Title
Causes and Consequences of Parasitism in the California Fiddler Crab, Uca crenulata

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Publication Date
2013

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Causes and Consequences of Parasitism in the California Fiddler Crab, *Uca Crenulata*

A Dissertation submitted in partial satisfaction of
the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Adrienne Brooke Mora

December 2013

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ACKNOWLEDGEMENTS

I owe a large debt of gratitude to the many people who contributed to the completion of this dissertation. First and foremost, I would like to thank my advisor, Dr. Marlene Zuk. Marlene, you always encouraged me to push myself and taught me to develop my own voice and mind. You were also a strong advocate for my research, and with your support I was inspired to reach beyond my comfort zone and take on new challenges that greatly contributed to my intellectual and personal growth.

I would also like to thank Dr. Daphne Fairbairn for her help and guidance throughout my graduate career. Daphne, you gave me great insights into the life of an academic, and helped me learn to think critically and set high standards for myself. When my working space was jeopardized, you jumped in and offered your lab space to me. I am forever grateful to you for your support during this time, as I would not have been able to complete my experiments were it not for you. I consider both you and Marlene to be great role models. You are both incredibly strong, brilliant, and witty women who have made me feel that I could achieve anything with hard work, creativity, and determination.

I would like to thank Dr. Bradley Mullens. Brad, you were always so enthusiastic and encouraging, offering helpful suggestions and advice along the way. In the academic world it is rare to find such a positive, uplifting person, and I am so thankful to have you in my corner.

I would like to thank the lab prep staff at UCR, especially Esther Valdez. You helped transform my research ideas into tangible realities, and made my day-to-day so much more pleasant! Thank you Tara Pastucha for helping me with all of my supply orders. Thank you Heather McDermott for all of your help with my receipts. I would also like to thank Melissa Gomez, who works very hard to make sure that things run smoothly in our department. Melissa, you wear so many hats and help so many people. You deserve a raise for all that you do!

I would like to thank Dr. Ryan Hechinger for his help, advice, and friendship throughout these years. It is great to have a friend who loves parasites as much as I do and find virtually everything about them exciting! I am extremely grateful for your help as I navigated through the trials and tribulations of statistical analysis, and for your encouragement when the challenges of graduate school got me down. Your bright mind always keeps me on my toes, and I thank you for all of our wonderful conversations.

I would like to thank Dr. Armand Kuris. Armand, every academic should strive to be more like you. You are an amazing scientist, teacher, and mentor. Thank you for taking me under your wing and introducing me to the wonderful world of parasites. You inspired me and gave me a chance when many would have turned me away. I'm not sure that I would have made it here were it not for you.

I would like to thank Dr. James Weaver. You are the best friend a person could ever have. You have always been there for me, no matter what the circumstances and no matter how incredibly busy you were. We have seen each other grow, from our beginnings at Santa Barbara City College to the glory days of UC Santa Barbara, to our labs at UC Riverside and Harvard. I am incredibly fortunate to have such a genuine friend who has never judged me. I will always cherish our adventures and your words of wisdom. And of course, I will never forget your oft-repeated advice: “Don’t back up!”

I would like to thank my lab mates Dr. Anne Jacobs, Dr. Brian Gray, Nadia Dimitrova, and Libby Swanger. I am lucky to have shared this formative time with you and I hope we all keep in touch as we continue on! Anne and Brian, thank you for all of the good times eating delicious food and having great discussions! You were both so helpful and encouraging whenever I had a question or needed a second eye for a paper or grant proposal. Anne, a special thank you for your help as I finished the dissertation. Your input and friendly phone calls from back east were greatly appreciated.

I would like to thank all of the undergraduates who made this work possible. You are all bright minds with much to offer and I wish you all the best in your future endeavors. Thank you Wil Splinter, Sarah Gomez, Arjun Patel, Michael Tsai, Autumn Farnik, Danyelle Vasquez, Danielle Rauch, Fatima Engineer, Michelle Hsueh, and Erik Rodarte.

I would especially like to thank Michelle Bruno-Castillo, an amazing undergraduate researcher and friend. You were integral to the completion of my dissertation and I owe much of my success to you. I have never met a more dedicated, hard-working, and kind person as you. I know you will go very far in life and I am so fortunate to have had your help and friendship through all of these years. I know you will excel in whatever you set your mind to, and you will continue to brighten the lives of those around you.

I would like to thank the Ecker family. You have always treated me like one of your own, and I am so thankful to have such caring, wonderful in-laws. Your generosity and unwavering encouragement helped me in the pursuit of my goals. Donna, you have become a scientist in your own right, and I have thoroughly enjoyed our many conversations about life and fiddler crabs over countless cups of coffee.

I would like to thank my mother, father, and brother for their love and support. You always encouraged me to succeed, but not lose sight of the bigger picture. Mom and dad, thank you for buying me my first microscope set, it completely changed my life and ignited a passion for learning that will remain with me for all of my days. Chris, keep being the awesome rock star brother that you are.

Lastly, I would like to thank my husband, Joe Ecker. Joe, I am very fortunate to have a partner who values my aspirations as much as his own. I owe much of this dissertation to your hard work, from helping me build crazy contraptions for my projects, to collecting sea water at all hours of the day and night. You were a real trooper through all of this, and I thank you for your unconditional love, sacrifice, and companionship. I have found with you what many cannot find in a lifetime.

As Charles Darwin so eloquently said, “There is grandeur in this view of life.” Evolution is a beautiful process that produces the most breathtaking results. I am so fortunate that I get to spend my time studying amazing animals and discovering the puzzles of life. I have learned a lot about myself and about the natural world around me, yet still have so much to explore. I can’t wait to begin the next journey.

ABSTRACT OF THE DISSERTATION

Causes and Consequences of Parasitism in the California Fiddler Crab, *Uca Crenulata*

by

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Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, December 2013
Dr. Marlene Zuk, Chairperson

Parasites can inflict serious fitness costs on their hosts, reducing growth, fecundity, and even survival. In response to these challenges, hosts have developed a wide range of behavioral defenses to combat infection. These strategies can significantly reduce parasitism and increase host fitness. However, some hosts are still more vulnerable to parasites, resulting in biased infection patterns. Males are often more susceptible to parasites due to behaviors that increase exposure and physiological differences that compromise their immune response. Host sexual signals in particular can attract parasites and preclude males from adequately defending themselves from infection. To circumvent host defenses, some parasites manipulate host phenotype to enhance their transmission and fitness. Manipulated hosts may perform more conspicuous behaviors that increase predation risk and parasite transmission. While many sexual signals are both conspicuous and risky, little is known about the potential for them to be manipulated by parasites. Here I examined the causes and consequences of parasitic infection in the California fiddler crab, *Uca crenulata*. Males have higher burdens with the *Probolocoryphe uca* trematode that are not predicted by body mass (Mora, unpub. data). I tested the hypothesis that the male bias in parasite intensity is caused by host sex differences in claw morphology and behavior and predicted that males would be less effective at parasite removal. Upon exposure to parasites, males groomed more often than females, yet harbored more trematodes. Grooming is an important antiparasite defense for males, as those that were allowed to groom experienced a 2.2-fold reduction in parasites compared with males prevented from grooming. While the major cheliped helps males acquire mates, it comes with a cost of reduced grooming ability, making them more susceptible to parasitism. I then hypothesized that parasites increase host sexual signaling to enhance their transmission to predatory final hosts. I predicted that male crabs with more *P. uca* parasites would produce more courtship displays and exhibit more claw brightness. In addition, crabs with *P. uca* infections should experience higher predation rates. Males harboring more *P. uca* parasites did display more frequently, however *P. uca* infection was not associated with total brightness of the major cheliped. In addition, males with the highest parasite intensities also produced the most courtship displays. These results support two competing hypotheses regarding parasitism and host sexual signaling. First, increased courtship displays may reflect parasite-induced manipulations of a conspicuous sexual signal to attract final host predators. Alternatively, infected males could be reproductively compensating for infection. I recaptured fewer experimentally infected crabs than control crabs from an open field pen exposed to predators, suggesting that parasitized crabs may be more vulnerable to predators. This is consistent with other

studies that suggest higher predation of parasitized animals, particularly when infected with parasites that depend on predation for their transmission to subsequent hosts. Contrary to my predictions, I recaptured significantly more males than females from the open pen, suggesting that females were more susceptible to predation. This observed female-biased pattern could be the result of sex differences in crab morphology and behavior. Because females lack the formidable major cheliped weapon wielded by males, predators may prefer them since they are easier to handle and pose less risk of injury upon ingestion.

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DISSERTATION INTRODUCTION

Parasites can have dramatic effects on host fitness by reducing host growth, vitality, reproductive success, and survival (Hart 1990, 1992, 1994; Moore 2002). A host's first line of defense in response to these challenges is often behavior, yet most research has focused on quantifying the efficacy of immunological over behavioral defenses (Hart 1990, Kiesecker et al. 1999, Ezenwa 2004, Råberg et al. 2009). A behavior can be considered a viable antiparasite strategy if it effectively reduces or eliminates a parasite that negatively affects host fitness (Hart 1992). Hosts have developed a wide variety of behavioral strategies to reduce or avoid infection. These defenses range from simple behaviors such as parasite avoidance, to more sophisticated methods such as self-medication (Hart 1990, 1992, 1994; Moore 2002).

While antiparasite behaviors can be robust ways to avoid and reduce infection, they are not the only factors that influence infection risk. Host sex differences in behavior, morphology, and physiology can cause considerable variation in a host's ability to ward off parasites (Poulin 1996, Zuk and McKean 1996, Duneau and Ebert 2012). For instance, vertebrate males may experience immunosuppressive effects from higher testosterone levels and be more prone to parasitism (Grossman 1985, Alexander and Stimson 1988, Folstad and Karter 1992, Zuk 1996, Zuk and McKean 1996, Duneau and Ebert 2012). In animals with extreme sexual dimorphism, the larger, more accessible host sex often provides an easier target for parasites, and exhibits higher parasite burdens (Zuk and McKean 1996, Schalk and Forbes 1997, Moore and Wilson 2002, Duneau and Ebert

2012). Additionally, larger animals generally have higher rates of food consumption and may increase their exposure to infective stages found in the diet (Zuk and McKean 1996).

Parasites also experience their own set of unique challenges, as they must seek hosts and ensure transmission for their survival and reproductive success. To overcome these obstacles, some parasites alter host phenotypes in ways that enhance their transmission and fitness, termed "host manipulation" (Holmes and Bethel 1972, Webster 2007). While manipulative parasites often induce subtle changes in host appearance or behavior, some parasites effect dramatic transformations. In one such spectacular example, ants infected with nematodes develop bright red abdomens and are manipulated to perch among clusters of red berries, awaiting predation by frugivorous final bird hosts (Yanoviak et al. 2008). In another classic example, snails infected with *Leucochloridium spp*. trematodes display marked changes in the size, shape, color, and movement of their eyestalks. The larval worms greatly distend the snail's colorful tentacles, and pulsate dramatically when exposed to light. These manipulated snails attract the attention of insectivorous bird final hosts, which may mistake the wriggling tentacles for insect larvae (Moore 2002, Poulin 2010). These parasite-induced alterations have been documented in a wide variety of taxa, and they generally increase parasite fitness through enhanced transmission and subsequent sexual reproduction (Moore 2002). Such modifications have been proposed to illustrate Richard Dawkins' "extended phenotype" concept, whereby the genes of one organism, the parasite, elicit phenotypic effects in another organism, the host (Dawkins 1982).

Trophically transmitted parasites have complex life cycles, requiring multiple hosts for life cycle completion. These parasites must achieve transmission from an intermediate to a final host for sexual reproduction, thus they are under strong selection to increase predation by final hosts. In these systems, manipulated intermediate hosts often exhibit more "risky" behaviors that attract predatory final hosts. For example, killifish infected with the trematode *Euhaplorchis californiensis* exhibited a four-fold increase in conspicuous swimming behaviors and were 10-30 times more likely to be eaten by avian final host predators (Lafferty and Morris 1996). Even minor changes in a phenotypic trait can have dramatic consequences for both the host and parasite.

Another way that trophically transmitted parasites can maximize their survival and reproduction is to manipulate hosts in accordance with their own development. Proper timing and type of host manipulation is crucial to parasite fitness, as premature transmission is often lethal to parasites. Parker et al. (2009) hypothesized that trophically transmitted parasites should evolve to suppress host predation before they are capable of establishing in final hosts, and enhance predation once becoming infective to final predatory hosts. Intermediate hosts may spend more time hiding or forage less when harboring developing parasites, and become more active or bold once their parasites have sufficiently developed to infect subsequent hosts.

In addition to imposing the pressures of natural selection on infected individuals through increased mortality, parasites can also affect sexual selection in hosts, through effects on sexual signals. Good genes models of sexual selection posit that sexual signals are condition dependent, and indicate the quality or health of an individual. Many parasitological studies support the idea that sexual signals are condition dependent, as parasitism has been shown to reduce signal quality and consequent mating success in infected individuals (Andersson 1994).

The Hamilton-Zuk hypothesis, a parasite-mediated model of sexual selection, contends that sexual signals have evolved to advertise health and genetic resistance to parasites. According to the hypothesis, parasite resistance is conveyed through traits such as bright ornaments or dynamic courtship displays. These traits must be heritable, and show variation in a population, depending on the degree of parasite resistance; otherwise genes for the preferred character would go to fixation, and negate any benefits accrued by female choice (Zuk 1992). Elaborate traits should be more pronounced in populations with a higher prevalence of parasitism, as they will encounter stronger selection for reliable indicators of pathogen resistance. Lastly, females must prefer to mate with bright, showy males, or alternatively, with less parasitized males, to ensure that offspring inherit resistance to infection, and achieve higher fitness as a result (Hamilton and Zuk 1982).

In this dissertation I propose a different way of thinking about how parasites affect the evolution of host sexual signals. Manipulative parasites, many of which are trophically transmitted, have evolved to exploit host phenotypes to enhance predation by final hosts, often through intensifying conspicuous characters. Although these effects are well documented in a variety of host traits, the manipulation of host sexual signals has received little attention. Sexual signals are highly conspicuous and pose risks to senders, as they often attract unwanted receivers, such as parasites and predators (Zuk and Kolluru 1998). It would benefit trophically transmitted parasites to intensify these conspicuous signals in intermediate hosts, especially if they draw the attention of predatory final hosts. In hosts, manipulation of sexual signals would produce conflicts between natural and sexual selection, as enhanced signaling would attract more mates, but also more predators. Consequently, infected hosts would experience a reduced lifespan, however these costs may be partially offset by the benefits of increased short-term mating success.

Parasitized animals may also increase reproductive effort to compensate for infection. Because parasitized animals may experience a shorter life span and reduced mating opportunities, they may enhance their sexual signaling to increase their residual reproductive value. While there are no documented cases of increased sexual signaling by infected hosts to my knowledge, parasitized animals are known to increase egg production (Pan 1965, Minchella and Loverde 1981, Thornhill et al. 1986, Krist 2001, Blair and Webster 2007, Heins 2012), nuptial gift quality (Hurd and Ardin 2003), and mating effort (Polak and Starmer 1998, McCurdy et al. 2000).

For my dissertation, I examined the causes and consequences of parasitic infection in the California fiddler crab, *Uca crenulata*. Fiddler crabs (genus *Uca*) are semiterrestrial invertebrates that live in mixed-sex colonies on mudflats within mangroves, beaches, bays, and estuaries (Crane 1975). Fiddler crabs exhibit extreme sexual dimorphism; females are generally cryptic and possess two small, isomorphic feeding claws, while males are brightly colored, have one feeding claw, and another, greatly enlarged claw for courtship displays and combat with rivals (Crane 1975, Backwell and Passmore 1996, Jordao and Oliveira 2001, deRivera 2003, 2005). This enlarged claw, or major cheliped, often exceeds the carapace width of the organism and can comprise up to 48% of the total body mass of an adult male (Christy and Salmon 1984, Rosenberg 2002). Research strongly suggests that the major cheliped is a product of sexual selection, and serves a dual function, both as an ornament and weapon (Crane 1975, Allen and Levinton 2007). Males use the major cheliped when fighting with rivals over territories and access to females (deRivera 1999, Latruffe et al. 1999). The major cheliped is also used during a visual courtship display, where males perform a series of elaborate, stereotyped movements that attract females to mate (Crane 1975, Pope 2000). Females prefer males that wave more frequently and exhibit more ultraviolet (UV) reflectance from their major claws (Backwell and Passmore 1996, deRivera 2003, 2005; Detto and Backwell, 2009).

U. crenulata are intermediate hosts to several helminth parasites, most notably the digenetic trematode *Probolocoryphe uca* (Sarkisian 1957, Hechinger et al. 2007, Mora, unpub. data). *P. uca* are small, parasitic flatworms that have a three-host life cycle. They undergo stages of their development within two intermediate hosts, and complete their life cycle via sexual reproduction within a final host (Lafferty 1997, Roberts and Janovy 2000, Deblock 2008). *P. uca* trematodes are characterized by an oral and ventral sucker, and an oval body that is covered with spines (Sarkisian 1957, Abdul-Salam and Sreelatha 2000). *P. uca* displays a highly characteristic spined collar anterior to the oral sucker. A spineless, conical projection is anterior to the collar (Sarkisian 1957, Abdul-Salam and Sreelatha 2000, Deblock 2008). They are relatively small, measuring approximately 0.50 millimeter in length, and their oral and ventral suckers are well-separated and unequal in size (Sarkisian 1957, Deblock 2008). Their short digestive tract has a bifurcated ceca that does not extend beyond the ventral sucker. An ovary is situated anterior to the testes, and opposite to the genital pore, and a genital pouch can be found anterior to the ventral sucker (Sarkisian 1957, Deblock 2008).

The life cycle begins when infected birds and raccoons excrete *P. uca* eggs with their feces into habitats where the first (California horn snail, *Cerithidea californica*) and second (California fiddler crab, *U. crenulata*) intermediate hosts cohabit. Grazing snails become infected when they inadvertently ingest *P. uca* eggs as they forage on mudflats, prompting the eggs to hatch and the larvae to replicate asexually within the snail (Lafferty 1997, Roberts and Janovy 2000). Through asexual reproduction *P. uca* will produce motile infective stages, known as *cercariae*. These cercariae are equipped with tails that enable them to exit the snail into water where they seek fiddler crab hosts. Parasitized molluscs regularly "shed" cercariae with periodicity that is prompted by environmental cues, such as water, temperature, and light (Fingerut et al. 2003, Koprivnikar and Poulin 2009).

After *P. uca* cercariae encounter a fiddler crab host, they will enter the gill chambers, and travel to the connective tissues where they encyst as *metacercariae* (Sarkisian 1957). At this stage, each worm becomes encased within a protective cyst that thickens over time as the worm matures inside. *P. uca* become infective to bird and raccoon final hosts after full encystment within a fiddler crab (Lafferty and Dunham 2005, Hechinger et al. 2007), however they must be eaten by a final host for life cycle completion. Once ingested, enzymes from the final host's gut will digest the protective wall that envelops the worms, thereby activating excystment of the trematodes. *P. uca* will travel to the intestines where they will sexually reproduce with other conspecifics, and produce eggs that are excreted with the feces, thus marking the beginning of another life cycle (Lafferty 1997, Lafferty 2002).

In the first chapter I review the evolutionary and ecological implications of antiparasite behavior in a wide range of animal taxa, discuss new developments, and propose future areas of research for advancement of the field. We know that behavioral defenses from parasites are widespread and diverse in nature, however we are just beginning to discover their significance in reducing infection and increasing host fitness (Daly and Johnson 2011, Milan et al. 2012. Kacsoh 2013). Additionally, behavioral and immunological defenses are often studied separately though they are linked. Recent work suggests that hosts trade-off investment in behavioral and immunological defenses depending on age, sex, and life-history stage (Zylberberg et al. 2013). More research on individual variation in the use of these defenses has important implications for how diseases emerge and spread through populations.

In chapter two I examine how sex differences in host morphology and antiparasite behavior influence infection patterns in fiddler crabs. Sexual signals are costly to the bearer, often increasing their risk to parasites and predators (Magnhagen 1991, Zuk and Kolluru 1998). Since infection with the *P. uca* parasite is associated with increased host mortality, I first explore how fiddler crabs use behavioral defenses to reduce infection. Second, I examine how host sex differences in behavior and morphology influence infection patterns. Lastly, I investigate whether possession of a sexual signal, the major cheliped, comes at a cost of increased parasitism risk as a function of reduced grooming efficacy.

In chapter three I investigate how *P. uca* parasites affect sexual signaling behavior in breeding male fiddler crabs. I tested the hypothesis that manipulative parasites intensify host sexual signals to increase parasite transmission and fitness. Through a field experiment using naturally parasitized crabs, I filmed courting males and quantified their display frequency and ultraviolet reflectance of the major cheliped.

In chapter four, I examine how host sex and parasite development influence predation rates of fiddler crabs. I hypothesized that parasites manipulate host predation risk according to their development. I predicted that immature parasites would suppress host predation risk to reduce premature transmission, and enhance risk once becoming infective to predatory final hosts. I additionally predicted that male crabs would be more susceptible to predation than females since they perform more risky behaviors that likely increase their visibility to predators.

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Chapter 1

Antiparasite behavior of animals: a synthesis and new directions

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INTRODUCTION

Parasites are ubiquitous and affect virtually every living organism on the planet. Organisms can suffer significant fitness costs from parasitism, ranging from reduced growth and fecundity to death (Smith 1988, Forbes 1991, Forbes and Baker 1991, Vincent and Bertram 2010). In response to these challenges, hosts have developed a wide array of defenses that help them avoid or mitigate the fitness costs of parasites. While immunological defenses have received much attention, behavioral responses are often ignored though they are widely used and are an important line of defense from parasites (Hart 1990, Moore 2002, Ezenwa 2004, Daly and Johnson 2011).

Hosts can employ behavioral defenses against parasites both before and after infection. Pre-infection defenses may involve behaviors that repel or kill parasites before attachment. This is achieved through various means, from fumigation of nesting areas to swatting parasites from the body. Additionally, animals may prevent infection by avoiding parasites altogether. They may evade infection by fleeing infested areas or timing activities during periods of parasite dormancy. After infection, hosts may groom ectoparasites from the body or procure compounds for self-medication. While other reviews have categorized these behaviors as either pre- or post-infection strategies, many are performed at both stages of the parasite encounter. For example, animals may prophylactically self-medicate to prevent infection (Christe et al. 2003, Valderrama et al. 2000, Weldon et al. 2003) and therapeutically medicate to reduce infection once parasitized (Villalba et al. 2010, Milan et al. 2012). Because antiparasite behaviors are not always distinctly performed before or after infection, we discuss them together in this review.

Hart (1992) proposed that two criteria must be satisfied for a behavior to be considered an antiparasite defense. First, the parasite in question must exact a negative effect on host fitness. This has been difficult to establish in many studies, possibly because the fitness effects of some parasites are only seen during periods of host duress, such as when nutrition is limited or when fleeing from predators (Hart 1990). Second, the host behavior must be effective in reducing or eliminating the afflicting parasite. Behavioral efficacy in parasite reduction has been well documented in many grooming studies (Murray 1961, Murray 1967, Chen et al. 2011, Mullens et al. 2010, Vincent and Bertram 2010), while other work has failed to quantify any therapeutic benefits or has reported mixed results (Clayton and Vernon 1993).

Here we review the different types of antiparasite behavior and discuss the broader implications of these defensive strategies. We then propose promising new avenues of research to move the field forward. Previous reviews have focused primarily on mammals and birds (Hart 1990, 1992, 1994; Clayton et al. 2010), but recent work has documented diverse antiparasite behaviors in a wide range of taxa, including invertebrates (Cremer et al. 2007, Milan et al. 2012, Chapuisat et al. 2013, Kacsoh et al. 2013). While behavioral defenses are widely practiced by many animals, gaps remain in our understanding of these strategies. First, little is known about how plasticity in antiparasite behaviors may enable animals to persist in rapidly changing environments. Animals with higher behavioral plasticity may be better at combating parasites in novel environments, and conserving more resources by "switching off" defensive behaviors in the absence of threats (Hughes and Cremer 2007). As a result, these individuals may be more resilient and competitively superior to others with more fixed behavioral patterns (Sih et al. 2011, Tuomainen and Candolin 2011, Sol et al. 2013). Second, more work is needed to understand how hosts may accrue fitness benefits from exposure to some parasites. Low-level infections with certain parasites may have modulatory effects on the host's immune system, reducing maladaptive responses that can cause serious pathology (Strachan 1989, Zuk 2007). Additionally, intentional exposure to parasites can promote pathogen-specific immunity (Ugelvig and Cremer 2007, Konrad et al. 2013). Lastly, we stress the importance of studying how individuals trade-off behavioral with immunological defenses to combat parasites. Recent work suggests that life history traits can strongly influence variation in investment in different pathogen defenses, and subsequent parasitism risk (Bouwman and Hawley 2010, Zylberberg et al. 2013). Understanding how these factors interact will give us new insights into how infectious diseases are transmitted, and which individuals are most susceptible (Hawley et al. 2011, Zylberberg et al. 2013).

BEHAVIORAL DEFENSES FROM PARASITES

Hygienic behavior

Hygienic behaviors include actions taken to reduce parasite contamination of the body, food, and habitats. Here we will focus on hygienic behaviors other than grooming that remove parasites from food and dwellings. Grooming is considered in a separate section.

For animals that occupy territories for long periods of time, parasitism can be a serious challenge. Nests and other long-term residences can accumulate parasites over time, imposing significant fitness effects on inhabitants (Oppliger et al. 1994, Allander and Bennett 1995, Fitze et al. 2004 a, b). Many animals routinely inspect nests for parasites and remove them upon detection. Birds often engage in "sanitation behavior", where the head is thrust through nest material in an active search of parasites (Clayton et al. 2010). Female blue tits spend more time sanitizing nests infected with blowfly larvae and fleas than unparasitized nests (Hurtrez-Bousses et al. 2000, Tripet et al. 2002). Similarly, great tits (*Parus major*) invest more time into nest sanitation of flea-infested nests than clear nests (Christe et al. 1996). Social insects also have a system for parasite detection and removal. Specialized workers regularly patrol nests and rid the colony of parasites and diseased members (Cremer et al. 2007).

In cases where effective parasite removal is not possible or is too costly, nests may be abandoned. Hatching success, mass, brood size, and survival can be significantly reduced in parasitized nests, thus abandonment can offset the fitness costs of infestation (Møller

1987, Oppliger et al. 1994, Fitze et al. 2004a, b). Great tits and barn swallows are known to desert nests and offspring infested with ectoparasites (Duffy 1983, Emlen 1986, Møller 1987, Oppliger et al. 1994). Bechstein's bats (*Myotis beichsteinii*) prefer uninfested to infested roosts and avoid re-occupying sites containing ectoparasitic fly larvae (Reckardt and Kerth 2007). While clear nests are generally preferred to parasitized nests, the decision to abandon an area is often limited by the availability of alternative habitat. Birds and social insects are more likely to abandon infested nests when unparasitized sites are available (Cremer et al. 2007). For instance, when given the choice between infested and uninfested nest boxes, great tits showed a strong preference for uninfected nests (Oppliger et al. 1994).

Waste localization is another type of hygienic behavior that reduces parasite transmission. Feces are concentrated and sequestered in areas that minimize risk of ingestion such as in latrines (Zuri et al. 1997, Irwin et al. 2004) or at the edges of territories (Ezenwa 2004). Fecal localization is commonly seen in animals that use dens, burrows, and regular rest areas, such as canids, felids, primates, ungulates, and social insects (Epsky and Capinera 1988, Bass and Cherrett 1994, Bot et al. 2001, Hart and Retnieks 2001, Hart and Ratnieks 2002, Ezenwa 2004, Ballari et al. 2007). When offspring are young and incapable of localized defecation they are often fastidiously cleaned by the mother, thereby keeping the living quarters clean (Hart 1990, 1992).

Collective hygiene: The social insects

Social insects face unique parasite pressure because potential hosts are abundant and share close spatial and genetic relatedness to each other, making them especially vulnerable to infection. While the behaviors performed by social insects are thought to increase colony efficiency, they are undoubtedly important in reducing disease transmission (Cremer et al. 2007). Nestmates that have succumbed to infection within the colony can pose a serious risk to the group. Ants, bees, and termites will often respond by sequestering infected corpses in "graveyards" away from the colony (Epsky and Capinera 1988). Specialized workers in charge of this task may additionally expose the corpses to ultraviolet light or dismember infected individuals to promote desiccation, both of which kill parasitic propagules such as fungal spores (Cremer et al. 2007, Wilson-Rich et al. 2007). Diseased brood are often removed from the colony or cannibalized (Woodrow 1942, Rosengaus and Traniello 2001).

Insects may socially exclude infected members from healthy individuals to reduce parasite transmission. Termites can build protective walls around infected members to isolate them (Fuji 1975, Epsky and Capinera 1988, Klein 1990). Additionally, they can produce vibrating alarm calls that warn nestmates to avoid infected individuals in the area (Rosengaus et al. 1999, Myles 2002). Honeybees have special guards at nest entrances; these workers attack and exclude infected nestmates, which is invariably fatal to the outcasts (Waddington and Rothenbuhler 1976, Drum and Rothenbuhler 1985). Colony members can also confine parasites to reduce infection risk. For example, Cape honeybees encapsulate invading parasitic beetles, essentially starving them to death (Neumann et al. 2001). Ants collect pathogenic fungal spores and bury them in the soil (Storey 1990, Pereira and Stimac 1992, Jacoud et al. 1999).

Social insects also practice very organized waste localization, which reduces nest contamination. Nest workers transport leftover food and other waste products to the periphery of the nest to be picked up by garbage workers. Moreover, garbage dumps are often placed downhill, preventing spillage back into the nest (Epsky and Capinera 1988, Bass and Cherrett 1994, Bot et al. 2001, Hart and Retnieks 2001, Hart and Ratnieks 2002, Ballari et al. 2007). The social structure of the colony members themselves is also thought to promote good hygiene and reduce disease transmission. Tasks are divided among caste members and spatially partitioned into compartments. Older nestmates with higher intrinsic mortality generally assume the role of garbage workers and foragers, which also have the highest risk of infection (Bot et al. 2001, Cremer and Sixt 2009). Nurse workers that tend to brood and the queen are younger and do not interact with colony members at the nest periphery (Holldobbler and Wilson 1990). The division of labor and compartmentalization of tasks minimizes interactions between different caste members and reduces chances of parasite transmission to more central areas of the nest (Hart and Ratnieks 2001, Naug and Smith 2007, Cremer and Sixt 2009).

Grooming

Grooming is another effective hygienic behavioral defense against parasites. Parasites are generally removed from the body surface through scratching, licking, chewing, and preening. While grooming behavior is believed to function in parasite control, it is also used in social interactions and for maintenance of pelage and plumage (Hart 1990, Spruijt et al. 1992). Individuals may groom themselves or others. The latter behavior, called allogrooming, may be directed towards conspecifics or members of another species.

Some of the strongest cases for grooming as an antiparasite mechanism come from animals that are prevented from self-grooming. Initial observations of natural populations reported significantly higher parasite loads in animals with impaired grooming ability (Clayton et al. 2010). Subsequent studies used experimental manipulations to quantify grooming efficacy. For example, beak-trimmed hens that were experimentally inoculated with either body lice or mites harbored at least 10 times more lice and mites than intact hens (Mullens et al. 2010). Crickets that survived exposure to parasitoid fly larvae groomed much more often than those that succumbed to infection (Vincent and Bertram 2010). Mice that were prevented from oral grooming harbored 60 times more lice than unimpaired cohorts (Murray 1961, Murray 1987).

While grooming mechanically removes parasites, saliva spread on the body during oral grooming can also reduce infections through anti-microbial activity. Saliva contains various components such as lysozyme, leukocytes, antibodies, lactoferrin, cationic proteins, and lactoperoxidase, which have bactericidal properties (Hart and Powell 1990). Post-copulatory genital grooming is commonly practiced in rats and carnivores and is linked to lower rates of sexually transmitted infections in rodents (Sachs and Barfield 1977, Hart 1985, Hart et al. 1987, Hart and Powell 1990). Wound licking is another hygienic behavior practiced by rats, canines, felids, and primates. The behavior usually begins immediately after injury, before initiation of the inflammatory response. As evidenced through studies on canines, saliva spread on wounds through licking can have antiseptic effects. Canine saliva was found to be bactericidal against *Streptococcus canis* and *Escherichia coli*, both of which were isolated from 10-20 percent of dog wounds (Hart and Powell 1990). Saliva also contains nerve and epithelial growth factors, which promote wound healing (Li et al. 1980, Niall et al. 1982). Mice that had their salivary glands experimentally removed exhibited delayed wound healing and subsequent application of these growth factors resulted in improved wound closure (Hutson et al. 1979, Li et al. 1980, Niall et al. 1982).

Grooming in social animals often extends to mates, offspring, and fellow group members in addition to themselves. While allogrooming facilitates social interactions such as pair bonding and conflict resolution, it is also important in parasite control. Allogroomers often target areas that animal being groomed cannot reach or visualize (Barton 1985, Borries 1992, Reichard and Sommer 1994, Franz 1999, Perez and Vea 2000, Lazaro-Perea et al. 2004, Singh et al. 2006, Lewis 2010). As a result, individuals that are exposed to allogrooming generally exhibit much lower ectoparasite burdens than solitary individuals. For example, unpaired penguins had two to three times more ticks than paired individuals (Brooke 1985). Brooke did not examine parasite loads prior to pairing, thus it cannot be determined if paired penguins found mates because they were less parasitized than single penguins, or if the lower tick burdens were the result of allogrooming partners. Solitary baboons and langurs exhibited much higher ectoparasite loads compared with individuals that had access to allogrooming (Clayton et al. 2010). While earlier work focused on allogrooming in mammals and birds, more recent work has documented the behavior in other taxa, such as social insects. For instance, mutual grooming in leaf cutter ants significantly reduced the prevalence of pathogenic fungi in the colony (Walker and Hughes 2009). Termites that removed fungal spores from each other exhibited longer lifespans (Rosengaus et al. 1999). For these animals, the incidence of allogrooming increases with group size (Cremer et al. 2007).

Parasite removal by different species of animals is known as heterospecific allogrooming. While conspecific allogrooming often has a social component (Spruijt et al. 1992, Stopka and Graciasova 2001, Kutsukake and Clutton-Brock 2006) heterospecific allogrooming provides parasite relief for the groomed animal and food for the groomer. Heterospecific grooming has been well documented in aquatic animals and in tick-eating birds. In aquatic environments larger parasitized fish will visit the cleaning stations or territories of cleaners to be groomed. The infected fish will present specific body parts to the cleaner, often opening the mouth to facilitate parasite removal from the gills and oral surfaces (Gorlick et al. 1978, Hart 1990, Poulin 1993). Cleaner animals, which are generally smaller fish and shrimp, will fastidiously groom the infected animals, ingesting bacteria, fungi, and ectoparasites from their bodies. Although cleaner species often resemble prey items of their larger clients, they do not eat them, supporting the idea that the cleaner-client relationship is mutualistic (Feder 1966, Hart 1990). Studies of fish before and after the removal of cleaner species highlight the importance of these animals in disease control. For example, client fish infected with gnathiid isopods exhibited a 4.5-fold reduction in parasite load after exposed to cleaner fish (Grutter 1999). Fish that remained in territories where cleaners had been removed exhibited a much higher incidence of pathology from parasites (Limbaugh 1961, but see Grutter 1997).

However, the mutualistic cleaning relationship can sometimes become parasitic. Cleaning "cheaters" have been observed in several marine species (Bshary and Grutter 2002, Bshary and Grutter 2003, Grutter and Bshary 2004). These animals exploit the sensory system of clients to obtain food at the client's expense. Instead of removing their ectoparasites, cleaning cheaters will instead consume the client's mucus coating, which harms the fish (Bshary and Grutter 2002, Ebran et al. 1999 and references therein).

Tick-eating birds form another group of heterospecific cleaners that dine on the parasites of others. Oxpeckers have been observed foraging on the tick-ridden bodies of buffalo, giraffe, zebra, eland, and rhinoceros (Hart 1990). Stomach content analyses of wild oxpeckers found an average of 400 ticks per bird. Moreover, experimental infections of cattle revealed that oxpeckers were able to remove 20 percent of tick larvae and 100 percent of adult ticks (Bezuidenhout and Stutterheim 1980). Animals in search of grooming will often assume specific postures, prompting the cleaner birds to inspect and forage on specific body regions (Hart 1990). For example, impala will lower their ears and close their eyes while oxpeckers remove ticks from the pinna and eyelids. Galapagos tortoises will extend their legs and neck, allowing Darwin's finches to inspect the body and consume ectoparasites (McFarland and Reeder 1974).

While grooming is effective in parasite removal, it is also costly to the individual. Grooming animals incur increased energy expenditure, decreased vigilance from predators, evaporative water loss, and reduced thermoregulation (Hart 1990). For instance, rats spend approximately 30% of their active time grooming, which accounts for one-third of their evaporative water loss (Bolles 1960, Ritter and Epstein 1974). Croll and McLaren (1993) reported a 200% increase in the metabolic rates of preening Thick-billed Murres compared with resting conspecifics. Moose infested with ticks groom more often and experience impaired thermoregulation as a result of fur loss from excessive cleaning (Samuel 1991). Allogrooming is reported to reduce vigilance to predators in birds, ungulates, and primates among others (Redpath 1988, Maestripieri 1993, Mooring and Hart 1995).

Parasite defenses against host grooming

Parasites have several behavioral and morphological adaptations that reduce grooming efficacy in hosts. Mites and lice have specialized clasping structures for attachment to host fur and feathers (Labrzycka 2006). Mobile stages can flee areas being groomed and establish on body regions that are difficult for the host to reach. For example, ticks and lice often inhabit the head, neck, and perianal regions of their hosts (Nelson and Murray 1971, Hart 1990, Hart 1994). Moreover, different tick species exhibit high specificity for certain body regions. While *R. evertsi* is found primarily in the perianal region of hosts, *R. appendiculatus* infests the head and ears (Howell et al. 1978). These regional tick distributions suggest selective migration of the parasites, and not simply byproducts of limited grooming ability.

Grooming and infection risk

Interestingly, grooming behavior may enhance the transmission of some parasites. For example, dogs and cats can become parasitized by the cestode *Dipylidium caninum* after ingesting a flea harboring an infective stage (John and Petri 2006). Similarly, mice become infected with the nematode *Heligmosomoides polygyrus* after ingesting infective larvae while grooming themselves and others (Hernandez and Sukhdeo 1995). Termites that groom nestmates infected with pathogenic fungi exhibited increased mortality rates (Kramm et al. 1982). Some bee viruses may also be transmitted through grooming by conspecifics (Waddington and Rothenbuhler 1976, Evans and Spivak 2010).

Parasite repelling behavior

Biting insects exact debilitating effects on their hosts through blood and weight loss, reduced feeding times, and increased disease transmission (Hart 1994, Moore 2002, Mooring et al. 2006). For example, horses may endure bites from as many as 4000 flies per day, resulting in a cumulative blood loss of 0.5 L (Tashiro and Schwardt 1949, 1953, Foil and Foil 1988). Additionally, hosts can contract a myriad of debilitating diseases from biting insects such as lyme disease, west nile virus, babesiosis, malaria, and equine encephalitis, among others (Hart 2011). Animals employ numerous repelling behaviors to reduce the pain and costs imposed by biting insects. Ungulates exposed to parasitic flies have been observed tail and ear switching, head shaking, leg stamping, and skin twitching (Espmark 1967, Okumura 1977, Harvey and Launchbaugh 1982, Hart 1994, Mullens et al. 2006). Elephants use branches as tools to swat flies away from the body (Hart and Hart 1994). Birds and small mammals respond to biting insects by tail and ear flipping, foot stamping, and face rubbing (Hart 1994). The frequency with which these behaviors are performed is strongly associated with ectoparasite density. Cattle, elephants, and woodrats are reported to increase repelling behaviors when biting insect densities are high (Edman and Kale 1971, Hart and Hart 1994, Mullens et al. 2006). Conversely, repelling movements are reduced in animals pretreated with insecticides (Harvey and Launchbaugh 1982, Harris et al. 1987, Woollard and Bullock 1987, Ralley et al. 1993). Repelling behaviors can be an effective means of deterring and dislodging ectoparasites. In a study on Asian elephants, swatting reduced median fly density by 43% (Hart and Hart 1994). Animals exhibiting higher rates of insect repelling activities generally suffer less blood loss from the parasites (Baylis 1996, Torr and Mangwiro 2000). Cattle that had their tails docked to prevent tail switching harbored more biting flies than intact cattle (Ladewig and Matthews 1992, Eicher et al. 2001).

While repelling behaviors may help ward off harassment from these parasites, they are not without their own set of costs. Animals often have to redirect time and energy away from resting and feeding toward parasite defense. Reductions in feeding and resting activity have been reported in caribou, reindeer, goats, and cattle exposed to tabanid flies (Espmark 1967, Brindley et al. 1989, Baylis 1996, Mörschel and Klein 1997, Mooring et al. 2007). Young cattle exposed to stable flies spend less time feeding, which results in a loss of weight gain (Hart 1990, Campbell et al. 1987, Campbell et al. 2001). Dairy cattle afflicted by the flies produce lower milk yields (Bruce and Decker 1958, Todd 1964, Miller et al. 1973, Harris et al. 1987) Smaller animals often exhibit the strongest repelling behaviors due to a higher cost of parasitism, termed the body size principle (Walker and Edman 1986, Edman and Scott 1987, Mooring et al. 2000, Mooring et al. 2006). Smaller animals have a greater surface to volume ratio, thus they risk losing a larger proportion of blood than larger animals. Incidences of higher programmed grooming rates in smaller animals have been reported in a variety of ungulate species (Mooring et al. 2000, Mooring et al. 2004, Mooring et al. 2006).

Avoidance behavior

Animals can reduce or prevent infection altogether by avoiding parasites. Individuals may vacate or avoid infested areas (spatial avoidance), seek refuge when parasites are active (temporal avoidance), and avoid contaminated food (dietary avoidance). Spatial avoidance of parasites may occur through migrations and shifts in habitat. Long-term or regular occupation of a site promotes parasite growth and propagation, thus moving away from infested areas reduces infection risk (Perrot-Minnot and Cezilly 2007). Several studies provide evidence that migratory behavior of herding animals can effectively reduce parasite burdens (Folstad et al. 1991). For instance, warble flies live in the skin of reindeer and can be lethal at high intensities. Migrating reindeer were less parasitized than those with higher site fidelity and fly intensities decreased with increased migratory distance (Folstad et al. 1991).

Potential hosts may select a different habitat to reduce parasite exposure. Habitat shifts are common in taxa that occupy sites for long periods, such as nesting or burrowing animals. For example, numerous studies demonstrate that animals avoid and abandon nests and roosts infested with ectoparasites (Duffy 1983, Emlen 1986, Møller 1987, Oppliger et al. 1994, Reckardt and Kerth 2007). For many animals, habitat selection often reflects a balance between the risks of parasitism and predation (Decaestecker 2002, Behringer and Butler 2010). For instance, sticklebacks will shift habitat preference in the presence of the hematophagous fish louse *Argulus canadensis*, which resides in vegetation near the bottom of ponds. In the absence of parasites, sticklebacks oriented near the bottom of tanks and swam in vegetation. However, when *A. canadensis* was added to tanks, the fish were more likely to surface and swim in open water areas (Poulin and FitzGerald 1989b).

Parasites often exhibit temporal patterns in host-seeking activity, thus animals can mitigate risk by scheduling activities when parasites are dormant. They may be more active during the day or during a particular season, as with vectored parasites and some infective stages, such as free-swimming trematode larvae (Combes et al. 1994, Esch et al. 2002). For example, the ant *Pheidole titanis* conducts diurnal raids on termite colonies during the dry season, but they shift their foraging schedules to night during the rainy season. This seasonal shift has been attributed to increased activity of a parasitoid fly (Diptera: Phoridae) that specializes on *P. titanis*. Dissections of *P. titanis* soldiers and workers indicate a parasite prevalence of less than 2%, indicating the strong ecological effect these parasitoid flies have on their hosts (Feener 1988). At least two cricket species subject to parasitism from the acoustically-orienting parasitoid fly *Ormia ochracea* shift calling behavior to avoid parasitism. *Gryllus integer* males sing more when *O. ochracea* is less active (Cade et al. 1996), and male *Teleogryllus oceanicus* from highly parasitized populations take longer to resume calling than males from less parasitized populations (Lewkiewicz and Zuk 2004).

Avoiding contaminated food is another mechanism by which animals can reduce parasitism risk. Optimal foraging theory predicts that animals should maximize their net unit consumption per unit time (MacArthur and Pianka 1966). However, when high quality diets are also associated with increased parasitism, animals may alter their feeding patterns. This is often the case with pasture contaminated by animal feces; while fertilized pasture is generally more robust and provides a higher nutrient intake to consumers, it is also more likely to harbor gastrointestinal parasites (Hutchings et al. 2003). A number of studies on domesticated herbivores document active avoidance of these contaminated areas. For example when taller, higher quality swards are infested with parasitic nematodes, sheep opt for shorter, less nutritious pasture (Hutchings et al. 2001, 2002). Similarly, this selective foraging behavior has been reported in horses, cattle, and kangaroos, among others (Michel 1955, Ezenwa 2004, Garnick et al. 2010).

While avoidance behavior has been studied in a variety of animals, the field is not without criticism. Perrot-Minnot and Cezilly (2007) argue that there are few proven cases where avoidance behavior is effectively involved in antiparasite defense. They maintain that the costs of being selective and potentially avoiding certain foods, territories, and mates are high when competition is strong. These costs cannot be offset unless the perception of infection risk is reliable, the probability of infection is high, and the parasite has high virulence. The authors add that host behavior to avoid parasites is less common than parasite behavior to reach their hosts. This is probably because selection pressure on the parasite to reach and establish within a host is stronger than that on the host to avoid the parasite (Combes 2001).

In essence, Perrot-Minnot and Cezilly (2007) make a similar argument to the life-dinner principle, described by Dawkins and Krebs in 1979. That is, predators and prey exhibit asymmetrical evolutionary outcomes from their interactions. During a predator-prey interaction, success for the predator means procuring another meal while success for the prey means survival. Further, if a predator fails they merely lose a meal while failure of the prey has much more dire consequences, often resulting in death. Thus coevolution between predator and prey would be highly asymmetrical, with prey experiencing stronger selection pressure to evade predators than for predators to circumvent prey defense (Dawkins and Krebs 1979, Vermeij 1994).

While parasites may generally experience stronger selection than their hosts, avoidance behavior can still evolve as an effective defense from infection. Animals are known to avoid parasites, even at the cost of seeking new territories, eating lower quality diets, and increasing susceptibility to predators (Duffy 1983, Emlen 1986, Møller 1987, Oppliger et al. 1994, Hutchings 2001, 2002, Decaestecker 2002, Reckardt and Kerth 2007). While failure to establish within a host results in death of the parasite, failure to avoid infection also has significant fitness consequences for the host, including sterilization and death (Minchella and Loverde 1981, Adamo et al. 1995, Moore 2002).

Parasites, the Selfish Herd, and Host Grouping Behavior

Parasites may have had a role in shaping social behavior in their hosts. However, the ways in which parasites can influence host group dynamics are many and varied, and depend on the parasites involved and their transmission routes. Different parasites may exert opposite selection pressures; when faced with some types of parasites, hosts may benefit from forming groups (Hamilton 1971, Mooring and Hart 1992), while other parasites may impose costs on social hosts (Rifkin et al. 2012). Specifically, animals may gain protection from staying together in large groups. There are many examples of such groups, including herds of cattle and deer, as well as large schools of fish. These groups are traditionally thought of as a way to spread out the risk of predation across many individuals. Mooring and Hart (1992) refer to this idea as the encounter-dilution effect because it relies on two major assumptions. First, Mooring and Hart assume that the rate at which the predator/parasite encounters a group of animals does not increase proportionally with group size (the encounter effect). Second, they assume that a predator/parasite will not attach or consume more prey/hosts in a large group, so that the individual risk of being attacked by a single predator is much lower when in a large group than when in a small one (the dilution effect). These principles have traditionally been applied to animals seeking to escape predators (Hamilton 1971); however, such tactics may work equally well in reducing the individual risk of parasitism.

Hamilton (1971) described the idea and potential geometry of the selfish herd with regard to predation risk. The basic idea behind the selfish herd is that a single predator will likely only kill one prey animal at a time, most likely the one closest to them. Prey animals can diminish their individual risk of being killed by moving closer to another individual, thus potentially putting that individual closer to the predator. In this scenario, the best spot a prey animal can occupy is the center of the herd, where it gains protection by having other potential prey around it.

While Hamilton's (1971) paper mostly dealt with predators, he did note that some parasites could induce this grouping response as well. Specifically, he described that in reindeer, animals at the outermost edges of the herd were more likely to be attacked by biting flies, similar to the situation seen with many predators. Thus, the idea of the selfish herd can apply to parasites as well, particularly ectoparasites such as blood sucking flies. Such parasites can impose a large cost on their hosts (Tashiro and Schwardt 1953). However, if hosts cluster together in a large group, the chances of a single individual being bitten by such flies is reduced due to the dilution effect described above. This may explain why cattle, when faced with the presence of biting flies, form dense groups in which most of the members turn their faces (a part of the body particularly vulnerable to biting insects) towards the center of the group (Mooring and Hart 1992).

Other examples point to the ability of parasites to promote group formation. While most work to date has focused on large mammals faced with biting flies, some work has looked at behavior in fishes. Poulin and FitzGerald (1989a) found that sticklebacks (*Gasterosteus spp*.) tended to form larger shoals when faced with the blood-sucking ectoparasite *Argulus canadensi*. Moreover, fish in larger shoals faced a lower risk of infection, consistent with the predictions of the dilution effect.

Most examples of selfish herd behavior occur in response to ectoparasites. However, some internal parasites may promote this behavior as well. Shoaling behavior in fish may also provide some protection against the infective cercariae of some trematode species. These parasites attack the host, burrow into the host's body and encyst there, and group formation may lower the likelihood that a given individual will be attacked (Stumbo et al. 2012). Moreover, individuals occupying the central position in the shoal gain the most protection, consistent with Hamilton's (1971) predictions (Stumbo et al. 2012). One should note that selfish herd behavior does not work equally well against all parasites. Parasites such as ticks and mites that spread easily between individuals may actually benefit from having hosts cluster together in close quarters. Threat of infection by such parasites can actually drive hosts to spend less time near conspecifics, a trend directly opposite that of the selfish herd behavior. For example, juvenile spiny lobsters normally prefer to take shelter in crevices with other members of their species. However, these lobsters will avoid sheltering with conspecifics infected with the lethal PaV1 virus, even though this avoidance behavior exposes them to greater predation risk (Behringer and Butler 2010). This demonstrates that some parasites also have the ability to shape host social dynamics, and such parasites may impose selection pressure against gregarious hosts, depending upon their mode of transmission. Which selection pressure will be stronger, the tendency to form groups or remain solitary, probably depends on which parasites infect the host and the virulence and infection risks for each parasite. In addition, other factors such as predation pressure, doubtless come into play.

Macroparasites could also potentially impose costs on social hosts, particularly if they are more likely to attack large groups, which Mooring and Hart (1992) speculate could happen if larger groups produce greater stimuli (such as carbon dioxide) that attract such parasites. Additionally, infection with some parasites may impair a host's ability to join a group. For example, rainbow trout (*Oncorhynchus mykiss*) infected with the trematode *Diplostomum spathaceum* show reduced shoaling behavior even in the presence of a simulated predator, possibly because the metacercariae cause cataracts and impaired vision when they encyst in the hosts. This poor vision could impact the ability of the fish to locate conspecifics with which to shoal (Seppälä et al. 2008). Trophically transmitted parasites may manipulate the behavior of their hosts in ways that make them more likely to be consumed by a predator (Moore 2002, Lefèvre et al. 2008). Such behavioral alterations could include a reduction in the amount of time the host spends in a group, particularly if staying in a group reduces predation risk. The trout example described above could tentatively fall in this category, as the trematode infecting them is trophically transmitted and might benefit from such a reduction in antipredator grouping behavior. However, in this case, the behavioral change is likely a byproduct of infection rather than a direct manipulation. Thus, caution must be used when testing this hypothesis (Poulin 1995).

In some cases, parasites may influence group dynamics in more subtle ways. For example, one would expect that many parasites, particularly those transmitted through close contact between hosts, would thrive when hosts form large groups (Rifkin et al. 2012). However, in some cases, in large groups, clusters of individuals can sometimes form smaller subgroups, and members of a given subgroup interact more closely with each other than they do with members of another subgroup. This makes it more difficult for a parasite to infect all the individuals in a larger group, as transmission is more likely to be confined to one or a few subgroups. Griffin and Nunn (2012) modeled this situation, and found that increased modularity (i.e. more subgroups within a larger group) decreased the overall number of hosts a parasite could infect. Moreover, primate species that had more modular groups tended to be infected by fewer socially-transmitted parasites, suggesting that hosts may form subgroups in part to reduce the risk of parasitism (Griffin and Nunn 2012). Thus, parasites have the ability to alter host social behavior in many different ways, some of them difficult to predict.

Self-medication

One of the most controversial forms of antiparasite behavior is self-medication, also known as zoopharmacognosy (Huffman 2003). Clayton and Wolfe (1993) defined self-medication as the use of medicinal compounds by organisms to defend against parasitism. Self-medication typically operates through one of four mechanisms: ingestion, absorption, topical application, or proximity (Clayton and Wolfe 1993). While much has been written about animal self-medication (for reviews, see Clayton and Wolfe 1993, Lozano 1998, Moore 2001), relatively few studies have actually demonstrated that self-medication is adaptive (Sapolsky 1994).

Clayton and Wolfe (1993) established three criteria that must be met in order to demonstrate the adaptive nature of self-medication. In addition to the deliberate contact of a medicinal compound by a host, the compound itself must prove detrimental to parasites when it is contacted and must also improve host fitness, generally as a result of the detrimental effect it has on parasites. More recently, Singer et al. (2009) developed their own criteria for adaptive self-medication (Table 1.1). The two sets of criteria share some commonalities. However, there are some critical differences. While Clayton and Wolfe developed criteria sufficient to allow most forms of prophylactic self-medication, Singer et al.'s third criterion precludes any pre-infection self-medication. Moreover, it's not entirely clear that self-medication should decrease host fitness when a host is not infected. In some cases, self-medication indeed reduces host fitness. In caterpillars, consumption of pyrrolizidine alkaloids confers resistance to parasitoid flies, but unparasitized individuals consuming these alkaloids suffer from reduced survival (Singer et al. 2009). Fruit flies also gain resistance to parasitoid flies by consuming alcohol, but consumption of high concentrations of alcohol prove detrimental to fruit fly survival (Milan et al. 2012). However, self-medication need not be detrimental and so we would not necessarily expect reduced fitness of uninfected medicators in cases of prophylactic self-medication. By definition, prophylaxis involves individuals without parasites, and self-medication in these cases would prevent or reduce, rather than cause, the loss of fitness by reducing or precluding parasite infection. For the purposes of this review, we employ Clayton and Wolfe's criteria, which allow the inclusion of prophylactic self-medication, which may be the most prevalent form of self-medication (Hart 2005).

The first criterion is the most easily met. A number of reports exist describing the use of plant or animal material by organisms (Janzen 1978, Hamilton et al. 1978). More importantly, these materials often do not comprise a normal part of the organism's diet, and animals are often observed contacting these substances during periods of increased parasite prevalence or when the animals themselves display the pathology of parasite infestation, such as lethargy or anorexia. Fulfilling the second and third criteria proves much more difficult. Of the nine examples referenced by Clayton and Wolfe, only one satisfies all three criteria, while two others potentially satisfy the criteria. Demonstrating that a particular substance negatively affects a parasite represents a difficult endeavor, though many researchers have attempted to get around this issue by testing a broad range of plant materials for potential antiparasitic properties. For example, Nègre et al. (2006) tested 16 of the 29 plant species that brown lemurs (*Eulemur fulvus)* were observed to eat during the mating season for antiparasitic properties *in vitro*. Of these, four plant species contained compounds that proved detrimental to parasites. However, numerous criticisms have been leveled at demonstrating antiparasitic properties *in vitro* as opposed to *in vivo* (Clayton and Wolfe 1993, Sapolsky 1994). For example, the phenomenon known as 'anting', in which a bird rubs an ant through its feathers, is thought to have antiparasitic functions. Tests on the efficacy of formic acid show that it is highly effective in killing lice and feather mites *in vitro* (reviewed in Clayton and Wolfe 1993). However, other experiments found that anting did not reduce lice infestations *in vivo* (reviewed in Clayton and Wolfe 1993; see also Clayton et al. 2010). Birds also perform a similar behavior with other substances, rubbing various objects, such as citrus fruit (Weldon 2004) through their plumage, but few studies have examined the efficacy of anting *in vivo*.

Further difficulties in establishing the adaptive nature of self-medication arise when the behavior is placed in the context of prophylaxis. In contrast to its use as a therapeutic treatment, organisms utilizing preventative self-medication are not ill. This makes Clayton and Wolfe's second criteria – that is, the medicinal compound should kill or prove detrimental to parasites – somewhat difficult to satisfy. Nevertheless, some studies suggest a prophylactic function for self-medication. Capuchin monkeys are thought to avoid mosquitoes by rubbing their body with millipedes (Valderrama et al. 2000, Weldon et al. 2003). These millipedes excrete benzoquinones, which are potent insect repellents. In the best-described case thus far, the wood ant *Formica paralugubris* seeks out and uses conifer resin in nests (Castella et al. 2008). The presence of this resin suppresses microbial growth (Christe et al. 2003) and increases ant survival (Chapuisat et al. 2007).

In addition to the prophylactic use of conifer resin by wood ants, other recent studies have attempted to fulfill the criteria set forth by Clayton and Wolfe (1993). Villalba et al. (2010) found that lambs infected with gastrointestinal nematodes may adaptively self-medicate. Infected lambs preferred alfalfa laced with tannins, whereas uninfected lambs preferred untreated alfalfa. Further, tannin consumption was linked to a decrease in fecal egg counts, though no measures of lamb fitness were included. Researchers have demonstrated adaptive antiparasite behavior – ethanol consumption – in *D. melanogaster* infected with endoparasitoid wasps (Milan et al. 2012). This system meets all of the criteria for adaptive self-medication in that infected fly larvae seek out ethanol, ethanol exposure kills wasp larvae *in vivo*, and fly larvae that utilize ethanol experience increased survival (Milan et al. 2012).

Perhaps the most extensively debated example of self-medication is that of nesting birds lining their nests with plant material or other compounds thought to ward off or kill parasites. Blue tits (*Cyanistes caeruleus*) actively incorporate aromatic plant material into their nests (Petit et al. 2002, Mennerat et al. 2009a). In this case, however, the material functions not to kill ectoparasites (Mennerat et al. 2008) but provides antibacterial defense (Mennerat et al. 2009b) and increases nestling fitness (Mennerat 2009b), satisfying all three of Clayton and Wolfe's adaptive criteria.

European starlings also line their nests with green plants, many of which contain volatile compounds with insecticidal and antibacterial properties (Clark and Mason 1985, reviewed in Clayton et al. 2010). However, Brouwer and Komdeur (2004) argue that green plant material is used in mate attraction rather than in nest defense. They found no effect of green plant material on ectoparasite numbers, but did find that only males carry green plant material, and generally only during nest construction rather than during rearing of nestlings (Brouwer and Komdeur 2004). In fact, a number of other studies of birds that line their nests with plant material have failed to find any effect of the plant material on ectoparasite prevalence or survival (Mason and Clark 1988, Fauth et al. 1991, Gwinner et al. 2000), casting doubt on the efficacy of this behavior as an antiparasite defense. Seeking to explain this, Gwinner et al. (2000) proposed the 'drug hypothesis', which maintains that animals seek out certain compounds not for any particular antiparasitic properties, but to enhance or stimulate immune function. This, in turn, would enable an organism to better combat parasitic infections. Gwinner and Berger marshal some evidence in support of this hypothesis. First, one study indicated that starling nestlings in nests lined with green herbs experienced an increase in basophil leukocyte counts (Gwinner et al. 2000), though this was not observed in a second study (Berger 2002, in Gwinner and Berger 2005). Second, starling nestlings reared in herb-lined nests attained greater body mass as fledglings than those reared in nests without herbs, regardless of ectoparasite load (Gwinner and Berger 2005). Taken together, Gwinner and Berger suggest that this indicates a potential role for herbs or other plant material to act as stimulants of the immune system.

The drug hypothesis remains extremely challenging to test. Vertebrate immune systems in particular are comprised of multiple parts that may operate independently of one another, and an increase in one branch may come at a reduction in another (Norris and Evans 2000, Adamo 2004, Lee 2006, Forsman et al. 2008). Studies of ecoimmunology demonstrate the extraordinary difficulty in determining what exactly an elevated immune response (or lack thereof) might mean (Salvante 2006). Do high-quality individuals mount greater or weaker immune responses? How do standing levels of immunity affect activated immunity? Combined with the lack of consistency in observed leukocyte counts in starlings (Gwinner et al. 2000, Berger 2002), the drug hypothesis requires further examination.

Finally, self-medication may include controlled exposure to parasites, a form of natural vaccination (Hart 1990, 2011). Carnivores in particular may expose their young to small doses of parasites by dragging fresh kills to their young, thereby contaminating the meat with small amounts of pathogens that prime the immune system (Hart 1990, 2011). However, ‘vaccination’ by this means currently lacks empirical support. We caution that such hypotheses need to be carefully and clearly defined so as not to convolute typical behaviors with more specialized antiparasite defenses.

BROADER IMPLICATIONS OF ANTIPARASITE BEHAVIOR

Ecological and Evolutionary Impacts of Antiparasite Behavior

Scientists have recognized parasites as potentially important players in ecological interactions for the last several decades. Anderson and May (1978) and May and Anderson (1978) developed mathematical models describing how parasitic infection can affect host populations. From this work, they conclude that parasites have the potential to regulate the growth of their hosts’ populations under certain conditions, mostly through parasite-induced mortality. These models considered many factors, such as the distribution of parasites within hosts and whether parasites can also affect host reproductive rate. However, if hosts have the ability to avoid infection via behavioral mechanisms, this may lessen the ability of parasites to limit host populations.

The ability of parasites to regulate host populations is impressive in its own right. However, depending upon the host species, parasite infections can affect other members of the community as well. In this respect, parasites can play a similar role to predators in ecological processes. Predators can affect plant and algal communities by altering herbivore densities in a process known as a trophic cascade (Shurin et al. 2002), and parasites can have similar effects. For example, bush lupines growing along the coast of California are frequently killed by larval ghost moths (*Hepialus californicus*). However, these moth larvae are infected by a pathogenic nematode, *Heterorhabditis hepialus*, which kills the larvae and thus protects the lupines (Strong et al. 1996). Thus, in a classic example of a parasite-mediated trophic cascade, lupine populations are greatly influenced by the presence and abundance of the nematode.

Like predators, parasites may induce host mortality and directly affect ecological processes through their impact on host populations. However, behavior may also play an important, and as yet mostly unexplored, role in regulating such processes as well. Sometimes predators can affect plant populations even without directly causing prey mortality, because prey alter their behavior in the presence of predators. These avoidance behaviors by prey can take the form of reduced foraging and/or habitat shifts, and can have a dramatic effect on plant abundance and community composition (Schmitz et al. 2004). For example, vegetation in Yellowstone changed dramatically after the reintroduction of wolves, and at least some of these changes are thought to have occurred because of habitat shifting by elk in the presence of predators (Fortin et al. 2005). Thus, the behavior of the prey species has the potential to directly affect plant communities.

The role of behavior in trophic cascades has been recognized and studied in predator-prey interactions (Schmitz et al. 2004). However, to our knowledge, no one has yet explored whether parasites may perform a similar function. Parasites may induce similar behavioral responses in their hosts, such as avoidance behaviors in which hosts, when confronted with the presence of parasites, seek out alternate areas (Rohr et al. 2009, sources). Whether or not these behaviors can also influence trophic structure remains to be seen. To date, few studies have explored the trophic effects of parasite avoidance behavior. A study on wild mammals suggests that animals forage less and consume less food in areas with a high density of ticks; however, this study looked at food placed by humans (Fritzsche and Allan 2012). More evidence is needed to show that avoidance behavior alters host foraging behavior and thus has far-reaching impacts on the rest of the food web. However, we suggest that such interactions probably exist and should be studied in more detail.

In addition, parasites may interact with predators to reduce the density of their hosts. In cases where antiparasite defenses trade off with antipredator behavior, hosts confronted with both parasites and predators may suffer higher mortality. Baker and Smith (1997) found that larval damselflies, when confronted with both parasitic mites and predatory fish, increased their antiparasite behavior (in this case, grooming) while simultaneously increasing the likelihood that the fish would see and eat them. Studies estimating attack rates by predators on prey in the absence of parasites may greatly underestimate prey mortality if they do not also consider possible interactions with parasites.

Tradeoffs between antipredator behaviors and other crucial behaviors are common (Creel and Christianson 2008). For example, organisms utilizing antipredator behavior may do so at the risk of increased susceptibility to parasites (Rigby and Jokela 2000, Decaestecker et al. 2002, Mikheev and Pasternak 2006). It stands to reason that the opposite may occur, and that antiparasite behavior may come into conflict with other behaviors. There is some evidence to support this idea. For example, allogrooming impala took longer to notice a simulated predator than non-grooming impala (Mooring and Hart 1995), suggesting a tradeoff between antiparasite behavior and vigilance for predators. Damselfly larvae also show an increase in antiparasite activity when in the presence of mites, and this is correlated with an increase predator attack rate and higher larval mortality (Baker and Smith 1997). Resource acquisition may also be affected by antiparasite behavior. In the arrowhead scale, *Unaspis yanonensis*, individuals utilizing ‘burrowing’ to defend against parasitoids suffer diminished growth rates due to a reduction in resource acquisition (Matsumoto et al. 2003). The ant *Pheidole titanis* responds actively to the presence of a parasitoid fly, stopping normal activity and seeking refuge under leaf litter or returning to the nest (Feener 1988). This dramatic change in activity reduces colony food intake by 65%, indicating a very high cost of antiparasite behavior. In a related ant species, *P. dentata,* colony defense is compromised by the presence of a specialist parasitic fly. The antiparasite behavior utilized by the ant is associated with a 50% decline in the colony’s ability to thwart attacks from enemy heterospecific ant colonies (Feener 1981).

Some researchers have suggested that antiparasite behavior plays a role in invasion biology (Hughes and Cremer 2007). The presence of tradeoffs between antiparasite and other critical behaviors implies a high cost of antiparasite behaviors, one supported by empirical studies (Dudley and Milton 1990, Baker and Smith 1997, Giorgi et al. 2001; reviewed in Hughes and Cremer 2007). However, when a species is introduced to a new area, it does so free from the parasites and predators found in its native range, and does not pay the cost of defensive behaviors, an idea captured in the enemy release hypothesis (Keane and Crawley 2002). If costly antiparasite behaviors are plastic in these species, then organisms can reallocate resources normally reserved for antiparasite defense to other areas, such as growth or reproduction (Hughes and Cremer 2007), facilitating a successful invasion.

The presence of parasites may have potent implications for selection. In fact, parasites may exert selective pressures equal to or greater than that of predators (Rohr et al. 2009), as is the case with zebra striping (Waage 1981; reviewed in Combes 2001). Therefore, behavioral mechanisms of parasite resistance become important for ecological and conservation purposes. If animals seek out plant and/or animal material for use as self-medication to ward off or treat parasitic infections, then we must pay more attention to the conservation of areas where such medicinal compounds can be found - a sort of medicinal refugia. This becomes more important in species where the typical forms of self-medication involve materials not normally found in their home range. Further, some organisms appear to migrate to leave or avoid parasite-infested areas. If this is the case, then protecting not only the home range of the species, but migration corridors and the areas used as refuge from parasites becomes critically important to the management of the species.

Parasites and Mate Choice

The idea that parasites play an important role in sexual selection and host mate choice behavior is not a new one. Hamilton and Zuk (1982) were the first to propose this, based on the idea that females would benefit by selecting parasite-resistant mates that could pass on genes for resistance to the offspring. Many studies support the idea that sexual signals reflect whether an individual is parasitized or not (e.g. Zuk et al. 1990, Møller 1990, Martín et al. 2007) and that females prefer to mate with less parasitized individuals (reviewed in Møller et al. 1999).

The original Hamilton-Zuk hypothesis proposes that females select parasite-resistant mates primarily for good genes (Hamilton and Zuk 1982). However, another possible explanation is that females that avoid parasitized males gain a more immediate benefit: preventing these parasites from infecting them as well. This idea is known as the transmission avoidance hypothesis (Clayton 1991). Previous reviews on antiparasite behavior have included female preference for less-parasitized males as a form of antiparasite behavior, citing both of these as behavioral responses that allow females to increase fitness in the face of parasitism (Hart 1990, Moore 2002).

While researchers have given thought to how female choice may constitute an antiparasite behavior, few, if any, have considered how other antiparasite behaviors may affect female choice. Many of the studies testing the Hamilton-Zuk hypothesis have implicitly assumed that females prefer less parasitized males because such males have superior immunity or some similar, physiology-based mechanism that allows them to fight off parasites (Møller et al. 1999). Thus, many researchers have looked for connections between female preference, sexual signals, and some indication of immunity (Jacobs and Zuk 2011), such as MHC genes (Milinski 2006). However, if females simply prefer the male with the fewest parasites, might not antiparasite behavior itself play an important role in determining which males are attractive? If a male can use antiparasite behavior to reduce his parasite load, or even avoid becoming parasitized in the first place, he could potentially appear equally attractive to a female as a male who had to rely on his immune response to lower his parasite load. The "good genes" passed on under this scenario would be the genes for antiparasite behavior. Given that antiparasite behavior may be as effective at lowering parasite loads as immune defenses (Daly and Johnson 2011), such genes have the potential to increase offspring fitness, as predicted by the Hamilton-Zuk hypothesis.

The Red Queen on Holiday

Van Valen (1973) was the first to describe the idea known as the Red Queen hypothesis, after Lewis Carroll's famous character. This hypothesis describes the evolutionary arms races that can occur when two species interact antagonistically and coevolve together.

Essentially, if one species evolves a feature that gives it an advantage over the other species, the other species will suffer a temporary loss of fitness. Any genotype that then allows the second species to get around that new feature will be favored, and thus the second species will in turn evolve some way of getting around the original improvement. Thus, no matter how much evolution occurs in each species, neither will gain an advantage over their adversary, and for all their "running" (or in this case, evolving), they end up in essentially the same place.

The Red Queen hypothesis has been invoked to explain the evolution and maintenance of sex (Lively 1987), and is perhaps best known for its association with this topic. However, the original Red Queen hypothesis has much broader applications. It can describe the coevolutionary battles that occur between competing species, between predators and prey, and between hosts and parasites. In the scenario dealing with parasitism, hosts are expected to evolve defenses that enable them to better fight off parasites. This genotype, once it appears, will spread rapidly through the host population, until the majority of the hosts can successfully resist infection. These defenses will lower the fitness of most parasites, as they can no longer infect as many hosts. However, if a genotype occurs in the parasite population that enables the parasite to evade or resist this new host defense, that genotype will confer much greater fitness to the parasites and will spread rapidly through the population until most or all of the parasites can once again infect the majority of the hosts.

While the idea of coevolutionary arms races is not new to most students of parasitology, it has rarely been applied to studies of behavioral defenses. Antiparasite behavior is, by definition, focused on the host and the ways in which hosts can use behavior to avoid or mitigate the costs of parasitism. However, less attention has been paid to how parasites respond to these host defenses. Antiparasite behavior reduces the fitness of the parasite; thus parasites should evolve counter strategies to get around these defenses. Yet surprisingly little work has focused on the coevolutionary responses of the parasites.

In one of the earlier reviews on antiparasite behavior, Hart (1994) describes a few instances of parasites employing counter-strategies to evade host behavioral defenses. Ticks, for example, seem to preferentially settle on areas of the host's body where they are least likely to be dislodged by grooming (i.e. areas the host cannot easily reach by itself, such as the head). In one rather spectacular example, lungworm larvae have been shown to use fungal spores to catapult themselves away from the fecal piles where they hatched (Robinson 1962). Hart (1994) suggests that this is a mechanism by which the parasite gets around the tendency of hosts not to forage too close to fecal piles.

These older examples illustrate that parasites can evolve their own strategies to get around host antiparasite behavior, but relatively few instances of this have been described. This lack is surprising, particularly when one considers the attention paid to other ways that parasites can get around host defenses. In particular, much attention has focused on how parasites interact with and/or evade the host immune system, and this antagonistic interaction between parasites and hosts has been likened to a war (Schmid-Hempel 2008). One would expect a similar war to be going on behaviorally, with parasites constantly evolving ways to circumvent host antiparasite behavior.

While studies of behavioral arms races between a host and a given parasite are fairly rare, we do see examples of other parasites co-opting some host defenses. For example, grooming is a common host defense against ectoparasites but can be used as an infection route by certain intestinal parasites (Waddington and Rothenbuhler 1976, Kramm et al. 1982, Hernandez and Sukhdeo 1995, Evans and Spivak 2010). Thus, parasites can evolve ways to make host behavior enhance their own fitness. However, these examples usually involve parasites co-opting defenses that were not intended to get rid of them, but rather to get rid of some other species of parasite.

One particularly relevant area in which researchers might seek counter strategies by parasites is in response to self-medication behaviors. In human medicine, drug-resistant parasite strains pose a huge problem. Might we not expect that the same is true in animals? If an animal uses a certain compound to kill parasites or reduce their fitness, one would anticipate that over time, parasites would evolve resistance to that compound, and the animal’s remedy would lose its effectiveness. Most studies of self-medication in animals seem to ignore this possibility, although a recent study in fruit flies suggests that this may indeed occur. Milan et al. (2012) examined the behavior of fruit flies when infected with parasitoid wasps. They found that parasitized larvae altered their diets to include more ethanol. Ethanol is toxic to the flies, but also to the wasps, and flies that consumed more ethanol were more likely to survive. However, this strategy was more effective when used against a generalist wasp species that infected several other species of fly. In contrast, a specialist wasp, which had coevolved with the fruit flies, suffered fewer negative impacts from the host self-medication.

We suggest that other examples of parasites evolving resistance to the compounds that hosts commonly use to mediate themselves are likely to exist. In cases where parasites have not evolved resistance to these compounds, it may behoove us to understand why they have not. Understanding the situations in which parasites do not evolve drug resistance, if indeed such situations exist, could greatly enhance human medical practices.

Implications for Human Activities

We often use natural enemies of pests to manage pest populations and protect economically or ecologically valuable crops, known as biological control (Hajek 2004). These pests are typically parasites or parasitoids and can be highly effective. For example, the use of an egg parasitoid as a biological control agent in French Polynesia reduced the population of the invasive glassy-winged sharpshooter, *Homalodisca vitripennis*, by more than 90% in a short period of time (Grandgirard et al. 2008). Antiparasite behavior, however, enables pests to avoid or combat biological control agents, potentially reducing the efficacy of biological control (Van Driesche and Bellows 1996, Gordh et al. 1999). The biological control literature includes several examples of pests successfully resisting attack from biological control agents (reviewed in Gross 1993). For instance, a number of lepidopterans exhibit thrashing, wriggling, or other evasive behaviors (Gross 1993) to avoid parasitoids. These behaviors are often seen in lepidoptera pupae. The pupa of the gypsy moth, *Lymantria dispar*, is surrounded by a webbing. The pupa rotates and arches itself when attacked by a parasitoid, so much so that the parasitoid often finds itself caught in the webbing. This behavior dramatically increases the handling time necessary for the parasitoid to successfully insert its ovipositor (Cole 1959, Gross 1993). Some species of hosts use behavioral defenses not to avoid parasitism, but to bite, strike, or otherwise attack both adult and immature forms of parasites, including parasitoids (Gross 1993). Outside of the biological control literature, there exist other examples of hosts successfully avoiding or combatting parasitism via behavioral mechanisms, especially in gryllids. Several species of gryllids are subject to parasitism from an acoustically-orienting parasitoid fly, *Ormia ochracea (*Gray et al. 2007, Sakaguchi and Gray 2011*)*. In response, the field crickets *Gryllus texensis* and *G. integer* shift periods of peak calling activity to times during which female *O. ochracea* are less active (Cade et al. 1996, Bertram et al. 2004) in order to avoid periods of high parasitism. The diel calling bouts of field cricket *Teleogryllus oceanicus* start and stop more abruptly in populations subject to parasitism from *O. ochracea* (Zuk et al. 1993), and the more heavily parasitized a population of this cricket is, the longer males take to resume calling after a disturbance (Lewkiewicz and Zuk 2004). Furthermore, *G. texensis* exposed to larval stages of *O. ochracea* groom more extensively, which reduces rates of parasitism and increases host survival (Vincent and Bertram 2010).

In short, antiparasite behaviors expand the suite of defenses that hosts may utilize to avoid or mitigate parasitism, and this may have detrimental effects on the efficacy of biological control. Studying antiparasite behaviors in agriculturally and medically important arenas and otherwise may prove a useful endeavor in preserving critical crops. As a result, research on antiparasite behavior can also be used to preserve economically important animals. Agricultural livestock are regularly afflicted with parasites that can cause significant blood and weight loss, reduced egg production, and lower milk yields (Bruce and Decker 1958, Todd 1964, Miller et al. 1973, Campbell et al. 1987, Harris et al. 1987, Hart 1990, Campbell et al. 2001). These reductions in livestock condition translate into substantial economic losses (Chen et al. 2011). Knowing how these animals ward off parasites can better inform livestock management practices to improve animal welfare and yields. For example, in some parts of the world tail docking of dairy cattle is a common practice to reduce contamination of udders from dirty tails. However, the tail is crucial for insect repelling behavior. Since docked cows cannot swat biting insects with their tail, they spend a greater amount of time performing parasite avoidance behaviors, which disrupts rumination behavior and milk production. Moreover, docked cows exhibit higher fly counts than intact cattle (Eicher et al. 2001). Similarly, hens often have their beaks trimmed to reduce feed waste, feather picking, and cannibalism, but this practice imposes another set of considerable costs. Beak-trimmed hens tend to have higher ectoparasite infections because they cannot properly groom themselves. Increased mite and lice burdens can wreak havoc on hen health and egg production, causing significant economic costs (Mullens et al. 2010, Chen et al. 2011). Poultry workers are often afflicted by the parasites, prompting the use of pesticides that impose health and environmental concerns (Mullens et al. 2004a, Chen et al. 2011). Pesticide use is also not ideal because parasites can develop resistance to these chemicals over time, greatly reducing their efficacy (Mullens et al. 2004b). Researchers have begun to use their knowledge of avian antiparasite behaviors to devise better solutions to these problems. Selective breeding for more docile hens may obviate the need for beak trimming and pesticide use by enabling hens to naturally reduce their parasite burdens with grooming (Chen et al. 2011). This proposed solution can improve animal and worker welfare while promoting production and industry earnings.

CONCLUSIONS AND FUTURE DIRECTIONS

Animals are exposed to threats from parasites and pathogens throughout their lives. These infectious agents can wreak havoc on host health and reproductive success, and can even be fatal. In response to these pressures, natural selection has favored behaviors that animals use to avoid or resist infection. These strategies are employed by a broad range of animal taxa and vary in complexity, ranging from simple avoidance to sophisticated methods of sequestering and destroying infected individuals. Antiparasite behaviors may occur before and/or after infection with varying degrees of success. While several studies indicate that these defenses can dramatically reduce parasite loads, fewer have quantified their effects on host fitness. This may be difficult to assess in wild and longer-lived animals or populations with higher dispersal rates and lower site fidelity. However, rigorous testing of behavioral efficacy is needed to more fully understand the evolutionary dynamics of host-parasite relationships.

Parasites have responded to host selection pressures by developing ways to circumvent their behavioral defenses. Some parasites even exploit antiparasite behaviors to enhance their own transmission, such as certain helminths that rely on host grooming to infiltrate the body (Hernandez and Sukhdeo 1995). Host herding behavior may dilute the effects of biting flies, however contact parasites such as mites, ticks, lice, and fleas greatly benefit from the close proximity of hosts (Hoagland and Sherman 1976, Hoagland 1979, Brown and Brown 1986, Hart 1994). Parasite counterstrategies can also be equally spectacular and complex as host defenses, such as lungworms that use fungal sporangiophores as catapults into new host territories (Robinson 1962). There are likely to be many more instances of fascinating parasite counterstrategies that have yet to be discovered. These coevolved adaptations have important implications for biological control, human and wildlife disease dynamics, and conservation management.

Even though antiparasite behavior is widely practiced among a variety of animals, its significance in disease dynamics is often overlooked. While some recent studies (Daly and Johnson 2011, Milan et al. 2012, Kacsoh et al. 2013) have begun to address this question, most research has focused on quantifying the efficacy of immunological defenses over behavioral mechanisms (Kiesecker et al. 1999, Ezenwa 2004, Råberg et al. 2009). While antiparasite behavior can incur energetic costs and reduce vigilance to predators, immunological defenses carry their own set of drawbacks. Mounting an immune response also requires energy and takes time to effectively eradicate infection. Behavioral responses often work more quickly to avoid or clear infections. Further, an overactive or misdirected immune response can cause considerable pathology and even death (Klasing and Leshchinsky 1999, Bonneaud et al. 2003, Graham et al. 2005). In some cases behavioral defenses may be more robust and cost effective ways of managing infection than immunological responses.

Future work should investigate how behavioral plasticity in antiparasite defense helps animals persist in rapidly changing environments. Virtually all animals occupy habitats that have been altered by human activities; as a result, they may encounter rapid alterations such as habitat loss, climate change, pollution, and the introduction of exotic species (Sih et al. 2011). While most animals have experienced environmental changes over their evolutionary history, anthropogenic perturbations dramatically speed up this process, forcing animals to quickly adapt if they are to survive and reproduce (Tuomainen and Candolin 2011). Behavioral plasticity is believed to play a major role in an animal's ability to persist after rapid environmental change, however little is known about how they behaviorally respond to parasites amid these perturbations (Sih et al. 2011, Tuomainen and Candolin 2011, Sol et al. 2013).

Animals that are more plastic could apply preexisting behaviors for new uses in novel environments. For example, with increased destruction of natural areas and growing urbanization, animals may need to adopt new ways to ward off parasites. Plants used for repelling parasites may be less accessible in city dwellings, posing a challenge for urban hosts. Recently, house sparrows and house finches from urban areas were discovered to be lining their nests with used cigarette filters. The nicotine and other noxious compounds contained in the used filters may act as parasitic arthropod repellants. Suárez-Rodríguez and colleagues (2013) discovered that nests containing more used cigarette filter material harbored fewer ectoparasites. Additionally, subsequent experiments using thermal traps found that traps with used filters attracted significantly less mites than traps containing unused filters. It appears that urban birds may be altering a preexisting self-medication behavior to repel parasites in cities.

Alternatively, urbanization may release animals from their natural parasites, conferring fitness benefits to more behaviorally plastic individuals that can "turn off" their behavioral defenses. There is evidence that some animals show plasticity in their antiparasite behaviors, and will adjust investment according to parasite risk (D'Ettore et al. 2004) These animals would have a competitive advantage over others as they could conserve resources for other activities, such as foraging and reproduction (Hughes and Cremer 2007). Maladaptive responses are more likely to occur when animals encounter conditions that were not part of their evolutionary history (Ghalambor et al. 2007). For example, the introduction of exotic parasites and pathogens that have not coevolved with their new hosts can pose a number of problems. First, individuals may not be able to recognize and effectively eradicate novel threats, placing them at increased risk (Sih et al. 2011, Sih 2013). Conversely, investing in behavioral defenses against novel pathogens that pose no threat can be costly, draining energy that could be used for more beneficial activities (Hughes and Cremer 2007). In both cases, animals that exhibit a higher degree of behavioral plasticity should be more successful in novel environments as they can elicit defenses when needed and conserve resources when those defenses are unnecessary.

In some cases animals may also gain fitness benefits from reducing behavioral defenses to increase their parasite exposure. While the immune system has evolved to defend the body from parasites and pathogens, it can also produce maladaptive responses that reduce fitness (Graham et al. 2005, Zuk 2007). The immune system may produce an overactive response, employ the wrong mechanisms to kill parasites, or be triggered by antigens from harmless substances in the environment (producing allergies), or from the host's own body (producing autoimmune diseases). These inappropriate responses have been implicated in many allergic and autoimmune diseases of humans and domestic animals (Okada et al. 2010). The "hygiene hypothesis" proposed by Strachan (1989) states that lower exposure to infectious agents is associated with increased immunologic disorders. It is believed that over evolutionary history, hosts coevolved with various infectious agents, many of which provide protection from a wide spectrum of immune related ailments (Okada et al. 2010). With the advent of modern hygienic and medical practices these organisms were reduced in the environment, contributing to subsequent immune malfunction. Indeed, there are now many reports of increased allergic and autoimmune diseases in industrialized countries where hygienic practices are high and antibiotics are easily accessible (Zuk 2007). In contrast, autoimmune diseases are much less common in developing countries where exposure to infectious organisms is presumably greater (Okada et al. 2010).

Recent work strongly suggests that parasites can have modulatory effects on host, immune systems, reducing maladaptive responses. This is the basis for “helminthic therapy”, which deliberately exposes patients to parasitic worms for the treatment of autoimmune diseases. The worms briefly colonize the human gut, but do not cause pathology since humans are not appropriate hosts for the parasites (Summers et al. 2005). It is believed that the worms down-modulate certain immune responses that contribute to the immunopathology observed in autoimmune diseases. For example, patients suffering from Crohn’s disease, a debilitating ailment of the gastrointestinal tract, experienced significant improvement or even complete remission after ingesting the pig whipworm *Trichiuris suis* (Summers et al. 2005). The worms appear to stimulate Th-2 activity, which down-modulates the overactive Th-1 immune responses observed in people with Crohn’s and other inflammatory bowel diseases (Kahn et al. 2002, Summers et al. 2005, Zuk 2007, Maizels and Yazdanbakhsh 2008). Helminths are increasingly being used in medical research for the treatment of autoimmune ailments such as ulcerative colitis, asthma, arthritis, and multiple sclerosis, among others (Summers et al. 2003, Zuk 2007, Fleming 2013, Kahn and Fallon 2013). Deliberate contact with some pathogens may also benefit animals by providing enhanced immunological protection against future infections. Recent work on social insects demonstrates that individuals may purposively expose colony members to infectious agents, thereby immunizing them from potentially lethal pathogens (Ugelvig and Cremer 2007, Konrad et al. 2013). For example, Konrad et al. (2013) observed intense allogrooming behavior between naïve ants and conspecifics exposed to a pathogenic fungus. The benefits of this behavior were twofold: grooming significantly reduced disease risk in exposed nest mates, while healthy individuals developed low-level infections that later conferred pathogen-specific immunity to the fungus.

More work is also needed to better understand trade-offs between investment in immunological and behavioral defenses. Studies of antiparasite defenses often examine each strategy separately though individuals likely employ both behavioral and immunological responses after parasite exposure. While both strategies aim to avoid or reduce pathogens, individuals may balance their investment depending on sex, life-history stage, or social context (Bouwman and Hawley 2010, Hawley et al. 2011, Zylberberg et al. 2013). For example, solitary individuals may not need to mount an immune response if they regularly avoid infested areas or infected conspecifics. Conversely, bolder or more social individuals that are more likely to encounter sources of infection may increase immune investment. Indeed, a recent study showed that house finches exhibiting lower behavioral avoidance of parasitized conspecifics also invested more into immune defense (Zylberberg et al. 2013). Variation in antiparasite defenses likely influences which individuals get sick, how quickly they recover, and the trajectory of an epidemic. A better understanding of these dynamics could be used to reduce the spread of infectious diseases in animals and humans.

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Table 1.1. Criteria for demonstrating the adaptive nature of self-medication.

Clayton and Wolfe (1993)	Singer et al. (2009)
1) Medicator must deliberately contact compound	1) Self-medication must increase fitness of medicator when infected
2) Compound must be detrimental to parasites	2) Self-medication should decrease fitness of medicator when not infected
3) Self-medication must increase host fitness	3) Infection should induce self-medication behavior

Table 1.2. A summary of antiparasite behaviors and their efficacy in reducing or eliminating parasitic infection as noted from the literature. "N.Q." indicates that behavioral efficacy in reducing parasitic infection was not quantified.

Self-medication				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Capuchin monkeys (*Cebus spp*.)	Lice, mites, ticks	Fur rubbing with various plant species	N.Q.	DeJoseph et al. 2002
Capuchin monkeys (*Cebus spp*.)	Mosquitoes	Fur rubbing with millipedes that excrete benzoquinones, which are potent insect repellents	N.Q.	Valderrama et al. 2000, Weldon et al. 2003
Various bird species	Various ectoparasites	"Anting": birds bathe in mounds of ants or anoint themselves with citrus fruits to cover feathers with formic acid	Ectoparasitic feather lice exposed to lime fruit slices exhibited higher mortality rates than lice from control treatments	Clayton and Vernon 1993
Cross-bred Lambs	Nematodes	Parasitized lambs prefer alfalfa with tannins; control lambs prefer alfalfa w/o tannins	Tannin-laced alfalfa reduced fecal egg counts over time	Villalba et al. 2010
Double-crested cormorant (*Phalacocorax auritus)*	Nematodes (*Contracaecum spp.)*	Swallowing small stones	Males with more stones in their digestive tracts tended to have fewer parasites	Robinson et al. 2008

Table 1.2. Continued

Self-medication (continued)				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
European starlings (*Stumis vulgaris*)	Lice, bacteria	Nest fumigation-starlings nest with plants that contain volatile compounds with insecticidal and antibacterial properties.	Hatching success of lice and growth of several bacterial strains significantly reduced when exposed to plant species preferred by starlings versus randomly selected vegetation	Clark and Mason 1985
Selfish herd and group resistance				
Sticklebacks (*Gasterosteus spp*.)	Fish louse (*Argulus Canadensis*)	Shoaling	Individual risk of parasitism decreased with increased shoal size	Poulin and Fitzgerald 1989a
Reindeer (*Rangifer tarandus*)	Warble flies (*Hypoderma spp*.)	Herding	Increased animal density associated with lower fly larvae abundance in calves	Fauchald et al. 2007
Fathead minnows (*Pimephales promelas)*	Trematodes (*Ornithodiplostomum ptychocheilus, Posthodiplostomum minimum)*	Fish decreased the area of shoals in presence of parasites	Shoaling fish harbored fewer metacercariae	Stumbo et al. 2012

Table 1.2. Continued

Avoidance				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Mule deer (*Odocoileus hemionus*) and elk (*Cervus canadensis*)	Horse flies	Hosts run away from flies	N.Q.	Collins and Urness 1982
Reindeer (*Rangifer tarandus*)	Warble flies (*Hypoderma spp*.)	Migration after calving	Warble flies decrease as reindeer migration distance increases	Folstad et al. 1991
Great tits (*Parus major*)	Hen fleas (*Ceratophyllus gallinae*)	Avoidance of roost sites and nests infested with fleas	Hatching success and brood size smaller in infested nests. Lay date, desertion, hatching success significantly affected by hen fleas	Christe et al. 1994, Opplinger et al. 1994
Birds	Mosquitoes vectoring malaria (*Plasmodium spp*.)	Birds roost with their heads and one leg tucked under their wings to reduce the area exposed to mosquitos	N.Q.	van Riper et al. 1986
Caribbean spiny lobster (*Panulirus argus*)	PaV1 virus	Avoid sheltering with infected conspecifics	N.Q.	Behringer and Butler 2010

Table 1.2. Continued

Avoidance (continued)				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Cliff swallows (*Petrochelidon pyrrhonata*)	Fleas, swallow bugs	Cliff swallows hover above old nests before entering, also appear to avoid parasitized nest sites	N.Q.	Brown and Brown 1986
Gypsy moth (*Lymantria dispar*)	Parasitoid wasp (*Brachymeria intermedia*)	Spinning when in contact with parasitoid, which causes the parasitoid to become tangled in webbing around pupa	When webbing is removed to expose the naked pupae, they become less responsive, and do not start spinning until after the ovipositor has been inserted	Rotheray et al. 1984
Fathead minnows (*Pimephales promelas)*	Trematodes (*Ornithodiplostomum sp*.)	Parasite experienced fish significantly reduced activity in the presence of cercariae, while controls and naïve fish increased activity.	Encounter rate and infection rate increased with fish activity	James et al. 2008
Pine weevil (*Hylobius abietus*)	Entomopathogenic nematodes (*Steinernema carpocapsae*, *Heterorhabditis downsei*)	Weevils avoided substrate infested with *S. carpocapsae* but not *H. downsei*	N.Q.	Ennis et al. 2010

Table 1.2. Continued

Avoidance (continued)				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Gypsy moth (*Lymantria dispar*)	Parasitoid wasp (*B. intermedia*)	Spinning when in contact with parasitoid, which causes the parasitoid to become tangled in webbing around pupa	When webbing is removed to expose the naked pupae, they become less responsive, and do not start spinning until after the ovipositor has been inserted	Rotheray et al. 1984
Fathead minnows (*Pimephales promelas)*	Trematode *Ornithodiplostomum sp*.	Parasite experienced fish significantly reduced activity in the presence of cercariae, while controls and naïve fish increased activity.	Encounter rate and infection rate increased with fish activity	James et al. 2008
Stickleback (*Gasterosteus spp*.)	Fish louse (*Argulus Canadensis*)	Stickleback swim near the water surface in the presence of fish lice which inhabit pond bottoms	N.Q.	Poulin and FitzGerald 1989b

Table 1.2. Continued

Grooming				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Cricket (*Teleogryllus oceanicus*)	Parasitoid fly (*Ormia ochracea*)	Crickets groom to remove planidia from cuticule	Crickets that groomed more often were less likely to succumb to infection	Vincent and Bertram 2010
Ants (*Formica selysi*)	Pathogenic fungi (*Metarhizium anisopliae*)	Ants groom colony mates when they return from foraging.	Ants groomed by their colony mates had fewer fungal spores adhering to them.	Reber et al. 2011
Rock doves (*Columba livia*)	Lice	Doves with minor bill deformities had highest parasite loads; it is thought that they could not effectively preen the ectoparasites off (although the high loads could be due to other factors)	N.Q.	Clayton et al. 1999
Pigeons	Lice	Pigeons fitted with "bits" to reduce grooming behavior	Pigeons fitted with "bits" had significantly high parasite loads than those without the bits	Clayton 1991, Clayton et al. 1999

Table 1.2. Continued

Grooming (continued)				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Domestic chickens	Various ectoparasites	Chickens mandibles were clipped to reduce grooming ability	Chickens with clipped mandibles exhibited much higher parasite loads than intact chickens	Kartman 1949, Nelson and Murray 1971, Brown 1972, DeVaney 1976
Pigeon	Lice	Experimental removal of the bill overhang to reduce grooming ability	Bill overhang removal of led to a dramatic increase in louse abundance. Regrowth of overhang was associated with significant louse reduction	Clayton et al. 2005
Green Woodhoopoe (*Phoeniculus purpureus*)	Ectoparasites	Preening and allopreening	Preening and allopreening are associated with lower ectoparasites loads	Radford and Du Plessis 2006
Domestic chickens	Fleas	Scratching the body	Scratching is associated with dead/damaged fleas on body	Suter, cited in Marshall 1981
Rock pigeons (*Columba livia*)	Flies	Preening the body	Preening birds killed twice as many flies as impaired birds	Waite and Clayton 2012

Table 1.2. Continued

Grooming (continued)				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Pine weevil (*Hylobius abietus*)	Entomopathogenic nematodes (*S. carpocapsae*, *H. downsei*)	Weevils groomed more in the presence of *S. carpocapsae* but not *H. downsei*	N.Q.	Ennis et al. 2010
Impala (*Aepyceros melampus*)	Various ectoparasites, but especially ticks	Reciprocal allogrooming, especially of head and neck regions	N.Q.	Hart and Hart 1992, Hart 2000
Male rats	*S. aureus*, various STIs, possibly *M. pulmonis*	Genital grooming post-copulation	Grooming reduced *S. aureus* infection; in vitro experiments showed antibacterial properties of saliva against gram-negative bacteria and *M. pulmonis*; however, no anti-bacterial properties against gram-positive bacteria	Hart et al. 1987

Table 1.2. Continued

Grooming (continued)				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Cats (*Felis domesticus*)	Fleas	Grooming	Grooming reduced flea numbers by 50%	Eckstein and Hart 2000
Impala (*Aepyceros melampus*)	Ticks	Grooming	Grooming reduced adult tick burdens to 1/20 that of controls	Mooring et al. 1996
Ecological and habitat shifts				
Fire ants (*Solenopsis sp.)*	Nematodes	Ants relocate nests more frequently in areas with higher abundances of nematodes	N.Q.	Oi and Pereira 1993
Crickets (*Teleogryllus oceanicus)*	Parasitoid flies (*Ormia ochracea*)	Crickets in heavily parasitized populations have greater latency to resume calling when disturbed	N.Q.	Lewkiewicz and Zuk 2004
Cricket (*Gryllus integer*)	Parasitoid flies (*Euphasiopteryx ochracea, Ormia ochracea*)	Male crickets shift calling peak to when flies are less abundant	N.Q.	Cade et al. 1996.

Table 1.2. Continued

Ecological and habitat shifts (continued)				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Ant	Phorid parasitoid flies	Ant foraging shifts from diurnal to nocturnal during rainy season, when flies are most active	N.Q.	Feener 1988
Birds	Mosquitoes vectoring malaria (*Plasmodium spp*.)	Birds roost at higher elevations and forage in areas with malaria during the day, when the vector is not active	N.Q.	Van Riper et al. 1986
Water fleas (*Daphnia magna*)	Microsporans	Inhabit middle of water column to avoid predators at top and microsporans at bottom of water column	Positively phototactic clones exhibited reduced parasitism	Decaestecker 2002
Feral horses	Biting flies	Horses avoid areas where biting flies are common	N.Q.	Powell 2006

Table 1.2. Continued

Hygiene				
Host	**Parasite**	**Behavior**	**Efficacy**	**References**
Bell miners (*Manorina melanophrys*)	Flies	Adult birds remove parasitic fly larvae from the nest and preen nestlings	N.Q.	Pacheco et al. 2008
Sheep	Nematode (*O. circumcincta*)	Avoid grazing in areas with parasites	N.Q.	Cooper et al. 2000
Wild ungulates	Nematodes	Selective avoidance of dung middens while foraging	N.Q.	Ezenwa 2004
Ants (*Linepithema humile*)	Nematodes (*S. carpocapsae*, *H. downsei*)	Avoid cannibalizing infected corpses	N.Q.	Zhou et al. 2002
Termites (*Reticulutermes sp.)*	Fungi (*Metarhizium anisopliae*)	Avoid cannibalizing infected corpses	Transmission reduced in groups where infected corpses were avoided	Kramm et al. 1982
Honeybees (*Apis mellifera*)	Bacteria	Workers remove infected larvae to reduce infection within hive	N.Q.	Rothenbuhler 1964
Ants (*Lasius neglectus*)	Fungus (*Metarhizium anisopliae*)	Exposed ants heavily groomed by healthy nestmates	Grooming reduces risk of getting sick, increases immunity to the fungus	Ugelvig and Cremer 2007, Konrad et al. 2013

Chapter 2

Anti-parasite behavior in the fiddler crab, *Uca crenulata*: efficacy and sex differences

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ABSTRACT

Although anti-parasite behaviors are a host's first line of defense against pathogens, they are relatively understudied despite being effective ways to and resist infection. The California fiddler crab (*Uca crenulata*) is host to several helminth parasites, most notably the trematode *Probolocoryphe uca*. Crabs exposed to *P. uca* exhibit increased mortality, thus anti-parasite behaviors may be important to host fitness. In addition, sex differences in host morphology and ecology could also affect infection rates in crab hosts. Fiddler crabs exhibit extreme sexual dimorphism; females possess two small claws for feeding and grooming, but in males, one claw is enlarged for use as an ornament and weapon. We predicted that crabs exposed to *P. uca* would exhibit antiparasite behaviors to reduce infection. Due to differences in claw morphology, we also expected that males would be less effective at grooming and ingesting parasites than females, resulting in higher parasite abundances. We exposed crabs to *P. uca* larvae, scored various behaviors, and dissected them for parasites. To quantify grooming efficacy in reducing infection, crabs were allowed to groom, stressed but allowed to groom, or had their minor claws glued to impair grooming and exposed to *P. uca* larvae, followed by dissection for parasites. Exposed crabs were 10 times more likely to feed and groom themselves than were unexposed controls, and males groomed almost twice as often as females. Males harbored more parasites than females and glued males had the highest *P. uca* abundances out of all treatment groups. Although males groom more often than females, they harbor more *P. uca* trematodes, consistent with the prediction that males are less able to behaviorally remove parasites.

INTRODUCTION

Parasites are ubiquitous and have been increasingly recognized as important contributors to host ecology and evolution (Moore 2002, Poulin 2007). Parasites can significantly reduce host vigor, reproductive success, and survival (Forbes 1991, Forbes and Baker 1991, Moore, 2002, Vincent and Bertram 2010). In response to these selective pressures, hosts have devised a wide range of behavioral defenses to reduce the fitness costs imposed by parasites. Hart (1992) stated that a behavior is an antiparasite defense if it significantly reduces infection by a parasite that negatively affects host fitness. Animals may perform behaviors such as grooming, self-medication, herding, and swatting among others to reduce or avoid contact with parasites (Gray et al. 2012, Hart 1990, Hart 1994, Moore 2002). While antiparasite behaviors are widely used and are an important first line of defense (Hart 1990, Hart 1994, Moore 2002, Ezenwa 2004, Daly and Johnson 2011), most research has focused on host immunity (Hart 1990, Relyea 2001, Ezenwa 2004, Råberg et al. 2009). In addition, studies quantifying the efficacy of antiparasite behaviors are lacking, though recent work shows that these defenses can be highly effective in reducing infection (Daly and Johnson 2011, Milan et al. 2012, Kacsoh et al. 2013).

Host sex differences in physiology, morphology, and behavior can produce sex-biased patterns of infection (Poulin 1996, Zuk and McKean 1996). For example, higher testosterone levels in vertebrate males can cause immunosuppression, making them more susceptible to parasitism (Grossman 1985, Alexander and Stimson 1988, Folstad and Karter 1992, Zuk 1996, Zuk and McKean 1996, Duneau and Ebert 2012). Additionally, studies have demonstrated higher parasite burdens and more fecund parasites in breeding males with higher circulating androgens (Luong et al. 2010, Luong et al. 2013). Sexual dimorphism in host body size can also influence the frequency of parasite encounters, resulting in sex-biased infections. Larger hosts may be easier targets for parasites due to increased body mass and foraging frequency (Zuk and McKean 1996). For example, male mammals are generally larger than females and they often harbor more parasites (Schalk and Forbes 1997, Moore and Wilson 2002). Larger animals also generally consume more food, and hence may increase their exposure to infectious stages in their diet (Zuk and McKean 1996).

Males and females also exhibit behavioral differences that can predispose them to varying degrees of parasitism. For example, male mammals often sniff the urine and feces of conspecifics to assess social hierarchy (Duneau and Ebert 2012). This sex-specific behavior can increase encounter rates with parasites and pathogens that are transmitted in animal waste (Baker 1998, Litvinova et al. 2010). Moreover, males often engage in behaviors that increase disease transmission. For instance, male domestic cats (*Felis domesticus*) are twice as likely to contract feline immunodeficiency virus (FIV) due to a higher propensity for biting during social interactions (Bendinelli et al. 1995). Similarly, male rats are more likely to engage in aggressive interactions and bite each other, which may help explain male-biased infections with hantaviruses, which are transmitted in saliva (Klein et al. 2004). Even antiparasite behavioral defenses can be sex-specific, leading to biased infection patterns. For example, shoaling has been proposed as a possible antiparasite strategy in fishes (Poulin and FitzGerald 1989a). Richards and colleagues (2010) found that female guppies (*Poecilia reticulata*) form shoals more often than males, and when shoaling, they form larger, tighter groups than males. These differences were linked to sex-biased infections with the *Gyrodactylus turnbulli* trematode, as females experienced higher transmission rates and parasite burdens than males after experimental exposure to the parasite (Richards et al. 2010).

California fiddler crabs (*Uca crenulata*) are ideal for testing how sex differences influence antiparasite behavior and parasitism risk. *U. crenulata* exhibit extreme sexual dimorphism: females possess two small claws for feeding and grooming, but males only have one, the other claw being greatly enlarged for use as an ornament and weapon (Crane 1975, Backwell and Passmore 1996). Past work has shown that males spend more time feeding, yet ingest the same amount of food relative to females (Valiela et al. 1974). This suggests that a secondary sexual character, the major cheliped, imposes a cost of reduced feeding efficacy. We suspected that the major cheliped would also reduce the efficacy of males to perform behaviors that could remove parasites from the body, such as grooming. *U. crenulata* regularly encounters parasites in their natural habitats, most notably the trematode *Probolocoryphe uca*. The *P. uca* trematode uses a snail, crab, and bird to complete its three-host life cycle (Sarkisian 1957, Lafferty and Dunham 2005, Hechinger et al. 2007). Snails become infected after ingesting *P. uca* eggs while grazing on mudflats. After asexually reproducing within the snail first intermediate host, free-swimming infective stages called cercariae leave the snail to seek and infect a fiddler crab second intermediate host (Lafferty and Dunham 2005, Hechinger et al. 2007). *P. uca* cercariae enter the crab through the gill chambers, migrating through the body until they encyst as metacercariae in the host's connective tissues (Sarkisian 1957). After a shore bird final host ingests an infected crab, *P. uca* will excyst and travel to the bird's intestines, where they will sexually reproduce and shed eggs in the host's feces, thus completing the life cycle (Lafferty and Dunham 2005). Infection with *P. uca* is associated with increased mortality of *U. crenulata* (Mora, unpub. data), hence we expect crabs to use antiparasite behaviors to reduce infection. We tested the hypothesis that *U. crenulata* perform antiparasite behaviors in response to parasites, and that males are less effective than females at parasite removal due to sex differences in claw morphology. We predicted that this sex-specific difference in claw morphology would hinder grooming ability, resulting in male-biased infection patterns.

METHODS

Measuring host behavioral response to parasites

To assess how fiddler crabs behaviorally responded to trematode parasites, we exposed fiddler crabs (*Uca crenulata*) to *Probolocoryphe uca* cercariae. We collected *U. crenulata* from Agua Hedionda lagoon (Carlsbad, CA) because this population naturally exhibits low parasitism rates (Mora, unpub. data). Crabs were maintained in the lab on a diet of crushed Tetramin (Tetra, Blacksburg VA) fish food, administered ad libitum for five days prior to testing. Male and female crabs were then randomly assigned to one of two experimental groups: unexposed to cercariae (n=30 males, 30 females) and exposed to cercariae (n=30 males, 30 females). Five males died prior to our experiment, resulting in a final sample size of 116 crabs.

We obtained trematode cercariae from infected *Cerithidea californica* snails collected from Batiquitos lagoon (Carlsbad, CA). We placed individual snails into 20-mL scintillation vials filled with seawater and identified released cercariae based on morphological characters described by Martin (1952) and Sarkisian (1957). We pooled all cercariae and 1-mL aliquots were individually added to plastic containers with 30-mL of seawater. Each aliquot contained approximately 500 or more *Probolocoryphe uca* cercariae. For the unexposed treatment we pooled unfiltered seawater from uninfected snails and administered 1-mL aliquots to plastic containers with 30-mL of seawater. We placed individual crabs into containers and filmed behavioral responses for a total of 13 minutes with a one-minute acclimation period. Following filming, we collected all crabs and dissected them for parasites. We scored films at two-minute intervals for various host behavioral responses including grooming (picking material from the body with the minor claws), eating, water avoidance, and leg rubbing (rubbing the walking legs together). All videos were scored blind to group identity.

Quantifying host grooming and infection success

To quantify the efficacy of host grooming in reducing infection rates, we randomly assigned 30 males and 30 females to each of three treatment groups, followed by exposure to *P. uca* cercariae: 1) Control, 2) Stressed control, or 3) Glued. Control crabs received minimal handling and were free to groom, while the stressed control crabs received additional handling and two drops of superglue on the carapace to account for any effects of stress on final infection success. Glued crabs had their minor claws glued shut to impair grooming behavior. To achieve this, we gently closed the claw with forceps and glued the dactyl to the propodus. Males have one minor claw for grooming and females have two, hence we glued either one or both claws, respectively. Methods of collection and husbandry follow those previously stated. All crabs were housed for three weeks prior to testing to ensure that all current infections were mature at dissection to distinguish new parasites obtained experimentally. Cercariae were pooled from infected snails and 500 larvae were counted and added to each container prior to testing. We exposed all crabs to cercariae for 31 minutes followed by transfer to individual home tanks. We dissected all crabs within one week of testing and quantified all juvenile *Probolocoryphe uca* parasites.

Statistical analyses

We performed all statistical analyses using JMP statistical software ver.10 (SAS Institute, Cary, NC), and significance was accepted at $p \leq 0.05$ for all tests. To evaluate how crabs behaviorally responded to the presence or absence of *P. uca* parasites, we rank transformed behavioral events data and performed a two-way analysis of variance including sex, treatment (exposed or unexposed), and the sex x treatment interaction in the model (Potvin and Roff 1993). To determine how claw treatment and sex affected variation in successful *P. uca* infections we used a generalized linear model (GLM) with a Poisson distribution, overdispersion parameter and a log-link function. We included sex, treatment (control, stressed, glued), and the sex x treatment interaction in the model. Initial analyses revealed that host mass was not a significant predictor of variation in successful *P. uca* infections, thus mass was not included in our final analysis.

RESULTS

Host behavioral response to parasites

Crabs exposed to *P. uca* cercariae engaged in more events of grooming ($F_{(1, 112)}$ =22.07, P<0.0001, n=116; Fig. 2.1), eating ($F_{(1, 112)}$ =26.53,P<0.000; Fig. 2.1), and leg rubbing ($F_{(1, 112)}$ =6.77, P<0.0001; Fig. 2.1) than unexposed crabs. Although on average exposed males engaged in more events of grooming and eating than exposed females, these differences were not significant (Grooming: $F_{(1, 112)}$ =2.14, P=0.1464; Eating: $F_{(1, 112)}$ =1.74, P=0.1904; Fig. 2.1).

Host grooming and infection success

Male crabs harbored significantly more *P. uca* parasites than females (Fig. 2.3, Table 2.1) and on average glued crabs had more parasites than crabs from both control and stressed treatment groups (Fig. 2.3, Table 2.1). Glued males had 2.7-fold more parasites than glued females, and 2.2-fold more parasites than control and stressed males (Glued males: $\bar{x}$ =7.3, n=30; Control and stressed males: $\bar{x}$ =3.3, n=60). Glued males had the highest infection rates out of all treatment groups ($\bar{x}$ =7.3, n=30; Fig. 2.3, Table 2.1) while glued females had the lowest *P. uca* infections out of all treatment groups ($\bar{x}$ = 2.86, n=30; Fig. 2.3). As expected, we found no differences in infection rates between control and stressed crabs ($F_{(1, 118)}$ =1.77, P=0.1863; Fig. 2.3).

DISCUSSION

Here we show that sex differences in secondary sexual characters can impose different costs of parasitism on male and female fiddler crabs. While females can use both of their claws to groom parasites from the body, males are only able to do so with one, resulting in male-biased infection patterns. Previous work has shown that males pay a cost of reduced feeding efficiency as a result of having one minor claw for procuring food (Valiela et al. 1974). Our study shows that males groom more often than females, yet are more susceptible to infection, especially when their minor claw is glued to inhibit grooming behavior. While grooming does not appear to significantly reduce parasite loads in females, males that were allowed to groom exhibited a significant reduction in infection rates compared with impaired males.

While we focused on how sex differences in grooming ability influence susceptibility to parasitism, other factors could have influenced our observed infection patterns. Although mass was not a significant source of variation in parasite abundance, it is possible that sex differences in respiration rates could cause variation in parasite exposure. The main source of entry for trematode parasites is through the hosts' gill chambers, thus higher respiration rates would likely draw more trematode larvae into the host. Males may have higher metabolic demands than females due to maintenance of the major claw. While one previous study revealed no sex differences in oxygen consumption rates (Valiela et al. 1974), other work found that males bearing an intact major cheliped exhibited higher metabolic rates than males without the claw (Allen and Levinton 2007).

Gluing the minor claws may have caused more stress to males than to females, resulting in higher parasite abundances. An increase in host stress prior to *P. uca* exposure could have decreased parasite resistance, or increased respiration rates, both of which could lead to increased infection rates. However, if claw treatment severely stressed glued males then we would likely see infection differences between the unmanipulated and stressed control groups and we did not observe this (Fig. 2.3).

Sex differences in host chemical cues could also produce variation in host infection patterns. Trematode parasites are known to use physical and chemical cues to detect hosts and they may be selected to infect the most susceptible animals (Carson and Wheeler 1968, Cohen et al. 1980, Feiler and Haas 1988, Haas et al. 1994, Haas et al. 1995, Campbell 1997, Haberl et al. 2000, Sears et al. 2012). Male fiddler crabs could be more susceptible hosts since they exhibit traits that may attract bird predators (Koga et al. 2001, Pratt et al. 2002, Ribeiro et al. 2003). Compared with female crabs, males are generally larger, more brightly colored, and use large claws to engage in highly conspicuous courtship behaviors and fights with rival males. If males are more susceptible to bird predation as males of other fiddler crab species are (Koga et al. 2001, Ribeiro et al. 2003) selection could favor parasites that use sex-specific chemical cues to locate and infect male hosts.

Lastly, it is also possible that sex-specific immune differences contributed to our male-biased infection patterns. Higher testosterone levels in vertebrate male hosts can have immunosuppressive effects, and male-biased parasite burdens have been reported in a number of taxa (Poulin 1996, Schalk and Forbes 1997, Klein 2000). However, invertebrates such as fiddler crabs lack testosterone and other potentially immunosuppressive steroid hormones (Sheridan et al. 2000). While male-biased parasitism has been observed in a wide range of vertebrates, this relationship is not prevalent in crustaceans (Sheridan et al. 2000).

Our study highlights the important role of host sex differences in disease dynamics and shows how sexual selection can produce different fitness consequences for males and females. While the major cheliped helps males to acquire mates and defend territories, it comes at a cost of increased risk of parasitism due to reduced grooming efficacy and possibly increased attack by parasites. Although most research on antiparasite defenses has focused on host immunity, our study underscores the importance of behavioral strategies in reducing infection. Future work should aim to quantify the efficacy of these behavioral defenses in reducing parasitism and increasing host fitness.

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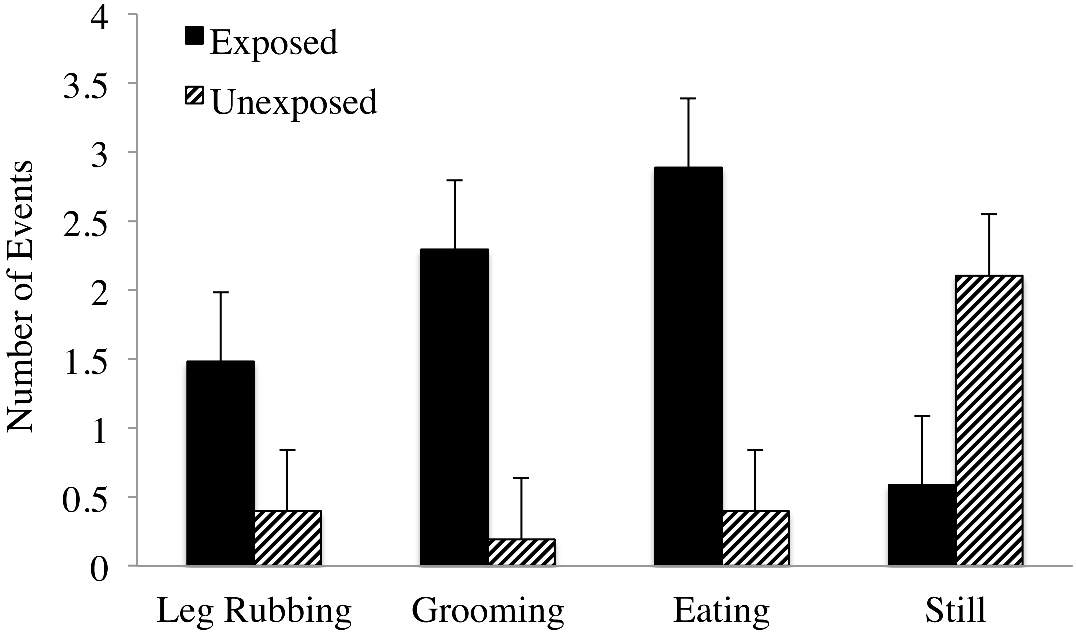

Figure 2.1. Number [mean + 1 standard error (SE)] of behaviors observed during 12 minutes of exposure to *P. uca* cercariae (dark bars) or exposure to unfiltered seawater from uninfected snails (hatched bars). N=116 (61 females, 55 males).

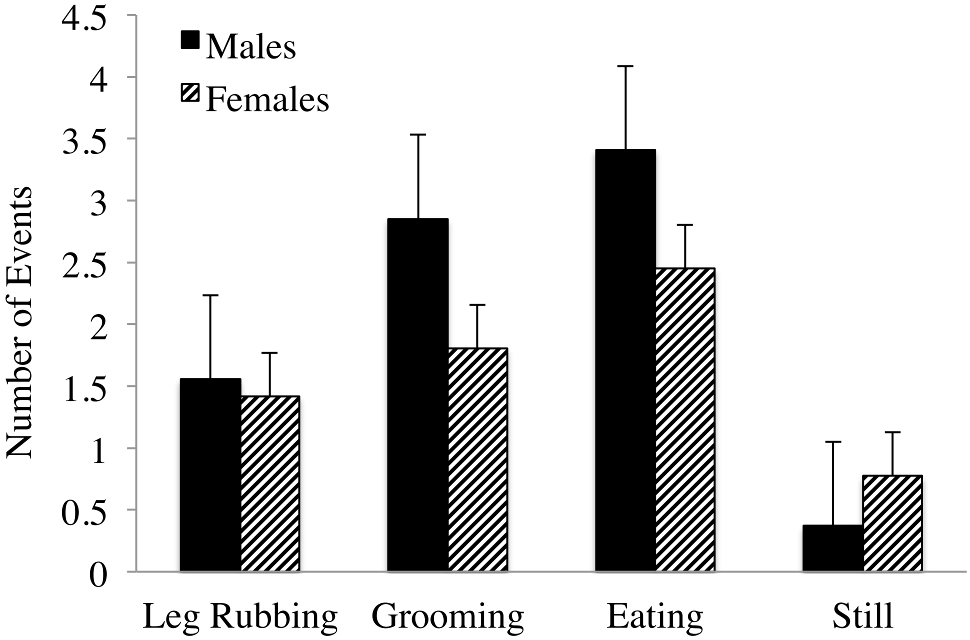

Figure 2.2. Sex differences in the number [mean + 1 standard error (SE)] of behaviors observed during 12 minutes of exposure to *P. uca* cercariae. N=58 (31 females, 27 males)

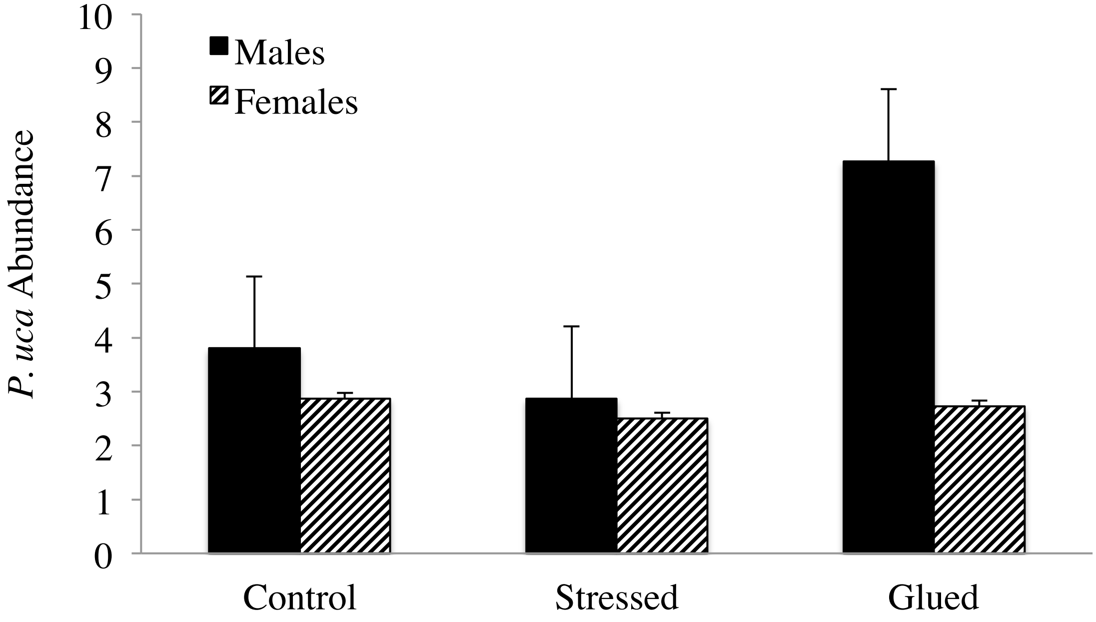

Figure 2.3. Sex differences in the number [mean + 1 standard error (SE)] of successful *P. uca* parasites recovered as a function of treatment. N=180 (30 females, 30 males per treatment).

Table 2.1. Generalized linear model testing the effects of sex and treatment on successful *P. uca* infections. Siginificant p-values are in bold.

	df	χ^2	*p*
Sex	1	11.864	**0.0006**
Treatment	2	9.559	**0.0084**
Sex*Treatment	2	8.083	**0.0176**

Chapter 3

Sick but sexy? Male fiddler crabs infected with trematodes show increased courtship effort

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ABSTRACT

The parasite manipulation hypothesis states that parasites are selected to alter host behavior to enhance the parasites' transmission and hence fitness. Altered hosts exhibit increased conspicuous or risky behaviors, often resulting in higher rates of host predation and parasite transmission. While many sexual signals are both conspicuous and risky, little is known about their potential for parasitic manipulation. We hypothesized that parasites increase host sexual signaling to enhance their transmission to predatory final hosts. To quantify parasitic effects on sexual signaling, we measured courtship displays and claw brightness in fiddler crabs (*Uca crenulata*) naturally infected with the *Probolocoryphe uca* trematode. We observed that males harboring more *P. uca* parasites displayed more frequently, but exhibited no differences in claw brightness. Males with the highest *P. uca* intensities also produced the most courtship displays. Our results support two competing hypotheses. First, infected males may be behaviorally manipulated by *P. uca* to enhance their sexual signaling to increase parasite transmission and fitness. Alternatively, parasitized males may be reproductively compensating for infection by enhancing courtship effort to acquire more matings and increase their residual reproductive value.

INTRODUCTION

Many parasites modify host phenotypic traits for the parasites' benefit, a phenomenon known as "host manipulation" (Holmes and Bethel 1972, Moore 2002, Poulin 2010). These morphological, behavioral, or physiological alterations can be subtle, such as an elevation in host activity levels, or extreme, such as performance of a complex, aberrant behavior (Poulin 1995, 2007; Moore 2002). The "manipulation hypothesis" states that parasites should be selected to manipulate host behavior in ways that enhance their transmission and fitness (Holmes and Bethel 1972, Webster 2007). Trophically transmitted parasites are especially likely to exhibit manipulation, as they depend on predation of their intermediate hosts by final hosts for survival and reproductive success (Lafferty 1999, Poulin 2010, Lafferty and Kuris 2012). Manipulated intermediate hosts often exhibit more risky or conspicuous behaviors or reduced escape responses that increase their vulnerability to predatory final hosts (Moore 2002). For example, killifish infected with the trematode *Euhaplorchis californiensis* exhibited a four-fold increase in conspicuous swimming behaviors and were 10-30 times more likely to be eaten by bird final hosts (Lafferty and Morris 1996).

Similarly, attracting a mate also often involves the display of risky behaviors and flashy ornaments (Andersson 1994). While these elaborate signals are used to attract potential mates, they can also attract the unwanted attention of predators or parasites (Magnhagen 1991, Zuk and Kolluru 1998). For example, acoustic sexual signals have been shown to attract both parasites and predators in crickets (Cade 1975, Sakaluk and Belwood 1984) and frogs (Tuttle and Ryan 1981, Ryan 1985, Bernal et al. 2006). Ornaments such as the elongated caudal fins of male swordtails attract cichlid predators (Hernandez-Jimenez and Rios-Cardenas 2012), while the flashing courtship signals of male fireflies make them more susceptible to a variety of predators (Woods et al. 2007). Male guppies (*Poecilia reticulata*) and pied flycatchers (*Ficedula hypoleuca*) displaying brighter coloration are at increased risk of predation (Slagsvold et al. 1995, Godin and McDonough 2003) and have been selected to exhibit more cryptic coloration (Endler 1980, 1982, 1983; Slavsgold et al. 1995). Parasites that manipulate sexual signals of their hosts might therefore be expected to have the counterintuitive effect of increasing short term mating success through more conspicuous signals, while at the same time decreasing long term survival through enhanced predator attraction. We propose the "sick but sexy" hypothesis to explain such situations; manipulative parasites that intensify host sexual signals will both increase the mating success of the host and make it more likely to be eaten by a predator, ensuring the parasite's transmission to its definitive host. This hypothesis provides an exception to the more traditional idea that infected animals produce lower-quality sexual signals and are less desirable mates.

Here we propose to test the sick but sexy hypothesis, which states that manipulative parasites intensify host sexual signals to enhance their transmission and fitness. We predict that parasitized hosts will increase sexual signaling as a result of parasitic manipulation. These hosts should experience an increase in short-term mating success, but also increased predation risk. Our hypothesis counters traditional models of parasite-mediated sexual selection that state infected animals generally produce lower quality sexual signals and are less desirable mates (Hamilton and Zuk 1982). Here we propose that hosts infected with manipulative parasites will exhibit intensified sexual signals that attract more mates before they are eaten by predatory final hosts.

We tested the sick but sexy hypothesis in the California fiddler crab (*Uca crenulata*). California fiddler crabs exhibit extreme sexual dimorphism; while females exhibit cryptic coloration and have two small, isomorphic feeding claws, males are more brightly colored and wield a greatly enlarged claw called the major chela (deRivera 1999). Males attract females by waving the major chela in a highly conspicuous courtship display (Crane 1975, deRivera 2003, deRivera 2005, Milner 2012). Females generally prefer larger-clawed males that wave at a faster rate (more waves per minute), and produce more leading waves (Backwell and Passmore 1996, Backwell et al. 1999, Reaney and Backwell 2007, Reaney 2009, Callander et al. 2012). Additionally, females preferentially orient to males that exhibit higher ultraviolet reflectance in the major chela (Detto and Backwell 2009).

Waving displays are costly to males both in energy expenditure and increased predation risk. In *Uca annulipes*, wave rate decreased when food was limited suggesting that displays exert substantial energy (Jennions and Backwell 1998). Male *U. lactea perplexa* displayed significant increases in blood lactate levels after bouts of vigorous waving (Matsumasa and Murai 2005). Courting *U. uruguayensis* males experienced higher predation rates by ruddy turnstones (*Arenaria interpres*) and black-bellied plovers (*Pluvialis squatarola*) compared with non-waving conspecifics, suggesting that displaying males may be easier targets for predators (Ribeiro et al. 2003). Male-biased predation by Great-tailed grackles (*Quiscalus mexicanus*) was also reported in *U. beebei* (Koga et al. 2001), further suggesting that male crabs are more conspicuous to predators than females.

In addition to its use in the waving display, the major claw may increase male susceptibility to visual predators (Jordao and Oliveira 2001, Koga et al. 2001). The major claw is substantial, comprising up to 50% of the total body mass (Crane 1975, Christy and Salmon 1984, Rosenberg 2002) and reflects significantly more light per unit surface area compared with the minor claws of males and females (Cummings et al. 2008). Contrast studies of fiddler crab anatomy revealed the major claw as the most visible body part against the dark substrates that fiddler crabs inhabit (Cummings et al. 2008).

California fiddler crabs are host to several helminth parasites, most notably the trematode *Probolocoryphe uca* (Hechinger et al. 2007, Mora, unpub. data). *P. uca* is a trophically transmitted trematode that utilizes three hosts in its life cycle. Adult worms sexually reproduce in the intestines of raccoons and birds, releasing eggs in the feces. The first-intermediate horn snail host (*Cerithidea californica*) becomes infected after ingesting parasite eggs while grazing on mudflats. The trematode parasitically castrates the snail and multiplies asexually, producing free-swimming cercariae that will infect a second-intermediate crustacean host and encyst in the connective tissues as metacercariae. Predation on an infected crustacean by a bird or raccoon final host followed by sexual reproduction of the parasite completes the life cycle (Martin 1971, Lafferty and Dunham 2005, Hechinger et al. 2007).

We examined courtship waving frequency, claw coloration, and trematode parasite burdens in a population of naturally-infected crabs. We predicted that males exhibiting more conspicuous signals would be more likely to be parasitized with trematodes.

METHODS

Study site and video observations

We studied a population of *Uca crenulata* at San Elijo lagoon near San Diego, California (33°00’35”N, 117°15’44”W) during the June 2012-August 2012 breeding season. This host population has a high prevalence and intensity of infection with the *Probolocoryphe uca* trematode (Mora, unpublished data). Surface-active males were haphazardly selected and filmed for 30 minutes before collection. Ambient temperature readings were recorded at five-minute intervals while filming and averaged to obtain a single temperature value for each trial. We additionally recorded time of day and date for each observational period. For each male we quantified total number of courtship waves, time exposed on the mudflat, and number of female visits within 30 minutes. We calculated courtship effort as total number of waves produced during the 30-minute observational period.

Measuring ultraviolet reflectance

When possible, we quantified ultraviolet reflectance of the major chela using methods described by Detto and Backwell (2009). We used a USB4000 ultraviolet-VIS spectrophotometer (Ocean Optics Inc., Dunedin, FL, U.S.A.) with a PX-2 pulsed Xenon lamp (Ocean Optics Inc.) to quantify total brightness (Σ 300-700 nm) of the major chela for each male. An area of approximately 2 mm^2 was illuminated at 90 degrees to the surface and reflected light was collected at 45 degrees to reduce specular reflections. Three measurements were taken from the manus of each male within 10 hours of collection and averaged before calculating total brightness values. Reflectance was measured relative to a white Spectralon standard (Ocean Optics, Inc.) and both light and dark reference standards were recorded before measuring each male. Previous work has shown that claw color and reflectance properties are stable and do not change with handling time (Zeil and Hofmann 2001, Hemmi et al. 2006).

Morphometric measurements and dissection procedures

We were able to record behavior and parasite abundance for 87 males. We measured all males for total body mass, major chela mass, carapace width, and major chela length prior to dissection. All morphometric measurements were made with digital calipers to 0.01 mm. Each crab was euthanized immediately prior to dissection by freezing for ten minutes. The back of the carapace was removed with the edge of a clean razor blade and all connective tissues and residual body fluids were removed and mounted on to slides with coverslips. All *P. uca* parasites were identified based on morphological characters, including a spherical metacercarial shape that measured between 0.22-0.25 mm in diameter, a spined body with an anterior collared region bearing a conical projection that lacked spines, and vitellaria arranged in a ring confirmation (Sarkisian 1957, Abdul-Salam and Sreelatha 2000, Deblock 2008, R.F. Hechinger, pers. comm.).

Statistical analyses

To determine how infection with *P. uca* influenced courtship effort, we performed a multiple regression analysis. Parasite abundance data were $\log_{10}$-transformed prior to analysis to meet assumptions of normality (Shapiro-Wilk: W=0.993, p=0.939, N=87). Since major chela mass, ambient temperature, and tidal cycle date can all influence courtship behavior, these were also included as predictors in the model. We calculated the variance inflation factors for all predictor variables to rule out multicollinearity in the model. To address the possibility that increased signaling was the result of crab age and not parasite intensity, we conducted a separate multiple regression analysis with carapace width (used as a proxy for age, Hayes et al. 2013), ambient temperature, and tidal cycle date included as predictors in the model. Two crabs were excluded from the analysis because they lacked burrows and did not perform displays. To determine the relationship between ultraviolet reflectance and parasite load for each male we performed a multiple regression analysis with $\log_{10}$-tranformed parasite abundance, major chela mass and date included as predictors in the model.

RESULTS

Infection and courtship displays

Our predictors explained 35% of the variance in waving display frequency (R^2=0.35, $F_{9, 86}$=4.688, p<0.0001). Consistent with our prediction, males harboring higher intensities of *P. uca* parasites performed more waving displays than did less parasitized males ($F_{1, 86}$=4.892, p=0.029, Fig. 3.1). In addition, males increased waving by 60% as parasite intensity increased, a relationship that remained after controlling for temperature, tidal cycle date, and major chela mass. Males with the highest parasite burdens exhibited a 1.6-fold increase in courtship displays over those with lower *P. uca* intensities. Increased signaling by more parasitized males was not influenced by host age ($F_{1, 86}$=3.652, p=0.0597). Males waved more frequently at higher temperatures ($F_{1, 86}$=8.476, p=0.005). Courtship effort decreased in males with heavier claws ($F_{1, 86}$=3.992, p=0.049, Fig. 3.2), and varied with tidal cycle date ($F_{5, 86}$=4.072, p=0.001, Fig. 3.3).

Infection and ultraviolet reflectance

Our predictors explained 24% of the variance in claw total brightness (R^2=0.24, $F_{7, 67}$=2.645, p<0.019). Infection with *P. uca* was not associated with total brightness of the major cheliped ($F_{1, 67}$=2.456, p=0.122, Fig. 3.4) but males with heavier claws exhibited greater total brightness ($F_{1, 67}$=10.949, p=0.0016, Fig. 3.5). Measurement date was not associated with total brightness of the major cheliped ($F_{1, 67}$=2.284, p=0.057).

DISCUSSION

The manipulation hypothesis predicts that parasites will be selected to alter host behavior to enhance their transmission and fitness (Holmes and Bethel 1972). Generally, these alterations involve an increase in behaviors that make hosts more visible and susceptible to predators. Traditionally, parasites drain host resources and reduce their reproductive vigor (Kolluru et al. 2002, Pai and Yan 2003, Dunn 2005). Here we show that male fiddler crabs with higher intensities of *P. uca* parasites actually perform more courtship waving displays. To our knowledge, this is the first study to suggest that a trophically transmitted parasite may manipulate host courtship signals and hence potentially enhance transmission. The increased wave rate observed in more heavily parasitized males should attract more females, but also more visual predators, such as bird final hosts. A study just completed on experimentally infected crabs demonstrated that parasitized crabs experienced increased predation rates, but females were eaten more often than males (Mora and Zuk, unpub. data). We propose that females may be more profitable prey items for certain predators because they lack a major cheliped, which increases handling time and risk of injury to predators (Wolf et al. 1975, Bildstein et al. 1989, Iribarne and Martinez 1999, Mora and Zuk, unpub. data). While we did not observe male-biased predation in our population, other studies have shown that sex-biased predation of fiddler crabs can vary by fiddler crab and predator species (Bildstein et al. 1989, Iribarne and Martinez 1999, Johnson 2003, Koga et al. 2001, Pratt et al. 2002, Ribeiro et al. 2003). Thus, it is possible that parasitized male fiddler crabs from other populations are more vulnerable to predation, especially in light of evidence that courting fiddler crab males of other species were eaten more often than non-courting crabs (Koga et al. 2001, Ribeiro et al. 2003).

Alternatively, the reproductive compensation hypothesis predicts that parasitized hosts should increase reproductive effort to offset the fitness costs of infection (Williams 1966, Minchella and Loverde 1981). Infected individuals with lower residual reproductive value may try to maximize their reproductive success to compensate for future losses due to illness or predation (Williams 1966, Roff 1992). Parasitized animals may be selected to maximize reproductive success by increasing egg production (e.g. snails, Pan 1965, Minchella and Loverde 1981, Thornhill et al. 1986, Krist 2001, Blair and Webster 2007; fishes, Heins 2012), nuptial gift quality (e.g., beetles, Hurd and Ardin 2003), mating effort (e.g., flies, Polak and Starmer 1998; amphipods, McCurdy et al. 2000), aggression to secure mates (e.g., milkweed beetles, Abbot and Dill 2001) and by reproducing earlier (e.g., water fleas, Chadwick and Little 2005). Despite male reproductive compensation for infection, females are generally able to discriminate among males of varying quality and prefer less parasitized mates (Hamilton and Zuk 1982, Millinski and Bakker 1990, Andersson 1994).

It is also possible that older males increased their courtship effort to compensate for reduced mating opportunities; in many taxa, older males produce higher quality signals (Proulx et al. 2002, Forsman and Hagman 2006, Gareamszegi et al. 2005, Kipper and

Kiefer 2010). Older males also have more time to accrue parasites, hence this relationship could have potentially confounded our findings. In fiddler crabs, body size is correlated with age, thus we were able to use carapace width in our analyses to explore this possibility (Hayes et al. 2013). However, we did not observe that larger, presumably older males displayed more frequently. Additionally, studies of other fiddler crab species have found that larger, older males do not reproductively compensate through increased courtship rate (Hayes et al. 2013).

Experimental infections of hosts followed by repeated measures of sexual signaling across parasite development are necessary to determine if increased courtship effort is due to parasitic manipulation or host reproductive compensation. Increased courtship effort observed soon after infection would support the reproductive compensation hypothesis, since the parasite could not successfully establish in a final host at this time. Under this scenario, males would be increasing courtship effort to offset the fitness costs of infection. Conversely, an observed increase in sexual signaling once the parasite is infective to final hosts would be more indicative of parasitic manipulation. Hosts would maximize their reproductive success by increasing mating effort soon after infection, while parasites would only benefit from increased signaling and predation after a sufficient period of growth and development within the intermediate host.

The higher display frequencies observed in more parasitized males may still favor parasite transmission regardless of the underlying mechanisms. Other fiddler crab species demonstrate male-biased predation with waving males at higher risk than non-waving cohorts (Koga et al. 2001, Ribeiro et al. 2003). If bird predators are more responsive to stronger prey signals, then highly parasitized males may be eaten more often than less infected conspecifics. While the *P. uca* parasite is detrimental to host fitness overall, the host-parasite relationship can still be maintained in populations due to possible mating advantages conferred on infected individuals. If parasitized males acquire more mates and predators as a result of infection and increased signaling, selection could favor a genetic predisposition to parasite infection susceptibility. Moreover, selection would also favor parasites that manipulate host courtship behavior if the altered host phenotype increases parasite fitness.

Conflicts between natural and sexual selection can maintain deleterious genes in populations if they confer mating advantages. For example, swordtail (*Xiphophorus cortezi*) populations have maintained an oncogene (*Xiphophorus* melanoma receptor kinase, *Xmrk*) for many generations due to female preferences for melanin based sexual signals. Males with the *Xmrk* oncogene develop malignant melanomas that appear as exaggerated melanin patches that females prefer. While inheritance and expression of the *Xmrk* oncogene significantly reduces life span, males with these cancerous lesions experience higher short-term mating success relative to healthy males (Fernandez and Morris 2008).

We propose a similar conflict of natural and sexual selection in the host-parasite relationship between *Uca crenulata* and *Probolocoryphe uca*. Males with *P. uca* infections may have reduced life spans due to increased morbidity and predation risk. However, these costs could be partially offset by enhanced short-term mating success if parasitized males are more attractive as a result of their intensified signaling. Future work investigating the underlying mechanisms of increased signaling in infected hosts would provide a better understanding of the relationship between manipulative parasites and the evolution of sexual signals.

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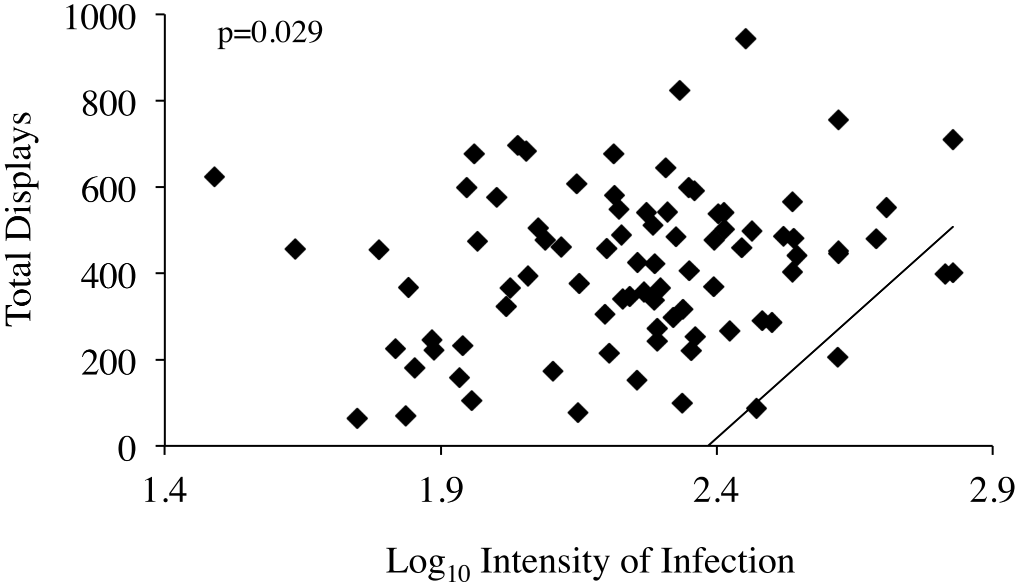

Figure 3.1. The frequency of waving displays each male performed in a 30-minute observation period in relation to the intensity of *P. uca* infection (number of metacercarial cysts per crab).

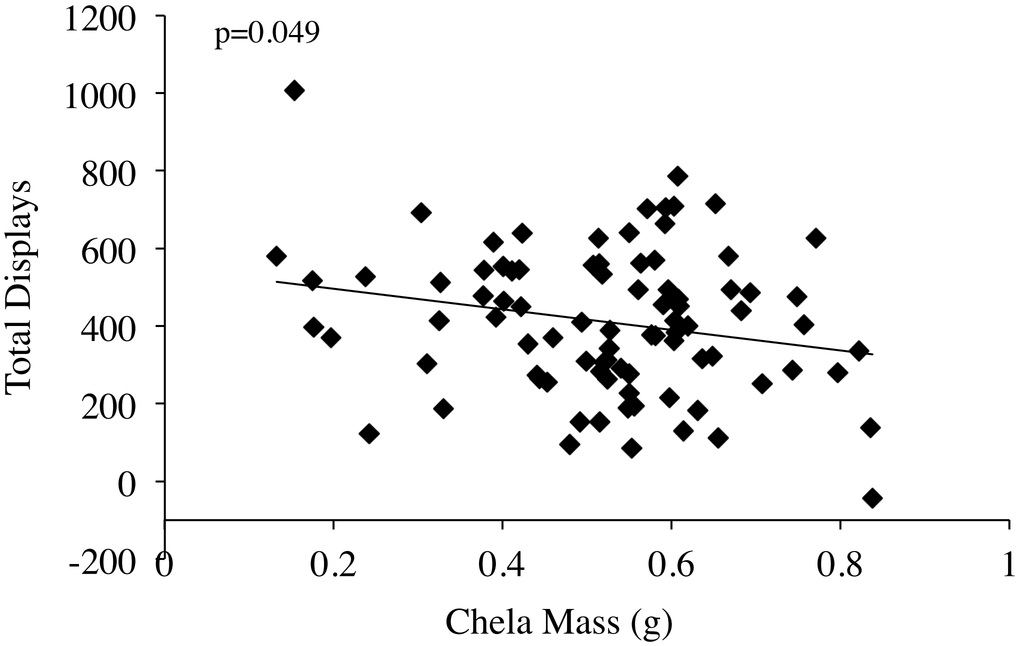

Figure 3.2. The frequency of waving displays each male performed in relation to major chela mass.

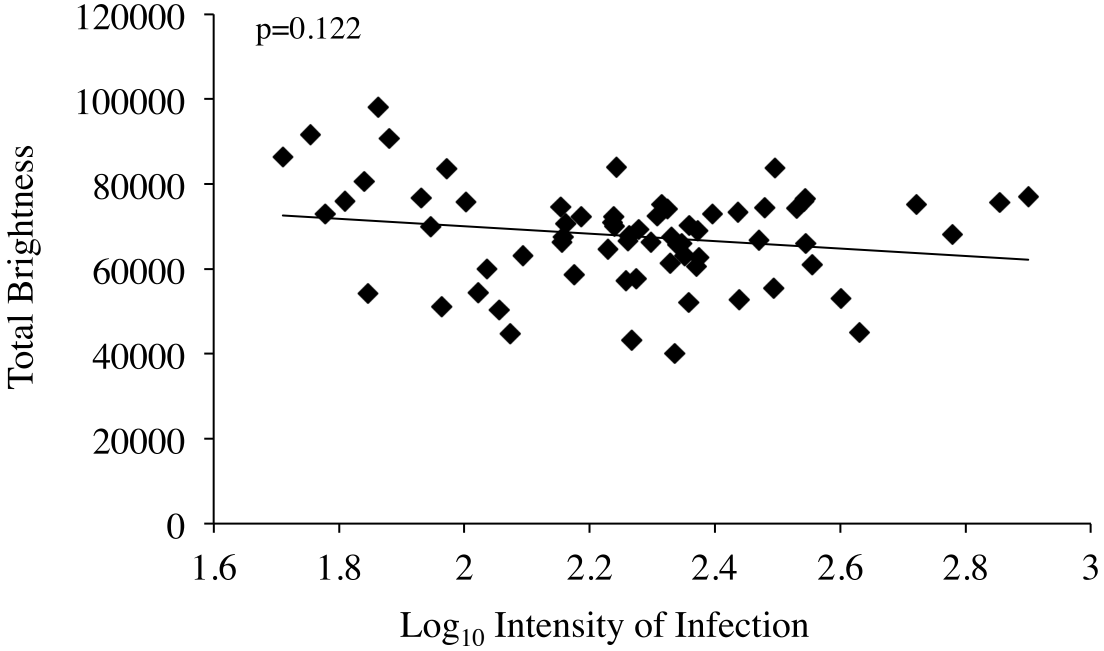

Figure 3.3. Total brightness of the major claw in relation to the intensity of *P. uca* infection.

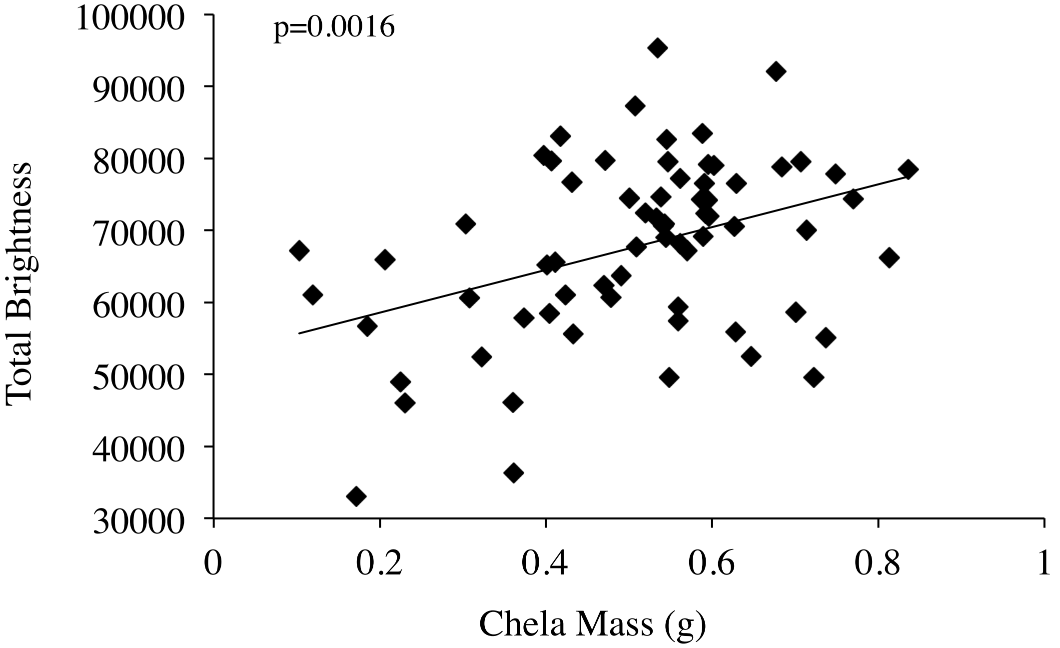

Figure 3.4. Total brightness of the major claw in relation to major claw mass.

Chapter 4

The effects of host sex and trematode infection on differential predation of fiddler crabs

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ABSTRACT

Trophically transmitted parasites have complex life cycles that require transmission via predation by a final host for life cycle completion. To address this challenge, these parasites often alter the host's phenotype in ways that increase host predation and subsequent parasite transmission, a process termed host manipulation. However, the timing of host manipulation is crucial to parasite fitness, as premature transmission is often lethal to parasites. Therefore, these parasites should be selected to manipulate host phenotype in accordance with their development. We tested the hypothesis that trophically transmitted parasites initially suppress host predation risk to reduce premature transmission; conversely, after such parasites have developed to become infective to final hosts, they should increase host predation risk. Manipulative parasites should also alter more susceptible hosts to maximize fitness benefits. We tested predation suppression and enhancement of fiddler crabs infected with the trophically transmitted parasite *Probolocoryphe uca*. We expected differential predation of crabs to vary according to parasite development; we also predicted that male crabs would be eaten more often than females due to sex-specific traits that may increase their predation risk. As expected, we recaptured significantly more unparasitized than parasitized crabs in the pen exposed to predators, suggesting that crabs experimentally infected with *P. uca* were more susceptible to predation. While our findings are consistent with other studies demonstrating higher predation rates of parasitized animals, we did not observe predation suppression and enhancement. Contrary to our predictions, we recaptured fewer females than males in the experimental pen, suggesting that females were more susceptible to

predators. While females are generally more cryptic than males, they may be easier targets for predators due to sex-specific differences in morphology and behavior. Because females lack the large, formidable claws that males possess, they may be easier to handle and ingest by predators. Additionally, during the breeding season females perform long mate searches away from the safety of their burrows, potentially reducing their ability to seek refuge when threatened by predators.

INTRODUCTION

Many parasites modify host phenotypic traits for their benefit, a phenomenon known as “host manipulation”. These morphological, behavioral, or physiological alterations can be subtle, such as an elevation in host activity levels, or extreme, such as performance of a complex, aberrant behavior (Moore 2002). The “manipulation hypothesis” states that parasites should be selected to manipulate host behavior in ways that facilitate transmission and fitness (Holmes and Bethel 1972, Webster 2007). Trophically transmitted parasites are especially likely to manipulate their hosts, as they largely depend on predation of their intermediate hosts by appropriate definitive hosts for survival and reproductive success. Manipulated intermediate hosts often exhibit more “risky” behaviors or reduced escape responses that increase vulnerability to predatory definitive hosts (Moore 2002, Zuk and Kolluru 1998). For example, killifish infected with the trematode *Euhaplorchis californiensis* exhibited a four-fold increase in “conspicuous” swimming behaviors and were 30 times more likely to be eaten by avian definitive hosts (Lafferty and Morris 1996).

While some parasites appear to manipulate hosts without much effort (Seppälä et al. 2004), host manipulation is generally thought to carry significant costs for parasites (Poulin 1997). First, it is energetically expensive to induce phenotypic changes in a host, as parasites may secrete neuroactive substances that react directly with the host’s nervous system (Thompson and Kavaliers 1997, Thomas et al. 2005 and references therein). Energy that is invested into altering host phenotype is diverted away from parasite growth, reproduction, and defense against the host immune system. In addition, manipulative parasites face increased mortality risk through ingestion by inappropriate hosts, premature transmission, and host immune attack (Mouritsen and Poulin 2003, Poulin 2007).

Because parasites encounter trade-offs when they manipulate hosts, Poulin (1994, 2010) hypothesized that they will evolve to optimize, not maximize, manipulative effort. He posited that selection should favor parasites that are able to maximize transmission while minimizing costs. Building upon Poulin's work, Parker and colleagues (2009) developed a model for the evolution of host manipulation by trophically transmitted parasites to predict when such parasites should evolve to manipulate hosts. They proposed two categories of manipulation by parasites, suppression and enhancement, which are predicted to occur at different stages of parasite development. When larval parasites are immature and not infective to final hosts, they should be selected to *suppress* predation risk of intermediate hosts to reduce premature transmission and death. After parasites have sufficiently developed to become infective to final hosts, they should be selected to *enhance* host predation risk to achieve transmission.

Parasites may also minimize costs by infecting and manipulating more susceptible hosts. Phenotypic differences in males and females may lead to sex-biased predation (Bildstein et al. 1989, Iribarne and Martinez 1999, Koga et al. 2001, Johnson 2003, Ribeiro et al. 2003), producing different costs and benefits to infecting and manipulating each host sex

(Duneau and Ebert 2012). Manipulative parasites should be selected to infect and alter hosts that will maximize their chances of transmission via predation.

We examined how host sex and parasitism influenced predation rates in a population of fiddler crabs (*Uca crenulata*) at San Dieguito lagoon (near San Diego, CA). *U. crenulata* exhibit extreme sexual dimorphism in both morphology and behavior (Backwell and Passmore 1996, Crane 1975, Johnson 2003). Compared with females, male fiddler crabs are more brightly colored, and they use an enlarged claw to perform a highly conspicuous courtship display. Studies of other *Uca* species suggest that these conspicuous traits make males more vulnerable to predators (Koga et al. 2001, Ribeiro et al. 2003, but see Iribarne and Martinez 1999, Johnson 2003). *U. crenulata* are also intermediate hosts to the trophically transmitted trematode parasite, *Probolocoryphe uca* (Sarkisian 1957, Hechinger et al. 2007, Mora, unpub. data). Male crabs with higher *P. uca* infections perform more courtship displays, which could make them more visible to predators (Mora and Zuk, unpub. data).

P. uca has a three-host life cycle, sexually reproducing in a bird or raccoon final host (Lafferty and Dunham 2005). *P. uca* eggs are shed with the final hosts' feces on to mudflats, where first intermediate snail hosts (*Cerithidea californica*) become infected after ingesting them while foraging. Within the snail, *P. uca* asexually replicates, producing free-swimming cercariae that enter the water to infect the second intermediate host, *U. crenulata*. *P. uca* will encyst as metacercariae in the connective tissues of the crab. Once inside of a fiddler crab host, *P. uca* often take up to three weeks to encyst as metacercariae (Sarkisian 1957, Mora, unpub. data), after which time they are infective to final hosts (Sarkisian 1957). Upon ingestion by a bird or raccoon final host, the worms will excyst and travel to the intestines where they will sexual reproduce with other conspecifics, thereby completing the life cycle (Sarkisian 1957, Lafferty and Dunham 2005, Hechinger et al. 2007).

We predicted that if *P. uca* manipulate *U. crenulata* hosts in accordance with parasite development, then host predation risk would be suppressed before post-infection day 21, and enhanced thereafter. Thus, we expected lower predation rates of parasitized crabs on post-infection day 21 as a function of host suppression by *P. uca*. At post-infection day 49, we expected higher predation of parasitized crabs due to host enhancement by *P. uca*. Since sex differences in host morphology and behavior likely make male crabs more conspicuous than females, we also expected that predators would consume males more often than females.

METHODS

Experimental groups

We collected 240 crabs from a mudflat in San Dieguito lagoon (near San Diego, CA) during the 2013 breeding season, which occurs from late May through late August (deRivera 2003, 2005). In an effort to use crabs with low natural parasite burdens, we chose this study site due to very low first intermediate snail host abundance and dissected all recaptured crabs to confirm infection status. Crabs were maintained in the lab on a diet of crushed Tetramin (Tetra, Blacksburg VA) fish food, administered ad libitum for five days prior to testing. Male and female crabs were then randomly assigned to one of two experimental groups: 1) Uninfected (n=30 males, 30 females per pen), exposed to unfiltered seawater from uninfected snails and 2) Infected (n=30 males, 30 females per pen), exposed to seawater containing *P. uca* cercariae.

Experimental infections

Snails infected with *P. uca* were individually placed into 20-mL scintillation vials filled with seawater and placed under warm, bright halogen lights to stimulate shedding of *P. uca* cercariae. We pooled all seawater from vials containing *P. uca* cercariae into one container for use. Prior to exposure, each crab was placed into a 118-mL clear plastic container with 10-mL of seawater and a lid to prevent escape. The water containing *P. uca* cercariae was stirred frequently to ensure a homogeneous mix of trematode cercariae. The number of *P. uca* cercariae contained within a 1-mL aliquot of seawater was quantified under a dissecting scope to determine an estimate of 500 or more *P. uca* cercariae. We then administered 1-mL aliquots of the water containing *P. uca* larvae to each crab container. Each crab was experimentally exposed to approximately 500 or more *P. uca* cercariae each day for six consecutive days prior to the predation experiment.

Predation experiment

We labeled crabs with identification numbers and released them into two field pens. Identification numbers were transcribed onto 6 mm x 3 mm strips of white electrical tape with a black Sharpie permanent marker. One identification label was affixed to the carapace of each crab using one drop of superglue. Field pens were installed next to each other on the mudflat to ensure continuity of sediment size, distance from the shore, and gradation. After field pen installation, the sediment inside of each pen was completely excavated to a depth of 0.6 m and all mud was sifted through by hand to locate all resident crabs. This depth was chosen to ensure that we excavated all crabs, as average burrow depths of *U. crenulata* are much shorter than 0.6 m (deRivera 2005). All resident crabs were removed and released on to the surrounding mudflat at low tide. Each field pen was constructed from a 1m x 2m wooden frame with attached hardware cloth. To prevent escape, a four inch strip of aluminum siding material was attached to the inside frame of each pen. One field pen served as a control to quantify any crab loss due to incidental mortality and escape. This control field exclosure had a hardware cloth roof attached to contain all control crabs. The second experimental pen was an open field enclosure that exposed our test crabs to vertebrate predators such as birds and raccoons. We released 120 crabs (Uninfected crabs: 30 males, 30 females; Infected crabs: 30 males, 30 females) into each pen on day seven of the initial exposure period. This fiddler crab density is well within previously observed *U. crenulata* population densities (deRivera 2003, deRivera 2005, Mora, unpub. data). To attract predators, we followed methods described by Kim et al. (2009) and baited each pen with two cups of dry dog food once a week for the duration of the experiment. We excavated the control and experimental pens to a depth of 0.6 m and recaptured and quantified all crabs on post-infection day 21 (suppression phase) and post-infection day 49 (enhancement phase). Additionally, we searched the surrounding crab colony for several consecutive days following each excavation to locate any escaped crabs bearing identification labels.

During the experiment, we observed willets (*Tringa semipalmata*), great egrets (*Ardea alba*), and western gulls (*Larus occidentalis*) foraging near the pens. These bird species are natural crab predators (Hechinger et al. 2007). Additionally, we observed tracks as evidence of raccoon (*Procyon lotor*) visits, another crab predator and final host for *P. uca* (Lafferty and Dunham 2005). Other potential predatory birds that were common at the lagoon during the experiment included: double-crested cormorants (*Phalocrocorax auritus*), great blue herons (*Ardea herodius*), light-footed clapper rails (*Rallus longirostris levipis*), long-billed curlews (*Numenius americanus*), whimbrels (*Numenius phaeopus*), marbled godwits (*Limosa fedoa*), greater yellowlegs (*Tringa melanoleuca*), long-billed dowitchers (*Limnodromus scolopaceus*), snowy egrets (*Egretta thula*), black-crowned night herons (*Nycticorax nycticorax*), little blue herons (*Egretta caerulea*), white-faced ibises (*Plegadis chihi*), and belted kingfishers (*Ceryle alcyon*).

Dissection protocol

We dissected all crabs that were recaptured at post-infection day 49. We followed dissection methods established by the Kuris lab at the University of California, Santa

Barbara (Appendix A). Briefly, we euthanized crabs immediately prior to dissection by freezing for 10 minutes. We used a razor blade to pry the carapace open and removed the connective tissues using fine forceps. We used a clean Pasteur pipet to remove all residual body fluids and connective tissue and mounted all samples on to slides with coverslips for examination at 100X using compound microscopy. *P. uca* parasites were identified based on various morphological characteristics, including metacercarial shape and size. We also identified *P. uca* based on the observation of a spined, gourd-shaped body, a collared anterior region with a conical projection that lacked spines, and vitellaria configured in a ring formation (Sarkisian 1957).

Statistical analyses

We performed all statistical analyses using JMP statistical software ver.10 (SAS Institute, Cary, NC), and with an alpha level of 0.05 for all tests. To test how sex, parasite treatment, and pen affected crab predation rates, we performed multiple logistic regression analysis and included pen, sex, treatment, and all interactions in the model. Initial analyses revealed that host mass was not a significant factor in predation, thus mass was not included in our final analysis. We additionally tested how host sex, host mass, and treatment affected total *P. uca* abundance of all recaptured crabs. We used a generalized linear model (GLM) with a Poisson distribution, overdispersion parameter and a log-link function. We included host sex, treatment (experimentally parasitized, unparasitized), host mass, and all interactions in the model.

RESULTS

Post-infection day 21

Our regression analyses revealed significant effects of pen and pen x treatment on the frequency of recaptured crabs (Table 4.1). As expected, we recaptured fewer crabs in the experimental pen that was exposed to predation (Fig. 4.1, Table 4.1). We recaptured more parasitized than unparasitized crabs from the closed control pen, however we recaptured more unparasitized than parasitized crabs in the open experimental pen (Fig. 4.1, Table 4.1). This suggests that parasitized crabs were significantly more susceptible to predation. We did not observe an effect of host sex on recapture rates (Fig. 4.1, Table 4.1).

Post-infection day 49

Our regression analyses revealed significant effects of sex, pen, sex x pen, and pen x treatment on the frequency of recaptured crabs (Table 4.2). We recaptured significantly more crabs from the closed control pen than from the open experimental pen (Fig. 4.2, Table 4.2). The effect of treatment depended on pen, with fewer parasitized crabs recaptured from the open experimental pen. The effect of sex depended on pen, with fewer female than male crabs recaptured in the open experimental pen (Fig. 4.2, Table 4.2). These results suggest that females and parasitized crabs were more susceptible to predation. We did not observe an effect of treatment, sex x treatment, or sex x treatment x pen on crab recapture rates (Table 4.2).

Experimental infection data

Crabs that were experimentally infected with *P. uca* harbored significantly more parasites than control crabs (Fig. 4.3, Table 4.3). We found no effects of sex, sex x treatment, mass, sex x mass, treatment x mass, and sex x treatment x mass on total *P. uca* abundance (Table 4.3).

DISCUSSION

Our results support the hypothesis that parasitized crabs are more susceptible to predation. Although we did not observe that host predation rates changed in accordance with parasite development, our findings are consistent with other studies suggesting higher predation rates of parasitized hosts (Lafferty and Morris 1996, Moore 2002). We are confident that our lower recapture rates of parasitized crabs were due to predation and not increased mortality from infection because we did not observe high mortality rates in the control pen. Further, we observed several discarded major chelae from males on the sediment surface in the experimental pen. In other fiddler crab predation studies, discarded chelae have been used as indicators of male predation, since bird predators often remove the major claws of males before ingesting them (Wolf et al. 1975, Iribarne and Martinez 1999, Johnson 2003). It is also possible that males lost their claws due to aggressive behavior. Although males regularly fight each other during the breeding season, it is uncommon for them to lose their major claws during agonistic encounters (Crane 1975, Ricketts et al. 1985). Additionally, if major chela loss was driven by aggressive interactions, we would have probably found discarded claws in the control pen, and we did not observe this. Additionally, it is unlikely that the crabs escaped from the pens for two reasons: first we observed that crabs were unable to traverse the aluminum sheeting strip that lined the inside of each pen and we also performed extensive searches for labeled crabs on the surrounding mudflats for several days following each recapture event.

Fewer females than males were recaptured in the open experimental pen, suggesting that females are more susceptible to predation even though males may be more conspicuous, particularly when parasitized (Mora and Zuk, unpub. data). It is possible that the major cheliped wielded by males makes them less attractive prey items. Studies with other *Uca* species have demonstrated female-biased predation (Bildstein et al. 1989, Iribarne and Martinez 1999, Johnson 2003, but see Koga et al. 2001, Pratt et al. 2002, Ribeiro et al. 2003). Bildstein and colleagues (1989) determined that ibises were four times more likely to eat females and males without major claws than intact males. Ibises also rejected intact males in burrows more often than females. Further, Bildstein et al. (1989) observed that intact males sometimes pinched the bills of ibis predators upon capture, causing them to be quickly released. In addition to the risk of injury from intact males, they also require more handling time by predators since the major claw is often removed before ingestion of the crab (Wolf et al. 1975, Iribarne and Martinez 1999). In contrast, female crabs are generally swallowed whole, potentially making them more profitable prey items (Wolf et al. 1975, Iribarne and Martinez 1999).

Sex differences in crab mate-searching behavior may have also influenced the female-biased predation that we observed. While courting males often display near their burrows, females can perform long mate searches during the breeding season (deRivera 2005). DeRivera (2005) reported that one *U. crenulata* female sampled 106 males before selecting a mate. Further, *U. crenulata* females conduct more and longer searches for mates in muddy-sand substrates with high crab density (deRivera 2003), as was the case in our experimental pens. Since breeding females spend more time away from their burrows, they may have less access to refuge from predators and may be more susceptible as a result.

Sex-specific traits carry different fitness costs and benefits for each sex. While possession of the major cheliped incurs fitness costs for males (Valiela et al. 1974, Koga et al. 2001, Ribeiro et al. 2003, Mora and Zuk unpub. data), it appears that in some situations it may afford them protection from certain predators (Wolf et al. 1975, Bildstein et al. 1989, Iribarne and Martinez 1999, Johnson 2003). For females, having two small feeding claws has the advantages of reduced foraging time (Valiela et al. 1974) and increased grooming efficacy (Mora and Zuk, unpub. data). However, the lack of a major cheliped may also increase their predation risk, since some predators may consider them more profitable prey items (Wolf et al. 1975, Bildstein et al. 1989, Iribarne and Martinez 1999, Johnson 2003).

Host sex differences likely produce different selection pressures on the parasites that infect and manipulate them for transmission. Future work should aim to investigate how manipulative parasites may be locally adapted to manipulate the more susceptible host sex. For example, in populations with high male-biased predation of hosts, parasites should be selected to infect males and manipulate sex-specific characters that increase their predation risk.

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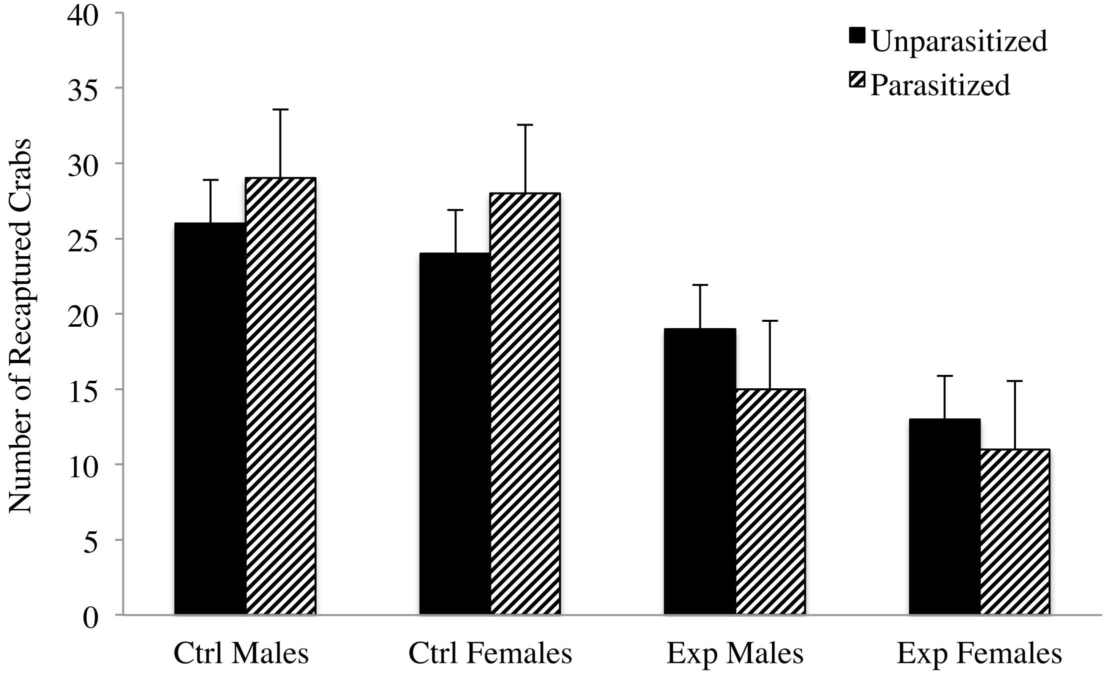

Figure 4.1. Number of male and female crabs recaptured (frequency + SE) in the control (Ctrl) and experimental (Exp) pens at post-infection day 21 as a function of treatment.

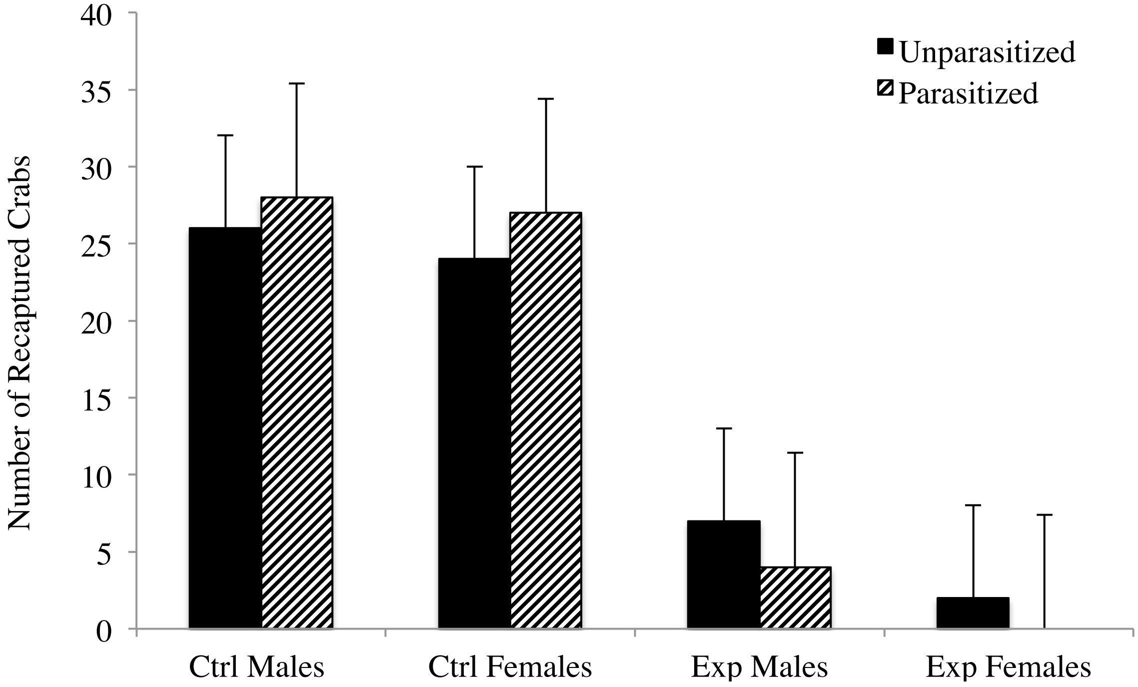

Figure 4.2. Number of male and female crabs recaptured (frequency + SE) in the control (Ctrl) and experimental (Exp) pens on post-infection day 49 as a function of treatment.

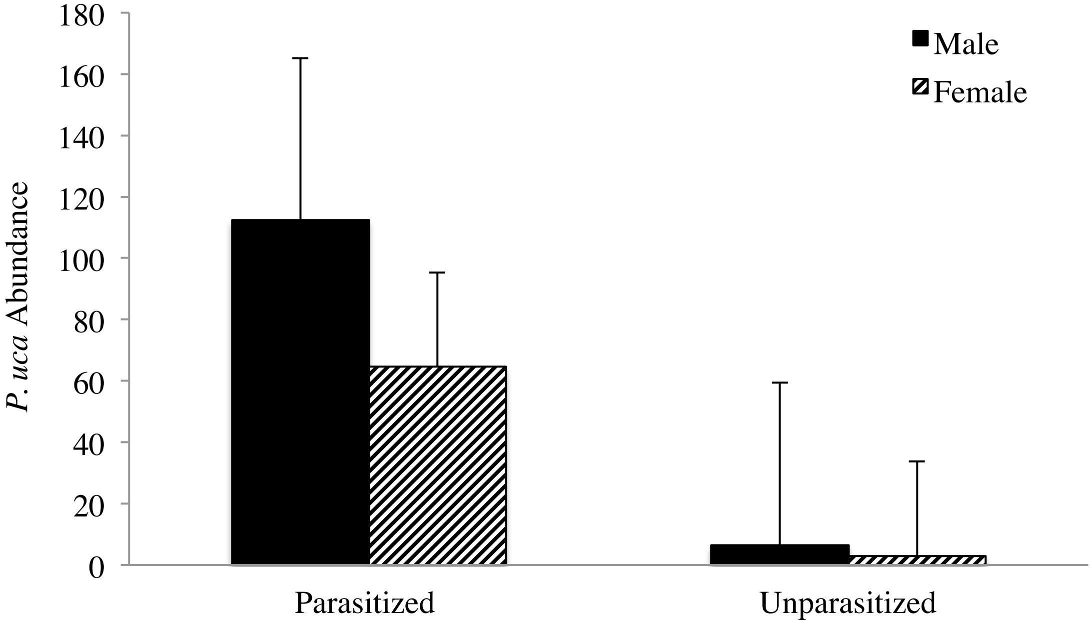

Figure 4.3. Average *P. uca* abundance (mean number of *P. uca* parasites in a host + SE) of male and female crabs as a function of treatment. Total n=118; parasitized males: n=32, parasitized females: n=27, unparasitized males: n=35, unparasitized females: n=24.

Table 4.1. Multiple logistic regression testing the effects of pen, sex, treatment and all interactions on frequency of recaptured crabs at post-infection day 21. Significant p-values are in bold.

	df	χ^2	*p*
Sex	1	1.650	0.1989
Pen	1	48.333	**<0.0001**
Sex * Pen	1	0.331	0.5649
Treatment	1	0.7359	0.3910
Sex * Treatment	1	0.2432	0.6219
Pen * Treatment	1	4.164	**0.0413**
Sex * Pen * Treatment	1	0.0156	0.9005

Table 4.2. Multiple logistic regression testing the effects of pen, sex, treatment and all interactions on frequency of recaptured crabs at post-infection day 49. Significant p-values are in bold.

	df	χ^2	*p*
Sex	1	7.241	**0.0071**
Pen	1	165.784	**<0.0001**
Sex * Pen	1	4.650	**0.0310**
Treatment	1	1.389	0.2385
Sex * Treatment	1	0.896	0.3440
Pen * Treatment	1	4.892	**0.0270**
Sex * Pen * Treatment	1	1.639	0.2005

Table 4.3. Generalized linear model testing the effects of host sex, treatment, host mass, and all interactions on total *P. uca* abundance. Significant p-values are in bold.

	df	χ^2	*p*
Sex	1	0.595	0.4406
Treatment	1	41.898	**<0.0001**
Sex * Treatment	1	0.014	0.9052
Mass	1	0.103	0.7488
Sex * Mass	1	0.252	0.616
Treatment * Mass	1	0.0006	0.979
Sex * Treatment * Mass	1	0.521	0.4706

CONCLUSIONS

The host-parasite relationship is complex, and each participant faces its own unique selection pressures. Hosts are challenged with the constant threat of parasitism in their natural environments. Parasites can substantially reduce host growth, reproduction, and survival (Hart 1990, 1992, 1994; Moore 2002). In response to these selection pressures, hosts can employ a number of behavioral defenses such as avoidance, grooming, and self-medication (Hart 1990, 1992, 1994; Moore 2002, Gray et al. 2012). Although most research has focused on immune responses to infection (Hart 1990, Kiesecker et al. 1999, Ezenwa 2004, Råberg et al. 2009), behavioral strategies are often the first line of defense and can be highly effective in reducing parasites and increasing host fitness (Hart 1990, Daly and Johnson 2011, Milan et al. 2012, Kacsoh et al. 2013).

I found that fiddler crabs become more active in the presence of parasites, and increase their frequencies of grooming, eating, and leg rubbing. In addition, males groom more often than females. This sex-specific difference is most likely attributed to the presence of the major cheliped in males, which is incapable of performing grooming behavior. While females can groom with both claws males can only do so with one, necessitating more grooming bouts. Grooming behavior appears to be an important antiparasite defense for males, as those that were allowed to groom *P. uca* parasites from their bodies exhibited a significant reduction in parasite burdens over those that were grooming impaired.

Sex differences in host morphology, behavior, and physiology can further complicate the host-parasite relationship, predisposing some individuals to a higher risk of infection. Consistent with other findings of male-biased parasitism, I found that male crabs were more susceptible to *P. uca* infection. Although they performed more grooming bouts, males harbored more parasites, and this result was not due to host mass. The major cheliped seems to hinder grooming ability, as male crabs had more parasites across all treatments and had the highest *P. uca* intensities when their grooming claw was impaired. Although the major cheliped aids males in mate attraction and the defense of territories, it significantly reduces grooming ability, thereby placing them at higher risk of parasitism. This finding is consistent with other studies that demonstrate fitness costs to bearers of elaborate and conspicuous sexual signals (Magnhagen 1991, Andersson 1994, Zuk and Kolluru 1998).

From the parasite's perspective, survival and reproductive success are fully dependent on transmission to appropriate hosts (Moore 2002). For trophically transmitted parasites this can be especially challenging, as life cycle completion must occur through predation of an intermediate host by a final host (Lafferty and Morris 1996, Moore 2002). To achieve this, such parasites will often change the appearance and behavior of intermediate hosts to facilitate transmission to final hosts, termed host manipulation (Holmes and Bethel 1972, Lafferty and Morris 1996, Lafferty 1999, Moore 2002, Poulin 2007, Webster 2007). Although host manipulation is known to confer fitness advantages for parasites, cases of manipulation that enhance components of host fitness are not known. I explored the novel idea that manipulative parasites intensify host sexual signals to increase their transmission and fitness. Under this scenario manipulated hosts would exhibit intensified signaling, and as a result experience increased short-term mating success, but also increased predation risk.

Consistent with my predictions, I found that males with more *P. uca* parasites produced more courtship waving displays, and males with the highest parasite burdens displayed with the highest frequency. Although older males tend to produce better sexual signals (Brooks and Kemp 2001, Proulx et al. 2002, Forsman and Hagman 2006, Garamszegi et al. 2005, Kipper and Kiefer 2010) and have more parasites (Poulin 2007), I did not observe this relationship; thus increased signaling seems to be driven by *P. uca* infection and not by host age. Additionally, this relationship remained after controlling for other factors such as temperature, tidal cycle, and major cheliped mass. These findings suggest that parasites may manipulate host sexual signals for fitness benefits. Alternatively, males could be increasing courtship effort to reproductively compensate for infection. If males experience reduced fitness as a result of *P. uca* infection, then enhanced courtship effort could increase their residual reproductive value. However, if males were compensating for future losses of mating opportunities, we may expect higher waving frequencies in older males, and I did not observe this relationship.

Crabs infected with *P. uca* parasites may experience higher predation rates, as I recaptured significantly more unparasitized than parasitized crabs from the pen exposed to predators. Contrary to my predictions, I recaptured fewer females than males in the open pen, suggesting that females were more susceptible to predation. While the major cheliped is a costly sexual signal (Valiela et al. 1974, Koga et al. 2001, Ribeiro et al. 2003, Mora and Zuk unpub. data), it may afford them protection from some predators. Several studies have documented female-biased predation in other fiddler crab species (Bildstein et al. 1989, Iribarne and Martinez 1999, Johnson 2003, but see Koga et al. 2001, Pratt et al. 2002, Ribeiro et al. 2003). The major cheliped wielded by males increases handling time, and may increase a predator's risk of injury (Bildstein et al. 1989, Wolf et al. 1975, Iribarne and Martinez 1999). While the major cheliped is often removed from the male before ingestion of the body, females are swallowed whole (Wolf et al. 1975, Iribarne and Martinez 1999). Thus, females may be more profitable prey items for some predators and as a result, may be targeted over males. This work highlights how sexual dimorphism can produce different fitness costs and benefits for males and females. While the major cheliped is a costly sexual signal that reduces grooming efficacy, and increases foraging time and infection risk, it may also deter predators (Valiela et al. 1974, Amin et al. 2011, Mora and Zuk, unpub. data). Conversely, female possession of two small claws helps them to feed and groom more effectively (Valiela et al. 1974, Amin et al. 2011, Mora and Zuk, unpub. data), however, the lack of a major cheliped weapon may also entice predators to selectively prey upon them.

Future work should aim to investigate how manipulative parasites influence host sexual signaling to increase their transmission and fitness. To most rigorously test this hypothesis, naturally unparasitized host animals should be experimentally infected with parasites followed by quantification of sexual signals at different stages of parasite development. If host sexual signaling increases soon after infection and before the parasites can establish in subsequent hosts, this would be more indicative of host reproductive compensation. In this case, hosts may be compensating for reduced residual reproductive value by increasing their courtship effort to acquire more mating opportunities. However, if sexual signaling increases only after the parasites have become infective to subsequent hosts, then this would support the sick but sexy hypothesis. In this scenario, parasites may manipulate conspicuous sexual signals of their hosts to facilitate their transmission and life cycle completion.

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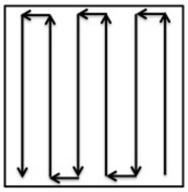
Appendix A

Dissection and microscopy procedures

All crabs were dissected following methods established by the Kuris lab (an ecological parasitology lab) at the University of California, Santa Barbara. I received training in crab dissection from Dr. Armand Kuris and Dr. Ryan Hechinger at the University of California, Santa Barbara. I euthanized crabs immediately prior to dissection by placing them into a standard freezer and chilling them for 10 minutes. This was sufficient to humanely euthanize the crabs and preserve their tissues for proper analysis. I began each dissection by using the edge of a clean razor blade to pry the carapace open and I removed the black connective tissues using fine forceps. Any residual connective tissues were removed by placing a few drops of seawater into carapace, gently detaching the tissue from the carapace with the tip of a clean Pasteur pipet, then suctioning up all residual body fluids, seawater, and connective tissues. I mounted all samples on to slides with coverslips for examination at 100X using compound microscopy.

Slides were analyzed in a highly systematic manner according to protocols established in the Kuris lab. I oriented the slide such that the lower, right corner of the slide containing a tissue specimen was directly in the field of view of the microscope eyepieces. I then slowly moved the stage of the microscope directly upward, scanning the tissues for parasites. All *P. uca* parasites were quantified using a tally counter. Once I reached the top right corner of the slide, I located a landmark on the slide on the far left field of view and moved the stage left until the landmark moved just out of view. This scanning method continued until I reached the end of the slide. A diagrammatic view of this procedure is illustrated below.



Parasite identification and quantification

P. uca parasites were identified based on various morphological characteristics described in the literature (Sarkisian 1957, DeBlock 2008). I received additional training in parasite identification from Dr. Armand Kuris and Dr. Ryan Hechinger at the University of California, Santa Barbara. *P. uca* metacercariae have a spherical shape and measure 0.22 mm-0.25 mm in diameter when under coverslip pressure (Sarkisian 1957). *P. uca* exhibit a spined, gourd-shaped body, an oral and ventral suckers, a collared anterior region with a conical projection that lacks spines, and vitellaria configured in a ring formation (Sarkisian 1957, Abdul-Salam and Sreelatha 2000, DeBlock 2008). When excysted, *P. uca* trematodes can additionally be identified through observation of a short digestive tract with a bifurcated ceca that does not extend beyond the ventral sucker. They also exhibit an ovary that is oriented anterior to the testes. All of these structures can be easily observed using a compound microscope at 100X.