Host Sex and Local Adaptation by Parasites in a Snail-Trematode Interaction

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ABSTRACT: One of the leading theories for the evolutionary stability of sex in eukaryotes relies on parasite-mediated selection against locally common host genotypes (the Red Queen hypothesis). As such, parasites would be expected to be better at infecting sympatric host populations than allopatric host populations. Here we examined all published and unpublished infection experiments on a snail-trematode system (*Potamopyrgus antipodarum* and *Microphallus* sp., respectively). A meta-analysis demonstrated significant local adaptation by the parasite, and a variance components analysis showed that the variance due to the host-parasite interaction far exceeded the variance due to the main effects of host source and parasite source. The meta-analysis also indicated that asexual host populations were more resistant to allopatric sources of parasites than were (mostly) sexual host populations, but we found no significant differences among parasite populations in the strength of local adaptation. This result suggests that triploid asexual snails are more resistant to remote sources of parasites, but the parasite has, through coevolution, overcome the difference. Finally, we found that the degree of local adaptation did not depend on the genetic distance among host populations. Taken together, the results demonstrate that the parasites are adapted, on average, to infecting their local host populations and suggest that they may be a factor in selecting against common host genotypes in natural populations.

Keywords: coevolution, local adaptation, meta-analysis, parasites, Red Queen hypothesis, sexual reproduction.

One of the more curious facts in evolutionary biology is that sexual reproduction persists in populations where asexual reproduction is known to occur. The observation is curious because reproductively isolated asexuals have an intrinsic reproductive advantage that stems from the fact that clonal females make only daughters, thereby saving on the cost of producing males (Maynard Smith 1971, 1978). Assuming that the asexuals are equally fecund and ecologically identical, they should rapidly replace the sexuals. The time for replacement depends on population size but is expected to be less than 60 generations for up to 10^6 individuals (Lively 1996). Similarly, an allele for self-fertilization in a population should go to fixation in a similarly short period (Fisher 1941; Williams 1975). Hence uniparental reproduction by parthenogenesis or self-fertilization should prevail if, in fact, all else is equal.

The widespread distribution of outcrossing suggests that all else is not equal in nature. The question, then, is what are the ecological and/or genetic mechanisms that ameliorate the costs of biparental reproduction? A number of hypotheses exist, which have been extensively reviewed (reviews in Bell 1982; Kondrashov 1993). One of the hypotheses that has received support relies on coevolution with biological enemies. At the core of this idea (known as the Red Queen hypothesis, following Bell 1982) is the assumption that parasites are under strong selection to infect the most common host genotypes. Virulent parasites are then expected to reduce the frequency of the most common host genotype, and a different host genotype then becomes most common. As such the parasite is never perfectly adapted to the host population with which it coevolves, but it may aid in preventing fixation of asexual clones and thereby contribute to the maintenance of host sex (Glesener and Tilman 1978; Bell 1982; Hamilton 1982; Antonovics and Ellstrand 1984; Lively 1987; Howard and Lively 1994). The same coevolutionary interaction can also contribute to selection for sexual reproduction in parasites (Lythgoe 2000; Galvani et al. 2001, 2003; Howard and Lively 2002).

One expectation of the Red Queen hypothesis is that the parasites should quickly become adapted to infecting their local (sympatric) host populations ("local adaptation"). Specifically, parasites are expected to become more infective to hosts from the same geographic location relative to allopatric sources of hosts (Dybdahl and Storfer

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Am. Nat. 2004. Vol. 164, pp. S6–S18. 0003-0147/2004/1640S5-40113$15.00.

Table 1: Sources of data

Study	Parasite population	Host population	Year	Source
1	Mapourika, Alexandrina	Mapourika, Alexandrina	1987	Lively 1989
2	Alexandrina, Mapourika, Wahapo	Alexandrina, Mapourika, Wahapo	1988	Lively 1989
3	Ianthe, Poerua	Ianthe, Poerua (A)	1997	Lively and Dybdahl 2000
4	Alexandrina, Poerua	Alexandrina, Poerua (A), Ianthe	2001	M. F. Dybdahl, J. Jokela, and C. M. Lively, unpublished manuscript
5	Alexandrina	Alexandrina, Poerua (A)	2001	Osnas and Lively 2004
6	Alexandrina, Ianthe	Alexandrina, Ianthe	2001	This study
7	Alexandrina, Mapourika	Alexandrina, Mapourika	2002	E. E. Osnas and C. M. Lively, unpublished manuscript
8	Mapourika	Mapourika, Alexandrina	2002	This study
9	Alexandrina, Ianthe	Alexandrina, Ianthe	2001	M. F. Dybdahl, unpublished data
10	Alexandrina, Mapourika	Alexandrina, Mapourika, McGregor (A)	2003	This study
11	Ianthe	Ianthe, Mapourika, Ellery, Paringa	2003	This study
12	Poerua	Poerua (A), Mapourika, Ellery, Paringa	2003	This study

Note: Year indicates the year that the study was conducted. "A" indicates an asexual host snail population; all other host populations contain a mixture of asexual and (mostly) sexual snails. See figure 1 for locations of populations.

2003). Theoretical models have been consistent with the expectation of local adaptation, but because of the inherent time lags in the system, local parasite adaptation is an average rather than uniform effect (Morand et al. 1996; Kaltz and Shykoff 1998; Lively 1999). In addition, simulation models have suggested that average local adaptation by parasites should be more likely when parasite virulence is high (Lively 1999) and when parasite dispersal is higher than host dispersal (Gandon 2002; Gandon and Michalakis 2002), but not so high as to swamp out the effects of local selection (Lively 1999; Gandon 2002; Gandon and Michalakis 2002). Moreover, contrary to conventional wisdom (Kaltz and Shykoff 1998), recent studies have found that average local adaptation by parasites does not require that they have faster generation times than their hosts (Lively 1999; Gandon 2002; Gandon and Michalakis 2002).

A range of results have been found in empirical studies of host-parasite interactions, ranging from strong local parasite adaptation (e.g., Parker 1985; Karban 1989; Ebert 1994; Mopper et al. 1995; Thrall et al. 2002) to nonsignificant differences between sympatric and allopatric host populations (e.g., Parker 1989; Strauss 1997; Imhoof and Schmid-Hempel 1998; Mutikainen et al. 2000) to results showing greater parasite infectivity on allopatric hosts (Kaltz et al. 1999; Oppliger et al. 1999). It is difficult to know all the sources of variation among results, but a recent review has shown that parasites with narrow host ranges are more likely to exhibit local adaptation than those with broad host ranges (Lajeunesse and Forbes 2002). The result makes sense because tracking of common local host genotypes would seem less complicated for parasites restricted to one host species (Lajeunesse and Forbes 2002), which is the situation most commonly assumed in spatial models of host-parasite coevolution (Morand et al. 1996; Lively 1999; Gandon 2002; Gandon and Michalakis 2002).

For parasites with narrow host ranges, the strength of local adaptation is expected to vary in space and time (Morand et al. 1996; Kaltz and Shykoff 1998; Lively 1999). The expectation stems from the fact that, in simulation models, parasite populations track local host populations but with a 90° time lag. If all the host populations have the same loci and alleles for resistance (but different multilocus genotype frequencies), then the strength of local adaptation depends greatly on the position of the allopatric host in genotype space, which varies over space and time. In fact, the model by Morand et al. (1996) suggests that parasites may periodically be more infective to allopatric than sympatric hosts (local "maladaptation"). Moreover, the model suggests that this outcome should be observed in a "significant minority" of cases and that it may occasionally be as strong or stronger than local adaptation (fig. 5*a* in Morand et al. 1996). Data from 48 studies on the infectivity of a trematode (*Schistosoma mansoni*) to its snail host showed two of 48 cases for which parasites were more infective to allopatric hosts, and both cases were highly significant (Morand et al. 1996). On the other hand (as the authors point out), the local snail species is believed to have been recently introduced in both of these cases. In the remaining 46 cases, 28 showed statistically significant local adaptation, and a sign test revealed this as a significant bias. One of the goals of our study was to determine the distribution of differences for infectivity on sympatric versus allopatric host populations in another

Figure 1: Locator map of the South Island, New Zealand, showing approximate locations of host and parasite source populations. Triangles represent the Southern Alps.

trematode-snail interaction for which the parasite has a narrow host range.

Models of host-parasite coevolution typically assume very tight specificity (genetic matching) for infection. Parasite genotypes that "match" the host are recognized as "self" by the host, and they are not attacked by the innate immune system. Mismatched parasite genotypes are recognized as "nonself," and these are killed by the host. There is some evidence for this kind of system from graft-rejection experiments in tunicates (Grosberg and Hart 2000), but it remains controversial. An alternative model for infection is the gene-for-gene (GFG) model, which has been favored by plant pathologists since the pioneering work by Flor (Flor 1956). Under this model, infection can occur in several ways: the host is susceptible at all loci involved; the host has resistance alleles, but they are matched by the appropriate "avirulence" alleles in the parasite; or the parasite has "virulence" alleles at all loci, which trump the resistance alleles of hosts. The GFG model has a broad base of support in agricultural systems (Parker 1994, 1996; Thrall and Burdon 2002). One interesting expectation generated by the simple GFG model is that host and parasite populations should vary in space for the frequencies of resistance and virulence alleles (Thrall et al. 2002). Such variation could break down local adaptation since some host populations would be composed of generally susceptible individuals and because some parasite populations would be composed of generally "virulent" (here meaning unconditionally infective) individuals. Indeed, Parker (1994) has shown in computer simulations that GFG genetics will not generally favor the evolution of recombination, and in another article (Lively 1999), it was found that the introduction of unconditional infectivity leads to the rapid breakdown of local adaptation. Using a meta-analysis, we tested the idea that host populations vary in resistance and that parasite populations vary in unconditional infectivity. We also determined the variance associated with host source, parasite source, and their interaction for eight reciprocal cross-infection experiments.

Material and Methods

The Study System

We analyzed all studies for which the digenetic trematode *Microphallus* was exposed to sympatric and allopatric populations of its first intermediate host *Potamopyrgus antipodarum*. Some of these studies were previously published, but some are reported here for the first time (table 1). No replicated studies were excluded, so there is no concern here regarding publication bias (see Rosenberg et al. 2000; Jennions and Møller 2002). One unreplicated study was excluded because we could not calculate a standard deviation, which is required by the method of meta-analysis we chose. This study nonetheless showed significantly higher parasite infectivity on the sympatric host population (Lively and McKenzie 1991).

For our purposes here, we define "study" as a set of comparisons that were made at the same time using the same methods. We define "comparison" as a contrast for one parasite population on its sympatric host versus one of the allopatric hosts within a study. In all, there were 12 studies and 32 sympatric-allopatric comparisons.

The exact experimental methods varied among studies, as did the number of replicates. However, all the studies had the following methods in common. The host snails were collected from the shallow-water margins of lakes on New Zealand's South Island. Parasites were collected from some of these snail populations. A total of six lakes were used as sources for parasite populations, and seven lakes were used for hosts. Host populations either were mixtures of mostly diploid sexual snails and triploid asexual snails ("mixed" populations) or they were composed of triploid all-female asexual snails (table 1; Dybdahl and Lively 1995). Following a variable period of habituation to lab conditions, we counted 40–120 of the snails into 2-L plastic containers that were about half full of water. The containers were then randomly assigned to receive parasites from a single source population. In all of our statistical analyses, these containers (not the snails) were treated as the experimental units.

Table 2: Results from model 2 ANOVA for the 12 infection experiments

Study	MS (para)	MS (host)	MS (para × host)	MS (error)	MS (contrast)	Mean ± SE sympatric	Mean ± SE allopatric
1	9,160 (1)	98 (1)	12,116 (1)***	231 (31)	12,700 (1)***	65.94 ± 5.43	27.83 ± 5.27
2	1,447 (2)	256 (2)	1,619 (1)***	87 (27)	6,321 (1)***	55.72 ± 3.81	27.61 ± 2.69
3	939 (1)	14 (1)	9,923 (1)***	49 (12)	9,923 (1)***	67.76 ± 3.72	17.95 ± 3.72
4	2,884 (1)	4,094 (2)	4,607 (1)***	78 (39)	17,958 (1)***	56.66 ± 3.36	14.28 ± 2.37
5		36,856 (1)***		39 (27)	36,856 (1)***	84.76 ± 1.36	5.00 ± 2.21
6	1,168 (1)	421 (1)	1,380 (1)***	95 (46)	776 (1)**	10.11 ± 2.33	2.17 ± 2.06
7	28 (1)	11 (1)	2,200 (1)***	38 (43)	2,200 (1)***	14.58 ± 1.68	1.04 ± 1.68
8		2,856 (1)***		76 (14)	2,856 (1)***	21.10 ± 2.66	4.20 ± 2.66
9	282 (1)	149 (1)	1,515 (1)***	32 (8)	1,515 (1)***	24.65 ± 3.39	2.18 ± 3.39
10	552 (1)	538 (2)	495 (1)***	7 (12)	1,557 (1)***	24.30 ± 3.46	4.57 ± 2.45
11		795 (3)**		88 (8)	148 (1)	28.53 ± 9.91	20.41 ± 5.72
12		1,117 (3)*		235 (8)	1,530 (1)*	51.27 ± 11.15	25.19 ± 6.41

Note: Host and parasite (para) were analyzed as random effects. Experiments 1–4, 6, 7, 9, and 10 were fully or partially reciprocal (shown in bold type). Parenthetic values in the table give the degrees of freedom. MS = mean squares. MS (contrast) is the linear contrast between sympatric and allopatric combinations of host and parasites.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

The parasites have two hosts in their life cycle. The definitive hosts are waterfowl or wading birds. The worms enter the birds as encysted larvae (metacercariae), which live within the tissue of living snails. The larvae "hatch" following ingestion by the bird. A small (0.2 mm) hermaphroditic worm emerges and matures in about 24 h. The worms then cross-fertilize (as indicated by population genetic data; Dybdahl and Lively 1996) and begin producing eggs. Embryonated eggs can be collected from the feces by about 36 h or less, postinfection. For all experiments, we substituted mice for the birds as the definitive hosts (as we have found that the worms mature and produce viable eggs in mice). Here we are interested in whether the eggs are more infective to sympatric versus allopatric host snails.

The parasite species is presently undescribed, as are most of the trematodes that infect New Zealand snails. However, genetic data show that *Microphallus* sp. is a single species with high levels of gene flow and low levels of population structure across New Zealand's South Island (Dybdahl and Lively 1996). In contrast, the host snail is highly structured, suggesting that gene flow among parasite populations is in excess of that among host populations (Dybdahl and Lively 1996). This is the kind of situation where local adaptation is expected in the parasite population, provided that gene flow does not swamp the effects of local selection (Judson 1995; Gandon et al. 1996; Lively 1999; Gandon 2002).

Parasites from a single lake were fed to one or more mice. We collected the fecal pellets from the mice (beginning 24–48 h postinfection) and distributed them at random to containers containing the snails (randomly assigned to parasite treatment). We did not measure the number of eggs per snail (dose), so dose is a source of variation among parasite sources (e.g., Osnas and Lively 2004), but it is not a source of variation among host sources within each parasite source. Successful infections can be detected after about 70 days, depending on temperature, as blastocercariae. Blastocercariae are relatively undifferentiated larvae that develop into metacercariae within the snail host. As such, the second intermediate host of many Digenea is skipped. The metacercariae become fully formed after 3–4 months postinfection, at which point they are transmissible to the final host. We were able to discriminate between infections gained in the field, before collection of the snails, and experimental infections because field infections are generally at the most advanced developmental stage. However, we also included containers of snails that were fed the fecal material of uninfected mice for determination of background levels of infection for most studies.

Statistical Analysis

ANOVA. We analyzed all 12 experiments using a separate ANOVA for each study. There were two basic kinds of studies. In one type, the cross-infection experiments were fully ($N = 6$) or partially ($N = 2$) reciprocal (table 1). These studies are the most valuable for detecting local adaptation because they allow for the calculation of host effects, parasite effects, and interaction effects. Unlike previous studies (Thrall et al. 2002), including our own (Lively 1989; Lively and Dybdahl 2000), we treated the host and parasite as random, rather than fixed, effects. We do not

Table 3: Results from variance components for the 12 infection experiments

Study	Var (parasite)	Var (host)	Var (parasite × host)	No. snails/replicate
1	176.06 (16.3)	<.01 (<.1)	673.87 (62.3)	17.1
2	23.48 (6.2)	<.01 (<.1)	269.55 (71.0)	19.7
3	<.01 (<.1)	<.01 (<.1)	894.00 (94.8)	60.8
4	<.01 (<.1)	4.51 (.7)	550.21 (86.9)	75.9
5		3,177.61 (98.8)		93.3
6	<.01 (<.1)	<.01 (<.1)	72.23 (43.2)	13.9
7	<.01 (<.1)	<.01 (<.1)	59.04 (60.7)	57.7
8		777.97 (91.06)		57.8
9	<.01 (<.1)	<.01 (<.1)	205.35 (86.4)	33.3
10	6.45 (3.5)	7.24 (4.0)	162.37 (88.5)	52.2
11		235.72 (72.8)		62.7
12		294.02 (55.6)		63.3

Note: Experiments 1–4, 6, 7, 9, and 10 were fully or partially reciprocal (shown in bold type). Variance components calculated using restricted maximum likelihood (REML). Parenthetic values give the percentage of variation explained. Variance (Var) due to error is given in table 2 as MS (error). The last column gives the mean number of snails per replicate used to calculate infection frequency. All calculations from SPSS 11.0.

mean to imply by this that the lakes were selected at random. They were, in fact, selected based on their accessibility and the presence of both host and parasite. Rather, the populations are random in the sense that they were not selected to give specific fixed intervals within the distribution of possible sites. One advantage of the random effects model (model 2) is that generalizations about the effects of host populations and parasite populations can be made (Sokal and Rohlf 1981; Underwood 1997), which was our present goal. One disadvantage, however, is that there is less statistical power to evaluate the main effects, but this problem is ameliorated by the meta-analysis described below. In addition, we constructed a linear contrast for each analysis that compared sympatric versus allopatric combinations. The contrast yields a test for the specific kind of interaction that is of biological interest here. It also gives the average effect of sympatry versus allopatry across the study (Thrall et al. 2002). Finally, we calculated the variance components for parasite source, host source, and their interaction using restricted maximum likelihood, REML (for an intuitive overview of REML, see Lynch and Walsh 1998). All calculations were made using SPSS (version 11.0).

The other type of study was not reciprocal but included only the eggs from one parasite population exposed to two or more host populations ($N = 4$). This design is less valuable because it confounds the host effect with the host-parasite interaction, but it nonetheless yields sympatric versus allopatric combinations for the meta-analysis. It also yields data for testing for the relationship between effect size and genetic distance. We analyzed these data using a one-way ANOVA to test for the effect of host, followed by a linear contrast that compared sympatric versus allopatric combinations.

Meta-analysis. We used standard meta-analysis to analyze the entire data set. Specifically, we used a random effects model to determine whether the average effect size (Hedge's d) was significantly different from 0. For all comparisons, we calculated Hedge's d as

$$d = J\frac{\bar{x}_{\text{sym}} - \bar{x}_{\text{allo}}}{\sigma_{\text{pooled}}},$$

where $\bar{x}_{\text{sym}}$ was equal to the mean across containers for sympatric hosts and $\bar{x}_{\text{allo}}$ was equal to the mean across containers for allopatric hosts (Gurevitch and Hedges 1993). Thus, a positive value indicates local adaptation by the parasite, and a negative value indicates that the parasite is more infective to allopatric than sympatric host populations (following Van Zandt and Mopper 1998). The denominator is the pooled standard deviation, and the weighting factor J corrects for bias due to small sample sizes (Gurevitch and Hedges 1993).

We also used meta-analysis to determine whether there were significant differences among studies, whether there were significant differences among parasite populations, whether there were significant differences among host populations in their resistance to allopatric parasites, and whether any such differences were related to the reproductive mode of the host population (mixed sexual and asexual vs. asexual). All analyses were treated as random-effects models and analyzed using MetaWin 2.0 (Rosenberg et al. 2000). Random-effects models are more conservative than fixed-effects models in meta-analysis

Table 4: Means, sample sizes, and standard deviations for 32 comparisons in 12 experimental infection studies

Study[a]	Comparison	Parasite and sympatric host source	Allopatric host	Reproductive mode of allopatric host[b]	*N*(sym)	*N*(allo)	$\bar{x}$ (sym)	$\bar{x}$ (allo)	σ (sym)	σ (allo)	*d*	σ^2 (*d*)
1	1	Alexandrina	Mapourika	Mixed	8	9	52.55	9.67	16.37	12.67	2.81	.47
1	2	Mapourika	Alexandrina	Mixed	9	9	79.90	45.58	12.91	14.81	2.35	.38
2	3	Mapourika	Paringa	Mixed	4	4	44.43	9.99	9.17	9.87	3.14	1.12
2	4	Mapourika	Wahapo	Mixed	4	4	44.43	24.36	9.17	9.35	1.89	.72
2	5	Paringa	Mapourika	Mixed	4	4	51.26	27.72	9.23	9.06	2.24	.81
2	6	Paringa	Wahapo	Mixed	4	4	51.26	30.78	9.23	9.35	1.92	.73
2	7	Wahapo	Mapourika	Mixed	4	4	71.60	34.58	9.17	9.17	3.51	1.27
2	8	Wahapo	Paringa	Mixed	4	4	71.60	38.47	9.17	9.35	3.11	1.11
3	9	Ianthe	Poerua	Asexual	4	4	59.81	11.02	7.62	6.91	5.83	2.63
3	10	Poerua	Ianthe	Mixed	4	4	76.38	24.27	6.69	7.13	6.56	3.19
4	11	Alexandrina	Ianthe	Mixed	6	6	40.68	1.60	7.56	8.16	4.59	1.21
4	12	Alexandrina	Poerua	Asexual	6	6	40.68	13.21	7.56	7.00	3.48	.84
4	13	Poerua	Alexandrina	Mixed	9	6	67.16	23.60	6.93	6.04	6.21	1.56
4	14	Poerua	Ianthe	Mixed	9	9	67.16	14.50	6.93	7.86	6.77	1.50
5	15	Alexandrina	Poerua	Asexual	21	8	84.98	4.89	6.27	6.08	12.51	2.87
6	16	Alexandrina	Ianthe	Mixed	15	15	4.69	.05	8.71	11.03	.45	.14
6	17	Ianthe	Alexandrina	Mixed	15	15	24.51	3.85	14.38	8.08	1.72	.18
7	18	Alexandrina	Mapourika	Mixed	12	12	15.73	1.39	7.66	10.05	1.55	.22
7	19	Mapourika	Alexandrina	Mixed	12	12	13.38	.71	9.59	7.83	1.40	.21
8	20	Mapourika	Alexandrina	Mixed	10	10	28.93	4.29	8.75	7.08	2.97	.42
9	21	Alexandrina	Ianthe	Mixed	3	3	17.05	1.09	5.40	6.02	2.23	1.08
9	22	Ianthe	Alexandrina	Mixed	3	3	33.33	3.55	6.19	5.60	4.04	2.03
10	23	Alexandrina	Mapourika	Mixed	3	3	37.19	9.95	2.99	2.32	8.15	6.21
10	24	Alexandrina	McGregor	Asexual	3	3	37.19	3.73	2.99	2.59	9.58	8.31
10	25	Mapourika	Alexandrina	Mixed	3	3	11.91	5.13	2.27	3.04	2.02	1.01
10	26	Mapourika	McGregor	Asexual	3	3	11.91	.00	2.27	2.88	3.67	1.79
11	27	Ianthe	Ellery	Mixed	3	3	28.19	3.10	12.67	13.92	1.51	.86
11	28	Ianthe	Mapourika	Mixed	3	3	28.19	41.02	12.67	12.13	−.83	.72
11	29	Ianthe	Paringa	Mixed	3	3	28.19	16.99	12.67	15.37	.64	.70
12	30	Poerua	Ellery	Mixed	3	3	51.66	8.14	12.38	13.01	2.74	1.29
12	31	Poerua	Mapourika	Mixed	3	3	51.66	42.66	12.38	12.70	.57	.69
12	32	Poerua	Paringa	Mixed	3	3	51.66	25.01	12.38	15.82	1.50	.85

Note: Sym = sympatric, allo = allopatric, *d* = Hedge's *d*.

[a] Number refers to the study number in table 1.

[b] Mixed indicates a mixture of asexual and (mostly) sexual females; asexual indicates asexual females only.

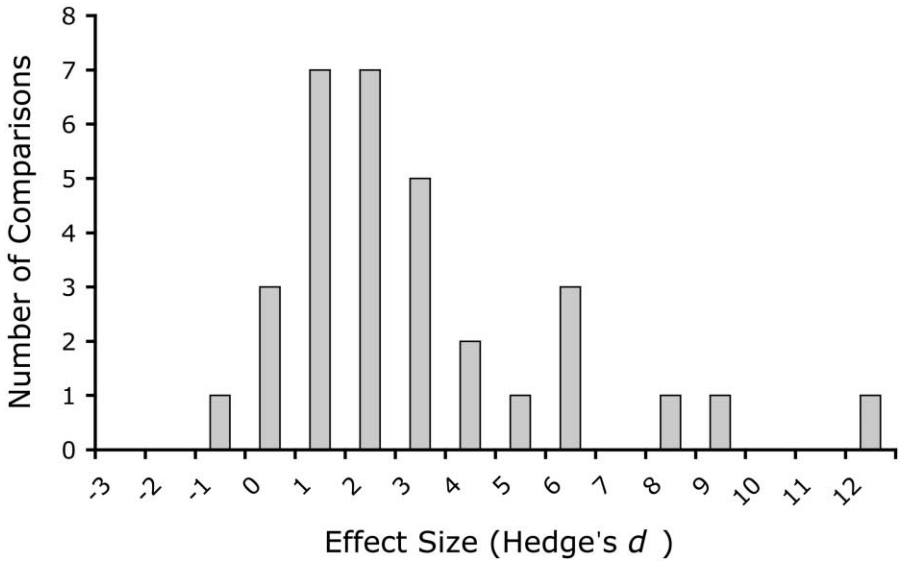

Figure 2: Distribution of effect sizes (Hedge's *d*) from meta-analysis

because they do not assume that each experiment is estimating the same mean (Gurevitch and Hedges 1993; Rosenberg et al. 2000). Variation among means would be expected here because the studies differed as a result of several key factors. For example, parasite dose, number of snails per container, condition of the snails, and feeding protocols were all sources of variation among experiments.

The raw data for the meta-analysis were prepared as follows. The untransformed percentages of lab-infected snails for each container were recorded. The data for each study were entered into an ANOVA using SPSS 11.0, where the percentage of infected snails was weighted by the number of snails in the sample. The means for the meta-analysis were the estimated marginal means from this analysis; the standard deviations were calculated from the standard errors of the estimated marginal means.

Finally, we repeated the meta-analysis to determine whether each host population was more susceptible to infection by sympatric parasites than by allopatric parasites. We have less confidence in this form of the analysis because the number of comparisons is fewer (table 1). In addition, differences in dose between sympatric and allopatric parasite sources represent a potential source of error variation. We include the analysis, nonetheless, because some authors prefer to define local adaptation as the difference in infectivity between sympatric and allopatric parasites on the same host (Ebert et al. 1998; Thrall et al. 2002) rather than as the difference in infectivity of the same parasite on sympatric versus allopatric hosts. The two approaches will tend to give very similar results, however, if the main effects of host source and parasite source are small relative to the interaction effect.

Genetic and Geographic Distances

We were interested in whether the genetic or geographic distances between host populations that were sympatric and allopatric with a given parasite population affected the strength of local adaptation by parasites. Host genetic distances were calculated from allozyme data for eight of the nine lakes in an earlier study (see Dybdahl and Lively 1996) and for the ninth lake (Ellery) in this study using the same methods (fig. 1). Briefly, we used cellulose acetate electrophoresis to distinguish diploid sexual and triploid clonal individuals and to determine allozyme frequencies for seven loci. For mixed snail populations (containing both sexual and asexual individuals), allozyme frequencies were calculated for diploid sexuals only. For all-asexual lakes (McGregor and Poerua), allozyme frequencies were computed by interpreting the individual banding patterns as diploid if two different alleles were present; all alleles were scored in rare cases where banding patterns indicated three different alleles. We repeated the earlier calculations of Nei's unbiased genetic distance between all pairwise combinations of the nine different lake populations using BIOSYS-1 (Swofford and Selander 1989). Geographic distances were measured between all pairwise populations in a stepping-stone manner (Dybdahl and Lively 1996).

Table 5: Results from meta-analysis

Model	df	Q	$P(\chi^2)$	P(rand)
A. Study:[a]				
Between	8	48.6912	.00000	.001
Within	21	22.9603	.34610	
Total	29	71.6515	.00002	
B. Parasite:				
Between	5	5.4013	.36889	.544
Within	26	40.1452	.03775	
Total	31	45.5465	.04453	
C. Allopatric host:				
Between	7	15.1518	.03410	.159
Within	24	33.5311	.09335	
Total	31	48.6829	.02261	
D. Reproductive mode:[b]				
Between	1	14.0938	.00017	.003
Within	30	44.7924	.04035	
Total	31	58.8862	.00182	

Note: Table grouped by study (A), parasite source (B), source of allopatric host (C), and whether the allopatric host was sexual or asexual (D). Q is the test statistic for heterogeneity among categories (e.g., studies in A; see Rosenberg et al. 2000). $P(\chi^2)$ is the probability value for a χ^2 test, and P(rand) is the probability value gained from randomization tests using MetaWin (Rosenberg et al. 2000).

[a] Two comparisons that had only one replicate were excluded.

[b] Reproductive mode of allopatric host: sexual versus asexual.

Results

ANOVA

In all experiments that were reciprocal in nature, we found no significant main effect of either host or parasite (table 2); in some cases the variance due to host and parasite was estimated to be 0 (table 3). In contrast, in all cases, the host-parasite interaction was highly significant (table 2), and the host-parasite interaction accounted for 43%–95% of the total variation (table 3). In addition, the mean squares (MS) for the linear contrasts comparing sympatric versus allopatric combinations were also all highly significant (table 2).

Similarly, the contrasts for sympatric versus allopatric combinations in the nonreciprocal experiments were significant in three of four studies (table 2). In the fourth study (11), where the overall sympatric versus allopatric comparison was not significant, we constructed linear contrasts to compare the sympatric combinations individually against the three allopatric combinations. One parasite population showed significant local adaptation (Ianthe/Ellery: $t = 3.34$; df = 8; $P = .010$); one showed a nonsignificant difference for which the sympatric host was more infected (Ianthe/Paringa: $t = 1.47$; df = 8; $P = .179$); and one showed a nonsignificant difference for which the allopatric host was more infected (Ianthe/Mapourika: $t = -1.64$; df = 8; $P = .139$; all t-tests were two-tailed).

Meta-Analysis

For the infectivity of parasite populations on sympatric versus allopatric hosts, the mean value for Hedge's d, $\bar{\bar{E}}$, was positive and significantly greater than 0 ($\bar{\bar{E}} = 2.898$; bootstrapped 95% confidence interval [CI]: 2.238–3.693); only one of the 32 comparisons showed a negative value for d (table 4), and this value was small (fig. 2). A virtually identical result was found for the meta-analysis comparing the infectivity of sympatric and allopatric parasites on the same host population ($\bar{\bar{E}} = 2.531$; bootstrapped 95% CI: 1.723–3.340; $N = 20$). It is interesting to note that the 95% CIs are very similar for the two analyses, indicating that differences in parasite doses, which undoubtedly occurred in the analysis of sympatric versus allopatric parasites on the same host, did not greatly affect the error variance. This surprising result, however, is consistent with a recent experimental dose-response study, which indicated little sensitivity of infection prevalence to dose, at least across the range of egg doses expected in the experiments reported here (Osnas and Lively 2004).

The meta-analysis also showed significant differences among the 12 different studies (table 5*A*). There were also differences among the seven host populations in their resistance to allopatric parasites (fig. 3; table 5*C*), but most of the variation was associated with host reproductive mode because asexual populations were more resistant to allopatric parasites than mixed (sexual and asexual) host populations (fig. 3; table 5*D*). This latter result suggests that there may be some inherent resistance to allopatric parasites associated with triploidy.

There were no differences in the strength of local adaptation by parasite populations (fig. 4; table 5*B*). In addition, we found no evidence that the strength of local adaptation depends on the genetic distance between the sympatric and allopatric host populations; specifically, there was no significant correlation in the analyses that either included or excluded asexual host populations (includes: $r = -0.230$; $P = .206$; $N = 32$; excludes: $r = 0.260$; $P = .255$; $N = 21$). Similarly, we found no significant relationship between geographic distance and the strength of local adaptation over all populations ($r = -0.119$; $P = .516$; $N = 32$). There was, however, a very strong correlation between stepping-stone geographic distance between host populations and genetic distance ($r = 0.813$, $P < .0001$, $N = 32$). Finally, the results indicated that the average variation in d for the three sympatric-allopatric comparisons that were made at three or more

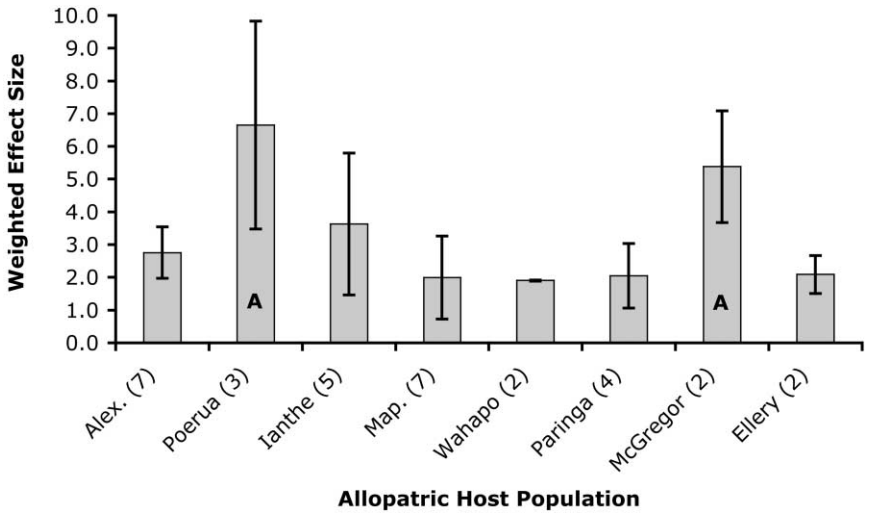

Figure 3: Mean (SD) of effect sizes by allopatric host population. Lakes marked with *A* contain asexual host snails. The other lakes contain mixtures of sexual and asexual snails. Numbers in parentheses are the number of comparisons involving each host.

different times (Var = 2.1) was similar to the variation generated by comparisons that were made only once at different spatial scales (Var = 2.2; $F = 1.05$, df = 1, 1, $P = .49$). Thus the variation in effect size over time was very similar to the variation in effect size in space.

Discussion

Meta-analysis is a valuable tool for determination of standardized treatment effects across different experiments. Most often, the experiments are conducted by different groups using different methods and different species (e.g., Van Zandt and Mopper 1998; Coltman and Slate 2003; Sheldon and West 2004). In this study, we used meta-analysis to evaluate 32 pairwise comparisons from 12 different studies on the same pair of species. We asked whether a parasite (*Microphallus* sp.) is more infective to sympatric than allopatric populations of its snail host (*Potamopyrgus antipodarum*). We found significant differences among studies, but the results showed very consistent local adaptation for all six parasite populations. The result is consistent with expectation under the Red Queen hypothesis because it indicates that parasites are tracking locally common host genotypes.

We found no support for the prediction that for some comparisons parasites would be significantly more infective on allopatric than sympatric host populations (Morand et al. 1996), as only one of 32 comparisons showed a negative value for Hedge's *d*, and the value was not significantly different from 0. The reasons for the discrepancy are not clear, but it could easily be due to small differences among host populations in the composition of alleles at resistance loci, especially given the marked population subdivision that is known for this snail (Dybdahl and Lively 1996). Whereas the models tend to assume that the compliment of resistance alleles is identical across host populations (but genotype frequencies vary), natural host populations may have unique alleles that preclude the occasional very close match by oscillating allopatric parasite populations (Kaltz and Shykoff 1998). On the other hand, we did find a distribution of differences from near 0 to strongly positive, suggesting that the strength of local adaptation varies in space and/or time, perhaps due in part to the oscillatory nature of the interaction (Morand et al. 1996; Lively 1999).

A great deal of discussion has focused on the genetic basis for infection. Much of the discussion has centered on the difference between GFG models favored by plant pathologists and the matching-alleles models favored by invertebrate zoologists. This study does not solve the issue, but the variance components analysis does show very clearly that much more of the variance in infection success is due to sympatry versus allopatry rather than the effects of parasite (or host) per se (tables 2, 3). In fact, the variance due to the main effects of parasites were surprisingly small (sometimes close to 0), especially given that dose was a potential source of variation in all of the fully reciprocal experiments. Similarly, the variance due to host was nonsignificant and very small, even though ecological and physiological variations seem likely to exist among host populations. In contrast, the variation due to the host-parasite interaction was very large and statistically signif-

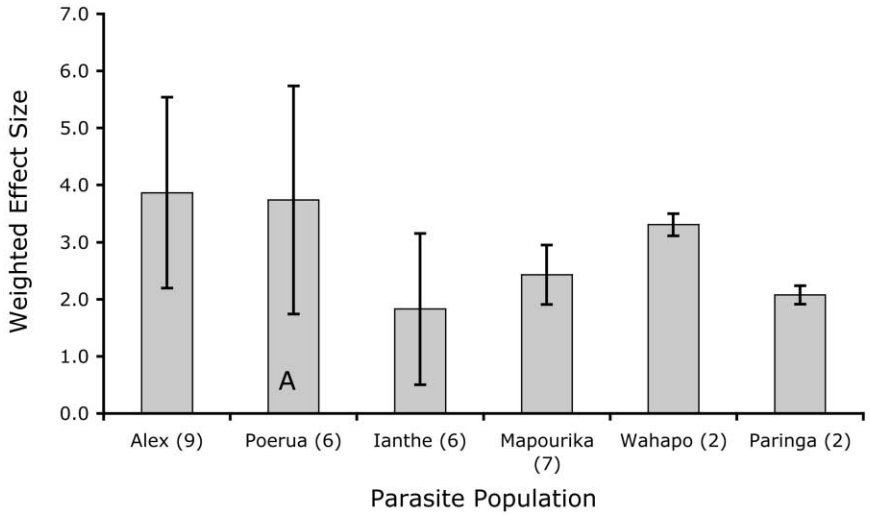

Figure 4: Mean (SD) of effect size by parasite population. Lakes marked with *A* contain asexual host snails. The other lakes contain mixtures of sexual and asexual snails. Numbers in parentheses are the number of comparisons involving each host.

icant across all studies, and most of this variation is attributable to greater infectivity of parasites on their local host sources (tables 2, 3). These results are completely consistent with expectation under a matching alleles type model, but it does not rule out GFG genetics. However, what it does seem to rule out (at least so far) is the idea that some populations of *Microphallus* parasites contain high frequencies of unconditionally infective genotypes. Such a situation would certainly undermine any local adaptation as well as any local advantage to recombination, since the production of rare progeny in a brood would not increase parasite resistance. In a sense, then, the struggle over the exact genetic details may not be profitable. The essential element for local adaptation and consequent local selection for recombination is some kind of tight specificity engendered by the infection genetics (see also Agrawal and Lively 2002). The present results indicate the presence of such a system. The results of an elegant hierarchal study of a flax rust metapopulation also seem to indicate a similar system (Thrall et al. 2002).

We found no indication that host genetic distance or geographical distance between populations affected the strength of local adaptation, as measured by Hedge's *d*. This result is also consistent with the findings of Thrall et al. (2002) and by Morand et al. (1996). The findings of all three studies suggest that gene flow by the parasites is not disrupting local adaptation on the scales that have been investigated in these studies. On the other hand, Ebert (1994) did find an effect of geographical distance among populations; specifically, he found that propagule production by a microsporidian decreased with increasing distance of the allopatric host population. We see no incompatibility in results here, since the systems are so different, and Ebert's metric of adaptation was a composite of infectivity and the reproductive rate of a parasite within a host. The rationale that gene flow can override local selection (Wright 1969; Li 1976; Thompson 1994) is not incompatible with our present results, although we find it surprising that populations in such close proximity are strongly locally adapted, in spite of the high parasite gene flow known to occur between the adjacent populations (Dybdahl and Lively 1996). The results are perhaps a testament to the strength of local selection on the parasite.

We did find a significant difference between mixed and asexual host populations in resistance to allopatric populations of parasites: triploid populations were more resistant on average. This result is supported by a recent experimental study showing that diploids from Lake Alexandrina were more susceptible than triploids to two remote sources of the parasite (J. Jokela, C. M. Lively, L. F. Delph, M. F. Dybdahl, and J. A. Fox, unpublished manuscript). The mechanistic reasons for the result are not clear at this time, but observations from other study systems have also suggested a role for host ploidy in parasite resistance. For example, polyploid goldfish have been found to be less resistant and have lower immune activity than diploids (Hakoyama et al. 2001). Polyploid salmon have also been found to have lower immune activity than diploids (Langston et al. 2001), while the converse has been found in other trout (Lapatra et al. 1996). In any case, the results suggest an immediate alternative hypothesis for our observations that sexual reproduction in the

snail is significantly and positively correlated with prevalence of infection (Lively 1987, 1992; Lively and Jokela 2002). It may simply be that parasites are more or less randomly distributed and that prevalence of infection depends only on the frequency of triploid individuals in the host population. However, such a situation would not lead to the strong local adaptation demonstrated by this study. In addition, the pattern of prevalence across New Zealand lakes does not show an even relationship with the frequency of sexual individuals, rather there is a discrete jump in the data, suggesting that at some threshold level of infection, there is a discrete shift to a predominance of sexual individuals, which is a unique prediction of the Red Queen hypothesis (Lively 2001).

The question of whether ploidy per se is controlling the prevalence of infection among lakes or whether ploidy and sexual reproduction are a consequence of localized selection by parasites can be addressed in one other way. The key is to know whether local populations of parasites can overcome the apparently greater innate resistance conferred by triploidy. In other words, can the parasites of triploid populations become locally adapted? The results of this study suggest that the parasites of triploid populations can indeed become locally adapted. The parasite population of Lake Poerua snails (an asexual host population) is as adapted to infecting the local triploid snails as are the parasites of the remaining sexual, diploid populations (fig. 4). Moreover, we have seen from experimental studies on Lake Poerua that parasites are tracking the most common local host clones within that lake (Dybdahl and Lively 1998; Lively and Dybdahl 2000). Hence it would appear that parasites can overcome any initial relative resistance that triploids may enjoy.

In summary, the results from this study indicate persistent local adaptation in both space and time, which is consistent with expectation under the Red Queen hypothesis. The results, of course, do not prove that parasites are responsible for the maintenance of sex, and there are good theoretical reasons to think that even if parasites contribute, they are unlikely to be solely responsible for host sex (Howard and Lively 1994, 1998). Nonetheless, local adaptation by a highly virulent parasite such as *Microphallus* indicates the capacity of this parasite to track locally common host genotypes, which would help to prevent fixation by numerically dominant clones. Results to the contrary would have been sufficient for rejection of the Red Queen hypothesis.

Acknowledgments

We thank A. Emblidge, G. Harp, B. Helman, S. Kane, K. King, B. Koskella, M. Rummel, and R. Steyn for help dissecting snails, changing water, and building equipment. We also thank A. Agrawal, J. Bever, D. Ebert, D. Harvell, and M. Woolhouse for critical reviews of the manuscript and J. Bever for helpful discussions on the statistical analyses. Many thanks again to the University of Canterbury for facilitating our work, with special thanks to J. McKenzie in Zoology and J. van Berkel at the Edward Percival Field Station in Kaikoura, New Zealand. Funding was provided by the National Science Foundation (DEB-9907373 and 0296049 to M.F.D.; DEB-9904840 to C.M.L., and DEB-0128510 to J.J. and C.M.L.), the Academy of Finland (202481 to J.J.), and the Marsden Foundation for Research, Science, and Technology, New Zealand (contract LL 0501 to L.F.D. and C.M.L.).

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