Doklady Biological Sciences, Vol. 401, 2005, pp. 116–119. Translated from Doklady Akademii Nauk, Vol. 401, No. 2, 2005, pp. 275–278.
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GENERAL BIOLOGY

Symbionts of Holothurians from South Vietnam: Intra- and Interspecific Interactions

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Presented by Academician D.S. Pavlov July 6, 2004

Received July 9, 2004

Large sessile marine animals (sponges, corals, ascidians, and echinoderms) are always colonized by various symbionts. Their composition varies widely, from protozoans to fish [1–3]. In a survey of the symbionts of echinoderms, 825 species associated with different representatives of this phylum were found [4]. An individual host may be colonized by one macrosymbiont or tens or hundreds animals from one or different species simultaneously. In the latter case, multispecies symbiotic associations are formed. Both intraspecific interactions between symbionts and interspecific interactions among symbionts and hosts as well as among different symbiotic species occur in these associations.

In symbiotic associations, the host serves as a buffer between symbionts and the environment, which decreases the influence of abiotic factors on the symbionts and makes biological interactions more distinct. Moreover, the symbiont–host systems have only few biotic connections and possess relatively simple organization. All these properties make symbiotic associations very suitable objects for studying of biological interactions.

Shallow-water tropical holothurians and their symbionts are an example of such multispecies symbiotic associations. Holothurians possess the richest symbiotic fauna among echinoderms [4]. However, in only two papers published by now holothurians and their symbionts were considered as an integrated community [5, 6], and only one of them dealt with biological (trophic) interactions between symbionts (copepods from the genus *Tisbe*) and their host (holothurians from the genus *Holothuria*) [5]. Recently, we studied trophic interactions between holothurians and one of their common symbionts, the polychaete *Gastrolepidia clavigera* Schmarda [7].

Here, we describe the set of species associated with shallow-water tropical holothurians living in South Vietnam and analyze their location on (in) the hosts, distribution on holothurians, traumatism, food spectrum, co-occurrence and, on the basis of these data, intra- and interspecific interactions.

The material, 984 holothurians from seven species and 1111 their symbionts, was collected in South Vietnam (Nha Trang city area) in the period from 1985 to 1990. The methods of sampling were reported earlier [7]. The location of symbionts was recorded either underwater during sampling (crabs, shrimp, and mollusks) or during the preliminary treatment of the material (fish). In addition, two series of observations in aquariums were performed to determine the location of crabs and polychetes. To analyze the distribution of symbionts on hosts, we used coefficient of variance (the ratio of the mean value to the dispersion, $K\delta^2$ [8]). The symbionts were observed with the help of a binocular microscope to detect traumas of the body and appendages. To study the food spectrum, we dissected 15 crabs *Lissocarcinus orbicularis*, 12 fish *Carapus homei*, and 7 shrimp *Periclimenes imperator.* The contents of the stomachs and intestines were placed into a 1 : 1 glycerol–alcohol mixture and examined under a light microscope. To analyze the peculiarities of the simultaneous occurrence of different symbiotic species, their actual distribution was compared with the theoretical one obtained in a model experiment using the random-number generator. The results were verified with the help of Fisher's exact test [8].

Taxonomic composition of associations. Holothurians from seven species were studied: *Actinopyga echinites* Jaeger, 1833 ($n = 65$), *Bohadschia graeffei* Semper, 1868 ($n = 12$), *B. tenuissima* Semper, 1868 ($n = 1$), *Holothuria atra* Jaeger, 1833 ($n = 118$), *H. leucospilota* Brandt, 1835 ($n = 43$), *Stichopus chloronotus* Brandt, 1835 ($n = 714$), and *S. variegatus* Semper, 1868 ($n = 28$). On (or in) these holothurians we found seven species of symbionts: polychaetes *G. clavigera* Schmarda, 1861 ($n = 820$), crabs *L. orbicularis* Dana, 1852 ($n = 109$) and *Chlorodiella* aff. *barbara* Borradaile, 1900 ($n = 1$), fishes *C. homei* Petit, 1934 ($n = 125$) and *C. mourlani* Richardson, 1844 ($n = 12$), shrimp *P. imperator* Bruce, 1967 ($n = 15$), and mollusks *Melanella* aff. *aciculata* Pease, 1861 ($n = 29$).

Location. Since the location of symbionts may reflect the peculiarities of their biology, we performed

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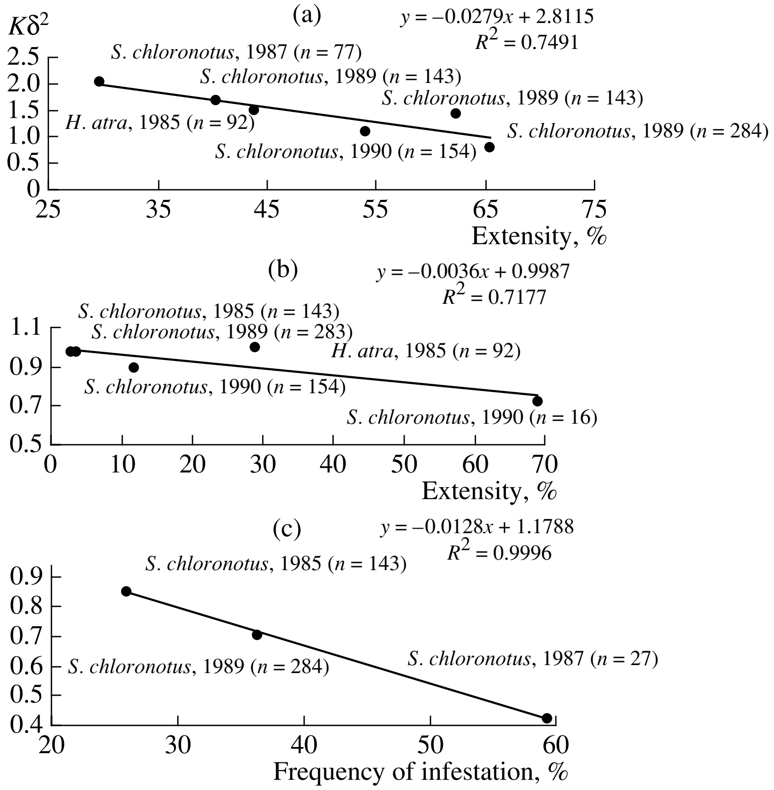

Fig. 1. Changes in $K\delta^2$ with an increase in frequency of infestation. Points show different samples of holothurians; sample sizes are indicated in parentheses. (a) *G. clavigera*; (b) *L. orbicularis*; (c) *C. homei.*

the quantitative analysis of location for the most numerous species. The polychaetes *G. clavigera* live on the body surface of holothurians. During the observations in aquariums, most *G. clavigera* were found on the posterior part of the body of holothurians (57.6% of observations, $n = 66$); 25.7 and 16.7% of them were found on the middle and anterior parts, respectively. Most crabs *L. orbicularis* (51.2%, $n = 41$) were found on the anterior part of the body (among oral tentacles and in the pharynx); 26.8 ($n = 11$) and 22.0% ($n = 9$) of the crabs were found in the cloaca and on the body surface, respectively. If two or more crabs occurred on the same host (four observations), they were located on different parts of the body surface of the holothurians. When we studied the distribution of a heterosexual pair (observations in an aquarium), only in 8 out of 29 cases (27.6%) both crabs were located on the same host and never, on the same part of the body. The fishes *C. homei* and *C. mourlani* were mostly located in the body cavity of holothurians (98.4%; $n = 184$); only 1.6% of the fish were found in respiratory tree. We did not quantitatively estimated the location of the shrimps *P. imperator* and mollusks *M.* aff. *aciculata.* All shrimps were found on the holothurian body surface; all mollusks, on the anterior part of the body and on oral tentacles.

Distribution over the hosts. The $K\delta^2$ values for general samples of the polychaete *G. clavigera*, crabs *L. orbicularis*, and fish *C. homei*, were low (1.10, 1.39, and 0.80, respectively), that indicated a random distribution or, in the case of fish, the tendency to a uniform distribution. Moreover, in the cases of the polychaetes *G. clavigera* and fishes *C. homei*, the coefficient of variance decreased as the frequency of infestation of holothurians by symbionts, i.e., the frequency of simultaneous occurrence of symbionts, increased (Figs. 1a, 1c). In the case of the crab *L. orbicularis*, the dispersion coefficient decreased in a sample consisting of adult animals only (Fig. 1b). The mollusk *M.* aff. *aciculata* formed aggregations, that was suggested by a high $K\delta^2$ (3.57).

Traumatism. Various traumas of the body and its appendages were observed in three symbiotic species: the polychaetes *G. clavigera,* the crabs *L. orbicularis*, and the shrimps *P. imperator.* In polychaetes, we found two types of traumas: minor (when parapodia, antennae, and/or dorsal cirri have been torn off and were regenerating) and major (when the posterior part of the body had been torn off and was regenerating), which were observed in 18.8% ($n = 602$) and 11.3% of polychaetes, respectively. The frequency of infestation of holothurians by polychaetes (an analogue of population density) was correlated with the frequency of minor traumas ($r = 0.98$, $n = 41$, $p < 0.02$), whereas major traumas did not exhibit a significant correlation ($r = 0.39$, $n = 39$, $p < 0.61$), which confirmed the suggestion

Observed and theoretically expected frequencies of the symbiont co-occurrence (above and below the diagonal, respectively)

Species	LO (53)	GC (304)	PI (5)	CH (118)	CM (12)
LO		20.00	3.00	5.00	0.00
GC	22.07^{r}		3.00	66.00	11.00
PI	0.72^{+}	2.45^{r}		1.00	1.00
CH	3.73^{r}	69.63^{r}	0.37^{r}		1.00
CM	0.46^{r}	7.27^{r}	0.27^{r}	3.34^{-}	

Note: The general sample of holothurians ($n = 984$) was analyzed. LO, *L. orbicularis*; GC, *G. clavigera*; PI, *P. imperator*; CH, *C. homei*; CM, *C. mourlani.* The occurrences of the respective species on holothurians are indicated in parentheses. The results of Fisher's test are indicated as superscripts at the expected frequencies: r, no deviation from the random distribution ($p < 0.05$); + and –, the simultaneous occurrence is higher or lower, respectively, than expected in the case of a random distribution.

that minor traumas result from intraspecific antagonism [9]. We observed traumas of the carapace and appendages in 6.4% of crabs *L. orbicularis* and traumas of the pereopods and chelipeds in 20% of shrimp *P. imperator.*

Food spectra. Various food particles were observed in the stomachs of 73.3% of crabs. These were fragments of the benthic crustaceans Ostracoda (in 18.2% of the crabs studied) and Harpacticoida (36.4%), gastropods (18.2%), and algae (45.6%), as well as detritus. Food remains were found in the digestive tracts of 83.3% of fishes *C. homei.* Fragments of crustaceans were the most frequent (75.0%). In three cases, they were identified as remains of shrimps and in one case, as *P.* aff. *imperator*, a symbiont of holothurians. Fragments of fishes (two findings or 16.7%) were identified as *Carapidae* gen. sp., apparently, *C. homei* or *C. mourlani.* Food fragments were found in the stomachs of 42.7% of shrimps *P. imperator.* All of them were remains of crustaceans. In two cases, we classified them as amphipods from the family Caprellidae. For none of these species we found any evidence that they fed on holothurian tissues. Two other symbiotic species, the polychaetes *G. clavigera* and the mollusks *M.* aff. *aciculata*, were shown to feed on tissues and coelomocytes, respectively, of their hosts [7, 10].

Simultaneous occurrence. When analyzing the distribution of different symbiotic species, we found that the observed frequency of co-occurrence was close to the theoretically expected one, that was confirmed statistically using Fisher's test (Table 1). The only exception was the pair of fish species (*C. homei* and *C. mourlani*). The decreased frequency of simultaneous occurrence of these species (the symbol minus in the table) indicates that they compete with each other.

A uniform distribution is usually related to the competition for resources and often appears as a result of intraspecific conflicts in various marine invertebrates (e.g., [11, 12]). In the given case, the tendency towards a uniform distribution of adult animals inmost symbionts studied (except of the mollusk *M.* aff. *aciculata*) and the decrease in coefficient of variance with an increase in frequency of infestation (density of infestation) apparently reflected intraspecific antagonism. Intraspecific conflicts in these species were confirmed by the traumas found in polychaetes, crabs, and shrimps; the presence of chaetae of conspecific animals in the stomachs of polychaetes [7]; cannibalism observed in fish; and the distribution of crabs living on the same host on different body parts. These data allow us to assume that most symbionts of holothurians (polychaetes, crabs, fish, and probably shrimp) exhibit behavior aimed at the defense of the host as their territory (territoriality). Apparently, territoriality is, in this case, a result of the competition for space and food [13] and is a mechanism protecting the hosts from overexploitation. Note that, in contrast to intraspecific interactions, interspecific ones (except for that between the morphologically and ecologically similar *C. homei* and *C. mourlani*) in the populations studied had no substantial effect on the distribution of symbionts on holothurians. Apparently, this is the result of the distribution of symbionts by different ecological niches. Thus, the food of crabs *L. orbicularis* consists of free-living benthic, mainly interstitial, organisms. The fishes *C. homei* and the shrimp *P. imperator* feed on free-living crustaceans; the polychaetes *G. clavigera*, on host tissues and crustaceans associated with them [7]. Mollusks from the genus *Melanella* are parasites feeding on coelomocytes of their hosts [10]. Thus, the feeding of *Melanella* and *Gastrolepidia* are practically confined to the bodies of their hosts, whereas crabs, fish, and shrimp feed on free-living benthic organisms. It is interesting to point out that the trophic differentiation of symbionts corresponds to the difference in the preferred parts of the host body. For example, polychaetes prefer the posterior part of the holothurian body; crabs are more often found among tentacles in the anterior part; and fish, in the coelomic cavity of holothurians. The distribution by different ecological niches was earlier demonstrated for symbionts of sea lilies [14]. Probably, this is an evolutionary adaptation of symbionts to the life on common hosts.

ACKNOWLEDGMENTS

We are grateful to the Russia–Vietnam Tropic Center for the possibility to perform these studies. We thank our colleagues Yu.Yu. Dgebuadze, O.V. Savinkin, A.V. Smurov, A.I. Buyanovskii, A.I. Azovskii, I. Eeckhaut, and C. Massin, who assisted us in preparing this study.

This work was supported by INTAS (project no. YSF 2001/2-0072).

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