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Host selection and preferences of coral symbiotic crab *Tetralia rubridactyla*

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ABSTRACT

Coral symbiotic crabs provide considerable benefits to their host corals. A comprehensive understanding of the association between these crabs and their hosts could help clarify the relationship, interaction, and importance of symbionts with coral reefs as hosts. In this study, the coral symbiotic crab *Tetralia rubridactyla* was test for host preference and fidelity. Five oceanic objects were provided to the crabs: common host corals (*Acropora hyacinthus* and *A. digitifera*), uncommon host corals (*Pocillopora damicornis* and *Stylophora pistillata*), and dead coral skeletons. The crabs were collected from the 2 source host corals *A. hyacinthus* and *A. digitifera* and subjected to an experiment comprising 7 treatments. Each treatment included 2 stages of no-choice and choice conditions to estimate the expected selection frequencies. The results revealed that the crabs chose any available object under the no-choice condition, and exhibited various preferences under the choice condition. Moreover, *T. rubridactyla* exhibited significantly higher frequencies to inhabit *Acropora* corals ($p < 0.01$, χ^2 test), than dead coral skeletons and uncommon host corals. In all the treatments, the preferences of the crabs from the 2 source hosts were similar. Present results demonstrated *T. rubridactyla* host selection conditioning as follows: (1) Under the no-choice condition, inhabit any choice object for shelter; (2) under the choice condition, if without a common host, randomly inhabit any uncommon choice object as a host; and (3) under the choice condition, if a common host is available, selecting the common host is the first priority because it could provide food and space. This study revealed that *T. rubridactyla* express neither fidelity nor preference between *A. hyacinthus* and *A. digitifera*. Thus, these results also suggested that the distribution of *T. rubridactyla* on *Acropora* corals in the reef is affected by an abundance of corals rather than the preferences of coral species.

1. Introduction

A host is a crucial ecological factor for symbionts that, affecting their existence and distribution. Many obligate symbiotic decapods in coral reefs exhibit a high degree of habitat specialization (Stella et al., 2011b). A host provides crucial benefits to its symbionts including protection from predators (Baeza and Stotz, 2001; Bruce, 1972; Castro, 1978; Huang et al., 2005), direct or indirect food sources (e.g., tissue, mucus, eggs, fat bodies, and associated detritus) (Barry, 1965; Bruce, 1972; Castro, 1969; Fautin et al., 1995; Huebner and Chadwick, 2012; Patton, 1994; Stimson, 1990), a substratum for burrowing and gall-forming decapods (Borradaile, 1921; Kropp, 1989; Wei et al., 2005, 2013), and a mating ground for seeking sexual partners (Huber, 1987; Ocampo et al., 2012).

Coral reefs are among the most complex marine ecosystems, with the highest biodiversity (Veron, 2000), and are predominantly inhabited by invertebrates (Stella et al., 2011b). At least 310 decapod crustaceans live in the spaces of a coral structure, particularly on the branching corals of the genus *Acropora* and the Pocilloporidae family (Abele and Patton, 1976; Bruce, 1998; Castro et al., 2004; Patton, 1966, 1994; Stella et al., 2011b). Brachyuran crabs of the genus *Tetralia* belong to the Tetraliidae family and Trapezioidea superfamily; these crabs are well-known obligate coral symbionts distributed throughout the Indo-West Pacific region (Castro, 1988; Galil, 1988; Patton, 1994; Poupin, 2008). The coevolution of trapezioid crabs with corals occurred in the Eocene (Schweitzer, 2005). Tetraliid crabs are one of the most coral-dependent animals worldwide; they exhibit distinct habitat specialization with corals of only the genus *Acropora* (Castro et al., 2004; Galil, 1988; Patton, 1994). These crabs rely on their hosts for refuge, feed on coral mucus rather than on coral live tissue and eggs, and use coral space as a breeding ground (Castro, 1988; Knudsen, 1967;

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Patton, 1994; Sin, 1999; Stimson, 1990). Few studies have reported an association between tetraliids and atypical hosts, such as *Pocillopora*, *Seriatopora*, and an alcyonarian (Castro, 2009; Chang et al., 1987; Garth, 1964, 1984; Knudsen, 1967).

Symbiotic *Tetralia* crabs are ecologically crucial for their coral hosts (Glynn, 2013). A previous study observed that corals exhibited decreasing growth rates and increasing bleaching and mortality after symbiotic crabs were removed, whereas corals with crabs remained healthy (Stewart et al., 2006). *Tetralia* crabs could help host corals clean sediments and protect the host from predators such as starfish (Pratchett, 2001; Rouzé et al., 2014; Stewart et al., 2006). Most studies on the ecological perspectives of crabs symbiotic with corals have focused on larger crabs of the genus *Trapezia* (Glynn, 2013; Glynn and Enochs, 2011). To date, few studies have reported on small symbiotic *Tetralia* crabs.

Choice experiments are commonly performed for examining the preference of animals exhibited for a particular resource (i.e., food and habitat). In a traditional experimental design, different choices have been presented to animals; however, with such an experimental design, preference can be confused with accessibility (Olabarria et al., 2002; Singer, 2000). Several recent researchers have demonstrated the appropriate methods and statistical analyses used for investigating the food and habitat preferences of animals (Jackson and Underwood, 2007; Olabarria et al., 2002; Underwood et al., 2004; Underwood and Clarke, 2005, 2006). Underwood and Clarke (2005) designed a protocol that includes a 2-stage test for determining the experimental preferences of animals. The 2-stage test design has an advantage over previous designs because it provides answers that are more accurate based on proper control rates of the Type I error, particularly with small samples (Underwood and Clarke, 2005). At Stage 1, a single type of habitat or food is provided to animals, followed by 2 or more choices at Stage 2. This analytical method has been widely applied in examining the preferences of various animals (Cacabelos et al., 2010; Hale et al., 2008; Mascaró et al., 2012; Pinna et al., 2012; Silva et al., 2010).

In the coastal areas of northeast Taiwan, where the southern Ryukyu arc meets the Coral Triangle, the diversity of symbiotic crabs living with *Acropora* corals is abundant. Limviriyakul et al. (2016) reported that *Acropora hyacinthus* (Dana, 1846) (60.0%) and *A. digitifera* (Dana, 1846) (30.6%) were commonly found in this area. The most abundant symbiotic crab was *Tetralia rubridactyla* Garth, 1971, which was more abundant and frequently associated with *A. hyacinthus* (68.6%) than with *A. digitifera* (23.1%) (Limviriyakul et al., 2016). Therefore, this area can provide a rich resource of symbiotic crabs and host corals for determining the coral–crab relationship and selection preference of the crab.

Relatively few studies have reported on the selection preferences of coral symbiotic crabs toward host corals. The present study adopted the methods by Underwood and Clarke (2005) and was conducted using a series of treatments involving *T. rubridactyla* and corals from northeast Taiwan. By examining the preferences for (1) an uncommon host coral and dead coral skeleton, (2) a common host coral and dead coral skeleton, (3) a common host coral and uncommon host coral, and (4) 2 host *Acropora* corals. The host selection behavior of *T. rubridactyla* in a reef environment was determined through a comprehensive statistical analysis.

2. Materials and methods

2.1. Collection of hosts and symbiotic crabs

The experimental crab *T. rubridactyla* (Fig. 1A) and corals *A. hyacinthus* (Fig. 1B), *A. digitifera* (Fig. 1C), *Pocillopora damicornis* (Linnaeus, 1758) (Fig. 1D), and *Stylophora pistillata* Esper, 1797 (Fig. 1E) were collected by scuba diving at a 2–3-m depth in the coastal area of western Fan-Zai-Aou Bay near Keelung, northeastern Taiwan, from April to August 2014. The sampling area is composed of a patchy reef and coral community on rocks exposed to strong wave action. Among the collected corals, *A. hyacinthus* and *A. digitifera* are typical host corals (Castro et al., 2004; Patton, 1994), whereas *P. damicornis* and *S. pistillata* are atypical host corals (Garth, 1984; Knudsen, 1967). Colony shapes of *A. hyacinthus* in the sampling area is varied from corymbose to small plate-like with slender branches, exhibiting mostly secondary branches (Fig. 1B), whereas *A. digitifera* is corymbose to digitate with shorter and thicker tapering vertical branches, rarely with secondary branches (Fig. 1C). Each coral colony was stored separately in a plastic zip-lock bag and immediately transported to the Chaojing Ocean Center (National Museum of Marine Science and Technology, Keelung, Taiwan). In the laboratory, the symbiotic crabs and all symbionts were gently removed from the corals by using flexible plastic rods. The total numbers of *T. rubridactyla*, including the adult and juvenile crabs in each colony, were recorded to evaluate the symbiont capacity of host corals. The taxonomic descriptions used for identifying tetraliid crabs and coral species were adapted from Castro et al. (2004), Wallace (1999), and Veron (2000). Furthermore, the coral colonies were wrapped tightly with Polyethylene food wrap film to measure the total volume by using the water displacement method (Vytopil and Willis, 2001). After the volume was recorded, the corals were stored in an aquarium for experimental use.

The crabs and corals were isolated in 2 aquaria (90 cm × 60 cm × 60 cm; containing 324 L of seawater). Each aquarium was connected to 2 separate recirculation filtration systems to prevent any chemical contact between the host corals and crabs during acclimatization. The crabs were individually separated in small perforated plastic boxes, held in the aquarium, and fed shrimp meat once daily. Furthermore, the aquaria were maintained at 25 ± 1 °C, 35 ± 1 salinity, and under a 12-h light–dark cycle of artificial lighting. Seawater was partially exchanged weekly and measured twice weekly by using ammonia portable photometer (Hanna HI93700), nitrate portable photometer (Hanna HI96728), phosphate portable photometer (Hanna HI96713), pH meter (Hanna HI-2211), alkalinity colorimeters (Hanna HI772), Calcium test kit (Salifert) and Magnesium test kit (Salifert). The water quality was maintained at approximately constant concentrations of ammonia, 0 mg L^{-1}; nitrates, 0 mg L^{-1}; phosphates, 0.02–0.04 mg L^{-1}; pH, 8.1–8.2; alkalinity, 8.0–8.3 dKH; calcium, 390–410 ppm; and magnesium, 1290–1350 ppm. The experimental animals used for the host preference test were acclimated in a laboratory for at least 1 week.

2.2. Experimental design

The experimental *T. rubridactyla* were divided into 2 groups according to their source host corals *A. hyacinthus* and *A. digitifera*. The "source host" refers to the species of acroporid coral from which the crab used in the experiment were collected; by contrast, the "non-source host" is another species of acroporid coral which the crab of that species has been reported to inhabit. The "common host" refers to the typical host corals of *T. rubridactyla*, commonly scleractinian corals of the genus *Acropora*, and the "uncommon host" refers to the reported atypical host corals of the crab, generally other genera than *Acropora*. Table 1 list of the hosts used in the experiments.

The trial comprised 7 treatments (Table 2), involving 5 choice objects: *A. hyacinthus*, *A. digitifera*, *P. damicornis*, *S. pistillata*, and dead coral skeletons. Dead coral skeletons were obtained by killing acroporid corals, allowing their tissues to rot, washing them with freshwater, and drying them in an oven. The crabs in both groups were subjected to the 7 treatments, each of which involved 48 replicates. Treatments 1–4 were designed to examine whether host selection depends on food or shelter, whereas Treatments 5 and 6 were designed to investigate the preference of an uncommon host. The final treatment was designed to determine the preference between source and non-source hosts. Based on the design by Underwood and Clarke (2005), all treatments involved 2 test stages. At Stage 1 (comprising Stages 1-1 and 1-2), all crabs