Oecologia (2016) 180:137–147
DOI 10.1007/s00442-015-3461-9

POPULATION ECOLOGY - ORIGINAL RESEARCH

Local adaptation to parasite selective pressure: comparing three congeneric co-occurring hosts

Carolyn L. Keogh[1,2] · **Martha E. Sanderson**[2,3] · **James E. Byers**[1]

Received: 11 March 2015 / Accepted: 20 September 2015 / Published online: 6 October 2015

Abstract Local adaptation may optimize an organism's investment in defenses in response to the risk of infection by spatially heterogeneous parasites and other natural enemies. However, local adaptation may be constrained if recruitment is decoupled from selective pressure experienced by the parent generation. We predicted that the ability of three intertidal littorinid snail species to defend against trematode parasites would depend on prior levels of population exposure to parasites and on larval dispersal mode, a proxy for population openness. In a common garden experiment, for two snail species with direct development and localized recruitment (*Littorina obtusata* and *Littorina saxatilis*), hosts from sites with high trematode infection risk were less susceptible to infection than hosts from low-risk sites. However, this relationship was not apparent for a third host species with broadcast larvae (*Littorina littorea*), suggesting that broad larval dispersal can impede local adaptation; alternatively, the lack of response in this species could owe to other factors that limited experimental infection in this host. Our findings support that locally recruiting hosts can adapt their defenses to scale with localized infection risk.

Keywords Ecoimmunology · Life history · Trematodes · *Littorina* · Coevolutionary arms race

Communicated by Pieter Johnson.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-015-3461-9) contains supplementary material, which is available to authorized users.

✉ Carolyn L. Keogh
clkeogh@gmail.com

Martha E. Sanderson
martha.sanderson@liu.edu

James E. Byers
jebyers@uga.edu

[1] Odum School of Ecology, University of Georgia, Athens, GA 30602, USA

[2] Shoals Marine Laboratory, Appledore Island, ME, USA

[3] Present Address: Biology Department, Long Island University-Post, Brookville, NY, USA

Introduction

All natural populations experience infection by parasites, which vary greatly in the degree to which they stimulate a defensive response from their hosts. Host responses to parasite infection risk are context dependent, and the relative levels of resistance expressed by individual hosts determine rates of disease spread and persistence at the level of populations and metapopulations (Ardia et al. 2011; Downs et al. 2014). Thus, understanding drivers of variation in susceptibility to parasite infection in populations under natural selective regimes is a key goal for the emerging field of ecoimmunology (Downs et al. 2014; Martin et al. 2011), and a step toward prediction and control of disease outbreaks in natural populations (Hawley and Altizer 2011).

While all wild hosts are expected to have some ability to defend against parasites, anti-parasite defenses are known to come at a cost to hosts, either by diverting energy from other fitness components, or via autoimmune reactivity (Hasu et al. 2009; Sheldon and Verhulst 1996). Higher defense investment is therefore only advantageous when resistance costs are outweighed by the cost of infection by the parasite, and when the risk of infection is consistent both spatially and temporally (Ardia et al. 2011; Barrett et al. 2008; Zuk and Stoehr 2002). Thus the existence of

transmission hotspots across a host range that is otherwise low in infection risk can give rise to divergent selection on traits for resistance, resulting in divergent adaptation of host traits to local levels of infection risk (Kawecki and Ebert 2004). For example, amphipods from natural populations that are naïve to infection have higher susceptibility to trematode or acanthocephalan parasites compared to populations where natural infection is common (Bryan-Walker et al. 2007; Hasu et al. 2009).

Although host immune investment may be expected to reflect the spatial mosaic of parasite-mediated selective pressure (i.e., local adaptation), the extent and effectiveness of any inherited mechanisms of adaptation may also be highly influenced by the amount of gene flow between host populations under different selection regimes (Gandon et al. 1996; Kawecki and Ebert 2004; Lenormand 2002; Slatkin 1987; Urban 2011). Some gene flow is necessary to maintain the variation on which selection acts, but too much mixing counteracts the effects of local selection and inhibits adaptation to local conditions (Lenormand 2002; Slatkin 1987). Given sufficient standing genetic variation, host populations that have high rates of local recruitment (i.e., with offspring retained in the parents' habitat) may be more likely to evolve phenotypes that reflect local selection pressure without the disruptive effect of gene flow from non-adapted populations. In contrast, hosts with broad propagule dispersal may be selected to maintain generalist phenotypes and greater phenotypic plasticity to ensure their success in a broader range of potential habitats (Warner 1997; Yamada 1989). Finally, because host-parasite interactions are coevolutionary in nature, the amount of dispersal and admixture of the parasites themselves may also be an important determinant of the hosts' ability to locally adapt (Gandon et al. 1996; Hoeksema and Forde 2008; Lively 1999; Roth et al. 2012).

Marine organisms provide ideal systems for testing these questions because of the diverse types of larval dispersal strategies (and their associated rates of gene flow) often found among sympatric, closely related species. We used a snail-trematode system to explore the interaction of historical parasite exposure (as a proxy for parasite-mediated selective pressure) and larval dispersal mode on the local adaptation of host defense against parasite infection. In the New England rocky intertidal zone, three congeneric species of *Littorina* snails occur in sympatry. *Littorina saxatilis* and *Littorina obtusata* are native to New England and Europe, while *Littorina littorea* is a naturalized invader from Europe that has inhabited New England rocky shores for over 150 years (Blakeslee et al. 2008). These species differ greatly in their reproductive strategies: *L. saxatilis* brood their young and fully developed juveniles emerge from the mother's brood sac; *L. obtusata* lays benthic egg masses that hatch as crawl-away juveniles; and *L. littorea* releases pelagic larvae which spend an estimated 4–7 weeks in the water column (Reid 1996). The amount of gene flow within each species conforms to expectations based on their life histories: *L. saxatilis* and *L. obtusata* have higher levels of population genetic structure, while *L. littorea* shows high levels of genetic admixture and little differentiation (Berger 1973; Johannesson and Johannesson 1996; Johannesson 2003; Schmidt et al. 2007; Snyder and Gooch 1973). *L. saxatilis* in particular has been found to have strong genetic clines over the scale of a few meters, and there is strong evidence of population isolation on separate but neighboring islands (<15 km distant) (reviewed in Johannesson 2003; Johannesson and Tatarenkov 1997).

Among the three snail hosts, spatially heterogeneous patterns of infection by castrating digenean trematode parasites have been well documented (Blakeslee et al. 2012; Blakeslee and Byers 2008; Byers et al. 2008, 2015; Granovitch et al. 2000). In New England, *L. littorea* is most commonly infected by the trematode *Cryptocotyle lingua*, while *L. saxatilis* and *L. obtusata* are more commonly infected with *Microphallus similis*, but there is considerable overlap of the trematode assemblages in all three host snails (Blakeslee and Byers 2008). Trematode parasites utilize sexually mature (adult) *Littorina* snails as first intermediate hosts in their complex life cycles, reproducing asexually in the region of the snail's digestive gland and gonad, and causing castration of the snail host (Robson and Williams 1970; Rothschild 1942). Free-swimming cercariae are released from the snail and penetrate a second intermediate host (fish for *C. lingua*; crabs for *M. similis*), which is consumed by a seabird definitive host [e.g., eider ducks, cormorants, and gulls (*Larus marinus, Larus argentatus*)]. As the infected birds forage, loiter or nest on rocky shores, they deliver trematode eggs via their guano to the vicinity of the snail hosts. *M. similis* and *C. lingua* eggs are ingested by grazing snails to complete the life cycle (Stunkard 1930, 1957). Thus, gull colonies, which are often found on uninhabited rocky islands, and gull attractors such as fishing piers, emerge as hotspots of trematode transmission to snails (>50 % prevalence in adult snails at some sites), while many other rocky intertidal sites exhibit low to no trematode prevalence (Byers et al. 2008; Hoff 1941; Matthews et al. 1985).

The unique features of this host-parasite system provide a valuable opportunity to test for the relative influence of local selective pressure and dispersal-mediated gene flow on host local adaptation. We carried out a common garden experiment to test for differences in susceptibility to trematode infection in wild-caught snails originating from locations with a history of a high risk (HR) or low risk (LR) of trematode infection, hypothesizing that snails from locations with high parasite selective pressure would show lower susceptibility to infection, reflecting local adaptation

of host defenses. We further hypothesized that this effect would only hold for the two snail species with local reproduction modes. We also tested the assumption that trematode parasites have high levels of genetic mixing in this system that might reduce their tendency to locally adapt to, or specialize on, differentiated snail genotypes.

Materials and methods

Snail collections

To test for an effect of parasite selective pressure on susceptibility to trematode infection, the three species of *Littorina* snails (*L. obtusata, L. saxatilis*, and *L. littorea*) were collected from multiple intertidal sites along the coast of New England, USA between 28 June and 1 July 2011 for use in a laboratory common garden experiment (Fig. 1). Specifically, we collected each snail species from three sites where at least one study over the previous 10 years had identified HR of trematode infection (i.e., >10 % trematode infection prevalence in at least one of the three species, and high densities of shorebird definitive hosts), and from three sites with known LR of trematode infection (<10 % infection rates in prior surveys of any species, and/or low density or absence of shorebirds) (Table 1). Not all snail species were present at all sites in sufficient numbers, so the six collection sites are not identical for each species (Fig. 1; Table 1). We sourced snails from multiple HR and LR sites to ensure broad characterization of each category and thus minimize the influence of isolated, site-specific effects on snail performance.

To determine the consistency of selective pressure acting on the current generation of snails at our snail collection sites, and thus to validate our categorization of a site as either HR or LR, we examined ~100 adult snails of each species at each site for trematode infections. Because snails live for multiple years and successful infections are usually permanent in the snails, measurements of trematode parasite prevalence in the adult snail population at each site, including larger, older snails, allowed us to infer infection risk integrated over multiple years, giving a fuller picture of the consistency of parasite infection risk at each site (Graham 2003). For these surveys, snails representing the full range of adult sizes were collected haphazardly at low tide from a broad area (>100-m length whenever possible, including

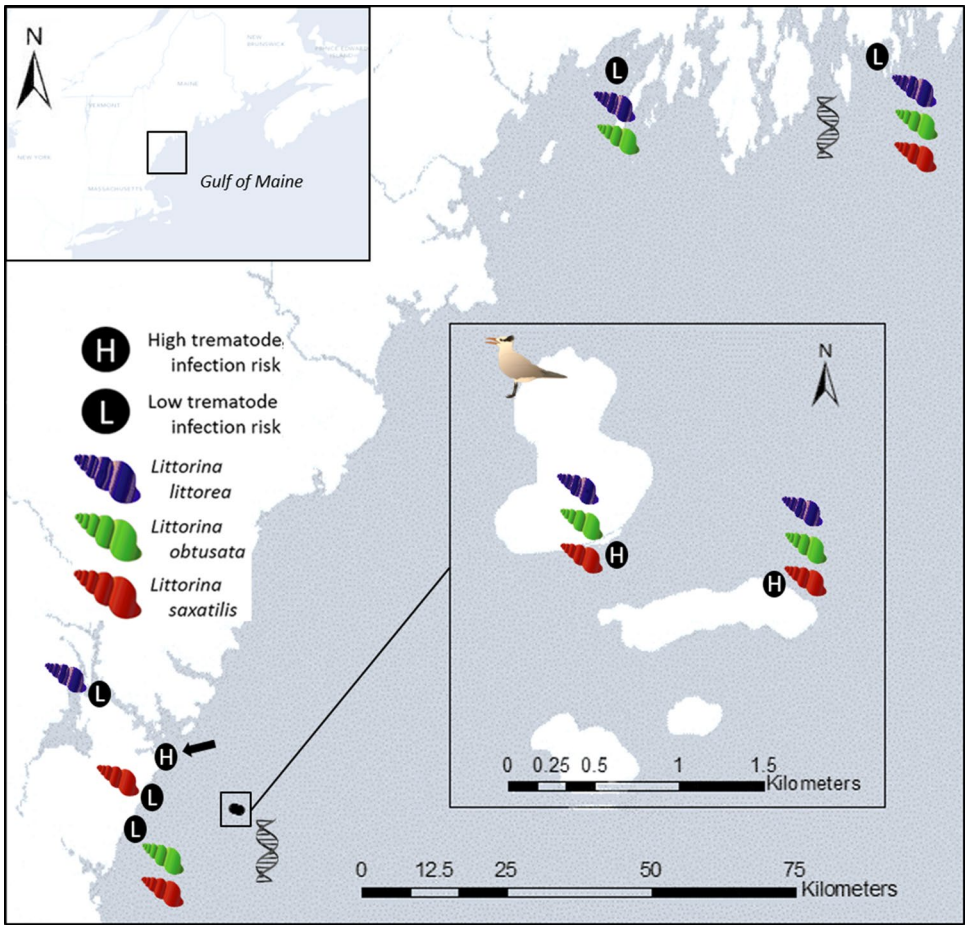

Fig. 1 Spatial distribution of snail-collection sites on the coast of the northeastern USA (New England). *Top left inset* Location of study area within New England. *Lower right inset* Two high trematode infection risk sites. *DNA helixes* denote collection sites for *Microphallus similis* DNA samples. *Bird icon* marks site of experimental guano collection. *Black arrow* points to proposed high trematode infection risk site that was ultimately excluded due to inconsistency in parasite pressure. *Littorina obtusata L. obtusata*, *L. saxatilis Littorina saxatilis*, *L. littorea Littorina littorea*

Table 1 Prevalence of trematode infection in each species of *Littorina* snails from each site of origin in 2011 and in prior studies

Site latitude/longitude	Species collected	Adult % prevalence (N)	Mean size (mm) ± SD	Prior % prevalence (*n*) and source
Appledore, ME[a]	*Littorina littorea*	27.43 (113)	15.51 ± 2.56	29.0 (279)[c]
42°59.09′N	*Littorina obtusata*	15.94 (69)	10.22 ± 1.08	
70°36.79′W	*Littorina saxatilis*	15.94 (69)	10.32 ± 1.42	
Smuttynose, ME[a]	*L. littorea*	23.47 (98)	15.58 ± 3.21	30.7 (205)[c]
42°59.01′N	*L. obtusata*	10.81 (111)	10.58 ± 1.42	
70°36.13′W	*L. saxatilis*	4.67 (107)	9.30 ± 1.60	
Boothbay, ME	*L. littorea*	4.85 (103)	16.42 ± 4.15	9.21 (76)[d]
43°50.55′N	*L. obtusata*	1.06 (94)	8.55 ± 0.94	0.00 (87)[d]
69°37.55′W	*L. saxatilis*	3.85 (52)	9.34 ± 1.29	3.85 (156)[e]
Hilton Park, NH	*L. littorea*	0.00 (78)	15.31 ± 3.55	1.9 (157)[c]
43°07.21′N				
70°49.63′W				
Merepoint, ME	*L. littorea*	1.06 (94)	15.77 ± 3.72	
43°49.41′N	*L. obtusata*	5.71 (105)	10.07 ± 1.02	
70°00.59′W				0.00 (90 *L. saxatilis*)[f]
N. Hampton, NH	*L. obtusata*	1.01 (99)	10.57 ± 1.10	NA
42°57.54′N	*L. saxatilis*	0.00 (81)	9.88 ± 1.35	
70°46.13′W				
Rye Harbor, NH	*L. saxatilis*	0.00 (120)	9.63 ± 1.41	4.96 (121)[g]
43°00.22′N				5.9 (169 *L. littorea*)[c]
70°44.99′W				
Odiorne Point, NH[b]	*L. littorea*	3.03 (99)	16.96 ± 3.80	11.8 (178)[c], 13.25 (317)[d]
43°02.59′N	*L. obtusata*	0.00 (105)	9.96 ± 1.42	8.08 (198)[d]
70°42.65′W	*L. saxatilis*	7.69 (78)	10.17 ± 1.82	26.21 (145)[d]

Not all species were present at sufficient densities to be sampled at each site, so more than six total sites are included

NA Not applicable

[a] Sites designated a priori as high prevalence based on data from prior year(s)

[b] Site designated a priori as high prevalence but dropped due to inconsistency

[c] Sampling carried out in summer 2002 (Byers et al. 2008)

[d] Sampling carried out in summers 2002–2005 (Blakeslee and Byers 2008)

[e] Sampling carried out in 2003 and 2008 (Blakeslee et al. 2012)

[f] Sampling carried out in 2010 (Blakeslee et al. 2012)

[g] Sampling carried out in 2004 (Blakeslee et al. 2012)

high and low intertidal). We measured the collected snails from the apex to the anterior tip of the aperture and then dissected them under a stereomicroscope and examined the gonad and digestive tissues for presence of trematode cercariae, sporocysts or rediae. Trematode species were identified using published keys and descriptions of trematodes infecting *Littorina* sp. (James 1968; Stunkard 1983).

Our estimates of trematode prevalence in each host species at each collection site were evaluated against data from previous studies to confirm or refute each collection site's a priori assignment to the HR or LR category (Byers et al. 2008; Blakeslee and Byers 2008; Blakeslee, unpublished data). Consistency of high parasite pressure was affirmed if trematode infection prevalence in at least one snail species exceeded 10 % in both a previous report and the current study (Table 1). We attempted to collect snails of each historical infection risk category (LR and HR) over as large a domain as possible; however, due to limitations in available qualifying sites, our range of collections was more geographically constrained for HR snails. Specifically, because of the uncertainty in the consistency of parasite-mediated selection pressure at one of our a priori designated HR sites (Odiorne, NH), we excluded snails from this site from our analysis. The two sites that showed consistent infection in the snails are both on gull nesting islands, where gulls and other sea birds are known to aggregate annually at high densities (Ellis and Good 2006), further supporting their categorization as HR sites. These two island sites are relatively geographically

close with the potential for greater connectivity by dispersal for the host species with pelagically dispersed larvae. For the two species with closed populations (*L. obtusata* and *L. saxatilis*), these two collection sites are still likely to represent separate demes, given they are on different islands and these two species can exhibit small-scale genetic differentiation (Johannesson and Tatarenkov 1997; Schmidt et al. 2007).

For the focal experimental snails used in the laboratory common garden experiment, we collected ~200 small-sized adult snails of each species at each site. Small adult size ranges were approximately 4.5- to 8-mm maximum spire height for *L. saxatilis*, 6–9 mm for *L. obtusata*, and 7–12 mm for *L. littorea*. Among sexually mature (adult) snails, smaller individuals were preferred for use in experiments because they are susceptible to trematode infections, but are less likely to harbor infections than larger, older adults which are more likely to have previously encountered trematode infective stages (Graham 2003). Collected snails were held in sea tables with running ambient seawater at the Shoals Marine Laboratory, Appledore Island, ME.

Common garden infection experiment

We used a common garden experiment to test whether a history of trematode infection risk (i.e., HR versus LR) affected susceptibility to trematode infection in each host species. A random subset of 50–100 snails of each species in the small-adult size class from each site was painted to denote their collection site using BriteMark paint pens. We combined these groups of snails in a single tray of a flowing seawater table, evenly distributing them into one of two identical 1.4-m^2 mesh bags (made from 0.33-mm^2 black screening material) populated with rocks and *Ascophyllum* seaweed to form a semi-natural habitat. The water was drained from the sea table tray once daily for a period of 2–3 h to simulate a low tide, and the tray was rinsed regularly to prevent the buildup of snail waste and algae. We exposed all experimental snails to infective trematode eggs by adding seabird guano, which contains trematode eggs from infected birds, to the common garden habitat. Bird guano was collected from a common seabird loitering area that was separate from any snail collection site (Fig. 1). Dissections of snails inhabiting this guano collection area over the 10 past years have consistently shown high levels of trematode prevalence in this area, suggesting that the site is a hotspot for transmission from infected birds to the snail first intermediate hosts (Byers et al. 2008, 2015). During the guano collection period, daily counts ($n = 11$) of seabirds utilizing the intertidal area (~100 m × 30 m) from which the majority of guano was collected averaged 27.5 ± 2.8 (mean ± SD) black back gulls (*Larus marinus*), 2.2 ± 0.7 double-crested cormorants (*Phalacrocorax auritus*), and 4.0 ± 1.3 herring gulls (*Larus argentatus*).

Fresh guano deposits were scraped off the rocky substrate into a 50-mL plastic tube, and diluted in a 1:2 ratio with seawater to form a homogeneous slurry. This slurry was either poured evenly across the habitat in both snail-containment bags during the simulated low tide each day or used to coat fresh seaweed (usually *Ascophyllum* with a mix of *Ulva* and *Fucus* sp.) which was then distributed in the habitat as a supplemental food source. For a period of 24 days, an average of 12.5 ± 1.3 mL guano day^{-1} was added to each bag (i.e., 37.5 mL per bag after seawater dilution), alternating between the two application methods. This volume of guano is substantially higher than would be encountered daily over a similar area in nature where the deposits are discrete and patchy; however, using the large diluted volume allowed us to ensure an even distribution across the bags to standardize access to infective stages for the experimental snails. After the inoculation period, the snails were maintained in the sea table habitat for an additional 4 weeks, and in aerated seawater tanks for an additional 2 weeks to allow any induced infections to reach patency. After this 6-week incubation period, all experimental snails were dissected to assess infection status.

The other half of the collected small adult snails were used to control for baseline levels of background field-acquired infection at each site, and were maintained in a separate sea table from the experimental snails to prevent parasite exposure. These control snails were maintained in Tupperware containers with mesh sides to allow water circulation, and were separated by collection site. The snails were provided with seaweeds collected from a cove on Appledore Island with low bird density and low natural trematode infection in snails to avoid introducing new infections. We subjected these snails to the same simulated tidal cycle and sea table cleaning schedule as the experimental snails. The snails were held for a minimum of 4 weeks to allow any field-acquired infections to reach patency, and then dissected over the course of 4 weeks. Though these control snails were necessarily maintained under slightly different conditions to prevent the acquisition of new infections, they should accurately quantify the baseline prevalence of pre-existing infections in wild-caught snails in the experimental size class.

Data analysis

Because induced infection levels were clearly quite different between the three snail species, and the species of trematode infecting one of the sentinel snail species was different, we analyzed each species separately. Each individual snail was considered an independent replicate, with a binomial response of infected or non-infected. Because one a priori HR site was dropped due to inconsistency in parasite pressure (Odiorne), our HR category encompasses

snails from two collection sites, and the LR category represents snails from three collection sites. To focus our analysis on the impact of parasite-mediated selection in determining each snail's response to exposure, we statistically accounted for collection site-level impacts by including individual collection site as a random variable in our model, and used the category of infection risk history (HR versus LR) as a fixed effect.

Thus for each species, our analysis consisted of a binomial generalized linear mixed effects model with experimental treatment (control or guano addition), infection risk history (HR or LR), and their interaction as fixed effects, and individual collection sites included as a random effect. The interaction term (experimental treatment × infection risk history) is particularly important because it relates whether experimental trematode exposure differentially affects snails with HR versus LR histories. Also, holding treatment constant (exposed), we calculated the differences in the log odds of infection for LR- relative to HR-sourced snails. Specifically, we used exponentiation for the sum of the generalized linear mixed effects model parameter estimates for the fixed effects of infection risk history and the interaction of risk history with experimental treatment. Analyses were carried out in RStudio version 0.98.1056 (RStudio 2012).

Trematode genetic structure

Our infection experiment assumed that the site of origin of the trematode eggs would not influence infectivity in snails from different sites due to relative genetic homogeneity of the trematode populations across the area of our study. To test this assumption, and to evaluate the overall amount of genetic admixture in the parasite population, we assessed population genetic structure of the most common trematode species in our study, *M. similis,* across two sites that span the geographic range of our study. In June 2012, trematode tissue samples were collected from all *M. similis*-infected snails originating at Appledore Ledges and from a site ~170 km away on Damariscove Island in Boothbay Harbor, ME, a geographically distant site that had a sufficient number of *M. similis* infections to conduct the test (Fig. 1). A single trematode DNA sample was collected from each *M. similis*-infected individual from Damariscove Island and from Appledore Island, and saved in 95 % ethanol. DNA extraction was carried out using Purgene (Gentra) reagents. We amplified a mitochondrial CO1 gene using primers published for a different microphallid species (Leung et al. 2009). Sequencing was carried out by Macrogen and alignment of the resulting sequences was carried out in Geneious version 6.1.2 (Biomatters). We used Hudson's nearest-neighbor statistic (Hudson 2000), which is robust to small sample sizes, to test for genetic differentiation between the two collection sites, using ten sequences from the Damariscove site, and six sequences from Appledore Ledges. Values near 1 indicate strong differentiation, while values near 0.5 indicate low heterogeneity or panmixis (Hudson 2000). The statistic and associated *p*-values were calculated in DNAsp (Librado and Rozas 2009).

Results

Common garden infection experiment

In our common garden infection experiment, naturally collected seabird guano produced trematode infections in all three snail species, especially *L. obtusata* and *L. saxatilis*. Infection prevalence in these two species increased from near 0 % in control snails to 54.5 and 82.9 %, respectively. Infection levels in *L. littorea* were markedly lower, increasing only by 6.5 % points overall (Fig. 2).

For the two direct developers (*L. obtusata, L. saxatilis*), our model indicated a significant interaction of infection risk history (HR or LR) and experimental exposure on infection (Table 2), after accounting for variation due to collection site-level differences. Experimentally exposed snails from LR locations had 2.13 times greater odds of becoming infected relative to HR history snails for *L. obtusata*, and 3.11 times greater odds of infection for *L. saxatilis*.

On the other hand, the interaction of infection risk history with exposure was not significant for the broadcast spawning species, *L. littorea*. Snails from both HR- and LR-history sites increased in infection, with HR snails trending toward higher infection than LR snails. Because the interaction of infection risk history and experimental exposure was not significant, we did not calculate the ratio of odds of infection for this species.

Many of the infections observed during dissections of experimentally infected snails were immature and did not possess mature cercariae with distinguishing morphological characteristics. However, toward the end of the 4-week period that was required to complete dissections of experimental snails, many infections had developed distinguishing characteristics, and the majority of these were identified as *M. similis* infections.

Trematode genetic structure

Samples of *M. similis* separated by 170 km represented 11 haplotypes (Supplemental Fig. 1) across the >800-base pair mitochondrial CO1 gene region we sequenced. There was no differentiation based on site of origin, suggesting high gene flow at the spatial scale of our study (Hudson's nearest-neighbor statistic = 0.64, $p = 0.08$).

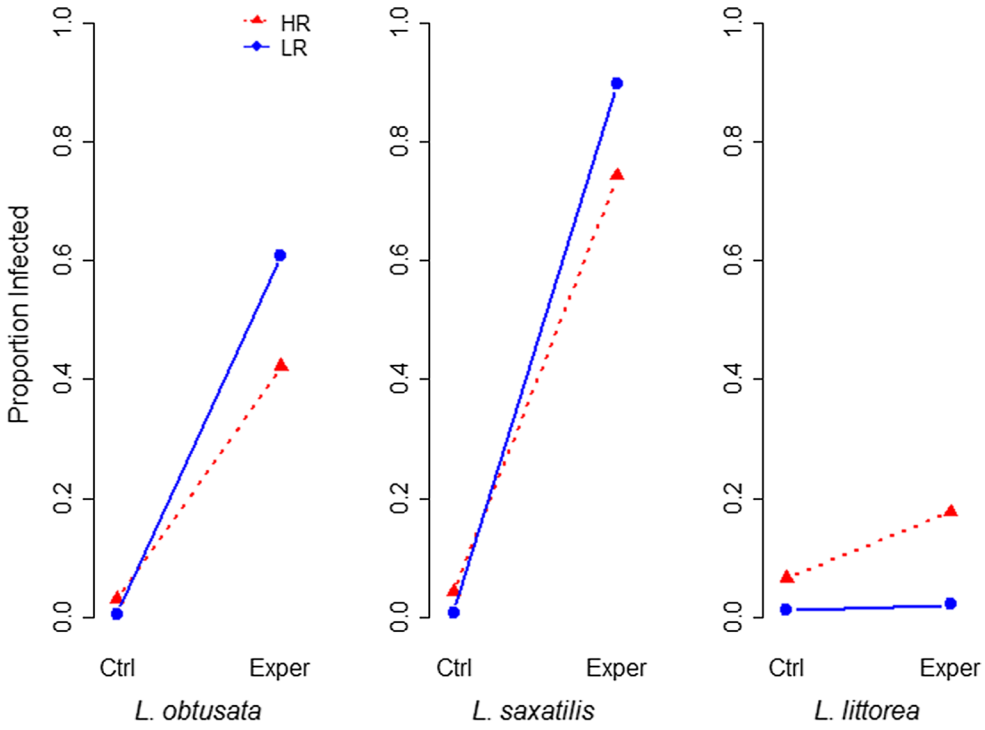

Fig. 2 Change in proportion of snails infected after experimental exposure in common garden (*Exper*) for each species relative to baseline infection prevalence (*Ctrl*), showing differences for each species in response among snails with a history of a high risk (*HR*; *red triangles*) and a low risk (*LR*; *blue circles*) of infection

Table 2 Results of binomial generalized linear mixed models for the outcome of infection for each species

	Parameter estimate	SE	*z*-value	*P* (>|*z*|)
L. obtusata				
Intercept	−3.46	0.41	−8.35	<0.001
Treatment (Exp.)	3.14	0.46	6.83	<0.001
Category (LR)	−1.85	1.08	−1.71	0.088
Treat. × Category	2.61	1.11	2.35	0.019
	Variance	Variance SD		
Site ID	<0.001	<0.001		
L. saxatilis				
Intercept	−3.11	0.41	−7.68	<0.001
Treatment (Exp.)	4.17	0.49	8.56	<0.001
Category (LR)	−1.96	0.82	−2.37	0.018
Treat. × Category	3.09	0.92	3.36	<0.001
	Variance	Variance SD		
Site ID	0.026	0.16		
L. littorea				
Intercept	−3.10	1.17	−2.64	<0.01
Treatment (Exp.)	1.18	0.34	3.47	<0.001
Category (LR)	−2.80	1.88	−1.49	0.14
Treat. × Category	−0.44	0.77	−0.57	0.57
	Variance	Variance SD		
Site ID	2.55	1.60		

Each model includes fixed effects of infection risk history [high risk (*HR*) or low risk (*LR*)] and experimental treatment (*Treat.*) [control or exposed (*Exp.*)], along with their interaction, and also includes collection site identity (*ID*) as a random effect. The reference categories are HR (risk history) and control (treatment)

Discussion

Adaptation of hosts to local parasite-mediated selective pressure is an important coevolutionary process with strong ramifications on disease dynamics in wild populations. Adaptation in the hosts is predicated on the strength of selection pressure exerted by the parasites relative to defense costs, the consistency of such pressure across a time frame relevant to the development of a response, and the isolation of the population under selection from disruptive introduction of maladapted genotypes. In our study system, castrating trematode parasites exert strong selective pressure on snail hosts to avoid or defend against infection. In agreement with our expectation, we found that for the two host species with relatively closed populations, snails from sites with consistently high parasite pressure were significantly less susceptible to infection than hosts that were more naïve to infection threats (Fig. 2). Our results contribute to a growing body of evidence that anti-parasite defenses involve trade-offs for hosts, such that strong defense is only selected for when the probability of infection is consistently high. The results also suggest that host gene flow plays a role in governing hosts' ability to locally adapt to parasite infection risk. The effect of prior exposure to reduce susceptibility was specific to our two host species with local recruitment. The one host species we examined with open populations, *L. littorea*, did not exhibit evidence of lower susceptibility among snails with higher prior exposure to trematodes, suggesting that they may not adapt to match local parasite risk. However, this result could also

stem from the lower overall susceptibility of this host species to the predominant trematode obtained from the field (*M. similis*), limiting the power of our experiment to detect adaptation in this species.

Effect of prior exposure

Consistent use of particular sites, such as our HR island sites, by seabird definitive hosts results in high levels of parasite infection risk for snails across generations (Byers et al. 2008; Fredensborg et al. 2006; Levakin et al. 2013), and this can impose directional selection on snail resistance mechanisms such as immune defenses (Gorbushin and Borisova 2014; Iakovleva et al. 2006). From the host perspective, consistent parasite-mediated selective pressure should enhance host immunity over generations via a combination of changes in frequencies of relevant alleles and epigenetic inheritance (Ardia et al. 2011). Our findings of higher defense among snails from HR-history sites are consistent with other studies indicating that immune defenses are only selected for when parasite infection risk is sufficiently high and is stable across generations (Bryan-Walker et al. 2007; Hasu et al. 2009; Kawecki and Ebert 2004), whereas defenses are selected against when infection risk is low because of costs of immune defense such as resource diversion or self-harm (Sheldon and Verhulst 1996). Among our experimental *L. obtusata* and *L. saxatilis*, we observed higher mortality during the experiment among snails originating from HR sites, which could reflect a cost of higher defense investment.

Non-immunological sources of defense such as behavioral avoidance, self-medication, beneficial symbionts, and fecundity compensation may also constitute important components of a host's response to parasites (Parker et al. 2011). For example, *L. littorea* are able to detect and behaviorally avoid seabird guano, the delivery medium of the trematode eggs, at exceedingly small concentrations (Davies and Knowles 2001). While this avoidance behavior may operate in habitats where guano is relatively rare, the extensive guano coverage achieved in our common garden experimental enclosures likely eliminated this defense option, instead enhancing reliance on other defense mechanisms, including immune defenses. If infection risk and immune defense costs are high, hosts demes experiencing consistent parasite pressure may be selected to tolerate some level of infection in favor of maximizing reproductive effort under the threat of castration. In White Sea populations of *L. saxatilis*, uninfected snails from HR locations compensate for the high likelihood of castration by having higher age-specific fecundity than uninfected females from LR areas (Granovitch et al. 2009). Different versions of this "fecundity compensation" have been observed in several other snail hosts that experience high trematode infection risk (Fredensborg and Poulin 2006; Ibikounle et al. 2012; Jokela and Lively 1995; Lafferty 1993). Whether this tolerance response operates in addition to higher defense responses in our host populations is another area for future study.

Effect of larval dispersal mode

High larval retention in the parent habitat that results from the direct development life history of *L. obtusata* and *L. saxatilis* promotes the adaptation of resistance in these two hosts to local levels of parasite risk. In some cases limited mixing can be a detriment, but only if genetic diversity is so limited that it minimizes the chance of adaptive alleles being present. Our data suggest that the restricted gene flow of the two direct developing snail species and the resulting minimal disruptive immigration of non-adapted immigrant genotypes gives them an advantage in adapting to their more broadly dispersed trematode parasites. It also implies costliness to maintaining resistance when it is not needed (Sheldon and Verhulst 1996). Our test for gene flow within the focal parasite species, *M. similis*, in agreement with prior studies of trematodes, shows a high admixture of parasite genotypes at the spatial scale of our study, reflecting its relatively broad dispersal by seabird definitive hosts (Dybdahl and Lively 1996; Keeney et al. 2008, 2009; Prugnolle et al. 2005).

The relatively high amount of genetic mixing in *M. similis*, in addition to its generalist tendency to infect multiple snail species, suggests that the collection site of the parasite should not strongly influence its infectivity in hosts from different locations. Indeed, in our study, if the relative proximity of the two HR sites to the guano collection site had positively influenced infectivity of the parasites from nearer sites (i.e., parasites performing better in sympatric hosts), we would expect the snails from these sites to have shown a greater increase in infection than snails from more distant sites. Instead, the fact that *L. obtusata* and *L. saxatilis* from sites closer to the parasite collection site had lower odds of experimental infection than more distant LR-site snails supports our overall hypothesis that the hosts have the advantage in the host-parasite arms race thanks to their local recruitment promoting adaptation to local parasite-mediated selection. Future experiments that reciprocally test the susceptibility of multiple HR-site snails to sympatric versus allopatric parasite populations would provide a more formal test of the coevolutionary arms race (Hoeksema and Forde 2008; Kawecki and Ebert 2004), building on our more general approach comparing host performance based on high versus low infection risk history.

From the parasite's perspective, a high level of mixing relative to their hosts does not necessarily beget a coevolutionary disadvantage (Gandon et al. 1996; Lively 1999),

and could even be advantageous if other aspects of the parasites' biology allowed positive new alleles to be quickly spread through the parasite population (e.g., fast generation times, direct transmission). However, in our particular host-parasite system there are several factors at play that may limit adaptation of the parasite to local host demes. First, the amplification of a subset of parasite genotypes that occurs when the trematode reproduces clonally within the first intermediate snail host may serve to reduce the effective population size of the parasite, reducing its evolutionary potential (Jarne and Theron 2001; Mulvey et al. 1991). Second, the complex three-host life cycle of the parasite may considerably lengthen the parasite's generation time, restricting its evolutionary pace relative to other types of parasites (Barrett et al. 2008). Third, because the primary trematode found infecting our experimental snails, *M. similis*, is thought to be a generalist species that infects multiple first intermediate host species, specialization by the parasite may be actively selected against in our system (Barrett et al. 2008; Gandon 2002; Roth et al. 2012). This final point may also be a key difference from other trematode-snail systems in which parasites appear to have the coevolutionary upper hand (Lively and Dybdahl 2000; Lively et al. 2004), or in which neither host nor parasite demonstrates local adaptation (Prugnolle et al. 2006).

Because *L. littorea* has pelagic larvae and thus higher genetic admixture within the metapopulation, we expected this species to act as an outgroup in terms of capacity for local adaptation when compared to the two direct-developing species. Indeed, although we did see a significant effect of treatment and a strong trend of influence of infection risk history (HR versus LR) on infection in *L. littorea*, we did not see an interaction effect indicative of local adaptation, perhaps because field-acquired infections contributed a relatively large proportion of ultimate infections in the HR snails. It is possible that the snail's likelihood of locally adapting to its trematode parasites could also be influenced by a disruption in their coevolutionary dynamics resulting from *L. littorea*'s invasion history. We think this explanation is unlikely to contribute heavily to the observed pattern of minimal local adaptation because: (1) *L. littorea* is still heavily infected by trematodes at sites within northeastern North America, including the two HR sites in the current study (Table 1) (Byers et al. 2008); and (2) the associations between *L. littorea* and its trematodes are not novel since all five of its trematode parasites in North America are found in the snail's native range in Europe (Blakeslee and Byers 2008). Overall levels of induced infection in *L. littorea* were much lower than the levels induced in the other two host species. While the relatively low amount of infection achieved in *L. littorea* may seem to suggest a high amount of defense by this host, the levels of infection experimentally induced in our three target species are not readily comparable because of the difference in trematode species that preferentially utilize these hosts. The majority of identifiable infections that were induced experimentally in *L. obtusata* and *L. saxatilis* were identified as *M. similis*, a species which usually occurs at very low levels in *L. littorea* in the field (Blakeslee and Byers 2008). The species of trematode that constitutes the majority of infections in *L. littorea* in the field [*Cryptocotyle lingua* (Byers et al. 2008)] did not seem to be amenable to our fecal manipulation. Thus a reduced chance of experimental infection may have reduced the power of our experiment to detect differences in adaptation between *L. littorea* from LR and HR sites. Further experiments that are successful in inducing *C. lingua* infection in *L. littorea* would allow for greater comparison of the defense abilities across the three snail host species.

Conclusion

Coevolutionary relationships between parasites and hosts are antagonistic and dynamic, providing a fertile testing ground for the ecological and evolutionary importance of local adaptation. The ability of individual hosts to resist or limit parasite attack has implications for disease emergence and persistence at population and meta-population levels. Our experimental approach demonstrates that, for two species with local recruitment, historical parasite exposure can alter hosts' susceptibility to infection. The expansion of this approach to include increased replication at the level of dispersal mode (i.e., host species identity) would provide a more robust test of the effect of dispersal on host local adaptation. Future studies may build on these findings to uncover potential feedbacks of host local adaptation to disease emergence and spread.

Acknowledgments The authors are grateful for the assistance of C. F. Malcolm, J. P. Wares, O. Miura, K. Bockrath, C. Ewers, and S. Budischak, and for helpful feedback from three reviewers. C. L. K. was funded by the National Science Foundation Graduate Research Fellowship Program, and additional funding for M. E. S. and C. L. K. was provided by the Shoals Marine Laboratory Research Internships in Field Science Program. J. E. B. provided funding through a grant from the University of Georgia Office of the Vice President for Research. This is contribution no. 171 of the Shoals Marine Laboratory.

Author contribution statement C. L. K. and J. E. B. designed the study. M. E. S. and C. L. K. executed the study. C. L. K. and J. E. B. analyzed the data. C. L. K. drafted the manuscript, and all authors contributed substantially to revisions.

References

Ardia DR, Parmentier HK, Vogel LA (2011) The role of constraints and limitation in driving individual variation in immune response. Funct Ecol 25:61–73. doi:10.1111/j.1365-2435.2010.01759.x

Barrett LG, Thrall PH, Burdon JJ, Linde CC (2008) Life history determines genetic structure and evolutionary potential of host–parasite interactions. Trends Ecol Evol 23:678–685. doi:10.1016/j.tree.2008.06.017

Berger EM (1973) Gene-enzyme variation in 3 sympatric species of *Littorina*. Biol Bull 145:83–90. doi:10.2307/1540349

Blakeslee AMH, Byers JE (2008) Using parasites to inform ecological history: comparisons among three congeneric marine snails. Ecology 89:1068–1078. doi:10.1890/07-0832.1

Blakeslee AMH, Byers JE, Lesser MP (2008) Solving cryptogenic histories using host and parasite molecular genetics: the resolution of *Littorina littorea*'s North American origin. Mol Ecol 17:3684–3696. doi:10.1111/j.1365-294X.2008.03865.x

Blakeslee AMH, Altman I, Miller AW, Byers JE, Gregory CEH (2012) Parasites and invasions: a biogeographic examination of parasites and hosts in native and introduced ranges. J Biogeogr 39:609–622. doi:10.1111/j.1365-2699.2011.02631.x

Bryan-Walker K, Leung TLF, Poulin R (2007) Local adaptation of immunity against a trematode parasite in marine amphipod populations. Mar Biol 152:687–695. doi:10.1007/s00227-007-0725-x

Byers JE, Blakeslee AMH, Linder E, Cooper AB, Maguire TJ (2008) Controls of spatial variation in the prevalence of trematode parasites infecting a marine snail. Ecology 89:439–451

Byers JE et al (2015) Opposing selective pressures decouple pattern and process of parasitic infection over small spatial scale. Oikos. doi:10.1111/oik.02088

Davies MS, Knowles AJ (2001) Effects of trematode parasitism on the behaviour and ecology of a common marine snail (*Littorina littorea* (L.)). J Exp Mar Biol Ecol 260:155–167. doi:10.1016/s0022-0981(01)00250-7

Downs CJ, Adelman JS, Demas GE (2014) Mechanisms and methods in ecoimmunology: integrating within-organism and between-organism processes. Integr Comp Biol 54:340–352. doi:10.1093/icb/icu082

Dybdahl MF, Lively CM (1996) The geography of coevolution: comparative population structures for a snail and its trematode parasite. Evolution 50:2264–2275. doi:10.2307/2410696

Ellis JC, Good TP (2006) Nest attributes, aggression, and breeding success of gulls in single and mixed species subcolonies. Condor 108:211–219. doi:10.1650/0010-5422(2006)108[0211:NAAABS]2.0.CO;2

Fredensborg BL, Poulin R (2006) Parasitism shaping host life-history evolution: adaptive responses in a marine gastropod to infection by trematodes. J Anim Ecol 75:44–53. doi:10.1111/j.1365-2656.2005.01021.x

Fredensborg BL, Mouritsen KN, Poulin R (2006) Relating bird host distribution and spatial heterogeneity in trematode infections in an intertidal snail-from small to large scale. Mar Biol 149:275–283. doi:10.1007/s00227-005-0184-1

Gandon S (2002) Local adaptation and the geometry of host-parasite coevolution. Ecol Lett 5:246–256. doi:10.1046/j.1461-0248.2002.00305.x

Gandon S, Capowiez Y, Dubois Y, Michalakis Y, Olivieri I (1996) Local adaptation and gene-for-gene coevolution in a metapopulation model. Proc R Soc B-Biol Sci 263:1003–1009. doi:10.1098/rspb.1996.0148

Gorbushin AM, Borisova EA (2014) *Himasthla elongata*: implantation of rediae to the specific iteroparous long-living host, *Littorina* littorea, results in the immune rejection. Fish Shellfish Immun 39:432–438. doi:10.1016/j.fsi.2014.05.035

Graham AL (2003) Effects of snail size and age on the prevalence and intensity of avian schistosome infection: relating laboratory to field studies. J Parasitol 89:458–463. doi:10.1645/0022-3395(2003)089[0458:eossaa]2.0.co;2

Granovitch AI, Sergievsky SO, Sokolova IM (2000) Spatial and temporal variation of trematode infection in coexisting populations of intertidal gastropods *Littorina saxatilis* and *L. obtusata* in the White Sea. Dis Aquat Org 41:53–64. doi:10.3354/dao041053

Granovitch AI, Yagunova EB, Maximovich AX, Sokolova IM (2009) Elevated female fecundity as a possible compensatory mechanism in response to trematode infestation in populations of *Littorina saxatilis* (Olivi). Int J Parasitol 39:1011–1019. doi:10.1016/j.ijpara.2009.02.014

Hasu T, Benesh DP, Valtonen ET (2009) Differences in parasite susceptibility and costs of resistance between naturally exposed and unexposed host populations. J Evol Biol 22:699–707. doi:10.1111/j.1420-9101.2009.01704.x

Hawley DM, Altizer SM (2011) Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. Funct Ecol 25:48–60. doi:10.1111/j.1365-2435.2010.01753.x

Hoeksema JD, Forde SE (2008) A meta-analysis of factors affecting local adaptation between interacting species. Am Nat 171:275–290. doi:10.1086/527496

Hoff C (1941) A case of correlation between infection of snail hosts with *Cryptocotyle lingua* and the habits of gulls. J Parasitol 27:539

Hudson RR (2000) A new statistic for detecting genetic differentiation. Genetics 155:2011–2014

Iakovleva NV, Shaposhnikova TG, Gorbushin AM (2006) Rediae of echinostomatid and heterophyid trematodes suppress phagocytosis of haemocytes in *Littorina littorea* (Gastropoda: Prosobranchia). Exp Parasitol 113:24–29. doi:10.1016/j.exppara.2005.12.002

Ibikounle M et al (2012) Life-history traits indicate local adaptation of the schistosome parasite, *Schistosoma mansoni*, to its snail host, *Biomphalaria pfeifferi*. Exp Parasitol 132:501–507. doi:10.1016/j.exppara.2012.09.020

James BL (1968) The distribution and keys of species in the family Littorinidae and of their digenean parasites, in the region of Dale, Pembrokeshire. Field Stud 2:615–650

Jarne P, Theron A (2001) Genetic structure in natural populations of flukes and snails: a practical approach and review. Parasitology 123:27–40. doi:10.1017/S0031182001007715

Johannesson K (2003) Evolution in *Littorina:* ecology matters. J Sea Res 49:107–117. doi:10.1016/s1385-1101(02)00218-6

Johannesson B, Johannesson K (1996) Population differences in behaviour and morphology in the snail *Littorina saxatilis*: phenotypic plasticity or genetic differentiation? J Zool 240:475–493

Johannesson K, Tatarenkov A (1997) Allozyme variation in a snail (*Littorina saxatilis*)—deconfounding the effects of microhabitat and gene flow. Evolution 51:402–409. doi:10.2307/2411112

Jokela J, Lively CM (1995) Parasites, sex, and early reproduction in a mixed population of freshwater snails. Evolution 49:1268–1271. doi:10.2307/2410451

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7:1225–1241. doi:10.1111/j.1461-0248.2004.00684.x

Keeney DB, Bryan-Walker K, King TM, Poulin R (2008) Local variation of within-host clonal diversity coupled with genetic homogeneity in a marine trematode. Mar Biol 154:183–190. doi:10.1007/s00227-008-0914-2

Keeney DB, King TM, Rowe DL, Poulin R (2009) Contrasting mtDNA diversity and population structure in a direct-developing marine gastropod and its trematode parasites. Mol Ecol 18:4591–4603. doi:10.1111/j.1365-294X.2009.04388.x

Lafferty KD (1993) The marine snail, *Cerithidea californica*, matures at smaller sizes where parasitism is high. Oikos 68:3–11. doi:10.2307/3545303

Lenormand T (2002) Gene flow and the limits to natural selection. Trends Ecol Evol 17:183–189. doi:10.1016/S0169-5347(02)02497-7

Leung TLF, Donald KM, Keeney DB, Koehler AV, Peoples RC, Poulin R (2009) Trematode parasites of Otago Harbour (New Zealand) soft-sediment intertidal ecosystems: life cycles, ecological roles and DNA barcodes. N Z J Mar Freshwater 43:857–865

Levakin IA, Nikolaev KE, Galaktionov KV (2013) Long-term variation in trematode (Trematoda, Digenea) component communities associated with intertidal gastropods is linked to abundance of final hosts. Hydrobiologia 706:103–118. doi:10.1007/s10750-012-1267-x

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452. doi:10.1093/bioinformatics/btp187

Lively CM (1999) Migration, virulence, and the geographic mosaic of adaptation by parasites. Am Nat 153:S34–S47. doi:10.1086/303210

Lively CM, Dybdahl MF (2000) Parasite adaptation to locally common host genotypes. Nature 405:679–681. doi:10.1038/35015069

Lively CM, Dybdahl MF, Jokela J, Osnas EE, Delph LF (2004) Host sex and local adaptation by parasites in a snail-trematode interaction. Am Nat 164:S6–S18. doi:10.1086/424605

Martin LB, Hawley DM, Ardia DR (2011) An introduction to ecological immunology. Funct Ecol 25:1–4. doi:10.1111/j.1365-2435.2010.01820.x

Matthews PM, Montgomery WI, Hanna REB (1985) Infestation of littorinids by larval Digenea around a small fishing port. Parasitology 90:277–287

Mulvey M, Aho JM, Lydeard C, Leberg PL, Smith MH (1991) Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. Evolution 45:1628–1640. doi:10.2307/2409784

Parker BJ, Barribeau SM, Laughton AM, de Roode JC, Gerardo NM (2011) Non-immunological defense in an evolutionary framework. Trends Ecol Evol 26:242–248. doi:10.1016/j.tree.2011.02.005

Prugnolle F et al (2005) Dispersal in a parasitic worm and its two hosts: consequence for local adaptation. Evolution 59:296–303

Prugnolle F, De Meeus T, Pointier JP, Durand P, Rognon A, Theron A (2006) Geographical variations in infectivity and susceptibility in the host-parasite system *Schistosoma mansoni/Biomphalaria glabrata*: no evidence for local adaptation. Parasitology 133:313–319. doi:10.1017/s0031182006000412

Reid DG (1996) *Littorina littorea*, *Littorina obtusata*, and *Littorina saxatilis*. In: D. G. Reid(ed) Systematics and evolution of Littorina.The Ray Society, Andover, Hampshire, UK, pp 95–120, 196–227,278–340

Robson EM, Williams IC (1970) Relationships of some species of Digenea with the marine prosobranch *Littorina littorea*. Part 1. The occurrence of larval Digenea in *Littorina littorea* on the North Yorkshire coast. J Helminthol 44:153–168

Roth O, Keller I, Landis SH, Salzburger W, Reusch TBH (2012) Hosts are ahead in a marine host-parasite coevolutionary arms race: innate immune system adaptation in pipefish *Syngnathus typhle* against *Vibrio* phylotypes. Evolution 66:2528–2539. doi:10.1111/j.1558-5646.2012.01614.x

Rothschild M (1942) A seven-year-old infection of *Cryptocotyle lingua* Creplin in the winkle *Littorina littorea* L. J Parasitol 28:350. doi:10.2307/3272976

RStudio (2012) RStudio: integrated develop environment for R. v. 0.98.1056, Boston

Schmidt PS, Phifer-Rixey M, Taylor GM, Christner J (2007) Genetic heterogeneity among intertidal habitats in the flat periwinkle, *Littorina obtusata*. Mol Ecol 16:2393–2404. doi:10.1111/j.1365-294X.2007.03323.x

Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317–321. doi:10.1016/0169-5347(96)10039-2

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792. doi:10.1126/science.3576198

Snyder TP, Gooch JL (1973) Genetic differentiation in *Littorina saxatilis* (Gastropoda). Mar Biol 22:177–182. doi:10.1007/bf00391781

Stunkard HW (1930) The life history of *Cryptocotyle lingua* (Creplin), with notes on the physiology of the metacercariae. J Morphol Physiol 50:143–191. doi:10.1002/jmor.1050500106

Stunkard HW (1957) The morphology and life history of the digenetic trematode, *Microphallus similis* (Jagerskiold, 1900) Baer, 1943. Biol Bull 112:254–266. doi:10.2307/1539203

Stunkard HW (1983) The marine cercariae of the Woods-Hole, Massachusetts region, a review and revision. Biol Bull 164:143–162. doi:10.2307/1541135

Urban MC (2011) The evolution of species interactions across natural landscapes. Ecol Lett 14:723–732. doi:10.1111/j.1461-0248.2011.01632.x

Warner RR (1997) Evolutionary ecology: how to reconcile pelagic dispersal with local adaptation. Coral Reefs 16:S115–S120. doi:10.1007/s003380050247

Yamada SB (1989) Are direct developers more locally adapted than planktonic developers? Mar Biol 103:403–411. doi:10.1007/bf00397275

Zuk M, Stoehr AM (2002) Immune defense and host life history. Am Nat 160:S9–S22