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Oriented Translocation of Energy in Grafted Reef Corals

B. Rinkevich and Y. Loya

Department of Zoology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

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Abstract. Colonies of the Red Sea reef coral *Stylophora pistillata* were grafted with alien branches (allografts), which had been labelled by $NaH^{14}CO_3$ in the light. The "cold" host-colonies translocated the ^{14}C-containing photosynthetic metabolites in an oriented pathway from the grafted branches into their own tissues. The highest accumulations of ^{14}C products were detected in specific branch-tips of the host, away from the contact zones. The "recipient" colonies utilize these energy-rich materials for their metabolic requirements. The $^{14}CO_2$ produced through respiration is consequently detected in the skeletal-carbonate of the tips as $Ca^{14}CO_3$. The purple morph of *S. pistillata* is found to be superior to the yellow morph.

Introduction

In many cases of naturally growing hermatypic corals, branches of different colonies of the same species, which settle beside one another, are subsequently fused (Lang 1973) and form lumps a few feet across (Gardiner 1931). Furthermore, it is well known that after heavy storm-activities (such as hurricanes), broken branches of different colonies are spread all over the reef zones and frequently are found lying in piles (Shinn 1976; Highsmith et al. 1980; Tunnicliffe 1981). Regeneration, fusion and growth of broken fragments often follow (Gilmore and Hall 1976; Tunnicliffe 1981). The phenomenon of formation of new reefs by broken branches has been also described for Pleistocene reefs at Barbados, as well as Holocene reefs at Curacao and Bonaire (Mesolella 1967; Focke 1978). The surprisingly fast recovery of corals and reefs (Shinn 1976; Highsmith et al. 1980) stimulates many questions on the mechanisms by which pieces of coral branches reestablish new colonies.

Similar to other branching corals in other localities (Shinn 1976; Gilmore and Hall 1976; Highsmith et al. 1980; Tunnicliffe 1981; Woodley et al. 1981), branches of the shallow water population of *Stylophora pistillata* at Eilat are scattered over the reef during severe southern winter storms. Consequently detached fragments of this species are often found "settled" within alien colonies of the same species. *S. pistillata* served as the experimental species in the present work, since it is one of the most abundant corals in the Gulf of Eilat (Loya 1972).

We describe here a newly discovered phenomenon of oriented translocation of photosynthetically fixed products between grafted branches of alien corals and host colonies, which suggests a competitive mechanism occurring in colonies brought into physical contact.

Materials and Methods

Branches of different colonies of *S. pistillata* were incubated in situ with ^{14}C-bicarbonate for 24 h. All incubations were carried out in front of the Marine Biological Laboratory in Eilat, Gulf of Eilat, Red Sea. The branches were attached to concrete plates before incubation. After an acclimation period of at least 24 h, they were enclosed within transparent plastic bags and $NaH^{14}CO_3$ was injected into the bags (final concentration of 0.05 μCi/ml). After incubation the bags were removed and the labelled branches were tied to host colonies by plastic filaments. The grafting procedure was simple and quick causing minimal damage to branches. Two types of segments were sampled: Tips (dome-like in shape) and fragments below the tips (cylinder-like). The branch samples were brought to the lab where surface areas of the segments (S) were calculated, using the formula $S = 2\pi rh$ for the cylinder samples, where $r = (l + w)/4$ (l = length, w = width and h = height of the segment), and for branch tips using $s = \pi(h^2 + (l+w)^2/16)$. All measurements were made with a caliper with accuracy of 0.1 mm. Coral branches were put into plastic vials and 0.5 ml of hydrogen peroxide (30%) was slowly added. After complete digestion of the tissue, the skeleton was removed and 0.1 ml of 5 N HCl was added to remove all unincorporated ^{14}C bicarbonate. Two replicates (0.2 ml each) were placed in separate minivials and 1 ml of distilled water followed by 2 ml of Instagel (Packard) scintillation cocktail were added. Activity of ^{14}C was determined by liquid scintillation spectrometer (Packard).

Acidification of the coral skeleton produces $^{14}CO_2$ and the only ^{14}C remaining is that of the skeletal matrix. Separation of ^{14}C found in these compartments was done by a special apparatus made of two plastic minivials connected by a polypropylene duct (Rinkevich 1982). Acidification was done by adding 0.5 ml of H_3PO_4 (25%) drop by drop. The $^{14}CO_2$ produced was collected by 1.5 ml of Carbosorb solution (Packard). Two aliquant replicates (0.2 ml each) were taken from the acidified skeletal-solution and 1 ml of distilled water followed by 2 ml of Instagel were added to each replicant.

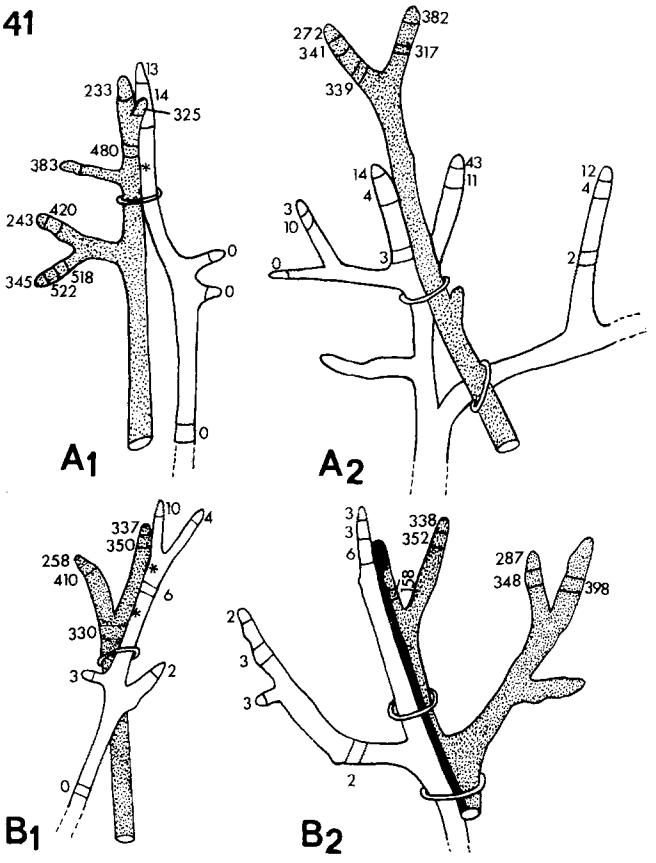

Fig. 1. *S. pistillata*-colony pair no. 41: Counts of ^{14}C materials detected in tissue fragments in reciprocally grafted colonies. A yellow morph (colony A) and purple morph (colony B) are tested. A_1 and B_1 are isografts (self grafts), A_2 and B_2 are allografts (alien grafts). *Dotted parts* represent the radioactive grafted branches, *blank parts* represent the nearest branches of the host "cold"-colony, *black parts* along a branch represent dead tissue, asterisk-areas of fusion. *Numbers* represent the level of radioactivity in CPM $(mm^2)^{-1}$ of surface area of a given segment. In cases of background levels or when recorded radioactivity is less than 1 CPM $(mm^2)^{-1}$, the results are designated as zero. In all cases of isografts complete fusion was recorded within one month in areas of attachment, while in allograft experiments fusion was evidenced in few cases only

In the Red Sea, *S. pistillata* exhibits a variety of colour morphs from pale yellow to purple (Rinkevich and Loya 1979). In field observations and experiments we found that the different colour morphs of *S. pistillata* exhibit ecological-hierarchy, where purple colonies are significantly superior to yellow colonies, when they come in contact (Rinkevich and Loya 1983). In the present work special attention was given to the colour morphs of the interacting branches.

Results

Three sets of experiments were conducted:

a) ^{14}C translocation in reciprocally-paired grafted-colonies: Four pairs of big colonies were chosen, and two branches were sampled from each colony for ^{14}C labelling. Then, one of the branches was replaced and tied onto its original colony (isograft), while the second was grafted onto the alien-pair colony (allograft). The attached branches remained for 4 to 8 weeks in situ and at the end of each experiment were carefully removed together with the surrounding branches of the host colony. Figure 1 summarises the results of a representative case in reciprocally-grafted colonies. The results of the other 3 coral pairs are similar to pair no. 41 (Fig. 1), and summarised in Table 1. From Fig. 1 and Table 1 it is concluded that: (1) In all the cases of allografts and isografts, ^{14}C fixed carbon was transferred to the host colony. When no significant damage was observed to either one of the partners, more ^{14}C was translocated to the host colonies in the cases of allografts as compared to isografts (141 and 195-fold difference in pairs nos. 11 and 20, respectively, in cases of superior colonies, Table 1). (2) In cases of partial death of one of the partners along contact zones, relatively low levels of translocated ^{14}C materials were recorded. (3) Radioactivity is found throughout the nearest host branches but is markedly accumulated only in the tips (upper 0.5 cm of the branches). In some cases, tips which were sampled approximately 10 cm or more away from the radioactive grafts showed the highest ^{14}C amounts. In all

Table 1. Summary of results in three pairs of reciprocally grafted corals within the first set of experiments: accumulation of ^{14}C into tissues. (A_1, A_2, B_1, B_2, see legend to Fig. 1). (b) base of branch; (t) tip

Pair no.	Colour type	Tested graft	The highest value of accumulated ^{14}C CPM $(mm^2)^{-1}$		Comments
			Nearest branch to contact zone	Far away branch from contact zone	
11	P[a]	A_1	4 (b)	1 (t)	Fusion
		A_2	8 (t)	141 (t)	Fusion
	P	B_1	8 (b)	6 (b)	
		B_2	22 (b)	23 (t)	
20	Y	A_1	6 (t)	3 (t)	Fusion
		A_2	5 (t)	5 (b)	Dead tissue in contact zones
	P[a]	B_1	12 (b)	1 (b)	
		B_2	12 (b)	195 (t)	High accumulation in two further away tips
43	P	A_1	0 (b and t)	1 (b)	
		A_2	2 (t)	13 (t)	
	P	B_1	4 (t)	1 (t)	Fusion
		B_2	9 (t)	2 (t)	Dead tissue in contact zones

[a] Superior colony

Table 2. Measurements of ^{14}C recorded within tissues, skeletal organic matrix and skeletal carbonate in two representative cases of allografts (colonies nos. 23 and 35). The host colonies and the allograft branches are purple morphs. The level of radioactivity is presented as CPM $(mm^2)^{-1}$ of surface area. The locality of the sampled segments is shown in Fig. 2. CPM $(mm^2)^{-1}$ values of the tissues in the alien branch grafted to colony 23 is about 150 and that of colony 35 is about 100

Sampled segment	^{14}C activity in					
	Coral no. 23			Coral no. 35		
	Tissue	Skeletal organic matrix	Skeletal carbonate	Tissue	Skeletal organic matrix	Skeletal carbonate
a	12	0	2	4	0	2
b	12	0	0	4	0	0
c	13	0	0	4	0	0
d	9	0	0	2	0	1
e	7	0	2	2	0	0
f	4	0	16	8	0	5
g	16	0	0	3	0	0
h	5	0	0	2	0	1
i	10	0	7	3	0	0
j	13	0	6	0	0	0
k	7	0	0	0	0	0
l	6	0	4	3	0	1
m				0	0	1
n				7	0	3
o				2	0	0
p				2	0	1
q				3	0	0

cases of undamaged allografts, only moderate or low levels of radioactivity were recorded in the nearest tip to the radioactive branch (141 CPM $(mm^2)^{-1}$ vs 8 CPM $(mm^2)^{-1}$ in pair no. 11 and 195 CPM $(mm^2)^{-1}$ vs 12 CPM $(mm^2)^{-1}$ in pair no. 20, Table 1). Only one or two tips in every tested colony contained the highest amount of ^{14}C materials. All other tips or bases accumulated only moderate amounts (Fig. 1).

Controls were run by fixing radioactive branches on concrete plates, 2–3 mm away from "cold" branches of the tested colonies. No radioactivity was recorded in the "cold" controls. We conclude therefore, that the translocation of ^{14}C materials is directional rather than diffused and occurs only when a physical contact exists between host colonies and grafts.

b) Utilization of ^{14}C products by allografted-colonies: Sixteen *S. pistillata* colonies were grafted at different times with ^{14}C labelled branches from other colonies. In 6 cases no ^{14}C activity was recorded in the host colonies up to 8 weeks after grafting. Four of these 6 unlabelled colonies belonged to the yellow morph while their attached "hot" branches were purple (Rinkevich and Loya 1983). In the other two colonies purple branches were attached to purple colonies. In the remaining 10 cases ^{14}C materials were translocated from the alien hot branches to the host colonies. In 7 of the latter colonies we also examined the relative accumulation of ^{14}C materials within the three major compartments of the coral: the tissue, organic matrix of the skeleton and the skeletal carbonate. The results concerning radioactivity in the coral tissues of the 10 colonies examined confirmed the conclusions of the first set of experiments, as well as the results of the controls (Fig. 1, Table 1). Figure 2 and Table 2 provide the results of colonies 23 and 35, two representative cases in the second set of experiments. These two colonies differ in the way of accumulation of ^{14}C within the skeletal carbonate. A significant amount of ^{14}C, up to 80% of the total radioactivity (sample f, colony 23), is accumulated within the skeletal carbonate, especially in tips. Colony no. 35 represents a case in which the highest amounts of ^{14}C are accumulated both in the tissue and skeletal carbonate of the same tip (segments f and n). Coral no. 23 represents a situation in which one tip accumulates the highest amounts

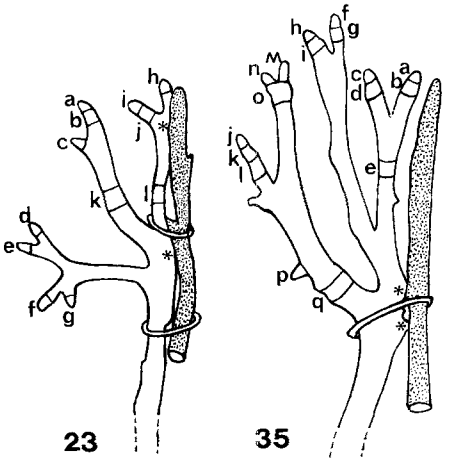

Fig. 2. Schematic drawing of allografts (colonies nos. 23 and 35) sampled 8 weeks after grafting. *Letters* denote sampled segments. Levels of radioactivity detected in the tissue, skeletal organic matrix and skeletal carbonate fragments are given in Table 2. For further explanation see legend to Fig. 1

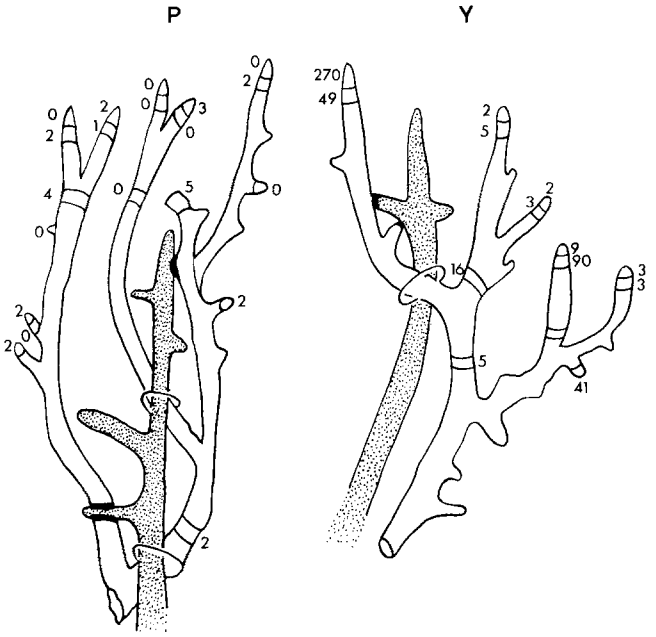

Fig. 3. Schematic drawing of ^{14}C labelled colonies: Purple colony (*P*) and yellow colony (*Y*). *Numbers* represent counts of ^{14}C materials in CPM $(mm^2)^{-1}$ detected in tissue fragments of purple "cold" branches grafted on the two experimental colonies. *Dotted branches* represent part of the ^{14}C labelled host colonies, *blank branches* represent the allografts, *black parts* represent dead tissue

of ^{14}C within the tissue (segment g) while another tip contains the highest amounts of radioactivity in the skeletal carbonate (segment f). In all the tested colonies the organic matrix did not accumulate any ^{14}C materials. In cases where traces of ^{14}C were found in the organic matrix, their values were always less than 1 CPM $(mm^2)^{-1}$ of surface area, which is negligible (perhaps from tissue residues). In the vast majority of the cases only background counts were recorded in the skeletal organic matrix.

c) The significance of the colour morphs: The first two sets of experiments suggest that the colour morph of a colony (which represents the aggressive hierarchy in *S. pistillata*, Rinkevich and Loya 1983) is significant in predicting the direction of the ^{14}C translocation. To strengthen this suggestion two mature colonies (one purple and one yellow) of *S. pistillata* were labelled with ^{14}C bicarbonate. After incubation the two colonies (Y and P) were grafted with "cold" branches taken from a third purple colony for one month. The results represented in Fig. 3 clearly demonstrate the correlation between the colour morph and the direction of the photosynthetic-metabolites. Very little amount of radioactive materials (no more than 5 cpm $(mm^2)^{-1}$) were detected in the "cold" purple branch, grafted to the purple colony (i.e., "superiority of the bigger", Rinkevich and Loya 1983). By contrast, considerable amounts of ^{14}C labelled materials were found in the tissue of the purple branch attached to the yellow colony (up to 270 cpm $(mm^2)^{-1}$ in a branch tip).

This observation further strengthens the previous results indicating that only one branch-tip is the most preferred area for the accumulation of the ^{14}C materials. Two other branch fragments contained much less ^{14}C materials, but still in considerable amounts (41 cpm $(mm^2)^{-1}$ in one tip and 90 cpm $(mm^2)^{-1}$ in a middle segment, Fig. 3).

Discussion

In a previous work (Rinkevich and Loya 1983) we have studied intraspecific interactions between the two basic colour morphs of *S. pistillata*. We showed that the purple colonies were superior to the yellow morphs and competitively excluded them, even when they were not physically touching. The present work concentrates on the physiological outcomes, when physical touch occurs between different morphs, further indicating the superiority of the purple morph in the intraspecific aggressive hierarchy within populations of *S. pistillata* (Table 1, Figs. 1, 3).

The results of the three sets of experiments described in the present work are the first evidence for oriented translocation of energy in grafted reef corals. Translocation of organic products of algal photosynthesis (such as lipids, glycerol and glucose) from coral bases to their tips, were found in the branching coral *Acropora cervicornis* (Pearse and Muscatine 1971; Taylor 1977). Similar intracolonial transport of soluble organic compounds were recorded in the massive coral *Montastrea annularis* (Taylor 1977). However, the present study demonstrates for the first time that such translocation occurs between neighbour-colonies of the same species, is oriented towards one or two specific sites of the recipient coral and that this phenomenon occurs only when physical contact is formed.

We suggest that the method of grafting ^{14}C labelled branches on "cold" colonies provides the opportunity of following the pathways of photosynthetically energy rich materials within and between different compartments of the coral. In addition, the results of this work point out the possibility of different rates of metabolism along a coral-branch. In the last years several models based on stable isotope ratios ($^{13}C/^{12}C$ and $^{18}O/^{16}O$ within a coral skeleton compared with sea water) have suggested that coral skeletal carbonate is derived from a mixture of seawater bicarbonate and respiratory CO_2 (Weber and Woodhead 1970; Goreau 1977). Earlier, Goreau (1961) proposed that ^{14}C which was incorporated within coral skeletons, was diluted by unlabelled carbonate from the coral tissue (CO_2 produced in respiration). Using an experimental procedure of feeding corals with ^{14}C labelled mouse-tissue (Pearse 1970), it was found that up to 9% of the radioactivity, accumulated in the skeletal carbonate during the following 13 days. We propose that the recipient coral utilizes the photosynthetic products derived from the "donor" coral for its metabolic requirements such as respiration, tissue growth, reproduction and perhaps most obviously for skeletal deposition. Particular tips on the recipient coral constitute the most active metabolic sites, where the highest amounts of calcium are being deposited (Rinkevich 1982). The $^{14}CO_2$ produced through respiration had evolved from utilization of the donor's ^{14}C photosynthetic-products and is detected in the skeletal carbonate as

$Ca^{14}CO_3$. Thus, the relatively low amounts of ^{14}C recorded in tip tissues in some cases, might be the result of high metabolism in these sites, rather than low ^{14}C accumulation (such as segment f colony 23, Table 2).

The movement of photosynthetic materials from grafted branches and their oriented translocation point to the possible ecological importance of this phenomenon in the re-establishment of coral reefs after severe storms. We suggest that the fusion between allogeneic branches (Gilmore and Hall 1976; Tunnicliffe 1981) lying in piles after such storms (Gilmore and Hall 1976; Shinn 1976; Highsmith et al. 1980; Tunnicliffe 1981; Woodley et al. 1981) and their fast regeneration (Shinn 1976; Highsmith et al. 1980) might be explained by the oriented translocation of energy. Thus, the chances of "recipient" branches for survivorship and development into colonies are enhanced, while "donors" might exhibit delayed mortality (Knowlton et al. 1981).

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