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ADAPTIVE ADVANTAGES OF THE "SYMBIOSIS" BETWEEN ALGAL CHLOROPLASTS AND SACOGLOSSAN MOLLUSCS

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SUMMARY

All available information is reviewed on the characteristics and the way the "symbiosis" between algal chromoplasts and sacoglossan molluscs works, using the existing bibliography and the authors' own studies. Emphasis is placed on the importance of the food subsidy received by the mollusc, which is one of the various benefits derived from the association, and on the possible benefit obtained from this consortium by the algal species involved and not by the retained chloroplasts, which are, after all, non-autonomic cell organelles. After making various theoretical considerations, and on the basis of the population dynamics of some pairs of alga-sacoglossan species, especially an *Acetabularia acetabulum* and *Elysia timida* population thoroughly studied by the authors, the following conclusions are drawn:

a) The establishment of a symbiotic relationship between sacoglossans and chromoplasts and the different levels of mutual adaptation and efficiency reached, are a direct function of the difficulty (seasonal or otherwise) experienced by the mollusc in obtaining food.

b) Depending on the moment of the biological cycle in which the animal receives it, the energy subsidy provided by the chloroplasts to the host mollusc acts either as a dietary complement, a dietary supplement or a partial substitute of the normally obtained algal food.

c) This subsidy is invested, successively and/or alternatively, in the mollusc's growth (Pg), in the shifting of its reproductive pattern from a lecithotrophic larval development to a direct one (Pr), or in increased exploratory and foraging activity (ΔR). All these entries represent a benefit for the mollusc, in allowing it to be more active, live longer, reproduce more efficiently and/or explore a greater area of the sea bed.

d) When the subsidy involves an energy complement for the sacoglossan species, it is plausible that the mollusc reduces its grazing rate on the alga species, in the same proportion at which it receives the subsidy. In this case the alga species also benefits, which would allow the relation between the alga and the sacoglossan to be classified as mutualistic. In all the other cases the benefit is exclusively for the mollusc.

KEY WORDS: Sacoglossa, chloroplasts, adaptation, symbiosis, *Elysia timida, Acetabularia acetabulum.*

"The close examination of any ecosystem reveals an infinity of comparable [trophic colateral] relations. The most obvious result of its evolution is that, in the end, a certain species forms a very strict relation with another defined species. The process of selection which has led to the establishment and maintenance of such relations has to be explained by some energy economy; in practice, however, the specificity of the relations between different organisms is more notable or more apparent than any general energy consideration, sometimes because it is unknown, and in most cases because once known, it is difficult to evaluate. In any case, the result is that traditional biology has always given more importance to the specificity of the relation than to the transfer or saving of energy that it implies". (MARGALEF, 1974: 543)

"Certain organisms can obtain "second hand" algae from their prey.... Stranger still is the survival, not of whole cells, but of Codium *and* Bryopsis *plastids in* Elysia, Tridachia *and* Placida, *and that these plastids continue to have a functional capacity...". (MARGALEF, 1974:538).*

INTRODUCTION

The Opisthobranchs are a group of marine gastropods which provide numerous examples of molluscs making use of morphological elements belonging to their prey species, such as plant and animal pigments (melanin, carotenoids, pterine and purine granules, etc.), sponge spicules, cnidarian nematocysts, etc. (EDMUNDS, 1966, 1974; THOMPSON, 1960, 1976; HAEFELFINGER, 1969; ROS, 1976, 1977; TODD, 1981; GREENWOOD & MARISCAL, 1984; CATTANEO, 1990; etc.)

The Sacoglossa (=Ascoglossa, Monostichoglossa) are an order of opisthobranchs of benthic habits. They are mainly herbivorous, and some species retain the chloroplasts of the algae that they consume (KAWAGUTI & YAMASU, 1965; TAYLOR, 1967, 1973; GREENE, 1970a; TRENCH, 1975; HINDE, 1983; MARÍN & ROS, 1988; MARÍN, 1991; etc.). The chloroplasts are not immediately digested, but remain active during weeks or even months in the cells of the digestive gland. The mollusc often houses so many chloroplasts that its colour perfectly matches that of the food alga (green, occasionally red), although sometimes these colours come from the ingesta and are seen by transparency, regardless of whether they correspond or not to functional chloroplasts. They may also be due to the animal's own pigments.

It has been known for over a century that some sacoglossans possess chlorophyllic pigments. DE NEGRI & DE NEGRI (1876) observed their presence in *Elysia viridis*, and BRANDT (1885) later described chloroplasts in the same species. Zooxanthellae were later observed in nudibranchs (NAVILLE, 1926, in *Aeolidiella alderi*), and YONGE & NICHOLAS (1940) described them in the sacoglossan *Tridachia crispata* (although they were really dealing with chloroplasts).

In the sixties, the electron microscope enabled easy identification of these organelles in *Plakobranchus ocellatus, Elysia atroviridis* and other sacoglossans (KAWAGUTI, 1941; KAWAGUTI & YAMASU, 1965; KAWAGUTI *et al.*, 1965; etc). Physiological and biochemical studies of various species (which included the measurement of oxygen produced and of the carbon fixed by photosynthesis) began shortly afterwards: TAYLOR (1967), GREENE (1970a, 1970b), TRENCH & SMITH (1970), GREENE & MUSCATINE (1972); etc.

These studies led to the first reviews and theoretical syntheses, which discussed the role of the chloroplast-sacoglossan relationship (TAYLOR, 1973, 1981; GREENE, 1974; TRENCH, 1975; TAYLOR 1973, 1981; HINDE, 1983; etc.). A relatively exhaustive list of the main recent works on the chloroplast-sacoglossan relationship can be found in the articles mentioned above and in WILLIAMS (1986), MARÍN (1988), MARÍN & ROS (1989, in press a, b). The synthesis by HINDE (1983) is particularly thorough.

The presence of plastids in their tissues has led these animals to be called cryptogamic molluscs and "leaves that crawl" (TRENCH, 1975). Many of the anatomical and behavioural characteristics of chloroplast-retaining sacoglossans cannot be explained if not as adaptations to this peculiar association: "Specialised tissues such as the large flat dorsal mantle of *Elysia [viridis]* which contains neat agricultural rows of chloroplasts, salvaged from *Codium*, are... a consequence of the importance of the symbiotic association" (DYER, 1989).

The functionality of the plant organelles enabled the term symbiosis to be used (as mentioned above) by the first researchers to look into this alga-animal relation (KAWAGUTI & YAMASU, 1965; GREENE, 1970a, 1970b; TRENCH, 1975), which has sometimes been qualified as chloroplast symbiosis (HINDE & SMITH, 1974; TRENCH, 1975). Such a symbiosis has always been interpreted as mutualistic (MARGALEF, 1974; AHMADJIAN & PARACER, 1986; DOUGLAS & SMITH, 1989), that is, with mutual benefit to the two species involved.

It has been effectively shown that the photosynthates produced by the chloroplasts are used, at least partly, by the molluscs (GREENE, 1970b; TRENCH *et al.*, 1970; GREENE & MUSCATINE, 1972; HINDE & SMITH, 1975; CLARK *et al.*, 1981; MARÍN & ROS, 1989; etc.). Individuals from different species of chloroplast-retaining sacoglossans live longer without eating than equivalent, non-retaining species, or grow faster if exposed to light than if they live in darkness; the animal consorts thus benefit from this unusual association.

The same cannot be said of the plant consorts, the chloroplasts. They are organelles separated from the algal cells and are, therefore, incapable of reproduction and of increasing their Darwinian fitness (which is one of the premises that has to be fulfilled in a mutualistic symbiosis). Then, strictly speaking, the relationship cannot be qualified as symbiotic (HINDE, 1983).

The same does not occur with the relationship established between cnidarian-eating nudibranchs and their symbiotic zooxanthellae, which are incorporated into the tissues of the predator (NAVILLE, 1926; KEMPF, 1984, 1991; MARÍN & ROS, 1991).

TRENCH (1980) proposed the term "foreign organelle retention" for the sacoglossan-chloroplast association (BLACKBOURN *et al.*, 1973), and CLARK & DeFREESE (1987) suggested "kleptoplasty", both terms in which the mutual benefit of the association is not implicit. "Chloroplast symbiosis" has also een used by some authors (HINDE & SMITH, 1974; CLARK & BUSACCA, 1978; CLARK *et al.*, 1981; WILLIAMS, 1986, etc.), while others use "symbiosis" (with quotation marks: HINDE & SMITH, 1974; MARÍN & ROS, 1989; etc.).

This paper, based on studies carried out by the authors on a score of sacoglossan species from the Spanish Mediterranean coast, contributes data and arguments which demonstrate that the symbiosis (the mutual association from which both partners benefit) is not between the mollusc and the plant organelle, but rather between the mollusc and the food alga.

At least in some situations, the benefit for the algal species lies in the reduction of its predation by the mollusc, in the same proportion in which the retained chloroplasts provide the mollusc with an energy subsidy based on photosynthates which are additional to the food consumed by the latter through the normal herbivory procedure. A very preliminary draft of the present arguments was advanced in MARÍN & ROS (1989).

SACOGLOSSANS AND THEIR ASSOCIATION WITH ALGAL CHLOROPLASTS

The Sacoglossans can be divided into three main morphological types (MORTON, 1979; THOMPSON, 1976; SCHMEKEL & PORTMANN, 1982, GASCOIGNE, 1985; see JENSEN, 1991). The most primitive are the testaceous, shelled forms of the suborders Juliacea, Volvatellacea and Oxynoacea, which apparently do not retain active chloroplasts.

There are two main types of more advanced morphological forms, naked and shell-less, of the suborders Elysiacea and Platyhedylacea, which can be slug-like, with parapodia and without dorsal appendages (families Elysiidae, Plakobranchidae, Boselliidae and Limapontiidae), or with cerata (dorsal papillae; families Polybranchiidae, Hermaeidae, Stiligeridae, Alderiidae, Platyhedylidae). Many of the species from these last two groups house functional

TABLE I. Systematic arrangement of the order Sacoglossa; the two suborders, the five superfamilies and the fourteen generally accepted families (GASCOIGNE, 1985; THOMPSON, 1976; MARCUS, 1982; JENSEN, 1983; LONG, 1983) are shown, along with the main world genera. Those genera are indicated in which chloroplast retention has been reported (full dots) and those in which retention does not exist (open dots, from the species studied). The species listed in standard type are those which, studied by different authors, have been shown to retain chloroplasts. The species listed in italics have been studied or are being studied by the authors (ROS & RODRÍGUEZ, 1985; MARÍN, 1988, 1991; MARÍN & ROS, 1988, 1989, in press a, b) and the preceding symbols indicate that they retain functional chloroplasts (•), that they do not retain them (°) or that it is not known whether they retain them or not (+; also see Table II). Note that many genera have not yet been prospected, and neither have the most primitive species (Juliacea, part of the Oxynoacea), or the most advanced ones, some of them egg-eaters (Alderiidae, Oleidae and part of the Stiligeridae).

SACOGLOSSA Bergh, 1876 (=ASCOGLOSSA v. Ihering, 1876; MONOSTICHOGLOSSA Pagenstecher, 1875)	
CONCHOIDEA Gascoigne, 1985	
Juliacea Boettger, 1963	
Juliidae E. A. Smith, 1885	
Julia Gould, 1862	
° Berthelinia Crosse, 1875	
Edenttellina Burn, 1960	
Tamanovalva Kawaguti & Baba, 1959	
Volvatellacea Marcus 1982	
Volvatellidae Pilsbry, 1895	
Volvatella Pease, 1860	
Cylindrobullidae Thiele, 1931	
Cylindrobulla P. Fisher, 1857	
Ascobulla Marcus, 1972	+ *A. fragilis*
Oxynoacea Boettger, 1963	
Oxynoidae Fischer, 1883	
Oxynoe Rafinesque, 1819	O. antillarum, *O. azuropunctata,* *O. olivacea,* O. panamensis
Lobigeridae Pruvot-Fol, 1954	
° Lobiger Krohn, 1847	° *L. serradifalci*
Lophopleurella Zilch, 1956	
Roburnella Marcus, 1982	
ACONCHOIDEA Gascoigne, 1985	
Elysiacea Bergh, 1872	
Elysiidae H. & A. Adams, 1854	
• Elysia Risso, 1818	E. atroviridis, E. australis, E. cauze, E. chlorotica, • *E. flava,* E. furvacauda • *E. gordanae,* E. hedgpethi, E. maoria, E. subornata, • *E. timida,* *E. translucens,* E. tuca, • *E. viridis*
Achillea Gascoigne, 1985	
Elysiobranchus Pruvot-Fol, 1930	
Elysiella Bergh, 1872	
Elysiopterus Marcus, 1957	
• Thuridilla Bergh, 1872	• *T. hopei*
• Tridachia Deshayes, 1857	T. crispata
• Tridachiella MacFarland, 1924	T. diomedea
Pattyclaya Marcus, 1982	
Plakobranchidae Rang, 1829	
• Plakobranchus Van Hasselt, 1824	P. ianthobapsus, P. ocellatus
Boselliidae, Marcus, 1982	
• Bosellia Trinchese, 1890-91	• *B. mimetica*
Polybranchiidae H. & A. Adams, 1854	
Caliphylla A. Costa, 1865(69)	
Mourgona Marcus, 1970	
Lobifera Pease, 1871	
Polybranchia Pease, 1860	
Cyerce Bergh, 1871	
Hermaeidae H. & A. Adams, 1854	
• Hermaea Lovén, 1844	• *H. bifida,* + *H. paucicirra*
• Costasiella Pruvot-Fol, 1951	C. lilianae
Aplysiopsis Deshayes, 1839-53	
Hermaeina Trinchese, 1874	
Stiligeridae Iredale & O'Donoghue, 1923	
Calliopaea D'Orbigny, 1837	° *C. bellula*
Stiliger Ehrenberg, 1831	
• Placida Trinchese, 1876	+ *P. cremoniana,* • *P. dendritica,* *P. viridis,* + *P. verticillata*
Ercolania Trinchese, 1872	+ *E. caerulea,* • *E. funerea*
° Olea Agersborg, 1923	
Limapontiidae Gray, 1842	
• Limapontia Johnston, 1836	° *L. capitata,* L. depressa
Alderiopsis Baba, 1968	
Alderiidae	
° Alderia Allman, 1846	
Alderella Odhner in Franc, 1968	
Platyhedylacea Rankin, 1979	
Platyhedylidae Salvini-Plawen, 1973	
Platyhedyle Salvini-Plawen, 1973	

chloroplasts from the food algae (Table I).

Almost all the Sacoglossan species are phytophagous suctorial feeders, that ingest suctorially the cytoplasm of caulerpalean algae and of other siphonalean chlorophyta and rhodophyta. For this reason the term chromoplast (coloured plastid) is preferred to chloroplast (green plastid), as some of the organelles retained are from red algae (rhodoplastids; TAYLOR, 1971; MARÍN & ROS, 1988). The term kleptoplast (stolen plastid) has also been used (WAUGH & CLARK, 1986; MARÍN & ROS, 1989).

Besides the morphology of the bucal apparatus, adapted for the perforation of the algal cells and the ulterior suction of their contents (THOMPSON, 1976; CLARK & BUSACCA, 1978; JENSEN, 1980, 1981, 1991; CLARK & DeFREESE, 1987; MARIN, 1988), these molluscs possess anatomical, physiological and behavioural adaptations designed for the efficient exploitation of the retained chloroplasts (Fig. 1). These adaptations range from the structure of the radula and the substitution mechanism of the only row of radular teeth, to the ramification of the digestive gland in the mantle (whose light-catching and gas- and nutrient-exchanging surface is increased by the presence of flaps or by cerata-like dorsal appendages; TAYLOR, 1981); and from the preference for certain intensities of light, to the synchronisation of the life cycle with that of the food alga (MARÍN & ROS, in press a), and to the animal behaviour, including syncopated ventilation movements of the mantle cavity that ensure the rapid renewal of the water

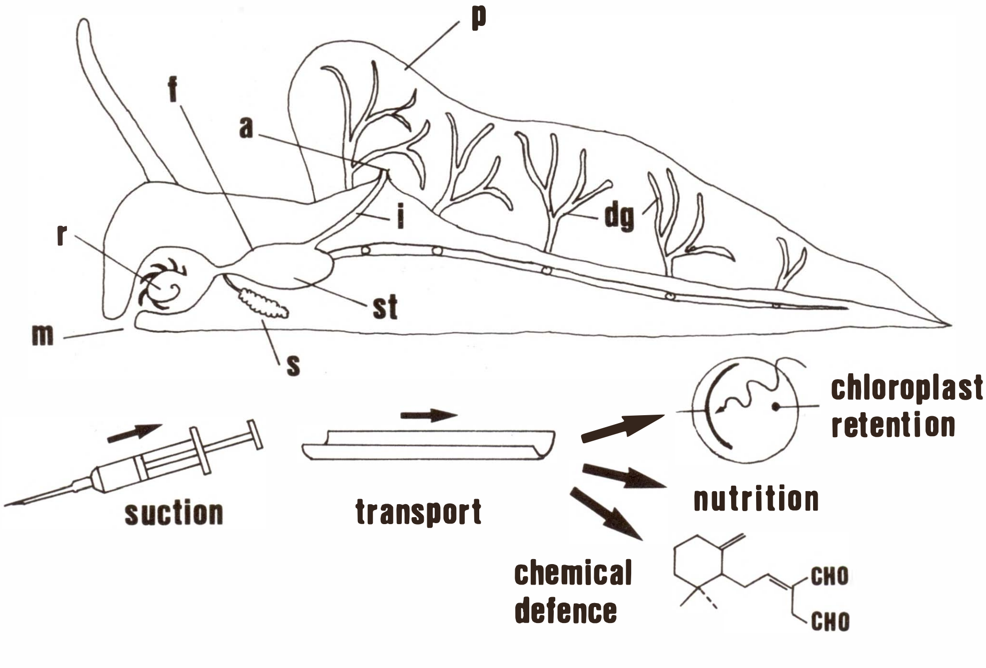


FIGURE I. Diagrammatic representation of the workings of a sacoglossan. Above, parts of the sacoglossan's digestive tube. Below, a functional and symbolic representation of the digestive tube. When it eats, the animal holds the food plant with its lips (m) and perforates the plant cells with the radula (r), while the pharynx (f) works as a suction pump for the stomach (st); also shown are the salival glands (s). The whole system works like a syringe (below). The plant cytoplasm ingested is distributed through the diverticles of the digestive gland (dg) (transport canal below). In the cells of the aforementioned digestive gland, the plant organelles and/or the molecules involved in the defence of the algae are retained intact, to be used later by the mollusc (screen to capture solar radiation and organic molecule below). The plant sap ingested is likewise used as food. The material which is not used is returned to the stomach, from where it is moved to the anus (a) through the intestine (i). This diagram shows a sacoglossan (an Elyisiidae, for example) with lateral parapodia (p), which in the Polybranchiidae and Stiligeridae are substituted for dorsal appendages (cerata).

TABLE II. Sacoglossan species from the south east of Spain studied by the authors, species of algae used as food and functional chloroplast retention in the molluscs. The symbols in the last column indicate that the existence of functional chloroplasts has been confirmed (+), that the mollusc does not retain them (-), or that it has not yet been possible to test for the presence or absence of such organelles (?). No attempt has been made to distinguish different degrees of kleptoplasty (in the sense of CLARK *et al.*, 1990). The references are identified in the bibliography; 0, this paper.

SACOGLOSSAN	ALGAL FOOD	CHLOROPLAST RETENTION
Ascobulla fragilis (Jeffreys, 1856)	Caulerpa prolifera (14, 15)	?
Oxynoe olivacea Rafinesque, 1819	Caulerpa prolifera (14, 15)	- (14, 15)
Lobiger serradifalci (Calcara, 1840)	Caulerpa prolifera (14, 15)	- (14, 15)
Elysia flava Verrill, 1901	Cladophora sp. ? (14, 15), Halimeda simulans (5)	+ (14, 15)
Elysia gordanae Thompson & Jaklin, 1988	Cladophora sp. (14, 15)	+ (14, 15)
Elysia timida (Risso, 1818)	Acetabularia acetabulum (14, 15, 21), A. calyculus (14, 15)	+ (14, 15, 20, 21)
Elysia translucens Pruvot-Fol, 1957	Udotea petiolata (14, 15)	+ (14, 15)
Elysia viridis (Montagu, 1804)	Codium fragile (12), C. bursa, C. tomentosum (2)	+ (12, 15, 26)
Thuridilla hopei (Vérany, 1853)	Cladophora vagabunda (14, 15), Pseudochlorodesmis furcellata, Derbesia tenuissima (0)	+ (14, 15)
Bosellia mimetica Trinchese, 1890	Halimeda tuna (14, 15, 19), H. simulans (5)	+ (14, 15)
Hermaea bifida (Montagu, 1815)	Griffithsia flosculosa (27), Lophosiphonia sp. (14, 15)	+ (14, 15, 27)
Hermaea paucicirra (Pruvot-Fol, 1954)	Codium fragile (22), C. tomentosum (2), Lophosiphonia sp. (14, 15)	+ (14, 15)
Placida cremoniana (Trinchese, 1892)	Unknown	?
Placida dendritica (Alder & Hancock, 1843)	Codium tomentosum, C. fragile (9), Bryopsis plumosa, C. vermilara (14, 15)	+ (10, 14, 15, 26)
Placida verticillata Ortea, 1982	Codium tomentosum, C. adhaerens (17), Bryopsis sp. (14, 15)	?
Placida viridis (Trinchese, 1873)	Bryopsis sp. (14, 15, 23)	+ (14, 15)
Calliopaea bellula D'Orbigny, 1873	Chaetomorpha linum (16)	- (14, 15)
Ercolania caerulea Trinchese, 1892	Valonia utricularis (28)	?
Ercolania funerea (A. Costa, 1867)	Chaetomorpha aerea, C. linum (14, 15), Cladophoropsis (5)	+ (14, 15)
Limapontia capitata (Müller, 1774)	Cladophora rupestris, Enteromorpha sp. (13), C. sericea (7); E. intestinalis, (8), C. arcta, C. vagabunda (14, 15)	- (9, 12, 14, 15)

that comes in contact with it (RAHAT & MONSELISE, 1979; CLARK *et al.*, 1981; ROS & RODRÍGUEZ, 1985; MARÍN & ROS, 1989),

The Sacoglossans are also characterised by a high trophic specialisation (Table II), which turns them into perhaps the most stenophagous of marine herbivores (CLARK & DeFREESE, 1987; MARÍN, 1988; MARÍN & ROS, 1988, JENSEN, 1991; MARÍN *et al.*, 1991), although the most highly evolved forms are egg-eaters. Their development is also characterised by a high proportion of lecithotrophic larval development and encapsulated metamorphosis (CLARK & GOETZFRIED, 1978; CLARK & JENSEN, 1981; ROS, 1981; MARÍN, 1988; MARÍN & ROS, in

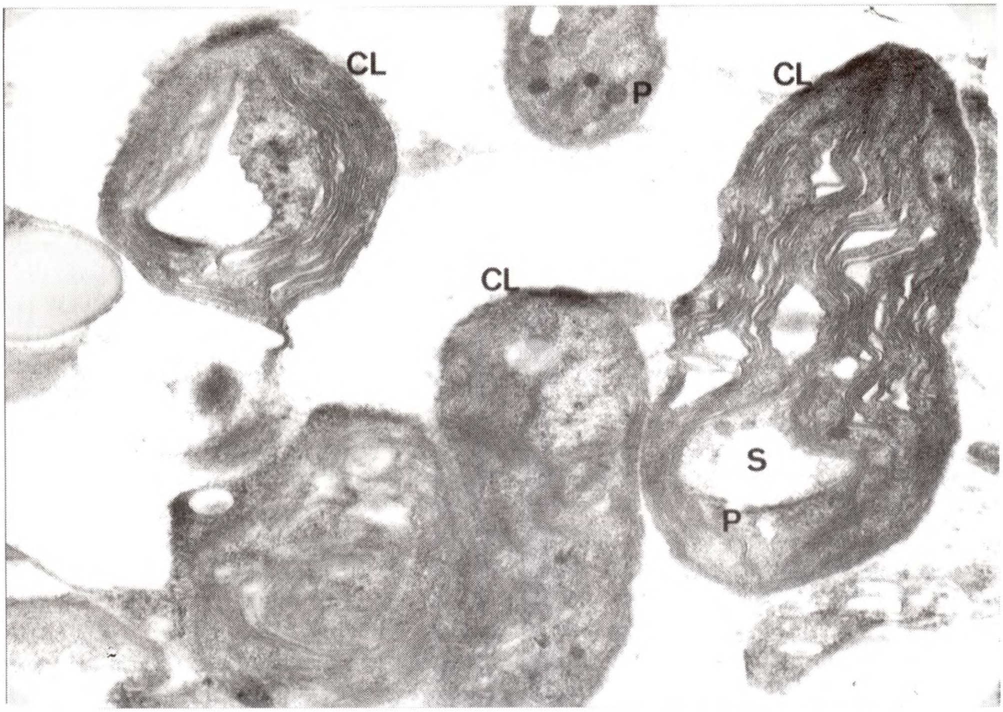


FIGURE 2. Chloroplasts of *Acetabularia acetabulum* (x6000). CL chloroplast; P, pyrenoid; S, starch.

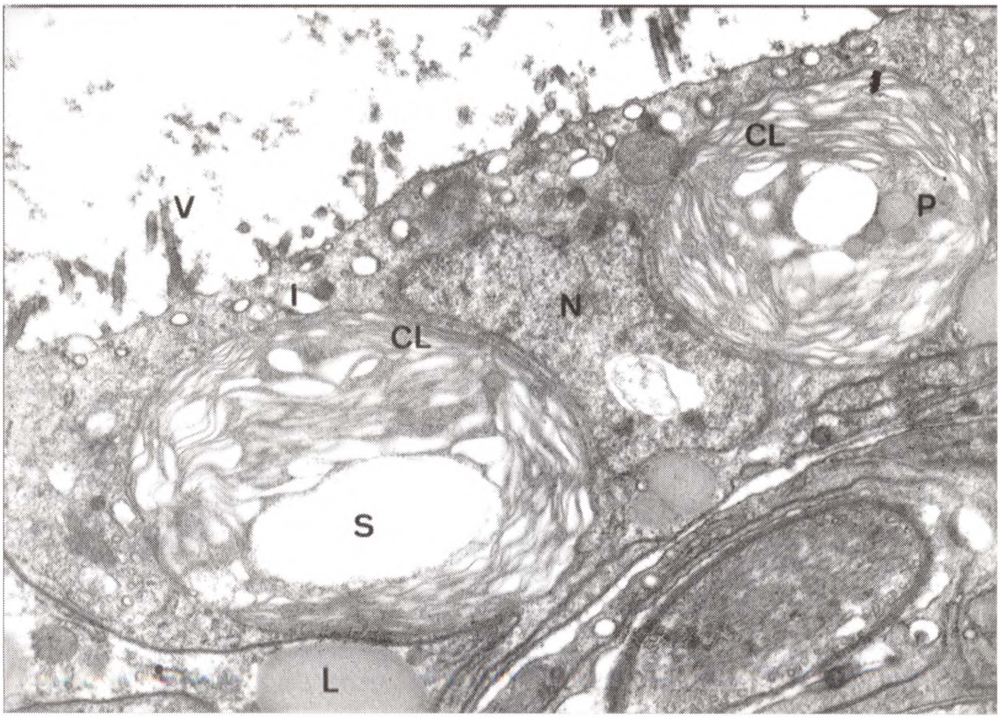


FIGURE 3. Digestive tube of *Elysia timida* showing a digestive cell with intact chloroplasts (x 6000). CL, chloroplast; I, vesicles along luminal border; L, lipid inclusions; N, nucleus; P, pyrenoids; V, microvilli.

press a).

In spite of appearing (like most nudibranch species) in relatively low densities in their natural habitats, as they exploit plant species which are chemically relatively well defended (PAUL & FENICAL, 1986), or calcified (CLARK & DeFREESE, 1987; MARÍN & ROS, in press a), the Sacoglossans perform a key role in the coastal ecosystems in which they live (CLARK & DeFREESE, 1987; MARÍN, 1988), as they make a fraction of the primary production of the marine and lagoon macrophytes (which is otherwise hard to obtain) available to the following trophic levels.

The digestive cells of the mollusc phagocyte the chromoplasts in the algal cytoplasm (Fig. 2; TRENCH, 1975; HINDE, 1973), which either remain free in the cytoplasm of the animal cells or are found inside a vacuole (MARÍN, 1988). The algal organelles and the cytoplasm which they carry stuck to them are surrounded by a host membrane (HAWES, 1979) in whose interior the chloroplasts remain undamaged (HAWES & COBB, 1980). The chromoplasts thus situated (Figs. 3 and 4) remain functional and continue to photosynthesise within the animal cell, in some species for at least as long as three months (HINDE & SMITH, 1974; MARÍN & ROS, 1989; CLARK *et al*., 1990).

The duration of the photosynthetic capacity of the retained chromoplasts is directly correlated to the difficulty in obtaining food, or in other words, to the degree of calcification of the food alga (Table III; CLARK & DeFREESE, 1987; MARÍN & ROS, 1989). The photosynthetic capacity of the kleptoplasts (generally measured as amount of radioactive carbon fixed per unit of chlorophyll) is usually

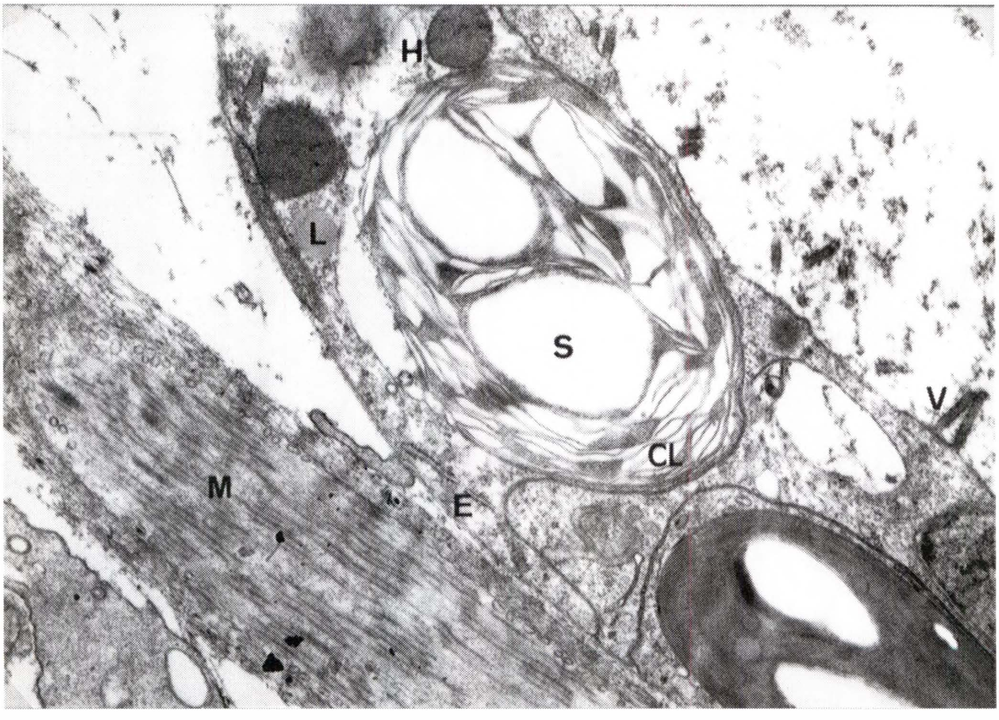


FIGURE 4. Digestive diverticula of *Elysia timida* showing single digestive cells containing intact chloroplasts (x 6000). CL, chloroplast; E, rough endoplasmic reticulum; H, mitochondria; M, smooth muscle fibre; S, starch; V, microvilli.

TABLE III. Ash content and organic matter content of the algal species eaten by the Iberian Sacoglossan species studied by the authors (ROS & RODRÍGUEZ, 1985; MARÍN, 1988; MARÍN & ROS, 1988, 1989). some figures have been estimated by the author of the reference (*) or by us (**). When there are two figures, they correspond to young and old plants, respectively. Note that, in general, the higher the ash content (or the lower the OM content) of the food alga, the longer are functional chromoplasts retained by the sacoglossan species. The references are identified in the bibliography. (Modified from MARÍN & ROS, 1989).

Algal species (eaten by sacoglossan species)	Ash, %	OM, %	Ref.
Caulerpa prolifera (Ascobulla fragilis, Oxyone olivacea, Lobiger serradifalci)	14.9	85.1	5
Griffithsia flosculosa (Hermaea bifida)	30*	70*	1
Chaetomorpha aerea (Ercolania funerea)	30**	70**	
Chaetomorpha linum (Calliopaea bellula, E. funerea)	30**	70**	
Cladophora vagabunda (Limapontia capitata, Thuridilla hopei)	31.7*	68.3*	1
Cladophora sericea (L. capitata, Elysia flava)	32*	68*	1
Bryopsis plumosa (Placida dendritica, P. viridis)	32.4*	67.6*	1
Pseudochlorodesmis furcellata (Thuridilla hopei)	32.4*	67.6*	1
Derbesia tenuissima (T. hopei)	32.4**	67.6**	1
Enteromorpha intestinalis (Limapontia capitata)	39*	61*	1
Cladophora arcta (L. capitata)	40*	60*	1
Cladophora rupestris (L. capitata, Elysia gordanae)	40*	60*	1
Codium fragile (Elysia viridis, Placida dendritica, Hermaea paucicirra)	42.0	58.0	18
Acetabularia acetabulum (Elysia timida)	19.2/ /73.1	26.9/ /80.8	4
Codium vermilara (E. viridis, H. paucicirra, P. dendritica, P. verticillata)	47.1/ /52.3	47.7/ /52.9	1
Udotea petiolata (Elysia translucens)	50.0	50.0	1
Codium bursa (E. viridis)	54.7	45.3	1
Codium adhaerens (Placida verticillata)	55*	45*	1
Valonia utricularis (Ercolania coerulea)	64.7	35.3	1
Halimeda tuna (Bosellia mimetica)	55.1/ /80.2	19.8/ /44.9	1
Halimeda simulans (B. mimetica, Elysia flava)	88.0	12.0	5

TABLE IV. Carbon fixation rates in several sacoglossan species, in relation to chlorophyll content and illumination. The references are identified in the bibliography (From MARÍN & ROS, 1989).

	Carbon fixation, μgC mgChl^{-1} h^{-1}	Illumination, μE m^{-2} s^{-1}	Ref.
A) Mediterranean species			
Elysia timida	144	200	15
Elysia viridis	95	162	11
Elysia translucens	68	100	15
Thuridilla hopei	40	100	15
Bosellia mimetica	45	100	15
Calliopaea bellula	0	-	14
Limapontia capitata	0	-	25
B) Non-Mediterranean species			
Costasiella lilianae	200-300	500	6
Elysia tuca	60	-	24

high (Table IV), sometimes as high as that of the food alga itself (BALLESTEROS, 1984; MARÍN, 1988; MARÍN & ROS, 1989; TERRADOS & ROS, in press).

The oxygen and the photosynthates produced by the chloroplasts (glucose and other carbohydrates, along with lipids and proteins) are released into the animal tissues (TRENCH *et al*., 1973b; HINDE & SMITH, 1975; HINDE, 1978, 1983; ROS & RODRÍGUEZ, 1985; MARÍN & ROS, 1989). In this way, the mollusc can satisfy some of its metabolic requirements with this organic matter subsidy of autotrophic origin. (Some species have also been shown to absorb amino acids dissolved in water; DeFREESE & CLARK, 1991). In short, the mucus produced in abundance by these gastropods contains as much as 36% of the ^{14}C fixed by the chloroplasts (TRENCH *et al*., 1970, 1972; HINDE, 1983; MARÍN & ROS, 1989), in the form of mucopolysaccharides or simple glucides such as galactose. The translocation of glucides, proteins and lipids from the chloroplast to the mollusc, and the fact that in some species up to a third of the carbon fixed photosynthetically is metabolised by the animal cells means that photosynthesis is an important source of organic nutrients for the sacoglossans with retained organelles.

The photosynthetic production of these molluscs is of the same order as that of the algae they eat (TRENCH *et al.*, 1973b; MARÍN, 1988; MARÍN & ROS, 1989). The sacoglossans, however, are not completely autotrophic, as in none of the cases studied is the algal herbivory completely substituted by the photosynthesis of the kleptoplasts; the degree of dependence varies according to the species. Through conventional feeding the molluscs also constantly manage to replace the chloroplasts that decay and become inactive. The chromoplasts cannot divide inside the mollusc because they are incapable of synthesising chlorophyll without the enzymes present in the algal cytoplasm. A sacoglossan is constantly renewing its supply of chromoplasts by feeding on new algae, but the feeding frequency seems to be above that which would be necessary if its only objective were the substitution of the non-functional chloroplasts. The sacoglossans, then, depend on photosynthesis for only a part of their metabolic requirements.

Other additional qualitative metabolic benefits have been suggested, apart from the simple quantitative contribution of nutrients. Thus, the molluscs could subsist on an unbalanced or incomplete diet (that of the algal sap or cytoplasm, which lacks the carbohydrates of the algal cell walls and thus constitutes a food input with a low

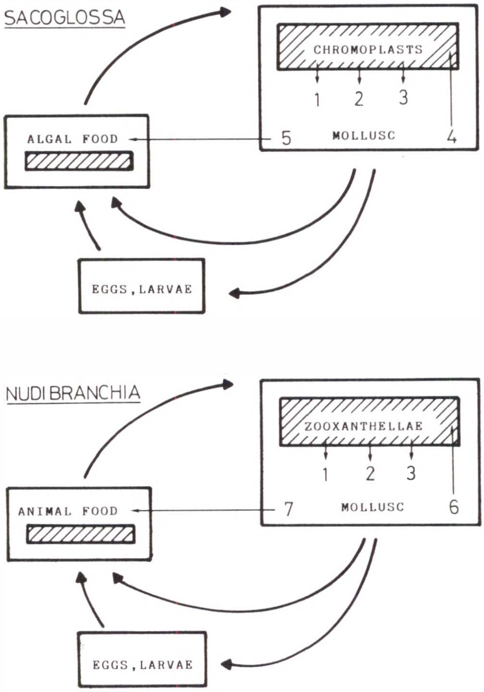


FIGURE 5. Diagrammatic representation of the benefits brought about by the association between algal chromoplasts and Sacoglossans (above) and between zooxanthellae and Nudibranchs (below). The organelles or the plant cells, depending on the case, are incorporated in the mollusc through the feeding process; the animal must eat continuously to ensure their replacement, although in the better established consortia the retention of plastids and/or cells may last weeks and even months. The eggs do not carry chromoplasts or zooxantellae and the larvae or the young acquire them when they first feed. The arrows going from the plant pseudosymbiont to the mollusc (above) or from the symbiont alga to the mollusc (below) indicate different types of benefits for the sacoglossan and the nudibranch, respectively: 1) Defensive (the animal is cryptic on its food species and on occasions the latter's defensive systems are used to the same ends by the mollusc); 2) Translocation of photosynthates (the passage of glucides, proteins and lipids has been demonstrated); 3) Other benefits, more speculative and not proven (which include the passage of oligoelements and vitamins, fixation of nitrogen by the algal consort and facilitation of the calcification processes in the mollusc). In the mollusc-algal consort direction there are no benefits (4) for the organelles, while the zooxanthellae, as well as obtaining a certain protection against external dangers, are dispersed (6), and with this can complete their biological cycle (see MARÍN & ROS, 1991). The benefit for the food species, on the other hand, is more apparent; the algae upon which the sacoglossans prey experience reduced grazing pressure in the same proportion in which its chromoplasts provide the mollusc with photosynthates (5). It has not been shown that the same (7) happens with the animal prey (cnidaria) which have symbiont zooxanthellae, on which dendronotacean and eolidacean feed and obtain, at the same time, secondary symbionts. From this diagram the conclusion can be drawn that only two of these four consortia are true mutualistic symbioses (alga-sacoglossan and zooxanthellae-nudibranch), the association between chromoplasts and sacoglossan and the one between cnidaria and nudibranch not fulfilling the requisite of mutual benefits.

C:N ratio), as the supplementary carbohydrates (in the form of polysaccharides necessary for the foot mucus and the jelly of the egg masses), would be contributed by the chloroplasts (HINDE, 1983). Epidermal uptake of dissolved organic matter has also been demonstrated (DeFREESE & CLARK, 1991), and together with nutritional transfer from kleptoplasts it may confer selective advantages to such specialized herbivores.

The possible passage of oligoelements and vitamins from plastids to host tissues (TAYLOR, 1973) and the fixation of nitrogen have also been mentioned (HINDE, 1983). Like other opisthobranchs, some species of sacoglossans are chemically defended, in many cases by products derived from substances produced by the food plant (GONOR, 1961; ROS, 1976; FAULKNER & GHISELIN, 1983; JENSEN, 1984; PAUL & FENICAL, 1986; CIMINO & SODANO, 1989; CIMINO *et al*., 1990, 1991, etc.). It is possible - although this has never been proved - that the secondary plant metabolites, precursors of the defensive animal substances, are also produced by the symbiotic chromoplasts.

Finally, it has been suggested that, similarly to what happens in the madrepore-zooxanthella interaction, the sacoglossan-chromoplast symbiosis would facilitate the calcification of the gastropod. While the supposed benefits mentioned above are plausible, even though they have not yet been demonstrated, this last one appears to be highly improbable, as testaceous sacoglossans (the only ones for which calcification would have any significance) do not appear to retain chloroplasts (Table I), and those which retain them lack calcified structures, with the exception of the radular teeth.

DOUGLAS & SMITH (1989) indicate the difficulty of proving the supposed mutual benefits that a "classical" endosymbiotic relation (between two complete species) involves for the two consorts. Likewise, the conceptual difficulty of accepting a benefit for an organelle has been mentioned (HINDE, 1983), while numerous studies exist which demonstrate the benefit for the animal host in the chloroplast-mollusc pseudosymbiosis or parasymbiosis. This is illustrated in figure 5, which shows the three main types of benefits which, at least in some cases, the sacoglossan can obtain by retaining functional chloroplasts in its tissues. For comparison, the benefits of the zooxanthellae-nudibranch symbiosis are also shown.

Firstly, there is the benefit of camouflage: the sacoglossan is cryptic on its food alga (PORTMANN, 1958; RIEDL, 1967; ROS, 1976, 1977, 1978; HINDE, 1983). Secondly, it uses either completely or partially the photosynthates produced by the plastids, which thus represent a complement to its herbivorous diet (GREENE, 1970b; GREENE & MUSCATINE, 1972; TRENCH *et al*., 1970, 1973a, 1973b; HINDE & SMITH, 1975; HINDE, 1978, 1983; TAYLOR, 1981; MARÍN & ROS, 1989; etc.). Thirdly, benefits have been drawn which, although plausible, have yet to be proved.

As regards the benefits that the algal consort may possibly obtain from the association, we shall first look at some theoretical aspects and then at a specific case which applies to the subject under discussion.

SIGNIFICANCE OF THE FOOD SUBSIDY FROM THE CHLOROPLASTS TO THE SACOGLOSSAN: FOUR POSSIBLE THEORETICAL SITUATIONS

The energy flow associated with the metabolism of a heterotrophic organism may be represented by means of a series of terms and symbols proposed by the IBP (PETRUSEWICZ & MACFADYEN, 1970; CRISP, 1984). We are interested in seeing how the production (the part of the assimilated food that is retained and transformed in growth and/or reproduction) differs in an organism receiving an energy subsidy, such as a sacoglossan that receives additional food inputs, as opposed to a typical, unsubsidised herbivore.

For a typical herbivore (a sacoglossan without kleptoplasts, for example), the following equations can be written (for an explanation of the symbols see Table V):

$$C = R + FU + P;$$

given that

$$P = Pg + Pr,$$

can be written:

$$C = R + FU + Pg + Pr,$$

which gives:

$$P = C - R - FU = Pg + Pr \qquad \text{(Eq. 1)}$$

For the case of the subsidised sacoglossan with functional kleptoplasts, the terms of the previous equations can be broken down as follows (see Table V):

$$C = Ca + Cc, \qquad \text{(Eq.2)}$$

$$R = Rs + Rc + \Delta Rs,$$

$$FU = FUs + FUc,$$

$$P = Pg + Pr + \Delta P.$$

Equation 1 then takes the following form:

$$P = Ca + Cc - Rs - Rc - \Delta Rs - FUs - FUc = \\ = \quad Pg + Pr + \Delta P \qquad \text{(Eq. 3)}$$

Rc is a loss which affects the chloroplast's photosynthetic efficiency, but not the mollusc, so it can therefore be ignored; the same goes, as regards this demonstration, for FUc, although these exudates may become part of Cc (in the form of simple compounds or oligoelements; see Fig. 5). ΔRs, or the metabolic cost of maintaining the chloroplasts, may also be negligible (if, as may be assumed, the organelle-sacoglossan symbiosis is old, both consorts have co-evolved and have experienced mutual adaptations; HINDE, 1983). Furthermore,

TABLE V. Meaning of the terms of the energy flux equation used in the text. (Based on PETRUSEWICZ & MACFADYEN, 1970; see also CRISP, 1984).

C	Consumption	Total intake of food
Ca	Alga consumption	Algal food obtained by herbivory or DOM uptake
Cc	Chromoplast consumption	Nutrient input from retained chromoplasts to mollusc
FU	Rejecta	Egesta of faeces (F) plus excreta (U): urine and other secreted material, such as exudates, mucus, etc.
FUc	Chromoplast "rejecta"	Rejecta of the healthy chromoplasts retained in the mollusc
FUs	Sacoglossan rejecta	Rejecta of the mollusc, including healthy chromoplasts or (more frequently) decaying ones
P	Production	That part of the assimilated food that is retained and incorporated in the biomass of the organism (Pg) or invested in offspring (Pr)
Pg	Growth production	That part of the neat production the animal invests in increasing its own biomass (growth)
Pr	Reproduction production	That part of the neat production the animal invests in offspring
R	Respiration	That part of the assimilated food that is metabolically converted into heat and lost
Rc	Chromoplast respiration	Metabolic losses by the chromoplasts
Rs	Sacoglossan respiration	Metabolic losses by the mollusc
ΔP	Production increase	That part of the production brought about by the additional input of nutrients from the chromoplasts
ΔRs	Respiration increase	That part of the respiration of the mollusc devoted to the chromoplasts maintenance (that is, metabolic investment in "cryptogamic" behaviour by the mollusc; see text)

there is no way of estimating the importance of ΔRs, as those sacoglossan species which are comparable from a metabolic viewpoint are all either retainers of chromoplasts or non-retainers; besides, no species of sacoglossan has been reported which throughout its adult life alternates periods of chromoplast retention with other periods without retention (see below). For this demonstration, then, it can be considered that $\Delta Rs = 0$ or, alternatively, that $\Delta Rs \ll Rs$; in both cases it is negligible.

Equation 3 then becomes:

$$P = Ca + Cc - Rs - FUs = Pg + Pr + \Delta P \qquad \text{(Eq. 4)}$$

If we compare Eq. 4 with Eq. 1 it is easy to see that $Cc = \Delta P$, that is, the energy subsidy that the chloroplasts contribute is transformed by the mollusc into an increase in production, which may be directed towards Pg, Pr, both items, or partly to subsidise Rs (including the hypothetical or negligible ΔRs).

There exist, however, four theoretically possible situations that affect the balance of Equation 2.

i) Cc AS A COMPLEMENT

In the theoretical case proposed, effectively,

$$C = Ca + Cc,$$

which means that C, the mollusc's total consumption (be it estimated either at the individual or at the population level), is provided for by means of the joint input of Ca (algal food obtained by herbivory) and Cc (nutritious contribution or "subsidy" from the chromoplasts; Fig. 6.1). Cc is a *complement* of Ca, so Ca may be less than C (the total energy that the animal must consume for its maintenance). In fact, the greater Cc, the less Ca will be. The relative proportions between Ca and Cc may vary during the life of the same species and from one species to another.

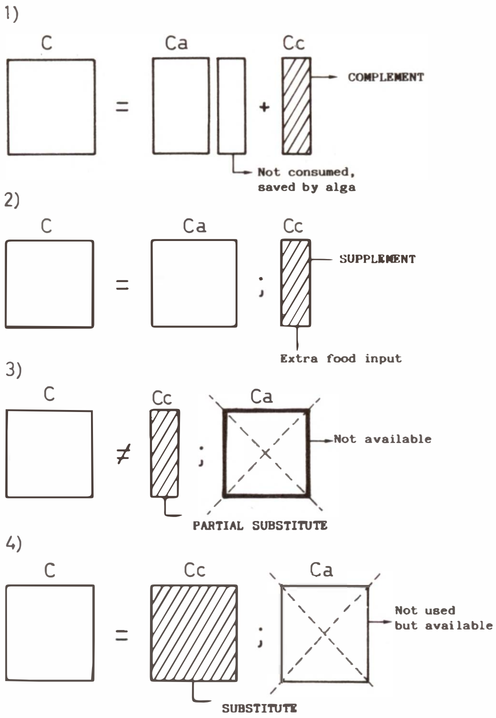


FIGURE 6. Diagrammatic representation of the four possible theoretical situations in which sacoglossans with functional kleptoplasts can take part in. 1) Complement. A part of the food requirements of the mollusc (C) is provided for by the energy contribution of the chloroplasts (Cc), which means that the mollusc's consumption of the alga population (Ca) is lower than it would be without the kleptoplasts complement (C); the alga population partly frees itself from herbivory pressure. 2) Supplement. Cc is an additional energy input, which can be wasted or invested in other metabolic needs; the basic metabolic requirements (C) are totally met by Ca. 3) Partial substitute. When Ca is not available (calcification, seasonal disappearance), the sacoglossan can thrive solely on Cc. As these energy inputs are less than C, the mollusc can only survive for a short time. At least in theory, it would be possible for Cc to provide for the greater part or all of the mollusc's energy requirements for a short period of several months (4. Total substitute), as happens in some cases of true symbiosis with zooxanthellae. No example of this kind has ever been reported for sacoglossans. See the explanation in the text.

The sacoglossan species that may be assumed to be most adapted to chloroplast retention (those which retain them the longest, and those in which the plant organelles feature the highest production) will have a notable decrease in Ca with respect to C. This is the case of *Elysia viridis* (HINDE & SMITH, 1974), *E. timida* (MARÍN & ROS, 1989) and *Costasiella lilianae* (CLARK *et al*., 1981), for example (Table IV).

In this case there is a clear benefit for the food alga, which is consumed in smaller quantities (since C > Ca, and a fraction of the potential tithe is saved by the alga). The mollusc, in turn, obtains part of its food without the corresponding foraging effort. It must be assumed that this energy saving is not compensated for by ΔR, the cost of keeping the chloroplasts active (convulsive movements, exposure of the mantle to the sun, migration in search of the light environment more suitable for the chloroplasts, etc.). This means that Rs - the respiration of a subsidised sacoglossan - is not much greater than R - the respiration of a non-subsidised sacoglossan; also applicable here are the considerations about ΔRs and Rs.

Given that the benefit is mutual when Cc is a complement to the diet, the mollusc-alga association in this case can clearly be qualified as a mutualistic symbiosis.

ii) Cc AS A SUPPLEMENT

It can also be supposed that in some situations in which the alga-mollusc association is more recent in evolutionary terms, or is for some reason less efficient (or is in the first evolutionary stages of the establishment of an association such as that described in i), it can be described thus:

$$C = Ca,$$

which means that Cc is a *supplement* to Ca, a subsidy that is obtained in addition to the energy intake necessary for the adequate functioning of the species (Fig. 6.2). This supplement may be "wasted" (in the same way that certain opportunistic, r-strategist species, both plant and animal, excrete an important part of the nutrients that they consume, or they ignore a considerable part of the food that they kill). Alternatively, it may be invested in P or R. In the first case, the energy supplement obtained by the mollusc may be the metabolic spur necessary in order for the sacoglossan species to evolve into a larger form (ΔP is invested in growth, Pg), with greater offspring production or with lecithotrophic or direct development (ΔP is invested in reproduction, Pr), or it may be invested in both entries. It has already been described how the sacoglossans are characterised by a high investment in reproductive output, often with intra- or extracapsular yolk (CLARK & GOETZFRIED, 1978; CLARK *et al*., 1979; CLARK & JENSEN, 1981; ROS, 1981; MARÍN, 1988). In the second case, Cc may provide for an increase in subsistence metabolism (an increase directed, for example, toward increasing the area explored when food is hard to find or difficult to eat; see below).

When Cc is a supplement the benefit is exclusively for the mollusc, which can grow more, reproduce more or forage more; there is no reduction in the herbivory pressure on the algal species (in fact, in almost all the situations this pressure would increase; larger animals consume more, as do those which forage over a larger area, etc.). In this way the sacoglossan species can, for example, evolve from a strategy which is more r to one which is more K (ROS, 1981, 1982; see below).

iii) Cc AS A PARTIAL SUBSTITUTE

The third possibility is that

$$Ca = 0, \text{ and } C > Cc,$$

which means that the mollusc only has available to it the energy input from the retained chromoplasts, either because the food alga has disappeared (due to its vegetative season having come to an end) or because it is unavailable to the mollusc (due to calcification of the thallus, for example; Fig. 6.3). In both cases, Cc is always less than C, and acts as a *partial substitute* for Ca; the animal subsists for some time in unfavourable conditions thanks to a partial energy subsidy.

There also exists here a benefit for the mollusc. As shown by the multiple survival experiments in starved conditions (HINDE & SMITH, 1975; CLARK *et al.*, 1979; MARÍN & ROS, 1989, etc.), the sacoglossan can survive for an extra period of time (up to three months) with a reduced, but vital, substitute subsidy (Cc).

iv) Cc AS A TOTAL SUBSTITUTE

Finally, a situation would be theoretically possible in which

$$C = Cc, \text{ being } Ca \neq 0,$$

which means that the mollusc would depend entirely on the food intake from the kleptoplasts (which would then represent a *total substitute*), in spite of having food available (Fig. 6.4). This would be the final theoretical stage of the symbiosis, similar to the situation which appears in more advanced consortia (for example, in hermatypic corals and in other cnidaria, or in molluscs such as *Tridacna*, in which, however, the animal consort complements the energy contribution of the symbiotic algae by occasionally ingesting zooplankton or necton; YONGE, 1957, 1963; MARGALEF, 1974).

No species of sacoglossan has been reported with such an independence from the algal food, and given the short life of the isolated chloroplasts and the impossibility of their reproducing inside the mollusc, it may be that such a high level of dependence on the kleptoplasts would be wholly unviable. This could be noted, however, as a theoretical possibility which deserves to be explored in further studies, on particular because *Elysia timida* may be in the way to reaching this fully autonomous state (see below).

THE EXPLOITATION OF SYMBIONT CHLOROPLASTS, A QUESTION OF SPACE AND TIME

Except for certain marginal references, the previous section looked at an alga-sacoglossan relationship invariable in space and time. Although it is not infrequent in ecology to take a classical Newtonian approach to the study of populations, in which the systems of reference are felt to be inertial, the ecosystems are not fixed systems and they undergo changes in space and time (MARGALEF, 1974).

Likewise, the changes observed in the sacoglossan populations that retain chloroplasts, the characteristics of their life cycles, and the degree of chloroplast exploitation all depend on space and time. Examples are given below of how the spatio-temporal scale determines the degree of "symbiosis" of the mollusc with the algal organelles.

i) SPACE

Herbivores, and predators in general, move around to find their food in a space whose size varies according to the characteristics of prey and predator and the abundance of food. The optimum forage area for each predator is that in which the energy used in foraging is not more than the energy obtained from the capture of food. The exploited surface will depend on this equilibrium between energy benefit and cost.

Any factor affecting the success of foraging will have a direct effect on the foraging area; if the success rate falls, the predator will need to move further to make the same number of captures (or perhaps even further still, to make up for the additional energy used in exploring the additional surface area). The final result is, therefore, an increase in the exploration and forage area, which can be described as a bending of space (Fig. 7).

The example of *Elysia timida* and *Acetabularia acetabulum,* which will be explained in greater detail below, fits especially well with this situation. As the alga calcifies, the mollusc's forage area increases; at some point between May (in which the forage area is ten times more than normal) and June (in which this area is virtually infinite), the equilibrium between benefit and energy expenditure gets out of order.

The population of a non-subsidised mollusc would disappear at this moment; the subsidised sacoglossan still survives for a supplementary period.

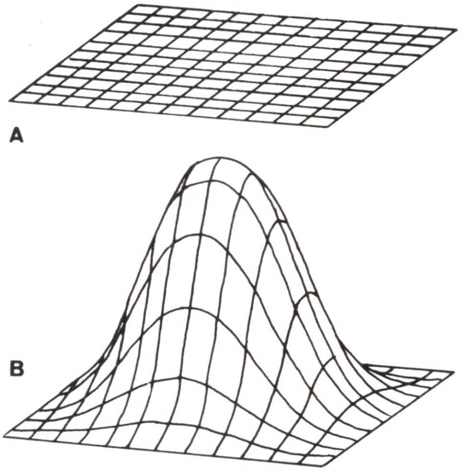


FIGURE 7. Bending of space of the alimentary forage area in a herbivorous mollusc. The animal explores an optimum space (A) when food is easily available and/or uniformly spread out. If, on the other hand, there is a factor which affects successful food capture (the food is not easily obtainable, for example), the effect is of an apparent deformation of the forage area, as the exploration surface necessary to obtain the same amount of food increases (B). If the food disappears entirely, then the exploration surface will become infinite (see Fig. 9D).

ii) TIME

The Mediterranean Sacoglossans feed on annual algae, or on algae which are distinctly seasonal in their production (BALLESTEROS, 1984, 1991; TERRADOS, 1986, 1991; TERRADOS & ROS, in press). The life cycle of these opisthobranchs is adapted to this seasonality of the food. The larval phases of the gastropods, according to the size and number of eggs and the importance of the yolk supply, adopt one of three feeding strategies. Some molluscs produce many small eggs that yield numerous larvae, which then feed for extended periods on the plankton (planktotrophy) and which are usually widely dispersed by ocean currents (THORSON, 1950; THOMPSON, 1976; ROS, 1981; TODD, 1981). Others produce fewer, larger eggs with more yolk - sometimes extracapsular - that develop as yolk-eating larvae, with limited dispersal abilities, either due to a short planktonic phase (lecithotrophy) or to a generally shorter benthic phase (direct development; THORSON, 1950; THOMPSON, 1976;

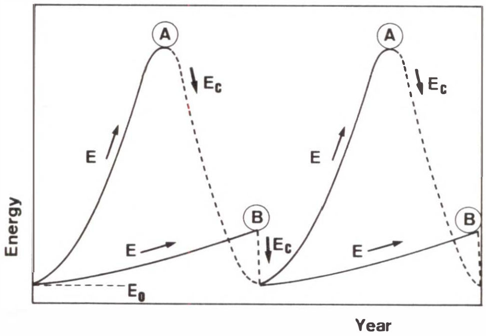


FIGURE 8. Diagrammatic comparison of the reproductive strategies in the sacoglossans, and implication of the use of chloroplast symbionts. A) Case of a typical herbivore, in which there is no energy subsidy from symbiosis (e.g *Oxynoe olivacea*). B) Situation in which the mollusc receives an energy subsidy from the algal symbiont (in this case, chloroplasts, e.g. *Elysia timida*). The continuous line represents the adult's benthic phase, and the broken line the embryonic and larval phases. See the explanation in the text.

ROS, 1981; TODD, 1981).

To sum up, we found some states of dispersion which feed on the means of dispersion (planktotrophic), while others depend on their reserves and do not feed on the means of dispersion (lecithotrophic and direct). The type of embryonary development shown by each sacoglossan species will be greatly influenced by the dynamics of the algal species used as food by the molluscs. The time that the larval phase has to remain in the plankton will depend on the spatial separation between the different food alga populations and on the period of time that these populations are functionally active.

We will now look at which theoretical situations are possible in terms of just how demanding the energy investment is which the mollusc dedicates to larval dispersal. Simplified, it can be expressed thus:

$$E = Eo + Ec,$$

in which E the is energy intake due to the food algae, Eo the threshold energy of each individual sacoglossan species and Ec the energy dedicated to larval dispersal. It is easy to see that the notation from the previous section could be used (E = C; Eo = R + FU + Pg + Pr [partially]; Ec = Pr [partially]), which we have not done so as not to complicate the reasoning that follows below.

It is known that the reproductive output depends directly on the food or energy input; for this case, there are two possible relationships between the energy dedicated by the mollusc to larval dispersal (Ec) and the total energy input (E).

iii) WHEN THERE IS PLENTY OF FOOD, AT LEAST SEASONALLY

When the food input is not limited, although it may have a distinct seasonal character, then E>> Eo, which means that investment in Ec may be important (Fig. 8). This situation corresponds to sacoglossan species that do not retain chloroplasts (like *Oxynoe olivacea*), or that retain them only for periods as short as a few hours, and so do not contribute to the energy economy of the mollusc (such as *Ercolania funerea*; MARÍN, 1988; MARÍN & ROS, 1988). *E. funerea* is a species distributed throughout the world, to be found in estuarine and coastal lagoon environments, where it feeds on the filamentous alga *Chaetomorpha linum*, whose high production is typical of the unstable environments in which it lives. The retention of *C. linum* chloroplasts by *E. funerea* never lasts longer than 24 hours (MARÍN & ROS, 1988). *Oxynoe olivacea*, which has a similarly extensive distribution, feeds on *Caulerpa prolifera* (JENSEN 1980; MARÍN & ROS, 1988), a perennial alga with a large biomass and production (TERRADOS & ROS, in press).

Both sacoglossan species have planktotrophic development, which at first seems to contradict the abundance of food. The environments in which *E. funerea* and *C. linum* live are very fluctuating and unstable, however, which results in a high larval mortality of the mollusc. *O. olivacea* feeds on the fronds of *C. prolifera*, which suffer a seasonal degradation cycle. The fronds develop in spring, then decay and fall in autumn. The mollusc's food disappears for 4-5 months; and this lack of food for such a long period is equivalent to a spatial separation of several kilometres. (If we assume that in one day a sacoglossan larva can move, say, 500m, the potential distance travelled in five months is some 75 km). When the alga used as food by a sacoglossan suffers bursts of high production and then suddenly vanishes, the herbivore anticipates the disappearance of its food species by producing a large quantity of planktonic larvae, capable of remaining in the plankton for prolonged periods. At the same time, the great abundance of the algal food, when present, makes the exploitation of symbiotic chloroplasts unnecessary.

iv) WHEN FOOD IS SCARCE OR DIFFICULT TO OBTAIN

When the food input is limited in adults (by calcification of the cell walls of the algae, for example), then E $\geq$ Eo, which means that the mollusc needs the active use of the organelles retained to obtain the energy which it must dedicate to Ec (Fig. 8). The type of larval development will depend in these cases on the seasonality of the food algae, which in turn will determine the greater or lesser diffusion of the larval phase. *Bosellia mimetica* feeds on *Halimeda tuna*, an alga which undergoes seasonal calcification (PORTMANN, 1958; MARÍN & ROS, 1988, 1989; BALLESTEROS, 1984). *B. mimetica* retains photosynthetically active chloroplasts and features planktotrophic development, allowing it to use food which is low in organic matter content, but of wide geographical dispersion and markedly seasonal.

An extreme case in this group can be illustrated by *Elysia timida*, which feeds on *Acetabularia acetabulum*, a seasonally calcified algae, low in organic matter content, but also low in geographical dispersion. *E. timida* alternates lecithotrophic development (when the food is scarce) with direct development (when food is abundant), in such a way that it substitutes a high dispersal phase for another of low dispersal, but capable of using abundant local or seasonal food (see below).

In *E. timida*, E has a value which is very close to that of Eo (Fig. 8). The "symbiosis" with chloroplasts allows energy input to be modulated and ensures the minimum number of offspring to colonise the alga's next vegetative cycle. Alternatively, exploitation of the retained chloroplasts prolongs the sacoglossans' benthic phase, which diminishes the uncertainty of the larvae's planktonic dispersion while also allowing greater energy investment in egg size, intra- or extra-capsular yolk and protection of the egg mass, which will ensure reproductive success.

A CASE STUDY: *ACETABULARIA ACETABULUM* AND *ELYSIA TIMIDA*

The theoretical situations explained above could have been successively described for a single species, during a prolonged process of alga-sacoglossan coevolution, up to the present, with different degrees of progressive "perfecting" of the consortium.

It is plausible that the typical herbivory relationship in a primitive sacoglossan could pass without too much difficulty (sturdiness of the plastids, transparency of the gastropod's body) through a first accidental phase of occasional use (as a supplement) of the products synthesised by the captive chloroplasts - it is reasonable to think that a fraction of them could continue photosynthesising during their stay in the digestive tract of the mollusc - and later towards the substitution of a part of the algal food intake for the complement represented by the photosynthates. This would have been encouraged by the progressive alimentary radiation of the group, from eaters of *Caulerpa* and other easily accessible algae, to exploiters of algal species which are more and more chemically defended or calcified, as mentioned above (CLARK & BUSACCA, 1978; CLARK & DeFREESE, 1987; MARÍN & ROS, 1989). It has already been mentioned that the final theoretical situation (in which the photosynthates of the chloroplasts would completely replace the algal food) has never been reported. On the other hand, this situation of total substitution is difficult to reach due to the limitations of the association itself, especially as it is impossible for the chloroplasts to self-reproduce.

What effectively happens is that distinct current sacoglossan species feature different levels of adaptation to the chloroplast symbiosis, and that this diversity seems linked to the taxonomic diversity of the group, as mentioned above (see for example CLARK *et al*., 1990). Different sacoglossan species which feed on algal species of different energy content, variable perforation difficulty, diverse levels of calcification or defensive substances content, and which obtain photosynthates from them in quantities which are also variable, can coexist in the same environment (BURN, 1966; CLARK & BUSACCA, 1978; TAYLOR, 1981; HINDE, 1983; GASCOIGNE, 1985; MURILLO *et al*., 1986; CLARK & DeFREESE, 1987; MARÍN & ROS, 1988, 1989; MARÍN, 1988, 1991; CLARK *et al*., 1990; De FREESE & CLARK, 1991).

The detailed study of a sacoglossan species from the Iberian Mediterranean (MARÍN, 1988; MARÍN & ROS, in press a) has revealed that, throughout the year, the use that the mollusc makes of the algal food and the food input from its kleptoplasts passes through the three phases described here as supplement, complement and partial substitute. Besides, certain biological characteristics of the two species, sacoglossan and alga, can seemingly only be explained as an adaptation to a life in common and to the chloroplast symbiosis.

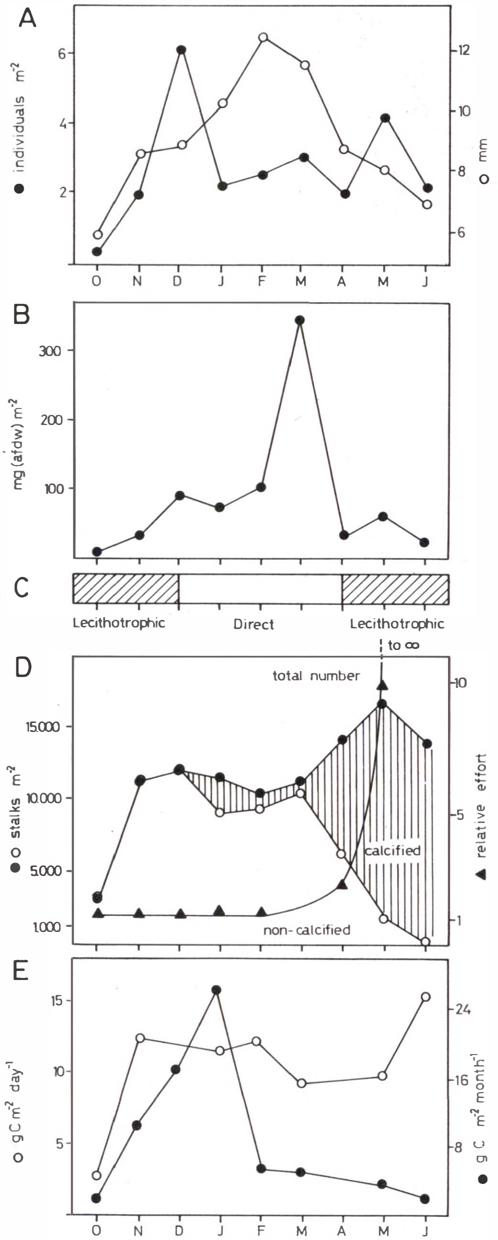
i) THE SETTING AND THE PLAYERS

The sacoglossan *Elysia timida* is an endemic Mediterranean mollusc that feeds on the green alga *Acetabularia acetabulum* (RAHAT, 1976; ROS & RODRÍGUEZ, 1985; MARÍN & ROS, 1988). The two species, the herbivorous mollusc and its algal food, were studied in their natural environment, which are the shallow, well-lit bottoms of the Mediterranean coast in south-east Spain (Mazarrón Bay), throughout an annual cycle (MARÍN & ROS, in press a). The object of this study was to investigate the main population parameters in natural conditions of both the mollusc and algal species: individual growth, population density and biomass seasonal pattern in *E. timida*, and phenology and calcification in *A. acetabulum.* Also studied were the herbivory dynamics, that is, the effect of the feeding by *Elysia* on the *Acetabularia* population, along with factors affecting photosynthesis in both algae and molluscs and the annual photosynthetic cycle. Furthermore, the development pattern of *E. timida* as a function of food abundance was established (MARÍN & ROS, in press b). Below is a summary of the results found (MARÍN & ROS, in press a).

The dasycladal *Acetabularia acetabulum* is a single-celled green alga widely used in morphogenetic studies (HÄMMERLING, 1930, 1963; BRACHET & BONOTTO, 1966). In Mazarrón Bay, the life cycle of the alga begins in October, when the young single-celled plant has two growing apices, one giving rise to the rhizoidal system that attaches the plant to the substrate, while the other growing apex becomes the erect thallus. From January, the cell walls of the thalli undergo progressive calcification; by April the base of the thallus is very highly calcified, although the apex continues its vegetative growth. The apex does not calcify until June (Fig. 9D), when the vegetative growth of the thallus stops and a cap of gametangial radial chambers is formed. The gametangial rays contain numerous cysts which when mature release pyriform biflagellate isogametes. The calcification process is not simultaneous; algae whose thalluses are calcified to different extents coexist in the same population at any one time, and different *A. acetabulum* populations also feature an unequal calcification period.

Elysia timida features a whole series of adaptations for optimum kleptoplast exploitation. *E. timida*'s photosynthetic production is one of the highest amongst the Mediterranean sacoglossans (Table IV), and in the laboratory it can survive for up to three months when starved (ROS &

RODRÍGUEZ, 1985; MARÍN & ROS, 1988, 1989, in press a and unpublished data). All these adaptations are related to the seasonal dynamics of the food used.



ii) THE PLAY

The young *E. timida* can be found on shallow rocks when the first *A. acetabulum* stalks appear in October. The population density of the sacoglossan species then increases to a maximum in December and a secondary maximum in May; the molluscs disappear in summer (Fig. 9A). The combination of number of individuals and individual growth produces a maximum sacoglossan biomass out of step with the population maximum (Fig. 9B). Calcification of the alga's cell wall first complicates browsing by *Elysia timida*, then impedes it altogether (Fig. 9D). Between January and June, the sacoglossan moves to the non-calcified apices of the algae (the only ones upon which it can feed), where it eats. From June, when all the thalli are totally calcified, the *E. timida* population falls drastically, until it disappears completely the following month.

Progressive calcification of the alga not only diminishes the food supply, but also makes it more difficult to find (Table VI). If we divide the total number of stalks of *A. acetabulum* found in a given month by the number of non-calcified stalks, we obtain a parameter measuring the energetic effort the mollusc must devote to obtain the same amount of food (F in Table VI). Even in March, virtually all attempts to find and eat a non-calcified stalk would have a positive result. In April, however, to obtain the same amount of food *E. timida* would have

FIGURE 9 Seasonal variation of some population parameters of the *Acetabularia acetabulum* and *Elysia timida* populations from Mazarrón Bay (SE Spanish coast). A) Population density (individuals m^{-2}; full dots and scale at left) and individual length (mm; open dots and scale at right) of *E. timida*. B) *E. timida* biomass, in mg (afdw) m^{-2}. C) Type of development of *E. timida*. D) Population density (stalks m^{-2}; scale at left) of *A. acetabulum* (full dots, total algae; open dots, non-calcified algae) and relative effort by *E. timida* to obtain the same edible algal diet (triangles and scale at right, see Table VI). E) Net carbon fixation rate by *A. acetabulum* (g C m^{-2} day^{-1}; open dots and scale at left) and by *E. timida* (g C m^{-2} month^{-1}; full dots and scale at right). (Modified from MARÍN & ROS, in press a).

TABLE VI. Some data on the *Acetabularia acetabulum* population in Mazarrón Bay (SE Spain coast) and on its consumption by *Elysia timida*, to show the effect of the calcification of the alga vegetative thallus on the efforts the mollusc must make to eat throughout its life cycle. A) Total number of *Acetabularia* stalks per m^2; B) Number of non-calcified stalks per m^2 ; C) Percentage of eatable *Acetabularia* stalks, (B/A) x 100; D) Number of stalks eaten per m^2 and month; E) Ratio of eatable *Acetabularia*, B/D; F) Relative effort by *Elysia* to obtain the same eatable diet. (Modified from MARÍN & ROS, in press a).

MONTH	A	B	C	D	E	F
October	2,764	2,764	100	89	31.0	1
November	11,333	11,333	100	510	22.2	1
December	12,000	12,000	100	368	32.6	1
January	11,555	9,111	78.8	285	31.9	1.3
February	10,444	9,555	91.4	521	18.3	1.1
March	11,333	10,666	94.1	1,809	5.9	1.1
April	14,222	6,222	43.7	143	43.3	2.3
May	16,950	1,753	10.3	312	5.6	9.7
June	13,998	0	0	103	0	∞

to double its efforts, and in May the effort necessary to obtain the same amount of food would be nearly ten times greater than in autumn and winter. The effort would become infinite in summer, when all *Acetabularia* stalks are calcified (Fig. 9D). This may explain the disappearance of the sacoglossans by mid-summer.

The monthly fixation of carbon by the chloroplasts retained by the sacoglossans amounts to 1% of the algae eaten during one month (Fig. 9E). This fraction is very low, but both the increasing difficulty in finding food as the year goes on and the relatively low abundance of the sacoglossans with respect to the algae must be borne in mind (Fig. 9A, D).

iii) THE PLOT (AS WE INTERPRET IT)

The reproductive pattern of *Elysia timida* is correlated to the annual growth cycle of its algal food. *E. timida* has a type 2 (of THOMPSON, 1976) development (lecithotrophic, with a short, non-feeding planktonic larval phase) in autumn and at the end of spring. In winter and early spring, however, the population changes to a direct, benthic development (type 3 of THOMPSON, 1976, without a planktonic phase; RAHAT, 1976; MARÍN & ROS, 1989, in press b; Fig. 9C). Direct development can be related to mollusc size and food availability: it occurs when the animal reaches maximum size (Fig. 9A) and *Acetabularia* is only partially calcified (Fig. 9D), thus allowing greater energy investment in yolk and accelerated development. Lecithotrophic development is restricted to less favourable periods as regards the energy investment pattern on the part of the mollusc (which is then of smaller average size) and/or the alga (which in summer is highly calcified).

An alternative interpretation is also possible, however: the planktonic lecithotrophic phase permits the mollusc to explore new areas when food is short (dispersion amongst the plankton ensures that a fraction of the larval population settles on stands of *Acetabularia acetabulum* which are not yet calcified). The direct, benthic development ensures that the population does not move far from the food-rich bottoms during the months in which the algal food is still available in large quantities (as it is either slightly calcified or not at all; Fig. 9D).

Recalling the theoretical situations mentioned above, it is easy to see that during its adult life, *Elysia timida* uses the subsidy supplied by the chloroplasts in three of the four ways possible. In its first stage (autumn), with a plentiful food supply, the energy subsidy is used as a complement; in fact, the juveniles of *E. timida* feed on *Cladophora* sp. in its first stage of development, but the chloroplasts of this green alga are not retained for long and the animal switches from the cladophoral to *Acetabularia* as soon (twelve days after its metamorphosis) as its radula enable it to perforate the cell wall of the dasycladal, which is more resistant than that of the cladophoral.

In winter and at the beginning of spring, the sacoglossan reaches its largest size (in the terms used above, Cc is invested in Pg). In synchrony with an efficient use of the chloroplasts (maximum photosynthesis; Fig. 9E), which leads us to believe that, at least in part, the photosynthates are now used as a supplement, it also changes to a metabolically more demanding type of development (Cc is invested in Pr). As spring goes on and summer draws near, the exploration effort of the sacoglossan requires greater energy expenditure, which may in part be subsidised by the kleptoplast supplement (Cc is invested in ΔRs and the mollusc reverts to lecithotrophy).

In summer, when more than 90% of *Acetabularia*'s stalks are calcified and the energy input contributed by the increasingly less successful forage trips dwindles beneath the subsistence threshold (Eo), *Elysia* can hardly survive using the usual herbivory process. Cc must then work as a partial substitute, in such a way that the typical laboratory experiment of keeping cryptogamic gastropods in starvation to determine their survival rate is to an extent reproduced in nature.

Research is needed to find those sacoglossan species (if they exist), which, even when using seasonal food, have an annual life cycle and do not disappear in the unfavourable period. If the possibility exists that Cc is used as total substitute it is in these species where such a situation would almost certainly occur. *Elysia timida* can endure up to three months in starved conditions in the laboratory, as has been stated. This is the nearest this species can go to filling the gap between June and October (Fig. 9A). Although we have not found a sole individual during this period (summer-early autumn) in Mazarrón Bay or in the remainder of the SE Spanish littoral, the possibility of this situation occurring (in this or in other sacoglossan species) cannot be entirely ruled out.

FOREIGN ORGANELLE RETENTION: CHROMOPLAST-SACOGLOSSAN "SYMBIOSIS", BUT ALGA-SACOGLOSSAN SYMBIOSIS

It therefore seems clear that the association between Sacoglossans and chromoplasts is an evolutionary solution directed toward avoiding the excessive dependency of the molluscs on food resources which are poor in energy, seasonally scarce or unobtainable for one reason or another. Other evolutionary solutions that have occurred in this order of opisthobranchs, characterised by notable polyphyletism and plasticity, have meant the appearance of oophagy in some species and families (JENSEN, 1980, 1981; see Table I), the absorption of dissolved organic matter (DeFREESE & CLARK, 1991) or the exploitation of lasting resources by others, that could have implied a secondary loss of chromoplast retention capacity (in *Limapontia capitata*, for instance; CLARK & BUSACCA, 1978).

The exploitation of the chloroplasts in their different degrees of adaptation and efficiency (structural and behavioural modifications of the sacoglossans, duration of the effective retention of the organelles, quantity of organic material synthesised by them and transferred to the mollusc, quality of photosynthates, etc.), seems to be closely correlated to the degree of difficulty in obtaining food (Table III). Moreover, the efficient exploitation of chromoplasts by sacoglossans that retain them requires various adaptations, some of which involve notable modifications of the basic structural, physiological or behavioural plan of a non-cryptogamic sacoglossan mollusc. (Incidentally, it is surprising that JENSEN, 1991, does not even mention the presence of chromoplasts in her comparative study of sacoglossan alimentary systems, as the digestive apparatus is one of the most modified by the chromoplast-sacoglossan association.)

The chromoplasts have also evolved during the sacoglossan evolution, in that they have increased their photosynthetic output (HINDE, 1983). We can clearly therefore speak of an alga-sacoglossan coevolution on a double level: specific (species-to-species) and organismic (chromoplast-to-individual mollusc).

If this is a clear case of coevolution, it is not so evident that the two consorts involved obtain mutual benefit. It has already been mentioned elsewhere that while the physiological consort of the mollusc is the "population" of chloroplasts, these do not have a genetic entity, a necessary premise to guarantee the passing on through inheritance of any evolutionary advantages produced by the mutual association (HINDE, 1983). We feel that it is necessary to pay attention to the mollusc's true consort, which is the food alga. Does the degree of alga-sacoglossan dependence shown, for example, in the high alimentary specificity or in the synchronisation of the biologial cycle of the mollusc with the phenological pattern of the alga, represent sufficient evidence of a mutualistic symbiosis? (If such an association has to be qualified as mutualistic or symbiotic it is not even clear to the specialists; see DOUGLAS & SMITH, 1989, for example. Here we adopt the traditional criteria of symbiosis: mutual benefit through the intimate physiological relationship between two species; CAULLERY, 1952; GEITLER, 1959; MARGALEF, 1974.)

Our hypothesis is that it is precisely the alga species involved in this coevolutionary "triangle" (sacoglossan-chromoplast-alga) which is the subject of the symbiosis. The alga, and not the chloroplast, is the consort which, at least in some cases, obtains benefits from the sacoglossan-chromoplast relation. Similarly, the whole algae are capable of evolving in response to the selection pressure exerted by the cryptogamic molluscs. However, while the molluscs and the cellular organelles have been studied in relation to a possible symbiosis, the same cannot be said of the chromoplasts' "donor" algae (TAYLOR, 1981). It would seem to be of greater interest to work out the plausible evolutionary changes of the algae in response to the aforementioned coevolution.

Two arguments which complement each other can be used to support the hypothesis of the algae as co-symbionts of the sacoglossans. First, an indirect reasoning, for which we shall use again the energy flux notations used above.

We could assume that the chloroplast-retaining sacoglossan has a metabolism higher than that of a sacoglossan species which does not retain them, because of the adaptations mentioned. The degree to which $Rs+\Delta Rs$ is higher than R may mean the partial or total use of the consumption increase brought about by the association. In the first case there would be no net benefit for the mollusc. A strategy in which

$$(Rs+\Delta Rs) - R > \Delta P$$

would be evolutionarily unviable. In other words, the selection of sacoglossans with captive and functional chromoplasts can only be understood if

$$(Rs+\Delta Rs) - R \leq \Delta P.$$

In this case, the symbiosis involves an increase in the evolutionary fitness of the molluscs, whatever they invest ΔP in.

Alternatively, a selection pressure towards the development of chromoplasts which are more resistant, longer lasting or more exploitable by the sacoglossans can only be explained if this involves an increase in the evolutionary fitness of the algae. Only the algae can be the object of selection for more efficient chromoplasts. The chromoplasts would be, then, simple thanatocretic instruments, such as the cnidocysts incorporated by the eolidaceans. The selection (in the sense of the eolidacean selecting actively the more urticant cnidocysts) produced by the eolidacean-hydrarian coevolution acts on these cnidaria and not on the cnidocysts themselves.

To sum up, if sacoglossans and algae are more efficient (the former because they obtain an input of energy additional to the input by typical herbivory, and the latter because they have the sacoglossans as an additional selection factor of more efficient chloroplasts), it is clear that this is a case of mutualistic symbiosis. This does not invalidate the possibility that the alga-sacoglossan coevolution has produced in the plant more efficient systems of defence (chemical, structural, etc.), or avoidance (calcification, seasonality, etc.) and a similarly more efficient herbivory in the animal.

Secondly, it has already been mentioned that, at least in one of the theoretical cases (Cc used as a food complement), the algae benefit in that they are freed from a fraction of the consumption by the herbivores. HINDE (1983) has already expounded this possibility of an alga-mollusc symbiosis. However, although she admits that "It is possible that selection pressure due to grazing by sacoglossans has produced chloroplasts capable of unusually extended survival in animal cells, thus decreasing grazing on the plants", she rapidly dismisses the possibility of such a relation thus: "If the plant from which the chloroplasts are obtained is considered to be the other member of the association, it is immediately clear that the relationship is simply a feeding one, not a mutualistic symbiosis. The animals feed on the algae, causing damage... that may... reduce the reproductive potential of the plants." However, she immediately admits that "There may have been some selective pressure on the food plants tending to produce chloroplasts that could survive longer in the Sacoglossa, thus reducing grazing on the algae." (HINDE, 1983: 104)

This decreased grazing is clearly of benefit to the algal population. The herbivory reduction may have been achieved, alternatively, by other means (those already mentioned of structural or chemical protection, for example). The final result is reduced grazing, achieved thanks to an alga-mollusc association (partial, given that only the chromoplasts are involved in it). A net benefit exists, therefore, which automatically qualifies the association as a mutualistic symbiosis.

As MARGALEF (1974) wrote, "The parallel evolution of the species united in a consortium.... is characterised, from the trophic point of view, by changes related to each other, within a general "progressive" direction. If a [subordinate] species finds itself at the limit of its turnover rate to subsist free, it can manage to subsist by increasing its energy flux.... or by reducing its mortality. Both modifications usually go hand-in-hand and frequently accompany a type of evolution in which a reduction in the turnover rate is permissible. Furthermore, due to the presence of the subordinate species, the principal species can reduce its turnover rate. The biochemical evolution has led to relations which are now indissoluble. It is necessary to consider not only the evolution of the isolated species, but also the evolution of groups of species bound in very intimate relations." (MARGALEF, 1974: 542).

Through the chromoplast "symbiosis", the alga experiences reduced mortality by herbivory, and its turnover rate also falls, as the pressure of exploitation that the mollusc exerts on it diminishes. The mollusc has a fraction of its metabolism subsidised, can invest in energetically expensive strategies and manages also to lengthen its life significantly, all of which also translates into a reduction of its turnover rate. The result is a true alga-sacoglossan symbiosis. The illustration which accompanies the above quotation (Fig. 15-16 in MARGALEF, 1974: 542), applies well to the situation described (MARÍN & ROS, 1989).

The chromoplast-retaining sacoglossans may be difficult experimental subjects when trying to establish reliable energy balances in the laboratory that universally confirm the assertions made here. In fact, this is one of the main difficulties for the precise delimitation of symbiosis situations (see the introductory quotation from MARGALEF, 1974; see also TAYLOR, 1981; HINDE, 1983; DYER, 1989; DOUGLAS & SMITH, 1989). The approach followed here, combining field data of population dynamics with theoretical arguments seems adequate, but leaves many paths open that demand further substantiation.

As the diet of the chromoplast-retaining sacoglossans is strictly herbivore and very specific, it is difficult to compare the metabolism of animals respectively with and without symbiotic chromoplasts (although the use of photosynthesis inhibitors may be a suitable method). On the other hand, the nudibranchs that retain zooxanthellae symbionts of the cnidaria that they consume (RIVA & VICENTE, 1978; RUDMAN, 1981, 1982; MARÍN & ROS, 1991), may be more suitable experimental subjects, as they can be fed with animal tissue either with or without algal symbionts. Whatever the immediate future of the research of the consortia in which sacoglossans and other opisthobranchs are involved, it will presumably provide as many surprises and as much satisfaction as in the last few years.

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