Available online at www.sciencedirect.com
ScienceDirect
International Journal for Parasitology 37 (2007) 539–545
www.elsevier.com/locate/ijpara

An experimental evaluation of host specificity: The role of encounter and compatibility filters for a rhizocephalan parasite of crabs

Armand M. Kuris [a,b,*], Jeffrey H.R. Goddard [b], Mark E. Torchin [c], Nicole Murphy [d], Robert Gurney [d], Kevin D. Lafferty [e]

[a] *Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA 93106, USA*
[b] *Marine Science Institute, University of California, Santa Barbara, CA 93106, USA*
[c] *Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panama*
[d] *Centre for Research on Introduced Marine Pests, CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia*
[e] *Western Ecological Research Center, c/o Marine Science Institute, University of California, Santa Barbara, CA 93106, USA*

Received 29 July 2006; received in revised form 1 December 2006; accepted 4 December 2006

Abstract

The encounter/compatibility paradigm of host specificity provides three qualitative pathways to the success or failure of a potential host-parasite interaction. It is usually impossible to distinguish between two of these (encounter and compatibility filters closed versus encounter filter open and compatibility filter closed) because unsuccessful infection attempts are difficult to observe in nature. We were able to open the encounter filter under experimental laboratory conditions. Our analytical system used the rhizocephalan barnacle, *Sacculina carcini*, a parasitic castrator of the European green crab, *Carcinus maenas*, and *Pachygrapsus marmoratus*, a native European crab that occurs with *C. maenas* but is not parasitized by *S. carcini* in nature. Penetration followed by unsuccessful infection of *P. marmoratus* crabs by parasitic barnacle larvae leaves a uniquely permanent record in the thoracic ganglion of the crabs. This provided us with a novel tool to quantify the encounter filter in a host-parasite system in nature. We demonstrated, in the laboratory, that the compatibility filter was closed and that, in nature, even where barnacle larvae were present, the encounter filter was also effectively closed. The closure of both filters in nature explains the failure of this potential host-parasite interaction, an outcome favored by selection in both host and parasite.

Keywords: *Carcinus maenas*; Host-parasite evolution; Host specificity; Host response; *Pachygrapsus marmoratus*; Rhizocephala

1. Introduction

Host specificity is a key concept, critical to our understanding of parasite community organization, evolution of host defenses and risk of transmission of infectious agents. Combes provides a conceptual approach to the analysis of host specificity, proposing a two-step encounter/compatibility paradigm (Euzet and Combes, 1980; Holmes, 1987; Combes, 2001). Competent infective stages of a parasite must pass through an encounter filter to reach a potential host. This includes appropriate spatial and temporal overlap and the ability to locate and enter the potential host. If the encounter filter is sufficiently open to initiate an infection, the parasite must also pass through a compatibility filter, determining that it can escape or deter effective defensive responses of the host and whether the host is nutritionally suitable for parasite development. An example is swimmer's itch. Here, avian schistosome cercariae have an open encounter filter for the bare skin of wading humans, but a closed compatibility filter prevents successful infection (despite the intense itch).

The presence of two filters, which can be open or closed, presents four qualitative outcomes. If both filters are open (1), the parasite occurs in the host. In the other three

* Corresponding author. Tel.: +1 805 893 3998; fax: +1 805 893 4724.
E-mail address: kuris@lifesci.ucsb.edu (A.M. Kuris).

0020-7519/$30.00
doi:10.1016/j.ijpara.2006.12.003

pathways, the infection fails. The host remains uninfected if (2) the encounter filter is closed and the compatibility filter is open, (3) the encounter filter is open and the compatibility filter is closed or (4) both filters are closed.

The nature of the compatibility filter is often amenable to experimentation. In laboratory experiments, infective stages of parasites are provided access to potential hosts. Parasite infectivity, growth, survivorship and reproductive outputs can be compared across host species, sexes, sizes, ages, etc. If a non-host is found to be compatible following experimental exposure, one can assume that a closed encounter filter prevents the parasite from exploiting the host in nature (scenario 2).

Unlike the compatibility filter, direct assessment of the encounter filter is difficult to quantify (Combes, 2001), meaning that scenarios 3 and 4 are usually difficult to distinguish from each other and their relative contributions to host specificity cannot be quantified. Examples such as swimmer's itch do show that encounter is possible and a closed compatibility filter prevents successful development of the parasite. However, these examples involve hosts (e.g., humans and ducks) that are neither closely related nor share the same habitat (excepting occasional and ephemeral human wading or swimming). In natural systems, distinguishing the roles of encounter and compatibility, particularly in a quantifiable way, has been intractable. This is particularly important when related hosts share overlap with respect to habitat. Such accessible conditions presumably drive the evolution of host specificity. For example, recent analysis of the dilution effect (Keesing et al., 2006) shows the importance of selective pressure to close the encounter filter to incompatible hosts.

The number of parasites that may have located a host cannot usually be estimated. There is rarely an assay available to quantify the rate of failed attack on an unsusceptible host. Hence, we are left with data that merely show whether a parasite was present (both encounter and compatibility filters were at least partially open) or absent. If absent, laboratory experiments may aid evaluation of the compatibility filter, but we are left to merely infer the operation of the encounter filter should a host, lacking a parasite in nature, be compatible with such a parasite in an experimental arena.

There are at least three important reasons to distinguish these scenarios. Firstly, it reveals which filter natural selection needs to open to create a new host-parasite association. Second, if the encounter filter is open, there may be damage to the host even if the compatibility filter is closed. These would include repair of damage due to the temporarily developing parasite and the cost of immune surveillance and defense. Third, if the encounter filter is open, then the defended host becomes a population sink for the parasite. This loss of parasites from the system, a dilution effect, diminishes the potential for such a parasite to impact its appropriate hosts in an ecosystem (Keesing et al., 2006).

Sacculina carcini, a rhizocephalan barnacle, is an ideal parasite for testing the encounter-compatibility model of host specificity. Its cyprid larva locates and attaches to the host. It then metamorphoses into a kentrogon that injects the vermigon stage, subsequently establishing the root-like parasitic interna (Høeg and Lützen, 1995). *Sacculina carcini* is a parasitic castrator of the European shore crab, *Carcinus maenas*. *Pachygrapsus marmoratus*, another shore crab, albeit in a different family, shares broad geographic and habitat overlap with *C. maenas* and *S. carcini* in the bays and estuaries of southern Europe. Despite this overlap, *S. carcini* does not infect *P. marmoratus* in nature. This does not imply that *P. marmoratus* has an innate resistance to rhizocephalans. Laboratory experiments demonstrate that *S. carcini* larvae can penetrate other crab species outside its native range (Goddard et al., 2005). These experiments included *Pachygrapsus crassipes*, a congener of *P. marmoratus*. However, these infections are not viable and generally result in the death of both the host crab and the immature parasitic interna (Goddard et al., 2005). Additionally, a congeneric rhizocephalan barnacle, *Sacculina benedeni*, successfully and exclusively infects Mediterranean populations of *P. marmoratus* (Perez, 1933; Øksnebjerg, 2000). This dichotomy led us to determine which of the three encounter-combatibility scenarios prevents *S. carcini* from using *P. marmoratus* as a host. In other words, does this parasite encounter *P. marmoratus* in nature but lack the compatibility to successfully infect it, resulting in a sink for the parasite population? Or, does this parasite not encounter the host even though it does encounter/infect sympatric *C. maenas* which can often be found under the same rock? The novel element for this evaluation was our ability to quantify, in nature, the frequency of failed infection attempts by *S. carcini* in *P. marmoratus*. Our evaluation combined results from field comparisons, laboratory experiments and genetic analyses.

The ability of arthropods to sometimes mount an effective cellular defense (haematocyte aggregation followed by melanization) against some infectious natural enemies is well known (Gotz, 1986; Karp, 1990). Factors affecting encapsulation have been extensively studied, particularly for insect hosts against internal parasitoids (Blumberg, 1997; Godfray, 2000). Rates of encapsulation have sometimes been measured in natural systems (e.g., Berberet et al., 1987). However, to our knowledge, no studies have used this as a record of failed attack in order to analyze the relative roles of the basic processes (encounter and compatibility) leading to host specificity in a natural host-parasite system.

2. Materials and methods

2.1. Experimental procedures

We exposed one to four individuals (depending on the availability of brood and crabs) each of *P. marmoratus* and *C. maenas* (held separately in two 500 ml containers) to the same brood of competent female cyprid larvae of *S. carcini*. For each crab, we recorded its sex, carapace

width (at the widest point, excluding lateral spines) and the stage in the molt cycle. The molt cycle was subdivided into early postmolt (Drach molt stages A_1–C_2), hard (C_3–D_1) and late premolt (D_{2-4}) (Drach, 1939; Hiatt, 1948).

We used *C. maenas* ranging in carapace width from 8 to 24 mm and *P. marmoratus* from 8 to 20 mm. We obtained *P. marmoratus* from the rocky shore at Mougas, 10 km south of Baiona, Galicia, on the Atlantic coast of Spain. European shore crabs, *C. maenas*, were present but uncommon on this shore. *Sacculina carcini* was absent. The closest population of *C. maenas* with a high prevalence of *S. carcini* was 40 km to the north and in the upper Ria de Vigo estuary. We obtained uninfected juvenile *C. maenas* from New England, where *S. carcini* does not occur.

Individual broods of *S. carcini* containing female larvae were mixed equally between containers in each trial, with densities of larvae between trials ranging from 1 to 75 cyprids/ml, depending on the size of the brood. Details of animal maintenance prior to experimentation, cyprid production from *S. carcini* externas, cyprid maintenance until competency and cyprid sexing are given in Goddard et al. (2005). We exposed crabs for 1–2 days and then, using a dissecting microscope, quantified settlement of parasite larvae by counting the number of attached cyprids on the dorsal surface of each crab. We then isolated exposed crabs in individual compartments, monitored them daily for survival and abnormal behavior, and fed them pieces of scallop and shrimp three times a week.

For dissections, we sacrificed crabs ranging from 5 days to 1 year post-exposure. We also dissected, within a day, any crabs that died in tanks after exposure to *S. carcini*. We preserved voucher specimens and samples for future studies in 70% or 95% ethanol or in Bouin's fixative. On post-mortem examination, we scored crabs as infected (early, spreading or extensive), uninfected, infection arrested (when all visible *S. carcini* rootlets were melanized by a host immune response), or no data (when a crab died less than 3–4 weeks after being settled on by *S. carcini*; too early to allow detection of the parasite) (Goddard et al., 2005). On the thoracic ganglion of exposed *P. marmoratus*, we discovered spherical, to slightly elongate, bodies encapsulated by a translucent brown to nearly opaque black layer of melanin. We suspected these spots were arrested *S. carcini* infections and thus counted the number on each ganglion we examined.

We used a standard linear regression model to examine the relationship between the number of settled cyprids per dorsal surface of each crab and the number of melanized bodies on its thoracic ganglion. We used X^2 to compare frequency of cyprid settlement on *P. marmoratus* and *C. maenas*. Logistic regression and analysis of variance (ANOVA) were used to compare the frequency and mean number of melanized bodies in *P. marmoratus* and *C. maenas* captured in the field. Analyzing crabs or containers as replicates yielded the same qualitative results, but data for individuals are presented for ease of interpretation.

2.2. Field observations

We selected the Mira River estuary (Furnas, south shore), across the river from Vila Nova de Milfontes, in Southern Portugal, to seek evidence of *S. carcini* attack rates on *P. marmoratus*. Our previous fieldwork at that location (Torchin et al., 2001) revealed a high prevalence of *S. carcini* in resident *C. maenas* (27%, $n = 57$, trapped) below the pier at Milfontes and 57% ($n = 24$, hand collected) at Furnas. We had also noted appreciable habitat overlap of *C. maenas* with *P. marmoratus*. To quantify habitat overlap, we collected crabs from three transects perpendicular to the shoreline that were 1 m wide and 40 m long. We dissected the *C. maenas* to estimate prevalence of *S. carcini*.

In, and immediately adjacent to, the zone of habitat overlap, we collected 60 *P. marmoratus* (7–29 mm carapace width) at Furnas and an additional 11 crabs at Milfontes (14–24 mm). These crabs were dissected and their thoracic ganglia were excised, mounted on a slide and examined under a compound microscope for the presence and number of melanized bodies or larger lesions. For control crabs (not exposed to potential attack by *S. carcini* cyprids), we collected and similarly examined 78 *P. marmoratus* (size range 8–30 mm) from the rocky intertidal zone along the exposed outer coast at Saõ Torpes. This location is about 20 km north of the nearest population of *C. maenas* parasitized by *S. carcini* (in the Mira River Estuary). We hypothesized that *P. marmoratus* in the Mira River Estuary would have a higher prevalence and intensity of melanized spots than would the crabs at Saõ Torpes because the former could have been penetrated more frequently by *S. carcini* larvae which would have died following a successful host defense response leading to melanization. Further, larger and, therefore, older crabs would contain a longer time record of exposure. This is because any other foreign objects, in addition to rhizocephalan parasites, would be permanently retained after melanization in the hemocoel and tissues of crabs (Kuris et al., 1980; Pichelin et al., 1998). Voucher specimens were preserved in alcohol, formalin and acetic acid (AFA) and 95% ethanol. Thoracic ganglia with spots from four of these crabs were also preserved in 95% ethanol.

2.3. Molecular genetics

As a qualitative confirmation that the melanized bodies we found in laboratory exposed crabs were evidence of infections by *S. carcini*, we conducted blind PCR tests on four exposed and two unexposed *P. marmoratus* along with infected and uninfected *C. maenas* controls. For this, we used voucher specimens that we had previously preserved in 95% ethanol. Additionally, we had preserved voucher specimens of thoracic ganglia (with spots) from *P. marmoratus* from our field sites (two from the Mira River Estuary and two from Saõ Torpes). We were able to use these samples to determine the presence of *S. carcini* using the diagnostic, species specific PCR primers SAC5 and SCAR1.

These primers were previously developed to amplify a 350 bp fragment of the Internal Transcribed Spacer 1 (ITS1) rDNA from *S. carcini* (Thresher et al., 2000). The amplification of a 350 bp fragment by PCR would demonstrate the presence of infection with *S. carcini* in screened crabs. Samples were run blind, with crab identity unknown during PCR and each sample given a consecutive number for analysis. A positive control of known *S. carcini* DNA from Sweden, negative controls of no DNA (Milli-Q water, Millipore, Melbourne, Australia) and the thoracic ganglia of another crab species, *Charybdis callianassa*, were also included in PCR analyses.

Tissue was crushed in 700 µl of CTAB extraction buffer (50 mM Tris, pH 8.0, 0.7 M NaCl, 10 mM EDTA, 1% hexadecyltrimethylammonium bromide, 0.1% 2-mercapotoethanol) using a pellet pestle mixer (Kontes, Melbourne, Australia), prior to incubation at 65 °C for 1 h with 100 µg ml proteinase-k. DNA was extracted using phenol/chloroform, precipitated in ethanol and eluted in 100–200 µl of Milli-Q water. A region of the ITS1 rDNA was amplified by PCR in 50 µl vols. using 10–100 ng DNA, 800 µmol deoxyribonucleotide triphosphate (dNTP) (Promega, Wisconsin, USA) and 20 pmol of a forward SAC5 (5′-GCTGGCTTCGGCCG-3′) and reverse SCAR1 (5′-CGATTGTGCATGAATGTG-3′) primer (Proligo, Lismore, Australia) used in PCR. Other PCR reagents were as supplied in a Perkin-Elmer kit (California, USA) and used in the following concentrations; 0.2 U AmpliTaq gold, 0.1 mM $MgCl_2$ in buffer (5 mM Tris–HCL, 50 mM KCl, pH 8.3). DNA was denatured at 95 °C for 60 s, primers annealed at 45 °C for 30 s, chains extended at 72 °C for 60 s for 30 cycles, with a final cycle of 72 °C for 7 min. Each PCR product was checked by electrophoresis in a 1% TBE agarose gel. Results of PCR tests were confirmed by repeat analysis and titrations of DNA concentration.

3. Results

3.1. Compatibility filter

In 26 simultaneous laboratory exposures of one to four *C. maenas* paired with one to four *P. marmoratus*, cyprids of *S. carcini* settled on at least one crab in 23 of these trials. At least one *C. maenas* was settled on in all 23 trials and at least one *P. marmoratus* in 18 trials. Among the individual crabs, cyprids settled significantly more on *C. maenas* (55 of the 61) than on *P. marmoratus* (42 of the 63) ($X^2 = 10.5$, $P < 0.005$, $n = 124$). The number of cyprids that settled on either crab species ranged from one to 40, with a mean of six cyprids per crab. Because the ability of cyprids to settle on *C. maenas* and develop to the interna stage in our experimental system was well established (Goddard et al., 2005), we only followed 20 of these crabs through to interna development. Of the *C. maenas* upon which cyprids settled, 17 of 20 (85%) became infected with internas. Two were held long enough that they ultimately produced virgin externas. Internas were never detected in the 42 *P. marmoratus* upon which cyprids had settled. Rather, small, apparently melanized, bodies were frequently observed in or on the thoracic ganglia of these crabs. Melanized bodies were absent on the gangla of 17 out of 20 *P. marmoratus* collected from the same locality (Mougas, Galicia Spain) and not exposed to *S. carcini* in the laboratory. The remaining three unexposed crabs had one, one and eight melanized bodies on their ganglia, respectively.

The melanized bodies in the thoracic ganglia of the experimentally exposed crabs were not observed in our numerous exposures of *C. maenas*, nor of four species of native California crabs (including a congener, *P. crassipes* (Goddard et al., 2005)). They were usually irregular, from 30 to 70 µm in greater dimension, and scattered about the thoracic ganglion (Fig. 1). The number of these bodies was highly correlated with the number of settled cyprids ($R^2 = 0.80$, $P < 0.0001$, $n = 58$, Fig. 2). Therefore, we considered it highly likely that these bodies represented early stages of *S. carcini* successfully killed by the cellular defense response of the host crabs, a supposition supported by molecular genetic results (see below).

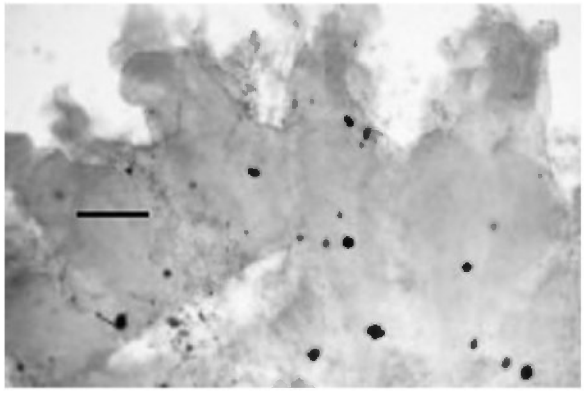

Fig. 1. Excised thoracic ganglion of *Pachygrapsus marmoratus* experimentally exposed to cyprids of *Sacculina carcini* in the laboratory. Melanized presumptive early stages of *S. carcini* internas are evident as spherical to irregularly shaped, opaque bodies. Scale bar = 0.5 mm. Bright field microscopy of fresh tissue.

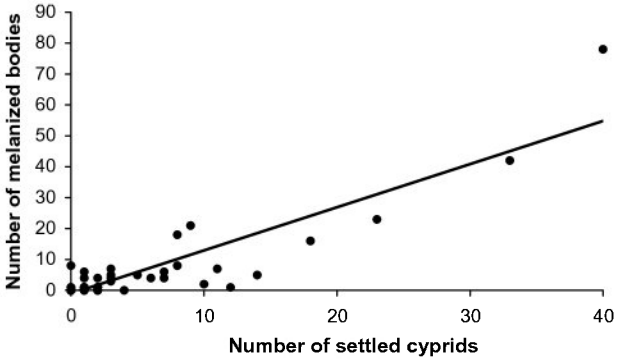

Fig. 2. Association between the number of *Sacculina carcini* cyprids settled dorsally and the number of melanized bodies in thoracic ganglia of *Pachygrapsus marmoratuus* exposed to cyprids of *S. carcini*.

All four exposed crabs tested positive for *S. carcini* DNA and the signals were proportional to the number of melanized bodies observed prior to preservation. Three *P. marmoratus* with five to 21 melanized bodies presented a weak signal, while the crab with 78 melanized bodies had a moderately strong signal. The unexposed control *C. maenas* lacked a *S. carcini* signal, while the two infected *C. maenas* gave one strong and one weak signal for *S. carcini* DNA. The latter *C. maenas* had died 37 days after exposure to *S. carcini* and contained an interna determined microscopically, prior to preservation, to consist of only a few cells.

3.2. Encounter filter

Pachygrapsus marmoratus and *C. maenas* occurred together along our transects, (transect 1 – six *P. marmoratus*, two *C. maenas*; transect 2 – three *P. marmoratus*, 22 *C. maenas*; transect 3 – 36 *P. marmoratus*, 25 *C. maenas*). These species were sometimes found under the same stones.

Examination and quantification of the melanized bodies in the thoracic ganglia of *P. marmoratus* from the Mira River Estuary and from Saõ Torpes employed a liberal detection standard. We chose to err with false positives rather than false negatives with respect to evidence of attack by *S. carcini*. The melanized bodies in crabs from Portugal were generally smaller than those in the experimental infections (Figs. 1 and 3). The frequency of crabs with melanized bodies and the mean number of bodies per crab were not significantly different between *P. marmoratus* from the Mira River Estuary and the control site, Saõ Torpes ($X^2 = 1.3$, $P = 0.24$, $n = 149$ and $F = 0.91$, $P = 0.34$, $n = 149$, respectively). There was a significant positive effect of crab size on the number of melanized bodies at both sites ($F = 17.3$, $P < 0.0001$, $n = 149$, Table 1). DNA extracted from the thoracic ganglia of four of these crabs with melanized spots (from Sao Torpes and the Mira River Estuary) tested negative for *S. carcini* DNA. Together, these data suggest that the encounter between *S. carcini* and *P. marmoratus* that we were able to force in the laboratory does not occur with detectable frequency in nature.

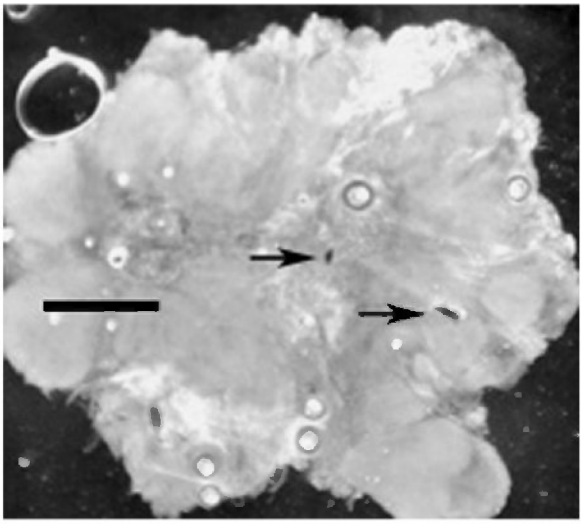

Fig. 3. Thoracic ganglion of *Pachygrapsus marmoratus* from the Mira River Estuary in Portugal, a site where *P. marmoratus* is potentially frequently exposed to the cyprids of *Sacculina carcini* from the large and heavily infected population of *Carcinus maenas* at that site. Arrows point to examples of the melanized bodies quantified in Table 1. Dark field microscopy of fresh tissue.

4. Discussion

Our discovery that the melanized bodies in the thoracic ganglia left a permanent record of *S. carcini* cyprid attack on a resistant host, *P. marmoratus*, provided a novel and unique tool to quantify the encounter rate for this parasite. This distinctive circumstance enabled us to examine the relative importance of the encounter and compatibility filters with respect to the concept of host specificity.

Our results indicate that, in nature, both the encounter and compatibility filters were effectively closed for *P. marmoratus* as a potential host for *S. carcini*. By opening the encounter filter, our laboratory experiment demonstrated that competent cyprids of *S. carcini* could readily settle on, differentiate into the inoculative kentrogon stage, and penetrate *P. marmoratus*. The inoculated vermigon stage parasites could make their way to the thoracic ganglion, the usual site of early interna development. Up to this point in the infection process, their performance was similar to that in their natural host, *C. maenas* (Goddard et al., 2005). Upon reaching the thoracic ganglion, the defense response of the crab closed the compatibility filter, the early interna was melanized and failed to develop. The strong correlation between the number of melanized bodies in the

Table 1
A size-specific comparison of the percentage of *Pachygrapsus marmoratus* with melanized bodies and the mean number of those bodies (in crabs where bodies were present)

Size class (mm)	Mira River			Saõ Torpes		
	n	% Crabs with melanized bodies	Mean number of bodies	*n*	% Crabs with melanized bodies	Mean number of bodies
7.0–11.9	17	0	–	18	6	1.0
12.0–19.9	41	9	1.2	40	18	1.4
20.0–30.9	13	31	1.8	20	35	2.3
Total	71			78		

Crabs were collected at the Mira River estuary and the rocky intertidal zone at Saõ Torpes, Portugal.

thoracic ganglion and the number of *S. carcini* cyprids that settled on crabs in our assay indicated that these bodies were likely early stages of the parasite killed by the host's defense response. Detection of *S. carcini* DNA in the thoracic ganglia of four of these crabs, but not in unexposed crabs, confirmed this conclusion. Interestingly, the post-exposure outcome for *P. marmoratus* is different from that which occurs when naïve crabs from California are exposed to *S. carcini* (Goddard et al., 2005). In the Californian crabs, including *P. crassipes*, a congener of *P. marmoratus*, infection generally progresses beyond the thoracic ganglion and internal roots are formed, ultimately resulting in the death of both the crab and the parasite (Goddard et al., 2005). These contrasting results may be due to the evolution of non-host *P. marmoratus* to defend itself against *S. carcini*, and to the evolution of *S. carcini* to avoid the non-host *P. marmoratus*. In contrast, *S. carcini* and the native Californian crabs lack any evolutionary history with each other and the infection is mutually fatal.

While the compatibility filter has long been amenable to experimental evaluation for many kinds of parasites, the inability to observe parasites following unsuccessful attack generally prevents a direct analysis of the encounter filter. Here, we were able to use the presence of the melanized bodies in the thoracic ganglion as an estimate of *S. carcini* cyprid attack on an inappropriate host found in the same habitat as its natural host, *C. maenas*. Because the numbers of melanized bodies present in wild crabs did not differ between crabs living near or far from populations of *S. carcini* and our genetic analyses did not detect *S. carcini* DNA in the thoracic ganglia of these crabs, we interpret the bodies found in the thoracic ganglia of the *P. marmoratus* from Portugal as melanization of other foreign bodies or of necrotic crab tissue, not as a record of encounter with *S. carcini*. Further, in our laboratory experiments, we found a few of these same melanized bodies in three out of 20 unexposed crabs. Hence, these melanized spots provide a means to assess the presence of foreign bodies, including unsuccessful infection by parasites such as *S. carcini*, in the thoracic ganglion. Our examinations disclosed several other types of parasites in these ganglia (Kuris et al., 2004), which may elicit melanization. Similarly, another study examining parasitism in an Asian crab (*Charybdis japonica*) found small melanized lesions in introduced populations in New Zealand (Miller et al., 2006). They attributed these lesions to cellular trauma or unsuccessful infection by unknown parasites. Although these spots are not necessarily specific to *S. carcini*, our laboratory experiments and genetic analysis demonstrate that they capture unsuccessful infection attempts by this parasite. Based on this, we were able to compare relative differences in encounter of *S. carcini* larvae by *P. marmoratus* at our two field sites, one where *S. carcini* is common in its natural host, *C. maenas* (where its larvae should be common in the water column) and another where *S. carcini* does not occur. Since there was no difference in the number of spots on the thoracic ganglia of *P. marmoratus* at the two sites, we inferred that in nature, even where *S. carcini* is common, it does not encounter *P. marmoratus*. Therefore, in nature, the encounter filter for this potential host-parasite system appears to be closed. Our experimental ability to permit an effective encounter suggests avenues for future experimentation concerning mechanisms. These include the role of chemical cues (which we may have provided by including *C. maenas* in the exposure arenas), overwhelming an effective defense, such as grooming, by providing more attacking cyprids in a brief period than any crab is likely to encounter in a lifetime, or by affecting scale-dependent searching mechanisms that became irrelevant in our small exposure containers. If encounter can be differentiated for hosts naturally in close proximity to each other, this implies that specific behaviors on the part of either or both the searching parasite and the alternative potential host may play a significant role in host specificity.

Considering both the field data and the laboratory experiment, it appears that *P. marmoratus* is well-protected from *S. carcini*, and this may be regarded as a coevolved response. The crab's defense appears to respond to *S. carcini* but not to its own crustacean parasitic castrators. For example, at the Mira River Estuary, *P. marmoratus* is sometimes infected by the entoniscid isopod, *Grapsion cavolini* (Kuris et al., 2004). Also, in the Mediterranean, it is parasitized by *S. benedeni* (Perez, 1933; Øksnebjerg, 2000). So, it can be infected by parasitic crustaceans in Portugal, and by its own host-specific sacculinid (at least its Mediterranean populations can be so infected). It is worth noting that the congeneric *P. crassipes* in Japan is also host to a sacculinid parasite (Nagasawa et al., 1996). Attack rates of *S. carcini* on *P. marmoratus* appear very low, perhaps nil, under conditions of considerable apparent opportunity. This could be due to considerable discriminatory power on the part of the searching barnacle cyprid; an adaptive feature given that the closed compatibility filter with *P. marmoratus* amounts to a population sink for the barnacle because the abundance of *P. marmoratus* and its habitat overlap with *C. maenas* probably satisfy the conditions of the dilution effect model (Keesing et al., 2006). Hence, only *C. maenas*, and not *P. marmoratus*, serves as a host for *S. carcini* where the ranges of the two crabs overlap.

Finally, while it is logical that parasites will often be selected to open the encounter filter (Combes 2001), this study shows that under conditions where the dilution effect may be important, a parasite, as well as a host, may be selected to close the encounter filter. This requires that the searching transmissive stage has the longevity and the sensory capabilities to continue to search for its appropriate host, conditions satisfied by the cyprid larvae of Rhizocephalans.

Acknowledgement

We thank T. Cruz and J. Castro for use of laboratory facilities at the Universidade de Evora Marine Laboratory

in Sines, and for information on collecting sites and local natural history in Portugal. P. Sadeghian assisted in the field. This research was funded by a grant from the National Sea Grant College Program, National Oceanographic and Atmospheric Administration (NOAA), US Department of Commerce under grant number NA06RG0142, project number R/CZ-162 through the California Sea Grant College system, and in part, by the California State Resources Agency. The views expressed herein are those of the author and do not necessarily represent the views of NOAA or any of its sub-agencies. The submitted manuscript has been authored by a contractor of the US Government; the US Government retains a non-exclusive royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for US Government purposes.

References

Berberet, R.C., Willson, L.J., Odear, M., 1987. Probability for encapsulation of eggs of *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae) by larvae of *Hypera postica* (Coleoptera: Curculionidae) and resulting reduction in parasitism. Ann. Entomol. Soc. Am. 80, 483–489.

Blumberg, D., 1997. Parsitoid encapsulation as a defensive mechanism in the Coccoidea (Homoptera) and its importance in biological control. Biol. Control 8, 225–236.

Combes, C., 2001. Parasitism: The ecology and evolution of intimate interactions. University of Chicago Press, Chicago and London.

Drach, P., 1939. Mue et cycle d'intermue chez les Crustacés Décapodes. Ann. Inst. Oceanog. Monaco 19, 103–391.

Euzet, L., Combes, C., 1980. Les problèmes de l'espèce chez les animaux parasites. Bull. Soc. Zool. France 40, 239–285.

Goddard, J.H.R., Torchin, M.E., Lafferty, K.D., Kuris, A.M., 2005. Experimental infection of native California crabs by *Sacculina carcini*, a potential biocontrol agent of introduced European green crabs. Biol. Invasions 7, 895–912.

Godfray, H.C.J., 2000. Host resistance, parasitoid virulence and population dynamics. In: Hochberg, M.E., Ives, A.R. (Eds.), Parasitoid Population Biology. Princeton Univ Press, Princeton, pp. 121–138.

Gotz, P., 1986. Encapsulation in arthropods. In: Brehelin, M. (Ed.), Immunity in Invertebrates. Springer-Verlag, Berlin, pp. 153–170.

Hiatt, R.W., 1948. The biology of the lined shore crab, *Pachygrapsus crassipes* Randall. Pac. Sci. 2, 135–213.

Høeg, J.T., Lützen, J., 1995. Life cycle and reproduction in the Cirripedia Rhizocephala. Oceanog. Mar. Biol. Ann. Rev. 33, 427–485.

Holmes, J.C., 1987. The structure of helminth communities. Int. J. Parasitol. 17, 203–208.

Karp, R.D., 1990. Cell-mediated immunity in invertebrates. Bioscience 40, 732–737.

Keesing, F., Holt, R.D., Ostfeld, R.S., 2006. Effects of species diversity on disease risk. Ecol. Lett. 9, 485–498.

Kuris, A.M., Poinar, G.O., Hess, R.T., 1980. Post-larval mortality of the endoparasitic isopod castrator *Portunion conformis* (Epicaridea: Entoniscidae) in the shore crab, *Hemigrapsus oregonensis*, with a description of the host response. Parasitology 80, 211–232.

Kuris, A.M., Torchin, M.E., Lafferty, K.D., 2004. Parasites in the thoracic ganglion of *Pachygrapsus marmoratus* (Brachyura: Grapsidae) from the coast of Portugal. Parasite 11, 425–427.

Miller, A., Inglis, G., Poulin, R., 2006. Comparison of the ectosymbionts and parasites of an introduced crab, *Charybdis japonica*, with sympatric and allopatric populations of a native New Zealand crab, *Ovalipes catharus* (Brachyura: Portunidae) New Zealand J. Mar. Fresh. Res. 40, 369–378.

Nagasawa, K., Lutzen, J., Kado, R., 1996. Parasitic Cirripedia (Rhizocephala) and Isopoda from brachyuran and anomuran crabs of the Pacific Coast of northern Honshu, Japan. Bull. Biogeog. Soc. Japan 51, 1–6.

Øksnebjerg, B., 2000. The Rhizocephala (Crustacea: Cirripedia) of the Mediterranean and Black Seas: taxonomy, biogeography, and ecology. Israel J. Zool. 46, 1–102.

Perez, C., 1933. Action de la sacculine sur les caractères sexuels éxtérieurs de *Pachygrapsus marmoratus*. Compt. Rend. Soc. Biol. Paris 113, 1027–1029.

Pichelin, S., Kuris, A.M., Gurney, R., 1998. Morphological and biological notes on *Polymorphus (Profilicollis) sphaerocephalus* and *Corynosoma stanleyi* (Polymorphidae: Acanthocephala). J. Parasitol. 84, 798–801.

Thresher, R.E., Werner, M., Høeg, J.T., Svane, I., Glenner, H., Murphy, N.E., Wittwer, C., 2000. Developing the options for managing marine pests: specificity trials on the parasitic castrator, *Sacculina carcini*, against the European crab, *Carcinus maenas*, and related species. J. Exp. Mar. Biol. Ecol. 254, 37–51.

Torchin, M.E., Lafferty, K.D., Kuris, A.M., 2001. Release from parasites as natural enemies: increased performance of a globally introduced marine crab. Biol. Invasions 3, 333–345.