Fine-Scale Spatial Covariation between Infection Prevalence and Susceptibility in a Natural Population

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ABSTRACT: The prevalence of infection varies dramatically on a fine spatial scale. Many evolutionary hypotheses are founded on the assumption that this variation is due to host genetics, such that sites with a high frequency of alleles conferring susceptibility are associated with higher infection prevalence. This assumption is largely untested and may be compromised at finer spatial scales where gene flow between sites is high. We put this assumption to the test in a natural snail-trematode interaction in which host susceptibility is known to have a strong genetic basis. A decade of field sampling revealed substantial spatial variation in infection prevalence between 13 sites around a small lake. Laboratory assays replicated over 3 years demonstrate striking variation in host susceptibility among sites in spite of high levels of gene flow between sites. We find that mean susceptibility can explain more than one-third of the observed variation in mean infection prevalence among sites. We estimate that variation in susceptibility and exposure together can explain the majority of variation in prevalence. Overall, our findings in this natural host-parasite system argue that spatial variation in infection prevalence covaries strongly with variation in the distribution of genetically based susceptibility, even at a fine spatial scale.

Keywords: infection prevalence, susceptibility, snail-trematode, spatial variation, *Potamopyrgus antipodarum*, *Microphallus*.

Introduction

The prevalence of infection varies in space. This heterogeneity is evident on a very fine scale for a wide range of host-parasite systems, including dengue virus (Yoon et al. 2012), anther smut (Burdon and Thrall 1999, fig. 2), cholera (Snow 1855), powdery mildew (Laine 2006), and human schistosomiasis (Rudge et al. 2008). The difference between nearby sites can be dramatic. For example, Woolhouse and Chandiwana (1989) measured infection prevalence of the freshwater

snail *Bulinus globosus* with the trematode agent of human schistosomiasis. At 22 sites along an 860-m stretch of river, they discovered that infection prevalence ranged from 0% to 60%. Their study exemplifies one of the motivations for documenting and explaining heterogeneity in infection prevalence: the density of snails and their frequency of infection at a given site determine a human individual's risk of contracting schistosomiasis (Woolhouse and Chandiwana 1990; Clennon et al. 2006).

Why does the prevalence of infection vary so much on small spatial scales? We are gaining a greater understanding of how ecological factors influence variation in infection prevalence. These factors include habitat structure (Grosholz 1993; Grosholz and Ruiz 1995; Penczykowski et al. 2014), competition (Grosholz 1992; Hall et al. 2009), enrichment and land use (Johnson et al. 2007; McKenzie 2007; King et al. 2010), resources (Duffy et al. 2012; Satterfield et al. 2015), temperature and climate (Linthicum et al. 1999; Stapp et al. 2004; Bruno et al. 2007), and the presence of roads (Altman and Byers 2014; Jousimo et al. 2014).

In contrast, we know much less about how infection prevalence is affected by genetic variation for susceptibility (as reviewed in Little 2002). A core assumption of many evolutionary hypotheses is that hosts evolve in response to parasite-mediated selection (e.g., maintenance of polymorphism at infection loci and the Red Queen; Haldane 1949; Jaenike 1978; Hamilton 1980; Hedrick 1994; Apanius et al. 1997; Dangl and Jones 2001; Hughes 2002; Watson et al. 2005), which requires standing genetic variation for resistance or susceptibility. However, variation due to environmental factors might overwhelm any contribution of genetic variation to variation in infection prevalence, weakening the response to parasite-mediated selection.

Studies in a few plant-fungal systems have documented extensive genetic variation in host susceptibility (as reviewed in Laine et al. 2011) and connected that variation in susceptibility to variation in infection prevalence within and between natural populations (Thrall and Jarosz 1994a, 1994b; Alex-

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ander and Antonovics 1995; Thrall and Burdon 2000; Thrall et al. 2001; Laine 2004, 2006). For example, Jousimo et al. (2014) recently demonstrated that powdery mildew was less likely to establish and persist in more resistant populations of its host, the ribwort plantain.

Similar studies are rare in animal systems, and results are contradictory. We might predict that the potential for significant mobility of animal hosts may reduce the contribution of genetics to spatial variation in infection prevalence. Indeed, Grosholz and Ruiz (1995) argued that high gene flow erodes genetic variation for susceptibility of xanthid crabs to castrating *Sacculina* barnacles. Though barnacle prevalence varied markedly between crab populations, they found no evidence of genetic variation in susceptibility. Even if susceptibility does vary, it may have little explanatory power: Scott (1991) found that laboratory mouse strains did not differ in nematode prevalence in seminatural mesocosms, even though they differed significantly in susceptibility. In contrast, studies of *Daphnia* (Little and Ebert 2000) and a wild sheep population (Hayward et al. 2014; Nussey et al. 2014) link estimates of increased genetic susceptibility to increased infection prevalence and mortality, respectively, in the field. Additional work on *Daphnia* demonstrates within-population variation in susceptibility to a fungal parasite (Duffy et al. 2008) and parasite-mediated selection on susceptibility (Duffy et al. 2008, 2012) that corresponds to a termination of epidemics (Duffy et al. 2009).

These mixed findings call into question the degree to which infection prevalence covaries with susceptibility at a fine spatial scale. By “fine,” we mean the scale at which gene flow between sites is expected to be high (Richardson et al. 2014). Snail-trematode interactions provide a promising avenue in which to develop this line of research. Infection prevalence is highly variable (Pesigan et al. 1958, pp. 568–571; Robson and Williams 1970; Anderson and May 1979; Curtis and Hurd 1983; Woolhouse and Chandiwana 1989; Jokela and Lively 1995b; Jokela et al. 1997; Smith 2001; Vergara et al. 2013), and genetic variation explains a great deal of variation in susceptibility (Newton 1953; Richards and Merritt 1972; Richards 1975; Basch 1976; Wakelin 1978; Webster and Woolhouse 1998; Negovetic and Jokela 2001). This is the case in our system, the freshwater snail *Potamopyrgus antipodarum* and its sterilizing trematode *Microphallus* sp. Laboratory assays consistently demonstrate that the relative susceptibility of hosts has a strong genetic basis (Dybdahl and Krist 2004; Krist et al. 2004; Jokela et al. 2009) that arises largely from the interaction of host and parasite genotypes (Lively 1989; Lively et al. 2004). Thus, any covariance of infection prevalence and susceptibility would likely reflect the contribution of genetic variation to variation in prevalence.

Here, we quantify fine-scale spatial variation in infection prevalence and susceptibility in this natural snail-trematode interaction. We then test the hypothesis that infection pre-

valence covaries with susceptibility. We conducted our study at a small New Zealand lake (6.4 km²) where gene flow between shoreline sites is sufficiently high that hosts and parasites from distant sites are not differentiated at neutral loci (Dybdahl and Lively 1996; Fox et al. 1996; Paczesniak et al. 2014). Ten years of field data demonstrate striking spatial and temporal variation in infection prevalence at 13 shoreline sites. Experimental inoculations of hosts from these sites reveal a similar degree of spatial variation in susceptibility. We found that mean susceptibility can explain more than one-third of the variation in mean field prevalence, with more susceptible sites having higher prevalence. Overall, our results strongly argue that genetic variation for susceptibility between sites persists in the face of high gene flow and makes a substantial contribution to fine-scale spatial variation in infection prevalence.

Methods

Natural History

Potamopyrgus antipodarum is a prosobranch gastropod that is abundant in New Zealand lakes and streams. It is the first intermediate host to at least 20 species of trematode parasites (Hechinger 2012). The best studied of these is *Microphallus* sp., which is highly virulent, sterilizing both male and female snails. Snails ingest parasite eggs while foraging and become sterilized as the developing infections replace the host gonads with larval metacercariae. The definitive host (ducks) ingests the infected snail while foraging, and *Microphallus* matures and sexually reproduces in the duck intestine. The parasite releases its eggs in the duck's feces, which are then dispersed in the environment (Hechinger 2012). We will hereafter use “host” to refer to the intermediate snail host.

We conducted this study at Lake Alexandrina, a 6.4-km² lake in the Mackenzie Basin of the central South Island of New Zealand. *Microphallus* is prevalent at this lake, with infection frequencies exceeding 60% at some sites (Jokela et al. 2009). Lake Alexandrina is home to several resident bird populations, including *Anas platyrhynchos* (introduced mallards), *Anas superciliosa* (native gray ducks), their hybrids, and *Aythya novaeseelandiae* (New Zealand scaup), the major definitive hosts of *Microphallus* sp. (Osnas and Lively 2011). Female *P. antipodarum* vary in reproductive mode at Lake Alexandrina, with asexual females coexisting with sexual males and females (Winterbourn 1970; Jokela et al. 2009). In this study, we investigate overall patterns across sexual and asexual lineages combined.

We focus on the lake's shallow shoreline habitat (<0.5 m deep). *Microphallus* prevalence in snails is higher here than in deeper habitats (Jokela and Lively 1995a, 1995b). The shoreline is easily accessible along the lake's southern half, making it ideal for investigation of fine-scale spatial patterns.

Consistent with *P. antipodarum*'s potential for passive dispersal (Hubendick 1950; Ribi 1986), gene flow between shoreline sites occurs readily, as evidenced by a lack of genetic structure at neutral loci (Fox et al. 1996; Paczesniak et al. 2014). There is also no structure at neutral loci for *Microphallus* (Dybdahl and Lively 1996), as we would predict for a parasite dispersed by mobile waterfowl.

Does Infection Prevalence Vary in Space?

We used a long-term data set to examine spatial variation in infection prevalence at Lake Alexandrina. Each year from 2006 to 2015, we visited 12 or 13 shoreline sites in mid-January to February. We sampled a large number of individuals at each site by sweeping a net along the bank and through vegetation. These samples were transported to the University of Canterbury's Edward Percival Field Station (Kaikoura, New Zealand). We performed dissections at the field station or after transportation to ETH Zurich (Switzerland) or Indiana University. For each site, we randomly selected approximately 100 snails and determined length (in millimeters), gender, and infection status. In 2014 and 2015, samples were sieved at >1.7 mm, excluding snails below ~ 3 mm.

We restricted analysis to female snails for consistency with susceptibility analyses (see the following section). We tested the hypothesis that infection prevalence varies between sites and years using a generalized linear model (GLM) with site, year, and their interaction as predictors of the probability of infection of an individual female (binomial with logit link function; SPSS v21, IBM). Each female's shell length was included as a covariate to control for age-related variation in prevalence. The cumulative risk of infection increases with age, and shell length correlates positively with age within habitats (Jokela and Lively 1995b). We determined whether the binomial distribution was appropriate for our data by testing for overdispersion. If a model's residual deviance is less than or approximately equal to the residual degrees of freedom, data are not overdispersed and the specified distribution of the model is appropriate (Crawley 2013). We found no evidence for overdispersion: the ratio of residual deviance to degrees of freedom was 1.

We used spatial analyses of infection prevalence to determine whether sites were independent of one another in space. The GPS coordinates and geographic distances between sites were obtained using Google Earth. For these analyses, we used length-corrected estimates of prevalence (estimated marginal means from generalized linear model above) with an arcsine transformation. First, we tested the hypothesis that nearby sites have similar prevalence with a Mantel test in the vegan package v2.3-0 (defaults = Pearson correlation, 999 permutations; Dixon and Palmer 2003) in R (R Core Team 2013). Specifically, we measured the cor-

relation of straight-line geographic distance with the absolute value of the difference in mean infection prevalence (equivalent of Euclidean and Manhattan distances for our data). Second, we tested the hypothesis that mean prevalence shows spatial autocorrelation by estimating Moran's I and its significance in the ape package v3.3 (Paradis et al. 2004) in R. Last, we tested the hypothesis that there is a geographic cline in prevalence using a generalized estimating equation (GEE) with latitude and longitude as predictors of prevalence (linear response variable) in SPSS. The GEEs were developed for analysis of longitudinal data and can account for the correlation between measurements taken at the same site through time (Liang and Zeger 1986; Zeger and Liang 1986). Site was included as a subject variable and year (2006–2015) as a within-subject variable with a first-order autoregressive variance-covariance matrix. This is the preferred correlation structure for longitudinal data (Wang and Carey 2003; Ziegler and Vens 2010; Vens and Ziegler 2012). We also tested for a correlation of mean infection prevalence in recent years (2013–2015) with latitude and longitude (Spearman's rank correlation due to deviations from normality).

Does Susceptibility Vary in Space?

To evaluate the hypothesis that variation in prevalence arises from variation in host susceptibility, we first tested the prediction that susceptibility varies between sites. We performed artificial inoculations to measure the susceptibility of juvenile hosts from different sites around the lake. Juvenile snails are ideal for artificial inoculations because they have experienced relatively little parasite exposure (Levri and Lively 1996) and are susceptible to infection (Krist and Lively 1998). We defined susceptibility as the infection rate obtained following standardized exposure to parasites in the lab. To ensure that infection rate in exposed replicates truly reflected susceptibility, we used high doses of parasites for inoculation. In doing so, our goal was to attain a saturating dose such that every host encountered a sufficiently large number of parasites to become infected if susceptible. It is possible that juvenile snails had acquired infection in the field prior to collection. To estimate the total frequency of susceptible snails at a site, we added to these field infections using artificial inoculations. In other words, the infection rate measured in exposed replicates (susceptibility) reflected infections gained both in the field and in artificial inoculations.

Our assay of susceptibility is not a direct measure of genetic variation in susceptibility: experimental snails were collected directly from the field, and thus susceptibility includes any variation due to maternal effects and early-life experience. Prior studies, however, strongly argue that genetic variation explains the majority of variation in susceptibility: relative susceptibility of host genotypes is affected by neither host condition (Dybdahl and Krist 2004) nor the upregulation of plastic

immune responses (Osnas and Lively 2005, 2006). Selection on host susceptibility results in a strong response to selection, which requires genetic variation for susceptibility (Koskella et al. 2011). Moreover, the interaction of host and parasite genotype explains the majority of variation in susceptibility (Lively et al. 2004), and outbreeding depression of hybrid parasites is consistent with nonadditive gene effects and genotype specificity for susceptibility (Dybdahl et al. 2008).

In February of 2013, 2014, and 2015, we collected snails from shoreline sites (2013: $n = 12$, Halfway excluded; 2014 and 2015: $n = 13$), as described above. We also collected duck feces from the lake shore to obtain parasite eggs that are infective to snails. In 2013 and 2015, we combined duck feces from multiple sites around the entire lake. In 2014, we made separate collections from a southern (source 1) and northern (source 2) site (fig. 1A, stars). We transported all samples to the Edward Percival Field Station. We sieved snail collections at <1.7 mm to obtain juvenile snails. The duck feces were repeatedly rinsed with freshwater to remove contaminants, homogenized, and sieved to remove debris.

For each site, we established replicates of 100 (2013) or 75 (2014 and 2015) snails each. Two replicates per site were not exposed to parasites (control treatment). The control treatment allowed us to measure variation in early-life exposure of juveniles from different sites. Control snails received a light feeding of spirulina in lieu of parasite exposure. For replicates in the exposed treatment, homogenized duck feces were added to the water of the containers over the course of 8 (2014 and 2015) or 10 (2013) days. In 2013 and 2015, four replicates were exposed to 1,500 and 2,900 eggs/snail, respectively. In 2014, three replicates were exposed to 800 eggs/snail of source 1 and three replicates to 2,100 eggs/snail of source 2. We used a Neubauer hemocytometer to determine egg concentrations. Such high doses are commonly used when exposing *P. antipodarum* to *Microphallus* (Osnas and Lively 2004; King et al. 2011), and mortality in exposed replicates did not exceed that in control replicates (see appendix). Doses used in different sources and years do vary substantially, but it is very likely that all doses were sufficiently high to exceed the minimum threshold required to infect all susceptible hosts (Osnas and Lively 2004).

We maintained our experimental replicates until 80 days post-exposure to allow for parasite development. We then dissected each snail to determine length, gender, and infection status. The subsequent analyses are restricted to female snails. They are preferable for evaluating susceptibility in artificial inoculations because males tend to be relatively rare at some sites (as little as 16% of juveniles) and vary significantly in size and behavior.

To evaluate susceptibility assays in 2013 and 2015, we used the function `glm` in R to fit generalized linear models with the number of infected and uninfected females in a rep-

licate as a binomial response variable (logit link function). We first determined whether artificial exposures increased the probability of infection by evaluating the treatment effect in a model with treatment, site, and their interaction as factors (years evaluated separately). We used the function `confint` to obtain confidence intervals for the odds ratios.

We found that artificial inoculations were successful (see “Results”), so we restricted analysis to the exposed treatment to evaluate variation in susceptibility (infection frequency in exposed replicates). We fitted logistic regressions with site as a predictor of the number of infected and uninfected females in exposed replicates. We used a likelihood ratio test to test the significance of the site effect, relative to an intercept-only model. To quantify the explanatory power of site, we calculated the likelihood ratio (McFadden’s pseudo- R^2):

$$R_L^2 = 1 - \frac{\ln(L_{\text{site}})}{\ln(L_{\text{int}})},$$

which is the proportional increase in log likelihood L (or decrease in $-2 \log L$) with inclusion of site (L_{site}), relative to an intercept-only model (L_{int} ; McFadden 1974). R_L^2 is analogous to R^2 , the ordinary least squares coefficient of determination, with L_{site} and L_{int} analogous to the residual sum of squares and the total sum of squares, respectively (Menard 2000). Values of R_L^2 between 0.2 and 0.4 indicate strong explanatory power (McFadden 1979). We similarly evaluated variation in the frequency of infection in control replicates, which reflects early-life exposure of juvenile snails prior to collection from the field. We applied the same statistical approach to the 2014 assay, evaluating each of the two parasite sources separately. We discuss comparison of susceptibility to the two sources in the final section of “Methods.” For all generalized linear models, we found no evidence of overdispersion, indicating that use of the binomial distribution was appropriate.

We examined the geographic distribution of susceptibility using the same spatial analyses described previously: Moran’s I and a Mantel test of geographic distance and overall mean susceptibility (mean of exposed replicates across all years, including both sources in 2014), a GEE with latitude and longitude as predictors (subject: site; within-subject: year, first-order autoregressive) of yearly mean susceptibility (mean of exposed replicates within a year; linear response variable), and Spearman rank correlations of yearly mean susceptibility with latitude and longitude for each year. Proportions were arcsine transformed.

Is Susceptibility Positively Correlated with Infection Prevalence?

We then tested the prediction that sites with highly susceptible juveniles in the lab also display higher infection prevalence in the field. First, we tested for correlations of mean suscep-

Figure 1: Prevalence of *Microphallus* varies in space and time on a fine spatial scale. **A**, Variation in space. Mean prevalence of *Microphallus* in female snails from 2013 to 2015 at 13 sites around Lake Alexandrina (2014 and 2015 for Halfway). Values are length-corrected estimates derived from a generalized linear model. Stars indicate sites where parasites were collected for artificial inoculations in 2014. **B–E**, Variation in time. Infection prevalence of adult female snails (>3.2 mm long) from 2006 to 2015 at 12 sites. Length cutoff was selected to standardize snail age between sites and years. Site Halfway is excluded due to inconsistent sampling.

tibility and infection prevalence (Pearson; overall and yearly means). We then tested whether overall mean susceptibility differed significantly from infection prevalence using a Student's *t*-test (H_0 : mean difference equal to 0). To examine the contribution of variation in exposure to variation in preva-

lence, we tested for a correlation of overall mean control infection rates with infection prevalence (Pearson). The infection rate in control replicates reflects variation in early-life exposure of juveniles from different sites. Because susceptibility may contribute to variation in infection rate in con-

trol replicates, we also performed a partial correlation of overall mean infection prevalence and mean infection rate in control replicates, controlling for susceptibility. This test provides an estimate of the residual variation in infection prevalence between sites that can be explained by variation in exposure alone, after accounting for variation in susceptibility. All proportions were arcsine transformed.

Characterizing Variation in Susceptibility

In 2014, we exposed hosts to two distinct parasite collections from southern (source 1) and northern (source 2) sites ~4 km apart (fig. 1A, stars). Comparison of susceptibility to these sources allows us to characterize the contribution of host and parasite effects to variation in susceptibility. We were particularly interested in the interaction of host site and parasite source; this interaction explains the majority of variation in susceptibility at larger scales (between lakes; Lively et al. 2004). Here, we test whether this interaction also has explanatory power at a fine spatial scale. We might expect it to be irrelevant given that gene flow within the shoreline habitat is high for host and parasite. We fit a generalized linear model (binomial with logit link function) with host site, parasite source, and their interaction as predictors of the number of infected and uninfected females in exposed replicates. We found no evidence of overdispersion. To test the significance of each effect, we performed likelihood ratio tests of models with and without the effect. Though doses for both sources were likely saturating, any main effect of parasite source may be partly attributed to the lower dose used for source 1. Last, we measured the correlation of mean susceptibility to parasite sources 1 and 2 at a site (Pearson, arcsine-transformed values). Data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.t89hc> (Gibson et al. 2016).

Results

Infection Prevalence Varies in Space

Each year from 2006 to 2015, we measured infection prevalence of *Potamopyrgus antipodarum* with the sterilizing trematode *Microphallus* at 12 or 13 shoreline sites around Lake Alexandrina, New Zealand. This long-term sampling revealed significant variation in a female's probability of infection between sites (minimum estimated marginal mean = 0.030 ± 0.006 ; 0.030 ± 0.007 SEM at Southwest End and West Bay, respectively; maximum estimated marginal mean = 0.280 ± 0.020 at JMS; fig. 1A) and between years (minimum = 0.080 ± 0.008 in 2014; maximum = 0.20 ± 0.014 in 2009; fig. 1B–1E). The interaction of site and year was also highly significant, indicating that changes in infection probability between years occur independently at different sites (table 1; fig. 1B–1E). A female's proba-

bility of infection increased significantly with shell length (coefficient = 1.397 ± 0.050 SEM), consistent with an increase in the cumulative risk of infection with snail age (table 1). There was no significant correlation between geographic distance and mean infection prevalence between sites (Mantel: $r = 0.141$, $P = .183$), indicating that sites are independent of one another in space with respect to infection prevalence. In addition, we found no evidence for spatial autocorrelation of mean prevalence (Moran's $I = 0.066$, $P = .118$). Relatedly, infection prevalence did not vary significantly with a site's location (GEE: latitude: Wald $\chi^2 = 0.162$, $df = 1$, $P = .688$; longitude: Wald $\chi^2 = 2.895$, $df = 1$, $P = .089$), indicating no spatial gradient to infection prevalence. Infection prevalence in recent years (mean from 2013 to 2015) was also uncorrelated with latitude (Spearman's $\rho = 0.363$, $P = .223$) and longitude (Spearman's $\rho = 0.286$, $P = .344$).

Susceptibility Varies in Space

For susceptibility to explain the observed spatial variation in infection prevalence, susceptibility to *Microphallus* must also vary around Lake Alexandrina. We evaluated this prediction through artificial inoculations of juvenile snails collected from sites around the lake. In 2013 and 2015, snails were exposed to a bulk field collection of local parasite eggs. Exposure to field-collected parasites increased the odds of infection with *Microphallus* by 2.2-fold in 2013 (95% confidence interval [CI] = [1.235, 4.190]; GLM, $z = 2.565$, $P = .010$) and by 3.5-fold in 2015 (95% CI = [1.504, 9.336]; $z = 2.742$, $P = .006$) relative to control replicates (fig. 2). Given that artificial inoculations were successful, we restricted our analyses to exposed replicates to evaluate variation between sites in susceptibility, measured as the infection frequency obtained in exposed replicates. We found that site contributed substantially to explaining variation in susceptibility, which ranged from 0.210 ± 0.009 SEM at SW End to 0.654 ± 0.038 at East Point in 2013 (GLM, likelihood ratio = 322.940, $df = 11$, $P < .001$, $R^2_i = 0.565$) and from

Table 1: Results of generalized linear model for field prevalence of infection

	Wald χ^2	df	P
Intercept	1,217.7	1	<.001
Site	338.1	12	<.001
Year	97.3	9	<.001
Site $\times$ year	270.2	100	<.001
Length	787.4	1	<.001

Note: Site, year, and their interaction are predictors of the probability that an individual female snail is infected with *Microphallus*. Snails were sampled at 12 or 13 shoreline sites from 2006 to 2015. Shell length is a covariate.

Figure 2: Host susceptibility varies in space. In 2013 (A) and 2015 (B), susceptibility (gray bars; mean infection rate in exposed replicates) differed significantly between the survey sites. Infection rates in the control replicates (white bars) reflect the initial level of infection in field-collected juveniles. These were significantly lower than infection rates obtained in exposed replicates. Susceptibility increased from south to north and from west to east around Lake Alexandrina, particularly in 2013. Sites are grouped by coast (West: left, white background; East: right, gray background) and ordered from southernmost to northernmost within coast. Results are presented for female snails only. Error bars are standard errors of the means.

0.150 ± 0.016 at Southwest End to 0.617 ± 0.007 at JMS in 2015 (likelihood ratio = 123.2, $df = 12$, $P < .001$, $R^2_L = 0.361$; fig. 2).

In 2014, snails were separately exposed to parasites collected from two different lake sites (fig. 1A, stars). Exposure increased the odds of infection with *Microphallus* by 1.4-fold for source 1 (95% CI = [1.250, 1.640]; GLM, $z = 5.154$, $P < .001$) and by 5.7-fold for source 2 (95% CI = [4.421, 7.499]; $z = 12.959$, $P < .001$), relative to control replicates (fig. 3). Restricting our analyses to exposed replicates, we also

found that site contributed substantially to explaining variation in susceptibility (source 1: GLM, likelihood ratio = 24.459, $df = 12$, $P = .018$, $R^2_L = 0.565$; source 2: likelihood ratio = 118.31, $df = 12$, $P < .001$, $R^2_L = 0.388$; fig. 3). In the final section of the results, we compare susceptibility to these two sources.

Site did not explain variation in infection rate of control replicates in 2014 (GLM, likelihood ratio = 11.063, $df = 12$, $P = .524$, $R^2_L = 0.121$) and did so marginally in 2013 (likelihood ratio = 19.239, $df = 11$, $P = .0570$, $R^2_L =$

Figure 3: Susceptibility varies by host site, parasite source, and their interaction. In 2014, artificial inoculations were performed with two distinct parasite sources, source 1 (southern, light gray) and source 2 (northern, dark gray). Susceptibility varied significantly with host site, parasite source, and their interaction. Infection rates in the control replicates (white bars) reflect the initial level of infection in field-collected juveniles and were significantly lower than infection rates obtained in exposed replicates of either parasite source. Sites are grouped by coast (West: left, white background; East: right, gray background) and ordered from southernmost to northernmost within coasts. Results are presented for female snails only. Error bars are standard errors of the means.

0.172). Site had significant explanatory power in 2015 (likelihood ratio = 30.617, $df = 12$, $P = .002$, $R^2_L = 0.260$), suggesting that juvenile snails from different sites did differ in exposure to parasites prior to collection from the field.

We further characterized variation in susceptibility between sites using spatial analyses. There was a significant correlation of overall mean susceptibility with geographic distance between sites (Mantel: $r = 0.318$, $P = .029$), suggesting that sites are not spatially independent with respect to susceptibility. We also detected a significant signal of positive spatial autocorrelation for overall mean susceptibility (Moran's $I = 0.234$, $P = .001$). Consistent with these findings, mean susceptibility varied significantly with latitude (GEE: Wald $\chi^2 = 6.349$, $df = 1$, $P = .012$) and longitude (Wald $\chi^2 = 11.305$, $df = 1$, $P = .001$), indicating a spatial gradient in which susceptibility increased from the southwest to the north (coefficient for latitude = 6.332 ± 2.513 SEM) and the east (coefficient for longitude = 11.480 ± 3.414). For individual years, we found mixed support for this spatial pattern: mean susceptibility was significantly correlated with latitude (Spearman's $\rho = 0.734$, $P = .007$) and longitude ($\rho = 0.608$, $P = .036$) in 2013 but marginally so in 2015 (latitude: $\rho = 0.533$, $P = .061$; longitude: $\rho = 0.500$, $P = .082$; fig. 2). In 2014, mean susceptibility to parasite source 2 increased significantly to the north (latitude: $\rho =$

0.610 , $P = .027$; insignificant increase to east, longitude: $\rho = 0.484$, $P = .094$). Mean susceptibility to parasite source 1, however, showed no spatial gradient (latitude: $\rho = 0.346$, $P = .247$; longitude: $\rho = 0.247$, $P = .415$; fig. 3).

Susceptibility Explains Observed Variation in Infection Prevalence

If variation in susceptibility explains the observed variation in infection prevalence, we would predict a positive relationship between the two variables. We indeed found a significant positive correlation between overall mean susceptibility to *Microphallus* of hosts and prevalence of *Microphallus* at that site (Pearson's $r = 0.603$, $P = .029$; fig. 4). Susceptibility can explain 36.4% of the observed variation in infection prevalence between sites. The correlation between mean susceptibility and infection prevalence varied for individual years: it was significantly positive in 2015, marginally so in 2014, and insignificant in 2013 (table 2). Overall mean susceptibility was significantly higher than mean infection prevalence at a site (one-sample t -test: $t = 9.127$, $df = 12$, $P < .001$; mean difference = 0.302 ± 0.033 SEM; fig. 4). This is consistent with the high dose of parasites in susceptibility assays relative to natural exposure levels.

Figure 4: Susceptibility explains spatial variation in infection prevalence. Overall mean susceptibility, measured as mean infection rate in exposed replicates from 2013 to 2015, is significantly positively correlated with mean infection prevalence. The line indicates a 1:1 relationship of susceptibility and infection prevalence. Susceptibility is consistently greater than infection prevalence in the field. Results are presented for female snails only.

Variation in infection prevalence may also arise from variation in exposure between sites, which we estimated as variation in *Microphallus* infection in control replicates (early-life exposure). We did find a significant positive correlation between overall mean rate of *Microphallus* in control replicates and prevalence of *Microphallus* at a site ($r = 0.597$, $P = .031$). Mean infection rate in control replicates can explain 35.6% of variation in mean infection prevalence between sites. After accounting for variation in susceptibility between sites, variation in overall mean infection rate in control replicates (field exposure) can explain a large and significant proportion of the residual variation in infection prevalence (partial correlation: $r = 0.809$, $P = .001$, $R^2 = 0.650$). This suggests that, together, variation in exposure and susceptibility (mean infection rates in control and exposed replicates, respectively) can explain the majority of variation in infection prevalence of hosts from different sites.

Variation in Susceptibility: Effects of Host, Parasite, and Their Interaction

In 2014, snails from our 13 study sites were separately exposed to parasites collected from southern (source 1) and northeastern (source 2) sites of Lake Alexandrina (fig. 1A, stars). By comparing host susceptibility to these two parasite sources, we could determine the proportion of variation in host susceptibility that arises from host main effects (site), parasite main effects (source), and their interaction. We found that both main effects and their interaction made significant

contributions to explaining variation in susceptibility (GLM: host site: likelihood ratio = 103.642, $df = 12$, $P < .001$; parasite source: likelihood ratio = 122.231, $df = 1$, $P < .001$; their interaction: likelihood ratio = 38.187, $df = 12$, $P < .001$; $R^2_L = 0.435$). The main effect of host site represents variation in susceptibility between host sites that is independent of parasite source. The main effect of parasite source could reflect inherent variation in infectivity between the two sources, but we cannot rule out the effect of dose, which was lower for source 1. However, the probability of infection was no greater under exposure to source 2 versus source 1 (odds ratio = 1.2, 95% CI = [0.601, 2.25]; $z = 0.459$, $P = .646$). Last, the significant interaction effect of host site and parasite source indicates that a proportion of variation in susceptibility arises from the interaction of host and parasite genotypes. Relatedly, host susceptibility at a site to parasite sources 1 and 2 were not significantly correlated (Pearson's $r = 0.481$, $P = .096$).

Discussion

Here, we tested whether spatial variation in infection prevalence is predicted by variation in snail susceptibility to trematode infection, a phenotype with a strong genetic basis (Dybdahl and Krist 2004; Krist et al. 2004; Lively et al. 2004; Koskella et al. 2011). With 10 years of extensive field samples, we demonstrate substantial variation in the prevalence of *Potamopyrgus antipodarum* snails infected with the sterilizing trematode *Microphallus* at shoreline sites distributed along the southern half of a small lake (table 1; fig. 1). With experimental inoculations replicated in 3 years, we demonstrate that susceptibility varies significantly between these shoreline sites (figs. 2, 3). We then show that variation in susceptibility contributes to variation in fitness in this natural population: variation in susceptibility can explain 36% of variation in mean trematode prevalence between sites (table 2; fig. 4). Our findings confirm that infection prevalence can covary with susceptibility at a fine spatial scale.

This covariance is particularly striking given the proximity of our study sites. Rates of migration and gene flow between

Table 2: Results of correlation of susceptibility and infection prevalence

	Pearson's r	P	R^2
2013	.443	.149	.196
2014	.495	.085	.245
2015	.645	.017	.416
Overall mean	.603	.029	.364

Note: Results for yearly means in individual years and the overall mean across all years. For 2014, all exposed replicates were included in estimation of susceptibility, regardless of parasite source.

them are sufficiently high to erode genetic variation at neutral loci (Fox et al. 1996; Paczesniak et al. 2014), yet the observed variation in susceptibility suggests that loci potentially under selection by parasites are differentiated between these same sites. We identified as much as 6.5-fold variation in the proportion of snails at a site that were resistant to local parasites (2014, source 2; fig. 3). This degree of variation is all the more surprising given that each snail was exposed to very high doses of parasites in our artificial inoculations (as in Osnas and Lively 2004).

The observed variation in susceptibility equals or exceeds that observed between snails from the shoreline and deep-water habitats of Lake Alexandrina (highly susceptible vs. highly resistant, respectively; Lively and Jokela 1996; King et al. 2009, 2011). Divergence in susceptibility between these two habitats is perhaps less surprising than the divergence observed here between sites within the shoreline habitat, because genetic divergence between snails from ecologically distinct habitat zones is high relative to that within a single habitat (Fox et al. 1996; Paczesniak et al. 2014). However, the mechanism driving variation in susceptibility is likely the same within and between habitats. Prior work proposes that the depth cline in susceptibility arises from the foraging habits of *Microphallus*'s definitive hosts: dabbling ducks forage in shallow water and so consume shoreline snails, allowing coevolution of shoreline parasites and hosts. They do not forage in deeper water (>4 m deep), preventing coevolutionary cycling and adaptation of local parasites to infect deep-water snails (King et al. 2009, 2011). Variation in duck foraging at shoreline sites may similarly generate a geographic mosaic of coevolution within the shoreline habitat, such that local parasites differ in the degree to which they are adapted to infect hosts from different sites (as suggested by Vergara et al. 2013).

The geographic covariance of susceptibility and prevalence suggests that parasites can exert strong selection in this system, because genetic variation in susceptibility has clear fitness consequences (e.g., frequency of parasitic castration at a site; fig. 4). We emphasize that we are inferring a genetic basis for variation in susceptibility. We could not exclude maternal effects and other variation in condition that might affect host susceptibility, as all experimental snails were collected directly from the field. However, snail susceptibility to trematodes generally has a strong genetic basis (Newton 1953; Richards and Merritt 1972; Richards 1975; Basch 1976; Wakelin 1978; Webster and Woolhouse 1998). In our system, prior studies show that variation in condition does not alter the relative susceptibility of snail genotypes (Dybdahl and Krist 2004; Krist et al. 2004). Additionally, the interaction of host and parasite genotype explains the majority of variation in host susceptibility (43%–95% of total variation; Lively et al. 2004), with host populations and clonal genotypes (Lively and Dybdahl 2000; Jokela et al.

2009) typically most susceptible to their sympatric (coevolving) parasites. The most likely explanation for such a pattern is that susceptibility is a product of the specific interaction of host and parasite alleles. Accordingly, hybridization of parasites results in outbreeding depression on sympatric hosts, the best explanation for which is that nonadditive gene effects and genotype specificity underlie susceptibility (Dybdahl et al. 2008). Similarly, experimental selection results in a rapid reduction in susceptibility of hosts that is specific to the experimental parasite population (Koskella et al. 2011). A response to selection is possible only if there is genetic variation for susceptibility. Importantly, there is no evidence that hosts collected from high-prevalence sites are inherently more susceptible to infection: hosts at high- and low-prevalence sites are equally susceptible to allopatric (non-coevolving) parasites (King et al. 2009, 2011).

Our results argue that the interaction of host and parasite can explain variation in susceptibility even at the fine spatial scale of our study. Earlier tests of the explanatory power of the host-by-parasite interaction were conducted between lakes (Lively 1989; Lively et al. 2004). The within-lake interaction observed here was unexpected given that rates of gene flow at neutral loci are high for both host and parasite (Dybdahl and Lively 1996; Fox et al. 1996; Paczesniak et al. 2014). Most notably, duck mobility results in little neutral genetic structure for *Microphallus* within and between lakes (Dybdahl and Lively 1996), and yet the within-lake interaction raises the possibility of divergence at infection loci of parasites sampled only 4 km apart. Both parasite sources are located where ducks breed (A. K. Gibson and C. M. Lively, personal observation), so localized duck movement during breeding season may promote differentiation of their parasites. Prugnolle et al. (2005) suggest that parasite dispersal may be reduced if migrants are maladapted to infect local hosts. Although local adaptation is evident between lakes, our within-lake interaction was not consistent with local adaptation: northeastern sites were most susceptible to the northeastern source 2 (most susceptible site = JMS: 0.598 ± 0.006 SEM), but southern sites were relatively resistant to the southern source 1 (least susceptible site = SW End: 0.090 ± 0.024 ; fig. 3). Similarly, host and parasite main effects explained relatively more variation in susceptibility than is typically seen at larger spatial scales (Lively et al. 2004).

We have provided evidence that hosts and parasites diverge geographically, manifesting as spatial variation in susceptibility. The critical question is then, does this variation in susceptibility actually matter for the distribution of infection in nature? Evidence has accumulated for a large role for environmental factors in determining variation in infection prevalence. It is reasonable to assume that environmental variation would overwhelm any contribution of susceptibility to variation in infection prevalence, particularly at fine spatial scales. This is not the case in our system: we

find that mean susceptibility can explain over one-third of the variation in mean infection prevalence between sites (fig. 4). The observed relationship is correlative, but it suggests a link between genetically based host and parasite traits and the distribution of a virulent parasite in a natural population.

Variation in susceptibility, of course, is not the sole explanation for the observed variation in infection prevalence (McNew 1960; Stevens 1960; Scholthof 2007). This is clear from the variable nature of the relationship between susceptibility and infection prevalence: in any given year, the relationship is not consistently significant (table 2), which may indicate variation in the influence of environmental factors. Most notably, snails at different sites are likely to vary in exposure to parasites, as wind, water movement, and duck behavior may patchily distribute parasite eggs. We find preliminary support for this idea in our examination of control replicates: sites vary somewhat in infection rates of control juveniles, indicative of variation in their exposure to parasites prior to collection from the field. This variation in exposure can explain most of the residual variation in infection prevalence after accounting for susceptibility. We therefore conclude that susceptibility and exposure together can explain the majority of fine-scale spatial variation in infection prevalence.

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APPENDIX

Mortality in Susceptibility Assays

Data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.t89hc> (Gibson et al. 2016). To de-

termine whether high doses of parasites caused snail mortality, we counted the number of snails that survived to the time of dissection (~80 days postexposure) in exposed and control replicates. Replicates were initiated with 100 snails in 2013 and 75 in 2014 and 2015. Any snails missing at the time of dissection were presumed dead. For each year, we fitted generalized linear models to mortality data using the function `glm` in R, with the number of dead and surviving snails as a binomial response variable (logit link function) and site, treatment, and their interaction as factors. The ratios of residual deviance to residual degrees of freedom exceeded 1, indicating substantial overdispersion (ratio = 3.040 in 2013, 1.886 in 2014, and 4.749 in 2015). Therefore, we refitted the models using the quasi-binomial distribution, which estimates a scale parameter to account for additional variance in the data (Crawley 2013). Based on these quasi-binomial models, we used odds ratios and their confidence intervals (function `confint`) to determine whether exposure increased the probability of mortality.

We found no evidence that exposure to parasites increased the probability of mortality. In 2013, exposure to field-collected parasites had no effect on the odds of mortality (odds ratio = 0.614, 95% confidence interval [CI] = [0.303, 1.264]; $z = -0.487$, $P = .186$). In 2014, exposure to the relatively low dose of source 1 decreased the odds of mortality (odds ratio = 0.415, 95% CI = [0.224, 0.761]; $z = -0.879$, $P = .006$), and exposure to the higher dose of source 2 had no effect (odds ratio = 0.663, 95% CI = [0.370, 1.187]; $z = -0.441$, $P = .171$). In 2015, exposure to parasites had no effect on the odds of mortality (odds ratio = 1.069, 95% CI = [0.456, 2.504]; $z = 0.067$, $P = .878$).

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Lake Alexandrina (left), a natural lake in the Mackenzie Country of New Zealand's South Island. The 13 study sites were located along the shoreline of the southern half of Lake Alexandrina. Lake Tekapo, a large hydrolake, is on the right, and Godley Peaks and the Southern Alps are in the distance. This photo was taken on the morning of January 15, 2013, from Mount John. Photo credit: Jukka Jokela.