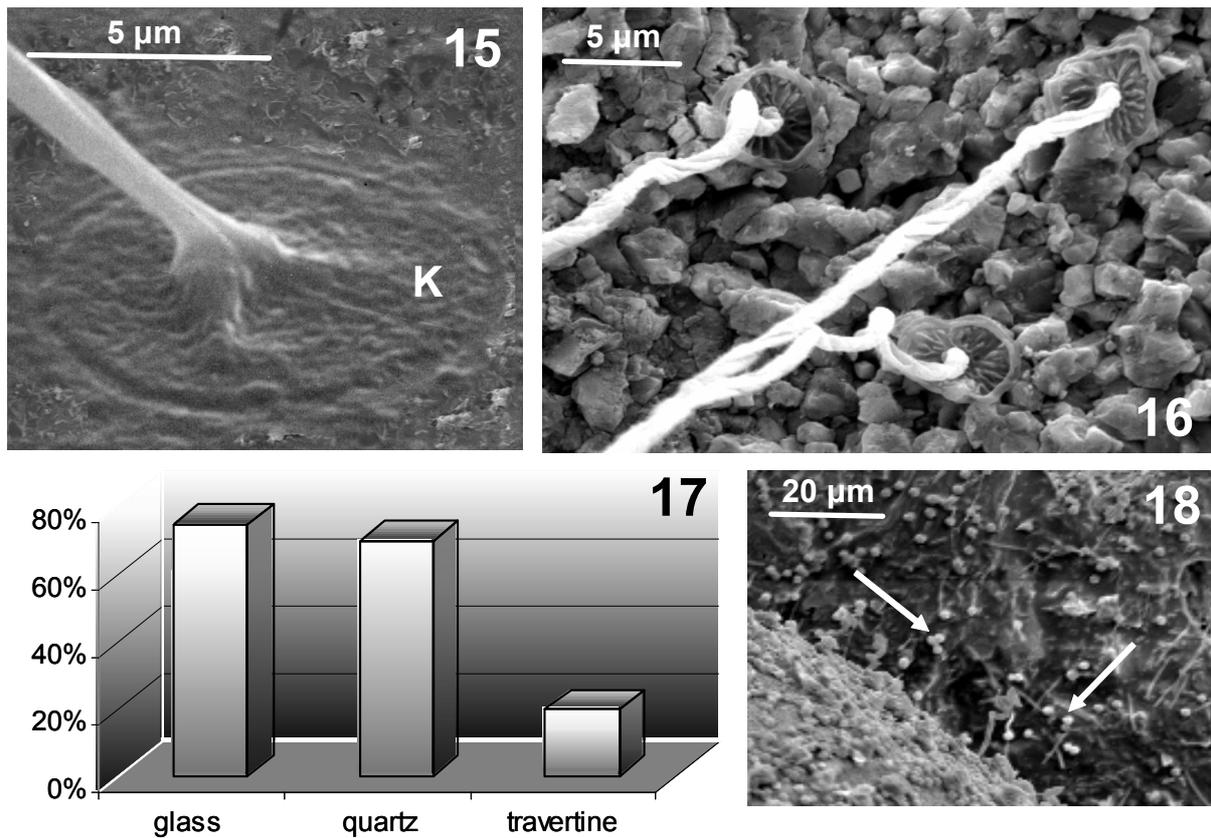


Fig. 15 – Tight adhesion of the knob (K) to the smooth surface of a quartz block; Fig. 16 – Partial adhesion of the knob (K) to the unevenness surface of a travertine block; Fig. 17 – Assay on the different adhesiveness of KCTs on the experimentally tested substrates. Note the gradual decrease in terminal knob adhesion from glass cover-slip, to quartz and travertine blocks; Fig. 18 – Adherent eggs slightly moved from the quartzitic surface on which they settled. Note the chorionic tubercles (arrows) scattered on the substrate.

Along with KCTs, chorionic tubercles play an adhesive function since any attempt to move settled eggs resulted in the detachment of these devices from the egg surface (Fig. 18).

Discussion

In Ephemeroptera follicle cell epithelium is competent for the synthesis of egg envelopes (vitelline envelope and chorionic layers) along with a variety of chorionic decorations (Mazzini and Gaino, 1988; Gaino and Mazzini, 1990). Presumably, follicle cells of *E. venosus* are involved in the elaboration of the KCTs since these devices are already completed inside the ovarioles before ovulation.

In laid eggs, the pathways of KCTs' uncoiling take place through a gradual folding of the terminal knob that causes thread extension, a feature already observed in the eggs of another heptageniid species (Gaino and Mazzini, 1987). The ultrastructural organisation of the KCTs of *E. venosus* confirms the organizational model evidenced for these devices in *Rhithrogena*

kimminsi (Gaino and Mazzini, 1988). The fine architecture of the knob is consistent with the mechanical unravelling of the KCTs after egg deposition in water. In fact, knobs are individually taken in place mainly by a ring of thin filaments connecting their central region to the thread beneath. The central region is strengthened by relieves arranged radial from the centre towards the peripheral border of the knob. This last is so thin that it tends to fold when eggs are still inside the oviduct. Therefore, the contact with the water at egg deposition acts in triggering KCTs' extension and further adhesion of the terminal knob to the substrate. Thread tension makes knob to slightly lift up, thus forming a sucker-like device. A smooth surface promotes a better adhesion, as proved by the eggs settled on the glass cover-slips. The different adhesiveness tested after egg settlement on travertine and quartz blocks could be mainly linked to the different surface texture of these substrates. In fact, calcareous surface is more irregular than the siliceous one. This feature could be responsible for a better fixation of the knob edge to the

siliceous surface, which causes a more tightly attachment to the bottom. Whereas KCTs act at a certain distance, chorionic tubercles intervene when the egg surface is in close contact with the substrate.

The relevance of the relationships between biological systems and minerals have been recently emphasised by Cerrano *et al.* (1998), who introduced the term bio-mineralogy to stress the influence of the mineralogical features of the substrate on the marine benthic communities.

Laboratory tests on a marine hydroid proved that planulae settle preferentially on carbonatic, rather than quartzitic, substrates (Bavestrello *et al.*, 2000). Field data suggested that the presence of quartz acts as an inhibiting factor in structuring marine epibenthic community (Bavestrello *et al.*, 2000). A different reactivity with regard to crystalline and amorphous silica has been demonstrated in a marine demosponge by following the crystalline quartz particle settlement on the sponge outermost surface (Bavestrello *et al.*, 1998). Whereas at the contact with the crystalline quartz particles, sponge cells contract and maintain a certain gap from the foreign material, amorphous opaline spicules elicit a positive motile response triggering cells to cover foreign material. These observations confirm that the different crystallographic texture of quartz have a different remarkable impact on the living organisms, which are able to recognize the crystallographic organization of inorganic matter. Therefore, it seems acceptable the hypothesis of an attraction to a particular substrate, which could also affect hard-bottom assemblages. In experimental conditions, case-building Trichoptera are able to select between quartz and travertine, and show a significant preference for supplied calcareous grains (Gaino *et al.*, 2002). In this regard, species corology could reflect the mineral texture of the substrate surface.

Biological systems interact with the substrate at their different level or organisation, and the effect of the mineralogical composition of the bottom changes when organisms produce non-living covers acting as a barrier interposed between them and the substrate (Bavestrello *et al.*, 2000). In this sense, chorion also constitutes a mechanical protection preventing egg surface from a direct interaction with the substrate.

Since egg survival in the environment constitutes the first step for species survival, egg adhesiveness plays an important role in allowing re-colonisation and biodiversity preservation. Chorionic decorations and /or mucous envelopes

having adhesive properties are involved in this activity. Connel and Slayter (1977) proposed three different mechanism of interaction between organisms colonising hard substrata: facilitation, inhibition and tolerance. We can recognise in smooth substrates a facilitation for egg adhesion by the intermediate of the KCTs .

It seems reasonable to think that no negative effect of quartz in the interaction with the knob occurs, and knob adhesiveness, likewise the uncoiling of KCTs, is a mechanical process efficient for space acquisition and maintenance.

As more species are investigated from this perspective, substrate characteristics may prove to be useful descriptors of spatial distribution and structure of benthic community.

In conclusion, KCTs uncoiling and further knob adhesion to the substrate, a process leading to the anchorage of settled eggs, depends on a mechanical mechanism, whose final efficiency is mainly linked to the smoothness of the surface. In this regard, the texture of the quartzitic substrates offers better opportunity than that of the travertine ones.

Acknowledgements

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