Evolutionary Ecology Research, 2011, **13**: 315–322

Experimental evolution of sexual host populations in response to sterilizing parasites

Britt Koskella, Daniela Vergara and Curtis M. Lively

Department of Biology, Indiana University, Bloomington, Indiana, USA

ABSTRACT

Hypothesis: Sexual host populations rapidly evolve specific resistance against parasites, and consequently diverge from populations under relaxed parasite-mediated selection or selection imposed by different parasite populations.

Organisms: The freshwater snail, *Potamopyrgus antipodarum*, and two different populations of a naturally prevalent sterilizing trematode, *Microphallus* sp.

Methods: Experimental populations of the host snail were exposed to one of the two parasite populations, or allowed to evolve in the absence of parasites. After three generations of experimental evolution, replicate hosts were exposed to parasites from one of the parasite source populations, and resistance was compared across parasite treatments.

Results: Host populations rapidly diverged as each evolved to their experimental parasite populations. This resistance was associated with an increased susceptibility, relative to the control and other parasite treatments, to the other experimental parasite population. Hence, resistance to infection by *Microphallus* is specific, rather than generalized.

Keywords: cost of resistance, host–parasite co-evolution, local adaptation, Red Queen hypothesis, specificity of resistance.

INTRODUCTION

Interactions between hosts and their parasites can play a major role in driving genetic divergence between populations and maintaining genetic diversity within populations (Haldane, 1949; Hamilton, 1982). The role of parasite-mediated selection in driving diversity depends, however, on several key factors, including the prevalence of parasites in the environment, the genetic specificity underlying infection, the virulence of the parasite, and the heterogeneity of the environment across which the interaction occurs. Specifically, when there is a genotype-by-genotype interaction underlying successful infection, such that one genotype can infect/resist a different subset of host/parasite genotypes than another (e.g. Lambrechts *et al*., 2005; Rauch *et al*., 2006), any parasite able to infect common host genotypes will have a significant fitness advantage and should increase in frequency over time (Haldane, 1949;

Correspondence: B. Koskella, Department of Zoology, University of Oxford, Oxford OX1 3PS, UK. e-mail: britt.koskella@zoo.ox.ac.uk

Hamilton, 1993). If these parasites decrease host fitness (for example, by sterilizing their hosts, or increasing host mortality), and reach high prevalence in the host population, they are likely to impose strong selection on their host population and cause significant population-genetic changes from one generation to the next. Under this scenario, parasite-mediated selection will lead to a rare-host advantage, as parasites adapt to infect common host genotypes (Haldane, 1949; Hutson and Law, 1981; Peters and Lively, 1999; Lively and Dybdahl, 2000; Jokela *et al.*, 2009; Koskella and Lively, 2009; Wolinska and Spaak, 2009), and the host population will evolve towards higher resistance to local parasites (e.g. Lohse *et al.*, 2006; Koskella and Lively, 2007).

In addition to maintaining diversity at the within-population level, parasites are also predicted to maintain between-population diversity, as co-evolution between host and parasite populations follows different trajectories across space (Thompson, 1999). One assumption of this prediction is that host and parasite populations are structured (i.e. are limited by gene flow) and/or that populations are spread across a heterogeneous environment, where separate populations are pushed along different trajectories due to local environmental conditions (Thompson, 2005). Parasite local adaptation, whereby parasites are found to be most successful on their local host population relative to other populations, is suggestive of divergent parasite-mediated selection across space; and there is robust evidence for this pattern from a number of systems (reviewed in Greischar and Koskella, 2007; Hoeksema and Forde, 2008). This pattern could indicate that parasites play a significant role in driving divergence among host populations or could reflect sorting of parasite genotypes across space according to host population composition. One powerful way to differentiate between these explanations is by experimental evolution. In this way, one can control for environmental heterogeneity and specifically examine whether parasites drive parallel evolution of host populations (i.e. select for general host resistance) or push host populations along divergent evolutionary trajectories (i.e. select for specific resistance to local parasites).

Here we examine the response of the freshwater snail, *Potamopyrgus antipodarum*, to selection by the sterilizing trematode, *Microphallus* sp., using a controlled laboratory experiment. We wished to determine whether initially similar populations of sexual hosts could be driven apart, with regard to their resistance phenotypes, based solely on the parasite populations to which they were previously exposed. The results from this simple experiment can help to address a number of questions, including: (1) whether independent parasite populations select for/against different subsets of host genotypes (which would indicate a high degree of specificity for infection); (2) whether the presence of parasites in a population leads to a general increase in immune function and resistance; and (3) whether the evolution of resistance to one parasite population changes a host population's ability to resist another parasite population.

METHODS AND MATERIALS

The host snail, *Potamopyrgus antipodarum*, is found in freshwater lakes and streams across New Zealand, and populations are typically made up of either asexual (apomictic) females or a mixture of asexual females, sexual females, and males (Dybdahl and Lively, 1996). The snail has a generation time of about 4 months under laboratory conditions, and it is the intermediate host for many sterilizing trematode parasites, including *Microphallus* sp., which excysts and sexually reproduces within the final host, waterfowl, following ingestion (Winterbourn, 1973). Embryonated eggs of *Microphallus* sp. are shed with the final host faeces

and then passively ingested by host snails, at which point the parasite reproduces asexually, leading to the production of hundreds of larval cysts and the sterilization of the snail host. In addition, there is strong evidence for a tight genetic specificity underlying infection. First, common clonal genotypes have been shown to become over-infected, relative to their frequency in the population, by co-evolving parasites (Lively and Dybdahl, 2000; Jokela *et al.*, 2009; Koskella and Lively, 2009). Second, a consistent degree of parasite local adaptation has been demonstrated, suggesting that parasites are able to adapt to the host genotypes in their local population (reviewed in Lively *et al.*, 2004). Finally, hybridization between parasite populations has been shown to break down infection success on host source populations, but not on non-host-source populations (Dybdahl *et al.*, 2008).

To examine the evolution of resistance by a sexual host population to different sources of parasites, we undertook an experimental co-evolution study in which replicate populations of sexual hosts were exposed either to no parasites, parasites from Lake Alexandrina or parasites from Lake Mapourika. To generate the host lines, we collected juvenile snails, measuring between 1.4 and 2.0 mm, from a laboratory population of sexual snails (originated from a single female and multiple males from Lake Alexandrina and maintained in the laboratory for over 5 years with no exposure to parasites). Juvenile snails were randomly split into five groups of 196 snails each, representing our five treatments (Fig. 1). In November 2008, we created four parasite inoculums by feeding 18 infected snails, collected from either the shallow or deep habitats (Lively and Jokela, 1996) from two lakes (Lake Alexandrina and Lake Mapourika on the South Island of New Zealand) to mice, which act as surrogate final hosts. Mouse faeces were collected three times a day for 3 days, starting 24 h after exposure, and washed thoroughly before inoculation. Snails were exposed to their respective parasite inoculum (or control inoculum) in 4-L bins for 2 weeks, at which point each population of snails was transferred into separate 500-L tanks for the remainder of the experiment. The water temperature of the tanks was maintained at approximately 17°C, and *Spirulina* algae were added to all tanks on an *ad lib* basis. All water added to the system was treated with Amquel® (Kordon, Hayward, CA) to remove harmful nitrate, nitrite, ammonia, chlorine, and chloramines.

After 7 months, a subsample of snails (between 50 and 130, depending on local population density) was collected from each tank to determine infection prevalence of experimental host populations after the first exposure to parasites. These samples were then used to generate the next generation of parasites for exposure by feeding infected snails (15 from Lake Mapourika shallow, 16 from Lake Mapourika deep, 19 from Lake Alexandrina shallow, and 19 from Lake Alexandrina deep) to one of four final mouse hosts. Then, in December 2009, all snails were removed from tanks and placed back into 4-L bins containing the appropriate inoculum (i.e. parasites from the same experimental population; Fig. 1). Due to low snail densities in the Lake Mapourika (shallow) tank after the first exposure, we were forced to combine the two host and parasite lines from Lake Mapourika into one. The population density of the Mapourika tank after the two lines were combined was estimated to be in between that of the Lake Alexandrina shallow tank, which had the largest population size apart from the control tank, and the Lake Alexandrina deep tank (estimates were based on volume of all snails in the population). After 2 weeks of exposure, host lines were put back into 500-L tanks.

In June 2010, we collected all snails from each tank and put them through a 1.4-mm sieve, so as to separate the adults from juveniles. Juvenile snails were divided into 2-L containers of 50 snails (for a total of 500 snails from each experimental population) and used in a

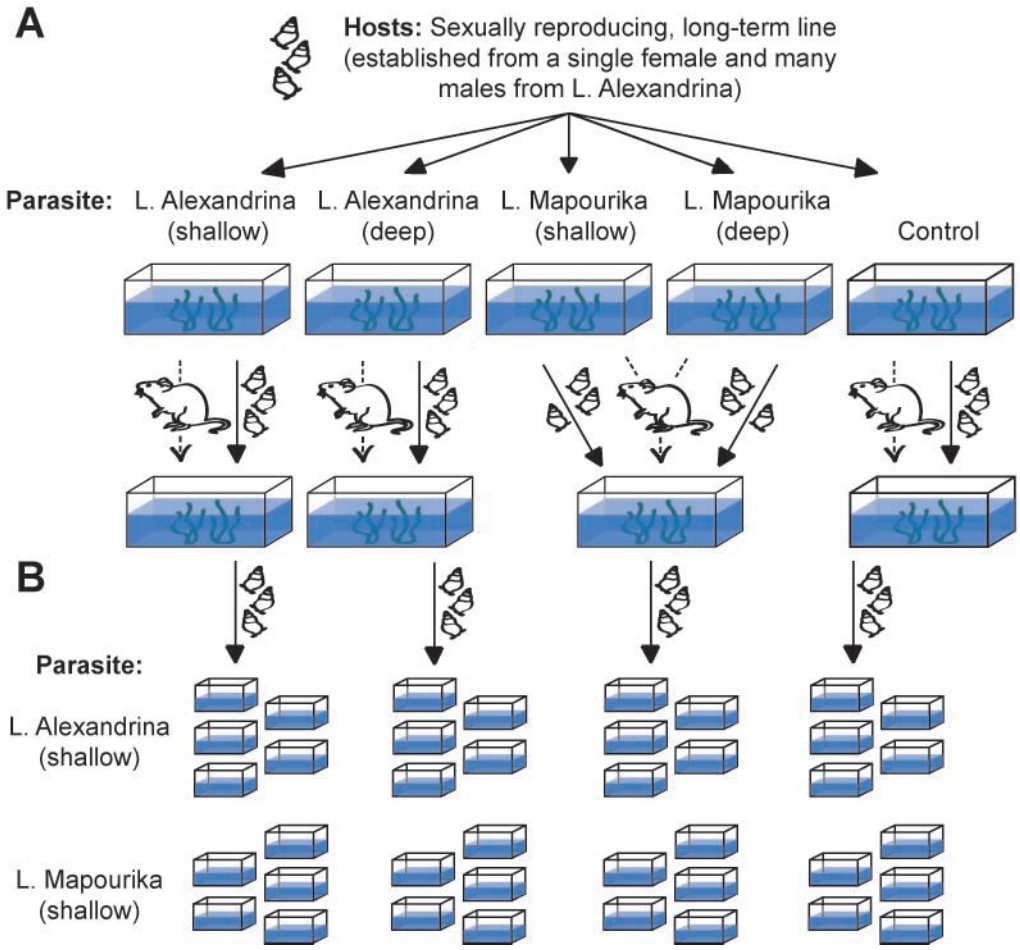

Fig. 1. Experimental methods for (A) the experimental co-evolution of snail and trematode populations and (B) the cross-inoculation run at the end of the study between experimental hosts and field parasites from the original source lakes.

cross-inoculation experiment. Specifically, five replicate containers from each host line were exposed to inocula generated from field-infected snails from either Lake Alexandrina or Lake Mapourika. We chose to use field rather than experimental parasites for two reasons: (1) the prevalence of experimental infections at the end of the experiment was low; and (2) we specifically wanted to examine host evolution in response to parasite populations, without potential confounding effects of parasite adaptation to hosts within the experiment. The inoculum was generated for each parasite source by feeding 30 infected snails, that had been collected 6 months earlier from the appropriate lake in New Zealand, to each of four mice. Mouse faeces were collected and cleaned as described above. Snails were kept on a water table for 3 months, with constant flow through of treated water, and then dissected to determine infection status.

We compared the prevalence of infection across treatments using a generalized linear model (GLM) with a logit link function and binomial errors. To compare the results of the

final cross-inoculation experiment, we used analysis of variance (ANOVA) in which the mean infection prevalence for each replicate container was arcsin-square root transformed and examined as the dependent variable, with the treatment tank of origin (i.e. the previous parasite exposure) used as a fixed factor. Unfortunately, the inoculum from Lake Alexandrina used in the final experiment did not result in any infections, regardless of host treatment, and thus only infection by parasites from Lake Mapourika was examined in this analysis. We then followed up with a series of planned linear contrasts to compare: (1) the two host tanks exposed to parasites from Lake Alexandrina to those that were not (i.e. the control tank and the tank exposed to Lake Mapourika parasites); and (2) hosts exposed to parasites from Lake Mapourika to those that did not receive parasites (controls).

RESULTS

Although the experimental host populations were all derived from the same sexual line (which had been kept in the laboratory for 5 years prior to the experiment), we saw marginally significant differences in susceptibility of the initial host line to each of the four parasite sources ($\chi^2_3 = 7.470$, $P = 0.058$). Specifically, after the first round of inoculation, the host population exposed to Lake Alexandrina 'deep' parasites had an infection prevalence of $22 \pm 4.4\%$ ($\pm$S.E.); the population exposed to Lake Alexandrina 'shallow' parasites had a prevalence of $15 \pm 3.2\%$; the population exposed to Lake Mapourika 'deep' parasites had a prevalence of $16 \pm 3.6\%$; and the population exposed to Lake Maporika 'shallow' parasites had a prevalence of $32 \pm 6.9\%$. This result suggests that there was relatively strong selection for resistance during the first round of exposure. Consistent with strong selection on the host, the infection prevalences after the second exposure were quite low, and did not differ across treatments ($\chi^2_2 = 0.503$, $P = 0.778$): 1.6% for the Lake Alexandrina deep treatment, 1.6% for the Lake Alexandrina shallow treatment, and 3.2% for the Lake Mapourika treatment (combined, as described above; Fig. 1), suggesting that most offspring from the parents that survived to reproduce after the first inoculation were more resistant than the ancestral population.

The results of the final exposure to parasites from Lake Mapourika showed that the experimental host populations had diverged over the course of the experiment in terms of their susceptibility to these parasites (Fig. 2). Host susceptibility to parasites from Lake Mapourika differed significantly across treatments (ANOVA: $F_{3,19} = 15.102$, $P < 0.001$) (Fig. 2). Specifically, the host population originally exposed to parasites from Lake Mapourika was more resistant to parasites from Lake Mapourika than the control host population ($t_{16} = 2.615$, $P = 0.019$). Also, the hosts originally exposed to Lake Alexandrina parasites were more susceptible to parasites from Lake Mapourika than hosts that had not been previously exposed to parasites from Lake Alexandrina (i.e. the mean of control tanks and Lake Mapourika-exposed tanks; $t_{16} = 6.189$, $P < 0.001$). Hence, generalized resistance did not evolve. Instead, it appears that the evolution of resistance to one parasite population increased the susceptibility to another parasite population.

DISCUSSION

There is strong evidence from both natural and experimental studies that parasites can exert strong selection pressure on their local host populations and that host populations can respond by evolving increased resistance to their local parasites (Buckling and Rainey, 2002; Koskella

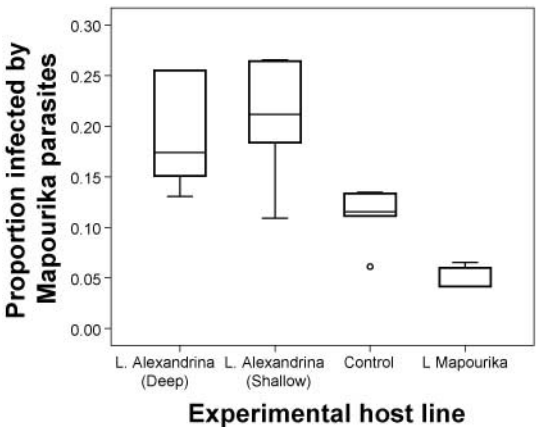

Fig. 2. Infection success of parasites from Lake Mapourika on experimental hosts, all derived from the same sexual line, evolved in either the presence of parasites from Lake Alexandrina (deep and shallow parasite sources), no parasites (control), or parasites from Lake Mapourika (shallow and deep parasites combined). Lines show median infection prevalence across the five replicates per experimental host line; boxes show 25% to 75% quartiles and whiskers show ranges.

and Lively, 2007; Zbinden *et al.*, 2008; Schulte *et al.*, 2010). Predicting how this increased resistance will structure populations over space and maintain diversity requires an understanding of the specificity of these evolutionary responses (Duffy *et al.*, 2008; Lazzaro and Little, 2009). For example, parasite-mediated selection might result in hosts that are very well adapted to resisting their local parasites, but are no longer resistant to other parasite populations. Alternatively, this selection might result in a host population having generally higher resistance to parasites regardless of their source. Experimental evolution provides a powerful tool to explicitly test the consequences of host–parasite interactions in that it allows for the examination of population-level change over time in specific response to the selection pressure of interest, while controlling for other selective and neutral evolutionary processes. Here, we used a simple experimental evolution approach (Fig. 1) to demonstrate a very rapid and specific response of sexual host populations derived from the same laboratory line to two geographically distinct parasite populations.

Our results show that sexual hosts populations can rapidly evolve resistance against parasites; after only two generations of exposure to sterilizing trematode parasites from Lake Mapourika, hosts were found to be more resistant to parasites collected from that lake than were either control hosts, which had not been exposed to parasites, or hosts that had been exposed to a different parasite source (Fig. 2). This result adds to a growing body of evidence (primarily from asexual host systems) that hosts evolve resistance in a parasite population-specific manner. For example, the freshwater protozoan, *Paramecium caudatum*, showed a marked increase in resistance to parasites during experimental co-evolution, and they were found to be more resistant to their own experimental parasite population than to parasites from other co-evolving lines (Lohse *et al.*, 2006). Similarly, populations of the bacterium, *Pseudomonas fluorescens*, showed increased resistance to local bacteriophages after experimental evolution (Buckling and Rainey, 2002).

We also found that host snails that were exposed to parasites from Lake Alexandrina were more susceptible to parasites from Lake Mapourika at the end of the experiment than

hosts from the other two treatments (i.e. no parasite controls and hosts exposed to Lake Mapourika parasites). Although we cannot definitively say whether these hosts evolved specific resistance to parasites from Lake Alexandrina (due to the failure of the inocula during the cross-inoculation), the results of the second exposure during experimental evolution suggest that resistance had evolved just as quickly in these tanks as it had in the tanks exposed to Lake Mapourika parasites. Thus, the most likely interpretation is that these hosts evolved increased resistance to their local experimental parasites but did not evolve a general resistance to parasites and may even have become more susceptible to foreign parasites. Importantly, the experimental results presented here relate to adaptation over a very short time scale and focus on host–parasite interactions in a closed system with no migration. However, the rapid response to parasite-mediated selection observed here confirms a key assumption of the Red Queen Hypothesis for the maintenance of sexual reproduction: that sexual host populations are able to adapt specifically to local parasites over very short time scales.

ACKNOWLEDGEMENTS

Funding for the project was provided by the US National Science Foundation (NSF-DEB 0754399 to B.K. and DEB-0640639 to C.M.L.).

REFERENCES

Buckling, A. and Rainey, P.B. 2002. Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. R. Soc. Lond. B*, **269**: 931–936.

Duffy, M.A., Brassil, C.E., Hall, S.R., Tessier, A.J., Caceres, C.E. and Conner, J.K. 2008. Parasite-mediated disruptive selection in a natural *Daphnia* population. *BMC Evol. Biol.*, **8**: 80.

Dybdahl, M.F. and Lively, C.M. 1996. The geography of coevolution: comparative population structures for a snail and its trematode parasite. *Evolution*, **50**: 2264–2275.

Dybdahl, M.F., Jokela, J., Delph, L.F., Koskella, B. and Lively, C.M. 2008. Hybrid fitness in a locally adapted parasite. *Am. Nat.*, **172**: 772–782.

Greischar, M.A. and Koskella, B. 2007. A synthesis of experimental work on parasite local adaptation. *Ecol. Lett.*, **10**: 418–434.

Haldane, J.B.S. 1949. Disease and evolution. *La Ricerca Scientifica*, **19** (suppl.): 68–76.

Hamilton, W.D. 1982. Pathogens as causes of genetic diversity in their host populations. In *Population Biology of Infectious Diseases* (R.M. Anderson and R.M. May, eds.), pp. 269–296. New York: Springer-Verlag.

Hamilton, W.D. 1993. Haploid dynamic polymorphism in a host with matching parasites: effects of mutation/subdivision, linkage, and patterns of selection. *J. Hered.*, **84**: 328–338.

Hoeksema, J.D. and Forde, S.E. 2008. A meta-analysis of factors affecting local adaptation between interacting species. *Am. Nat.*, **171**: 275–290.

Hutson, V. and Law, R. 1981. Evolution of recombination in populations experiencing frequency-dependent selection with time delay. *Proc. R. Soc. Lond. B*, **213**: 345–359.

Jokela, J., Dybdahl, M.F. and Lively, C.M. 2009. The maintenance of sex, clonal dynamics, and host–parasite coevolution in a mixed population of sexual and asexual snails. *Am. Nat.*, **174**: S43–S53.

Koskella, B. and Lively, C.M. 2007. Advice of the rose: experimental coevolution of a trematode parasite and its snail host. *Evolution*, **61**: 152–159.

Koskella, B. and Lively, C.M. 2009. Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. *Evolution*, **63**: 2213–2221.

Lambrechts, L., Halbert, J., Durand, P., Gouagna, L. and Koella, J. 2005. Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitoes to *Plasmodium falciparum*. *Malaria J.*, **4**: 3.

Lazzaro, B.P. and Little, T.J. 2009. Immunity in a variable world. *Phil. Trans. R. Soc. Lond. B*, **364**: 15–26.

Lively, C.M. and Dybdahl, M.F. 2000. Parasite adaptation to locally common host genotypes. *Nature*, **405**: 679–681.

Lively, C.M. and Jokela, J. 1996. Clinal variation for local adaptation in a host–parasite interaction. *Proc. R. Soc. Lond. B*, **263**: 891–897.

Lively, C.M., Dybdahl, M.F., Jokela, J., Osnas, E.E. and Delph, L.F. 2004. Host sex and local adaptation by parasites in a snail–trematode interaction. *Am. Nat.*, **164**: S6–S18.

Lohse, K., Gutierrez, A. and Kaltz, O. 2006. Experimental evolution of resistance in *Paramecium caudatum* against the bacterial parasite *Holospora undulate*. *Evolution*, **60**: 1177–1186.

Peters, A.D. and Lively, C.M. 1999. The red queen and fluctuating epistasis: a population genetic analysis of antagonistic coevolution. *Am. Nat.*, **154**: 393–405.

Rauch, G., Kalbe, M. and Reusch, T.B.H. 2006. One day is enough: rapid and specific host–parasite interactions in a stickleback–trematode system. *Biol. Lett.*, **2**: 382–384.

Schulte, R.D., Makus, C., Hasert, B., Michiels, N.K. and Schulenburg, H. 2010. Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. *Proc. Natl. Acad. Sci. USA*, **107**: 7359–7364.

Thompson, J.N. 1999. Specific hypotheses on the geographic mosaic of coevolution. *Am. Nat.*, **153**: S1–S14.

Thompson, J.N. 2005. *The Geographic Mosaic of Coevolution*. Chicago, IL: University of Chicago Press.

Winterbourn, M.J. 1973. Larval Trematoda parasitizing the New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Mauri Ora*, **2**: 17–30.

Wolinska, J. and Spaak, P. 2009. The cost of being common: evidence from natural *Daphnia* populations. *Evolution*, **63**: 1893–1901.

Zbinden, M., Haag, C.R. and Ebert, D. 2008. Experimental evolution of field populations of *Daphnia magna* in response to parasite treatment. *J. Evol. Biol.*, **21**: 1068–1078.