Evolution, 60(1), 2006, pp. 31–38

PARASITE-HOST SPECIFICITY: EXPERIMENTAL STUDIES ON THE BASIS OF PARASITE ADAPTATION

TOM J. LITTLE,[1,2] KATHRYN WATT,[1] AND DIETER EBERT[3,4]
[1]*Institute of Evolutionary Biology, School of Biology, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JT, Scotland*
[2]*E-mail: tom.little@ed.ac.uk*
[3]*Zoologisches Institut, Universität Basel, Vesalgasse 1, CH-4051 Basel, Switzerland*
[4]*E-mail: dieter.ebert@unibas.ch*

Abstract.—Specificity in parasitic interactions can be defined by host genotypes that are resistant to only a subset of parasite genotypes and parasite genotypes that are infective on a subset of host genotypes. It is not always clear if specificity is determined by the genotypes of the interactors, or if phenotypic plasticity (sometimes called acclimation) plays a larger role. Coevolutionary outcomes critically depend on the pervasiveness of genetic interactions. We studied specificity using the bacterial parasite *Pasteuria ramosa* and its crustacean host *Daphnia magna*. First, we tested for short-term adaptation of *P. ramosa* lines that had been rapidly shifted among different host genotypes. Adaptation at this time-scale would demonstrate the contribution of phenotypic plasticity to specificity. We found that infectivity was stable across lines irrespective of recent passage history, indicating that in the short term infection outcomes are fixed by genetic backgrounds. Second, we studied longer-term evolution with two host clones and two parasite lines. In this experiment, *P. ramosa* lines had the possibility to evolve adaptations to the host genotype (clone) in which they were serially passaged, which allowed us to test for a genetic component to specificity. Substantial differences arose in the two passaged lines: one parasite line gained infectivity on the host clone it was grown on, but it lost infectivity on the other host genotype (this line evolved specificity), while the other parasite line evolved higher infectivity on both host clones. We crossed the two host genotypes used in the serial passage experiment and found evidence that the number of host genes that underlies resistance variation is small. In sum, our results show that *P. ramosa* specificity is a stably inherited trait, it can evolve rapidly, and it is controlled by few genes in the host. These findings are consistent with the idea of a rapid, ongoing arms race between the bacterium and its host.

Key words.—Acclimation, coevolution, genetic variation, habituation, pathogen, phenotypic plasticity, resistance, selection, serial passage.

Received June 13, 2005. Accepted October 23, 2005.

While some parasites are generalists, many others are specialists, able to infect only one or a few host species (e.g., Lajeunesse and Forbes 2001). However, specialization may be more finely bound than this: it also occurs within species, as evidenced by local adaptation of parasites to their sympatric host populations (reviewed in Kaltz and Shykoff 1998), and within populations, as shown by parasite strains that are specialised to particular local host genotypes (Thompson and Burdon 1992; Wedekind and Ruetschi 2000; Carius et al. 2001; Lambrechts et al. 2005). The cause of specialization, or, conversely, what prevents a parasite from expanding its host range, is of considerable interest. For example, parasitism may be the principle determinant of rates of genetic recombination (Haldane 1949; Hamilton 1980; Lively 1987; Hamilton et al. 1990), but the impact of parasitism on reproductive systems crucially depends on the nature or degree of specialization (Frank 1992; Clay and Kover 1996). Aspects of specialization are also of practical interest: understanding what limits host range may aid in designing control measures for diseases or pests or may help to explain why the spread of disease is sometimes associated with increasing virulence (Read et al. 1999).

In most studies of parasite-host interactions, specificity is assumed to have a genetic basis. Alternatively, however, specificities can be based on phenotypic plasticity. Specialization with a large plastic component has, for example, been demonstrated in plant-herbivore interactions where insect herbivores adapt physiologically to the deterrent allelochemicals produced by their hosts (e.g., Jermy 1990; Akhtar and Isman 2004). Plasticity, sometimes called phenotypic acclimation, is also frequently assumed in studies of microorganisms (Bennett and Lenski 1997; Dillon et al. 2003; Rainey 2004). In many cases, it may be difficult to determine when specialization is due to genetic differences or differential patterns of expression among strains with the same genetic background. A crucial difference between plastic and nonplastic specificity is the speed with which infectivity patterns may change. Adaptation due to phenotypic plasticity would be expected to occur rapidly, because it involves only changes in expression levels within one generation. By contrast, nonplastic changes (i.e., evolutionary events) involve changes in genotype frequencies, which requires at least two generations, though typically more.

To establish the basic features governing the evolution of specialization, it is helpful to study simple experimental systems. The present study concerns specialization of the bacterial parasite *Pasteuria ramosa* to its crustacean *Daphnia magna*. Previous studies revealed considerable genetic variation for resistance and specificity in this interaction (Little and Ebert 2000; Carius et al. 2001). Some of this variation could be attributable to genetic factors, but plasticity in *P. ramosa* can not be ruled out. Here we present the results of experiments designed to gauge the importance of short-term phenotypic plasticity of *P. ramosa* relative to the role played by host and parasite genetic background. There were two main experiments: one experiment varied the recent passage history of the parasite to test for adaptation based on phenotypic plasticity, and a second experiment kept passage his-

tory constant over many passages to examine the role played by genetic background. As adaptation to host genotypes may be lost upon genetic recombination of the host, we also tested whether parasite specificity changes when the host clones are genetically crossed.

Organisms

Daphnia magna Straus is a planktonic freshwater crustacean found in shallow ponds in temperate regions. It is attacked by a variety of bacterial, microsporidial, and fungal parasites (Green 1974; Stirnadel and Ebert 1997; Little and Ebert 1999). Prevalence of parasites can be high (up to 100%), and these often have a large impact on *Daphnia* fitness (Stirnadel and Ebert 1997; Little and Ebert 1999). *Daphnia* reproduce by cyclical parthenogenesis and can be kept in the laboratory in the state of clonal reproduction, which greatly aids the experimental dissection of genetic and nongenetic effects.

Pasteuria ramosa Metchnikoff 1888 is a bacterial obligate endoparasite of *Daphnia* (Ebert et al. 1996). Infection occurs through contact of waterborne spores by the host and greatly reduces the fecundity of the host (Ebert et al. 2004). Parasite transmission requires host death, as spores are only released from decaying cadavers. The *Daphnia-Pasteuria* system is one of the few naturally occurring interactions that is amenable to experimental manipulation; thus, our studies are well positioned to gain insight into naturally coevolving systems.

Materials and Methods

Initial Host and Parasite Isolation

Daphnia magna individuals infected with *P. ramosa* were collected from a pond in northern Germany (Gaarzerfeld Pond; Carius et al. 2001; Little et al. 2002), brought into the laboratory and placed singly in jars filled with 100 ml synthetic pond water (Klüttgen et al. 1994) and were fed with the green algae (*Scenedesmus* spp., 5×10^6 cells/*Daphnia*/day). Nine infected *D. magna* individuals produced viable offspring before the parasite curtailed host reproduction. These offspring, which are genetically identical to their mother but uninfected (*P. ramosa* is not vertically transmitted), were collected and maintained as uniclonal lines in the laboratory. Allozyme electrophoresis revealed that each of the nine clones had a unique multilocus genotype (Hebert and Beaton 1993). The nine infected mothers of these clones were the source of the parasite isolates, and these were kept until their death and then placed singly into Eppendorf tubes and frozen at −20°C.

Single Passages: Testing for Short-Term Phenotypic Plasticity

Each of the nine parasite isolates were initially passaged twice through the same host clone from which it was isolated. We refer to one passage as the process of infecting a young host by exposing it to a spore suspension to the death of this host. At death, an infected host is filled with *Pasteuria* spores. These spores can be frozen or used directly to initiate the next passage. Parasite-induced death under laboratory conditions takes place about 40 days postinfection, but spores

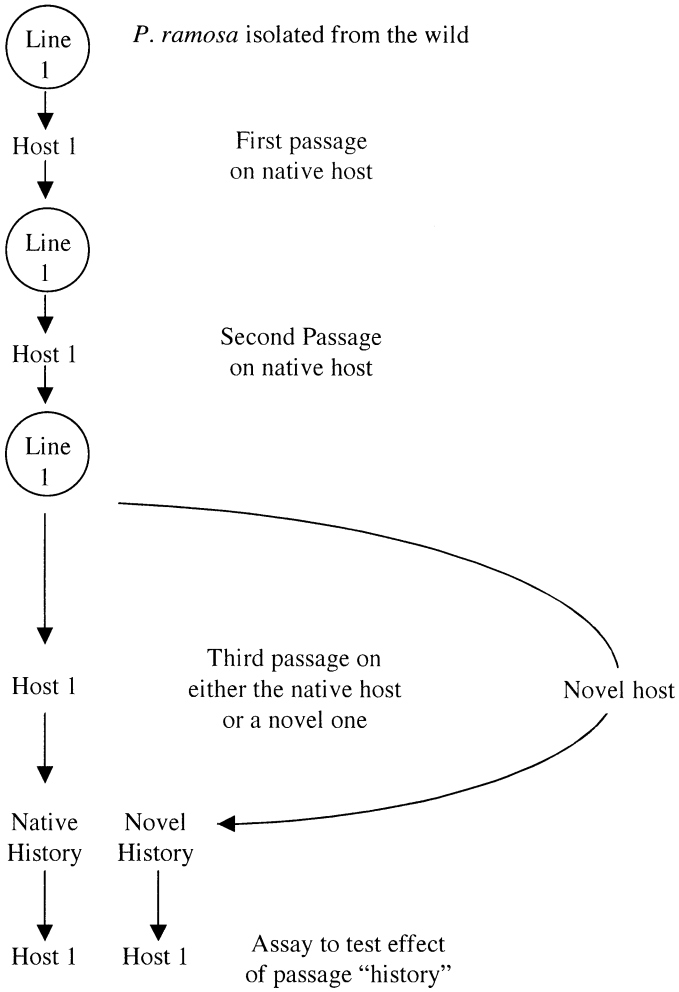

FIG. 1. Schematic outlining the design of the experiment to test for short-term phenotypic adaptation of *Pasteuria ramosa*. Each isolate of *P. ramosa* was twice passaged through one host clone, then for a single passage through either the same (native) host clone or a novel one. Lines that have passed through only one host clone are said to have a native history, while those that have experienced a foreign host have an novel history. The figure illustrates the procedure for one parasite line–host clone combinations, but a total of nine were studied.

can be harvested from killed hosts as early as 25 days. For this experiment, spore suspensions were prepared by grinding up the dead host in 1 ml of medium and then adding this suspension to a jar containing 30 *Daphnia* juveniles. The *Daphnia* were then screened daily, and dead infected *Daphnia* were removed and frozen. These infected *Daphnia* were then used in a second passage. The concentration of spores in the suspension (spore dose) was not controlled at this stage.

Following the two initial passages, each line was then passaged: (1) again through the host clone it was initially isolated and propagated on (native passage); or (2) on a different host clone (novel passage). Figure 1 presents a schematic of the experimental design. Infections for this passage used one *Daphnia* per 100-ml jar and spore dose was controlled at 1×10^6 transmission spores per jar. Infected hosts were maintained until they died and were then frozen. We then assayed these *P. ramosa* lines on their original host clone to test if

it mattered whether *P. ramosa* had spent one generation on a foreign host clone (novel passage history, Fig. 1) or had consistently been passaged through the same host clone (native passage history, Fig. 1). Novel combinations first were chosen based on previous knowledge that the particular host clone-parasite line combination resulted in infection. Second, we sought to perform the novel passage on a host that was as different as possible from the native host. This was possible because in a previous study on specificity we generated resistance profiles for each of the nine clones involved in the present study (Carius et al. 2001). With these criteria, five different host clones were used for novel passages.

For the assay, we applied a high (1×10^6 spores/*Daphnia*) and a low spore dose (0.2×10^6 spores/*Daphnia*), and there was one *Daphnia* per jar. For this and all assays, host replicates were kept under standardized conditions (5×10^6 algal cells/*Daphnia*/day, with medium changed every other day) in an incubator (20°C, 16h:8h light:dark cycle) for three generations prior to the experimental infections to randomize environmental and maternal effects. Parasite spores were kept frozen throughout this period of host preparation. Offspring of the third clutch of the third generation of replicate host lines were used in infection assays. *Daphnia* were kept in the jars containing spores for 5 days and then were transferred to fresh medium without spores. Following this infection period, medium was changed every other day. The experiment was terminated at day 25 postinfection and the infection status of hosts was determined by eye under a dissecting scope. Food levels during the infection period were 1×10^6 cells of the algae *Scenedesmus* spp. per day during the infection period and 5×10^6 algal cells at all other times. The lower food during the infection period encourages browsing on the jar bottom, which increases contact with parasite spores.

For data analysis, we used binary logistic regression to determine if the proportion of hosts that became infected was explained by history (two levels: novel or native), host genotype (nine levels), or spore dose (two levels). Similarly, and because the main fitness consequence of *P. ramosa* infection is a loss of reproduction, we used analysis of variance (ANOVA) with the same independent variables as factors to study the number of clutches produced by each host as dependent variable.

Longer-Term Passages

To study longer-term evolution of *P. ramosa*, we chose two parasite isolates (designated P1 and P7) and two host clones (designated H4 and H5) that differ in infectivity (see Results and Carius et al. 2001). In particular, host H4 is generally more susceptible than H5, and parasite line P7 is generally more infective than P1. Line P1 was passaged five times on H4 hosts, and P7 was passaged five times on H5 hosts. Each passage was performed in 200-ml jars with 10 hosts per jar, and there were 10 replicate jars per passage. Each new round of infections was initiated with 1×10^6 spores/*Daphnia*. Infected hosts were grown until they died, at which point they were pooled and frozen for use in the next passage. After the five passages, infectivity was assayed in a reciprocal cross infection experiment where both host types were exposed to both parasite lines, and there were original (which had been stored in the freezer) and evolved lines for each parasite line. For this assay, five newborn *Daphnia* from each replicate were placed in a 100-ml jar with 1×10^6 parasite spores (P1 or P7) per *Daphnia*. There was also a small quantity of purified sand in the bottom of each jar, which mimics pond sediments and enhances the probability of infection. Other conditions were identical to the assays described above. For data analysis, we used logistic regression to determine if the proportion of hosts that became infected was explained by host genotype (two levels: H4 and H5), parasite line (two levels: P1 and P7), or history (two levels: original or evolved).

Crossing Hosts

Host genotypes were crossed by placing H4 males in a jar with H5 females. Parthenogenetically produced offspring were removed every day, and thus it was not possible for H5 sons to mature and fertilize their mother. Jars were checked for sexually produced eggs (i.e., ephippia), which are visually distinct black structures. When found, ephippia were frozen for 6 months to simulate winter conditions. Hatching of ephippia was then accomplished by exposing them to 24 h light. Twenty independent F_1 clones were obtained in this way. These clones are related as full siblings. Twelve replicate jars (five *Daphnia* per jar) of each of the F_1 clones were assayed, plus replicates of each of the parental clones. For infections, five newborn *Daphnia* from each replicate were placed in a 100-ml jar with purified sand and parasite spores (P1 or P7, evolved lines) at either a high (1×10^6 spores/*Daphnia*) or a low spore dose (0.2×10^6 spores/*Daphnia*). The variable ''infected'' was analyzed with logistic regression using the explanatory variables ''cross'' ($n = 20$), ''parasite line,'' and ''dose'' using a binary error.

RESULTS

Short-Term Phenotypic Plasticity

We first tested for short-term adaptation of *P. ramosa* lines that had been rapidly shifted among different host genotypes (Fig. 1). Adaptation at this time scale would demonstrate the contribution of phenotypic plasticity to specificity. The proportion of hosts that became infected did not depend on the recent passage history of *P. ramosa* (Table 1, Fig. 2). Nor did passage history influence the level of parasite-induced fecundity losses (Fig. 3). The effect of genetic background was significant for both infectivity and fecundity (Table 1), which is in agreement with previous studies on these host and parasite genotypes (Carius et al. 2001).

Longer-Term Passages

We next studied longer-term evolution with two host clones and two parasite lines. In this experiment, *P. ramosa* lines had the possibility to evolve adaptations to the host genotype (clone) in which they were serially passaged, which allowed us to test for a genetic component to specificity. In these longer-term passages, the two parasite lines evolved higher infection prevalences on the host clone they were passaged on (Fig. 4). Based on an overall mean, it could appear

TABLE 1. Likelihood-ratio chi-square statistics for the proportion of *Daphnia magna* hosts becoming infected with *Pasteuria ramosa* during experimental infections assays. Results are from three separate experiments.

	Factor	Test statistic	df	*P*
1) Single passages: plasticity	history	0.68	1	ns
	dose	71.6	1	***
	host clone	128.1	8	***
2) Longer-term passages	history	3.83	1	ns
	line	235.1	1	***
	host clone	248.9	1	***
	history × line	101.9	1	***
	history × host clone	48.2	1	***
	host clone × line	88.3	1	***
	history × host clone × line	43.6	1	***
3) Crossing hosts	cross	259.7	19	***
	line	210.3	1	***
	dose	0.2	1	ns
	line × dose	11.7	1	**

** $P < 0.01$; *** $P < 0.001$; ns, not significant. Interaction terms that were not significant were omitted.

that *P. ramosa* showed only a very modest amount of evolution (nonsignificant effect of history, Table 1). However, this was because evolution strongly depended on the parasite line and the host clone involved, thus the strong interaction effects involving the factor origin (Table 1). Figure 2 indicates that isolate P1 gained infectivity on host H4 (the host it had been passaged on), but it lost the capacity to infect host H5. By contrast, P7, which had been passaged only through host H5, gained infectivity on H5 and may have gained infectivity on the other host also (Table 1, Fig. 4).

Crossing Hosts

We crossed the two host genotypes used in the longer-term serial passage experiment. Twelve of 20 of the F_1 host clones responded to the two parasite lines in a manner similar to the parental genotype H5 (Fig. 5, crosses 1–12), while the remainder showed susceptibility that was unlike either parental genotype (Fig. 5, crosses 13–20). Thus there were strong parasite line-specific differences among F_1 hosts in terms of overall susceptibility (Table 1).

This assay of F_1 offspring was a repeat of an earlier one

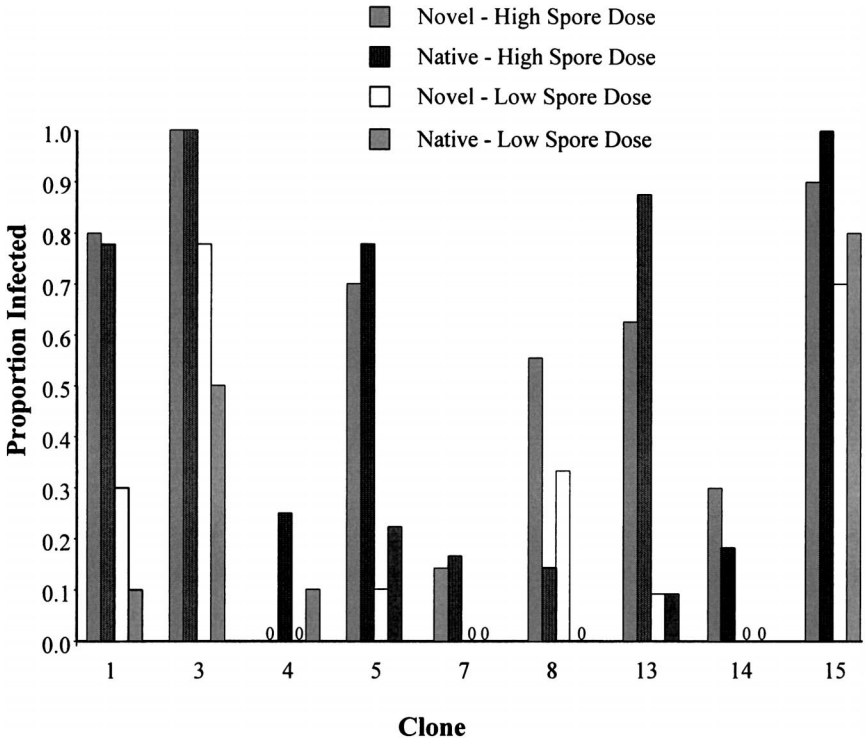

FIG. 2. Results of a test for short-term adaptation of *Pasteuria ramosa* to particular host clones of *Daphnia magna*. The proportion of hosts that became infected (infectivity) did not depend on the passage history (Table 1) at either of two spore doses.

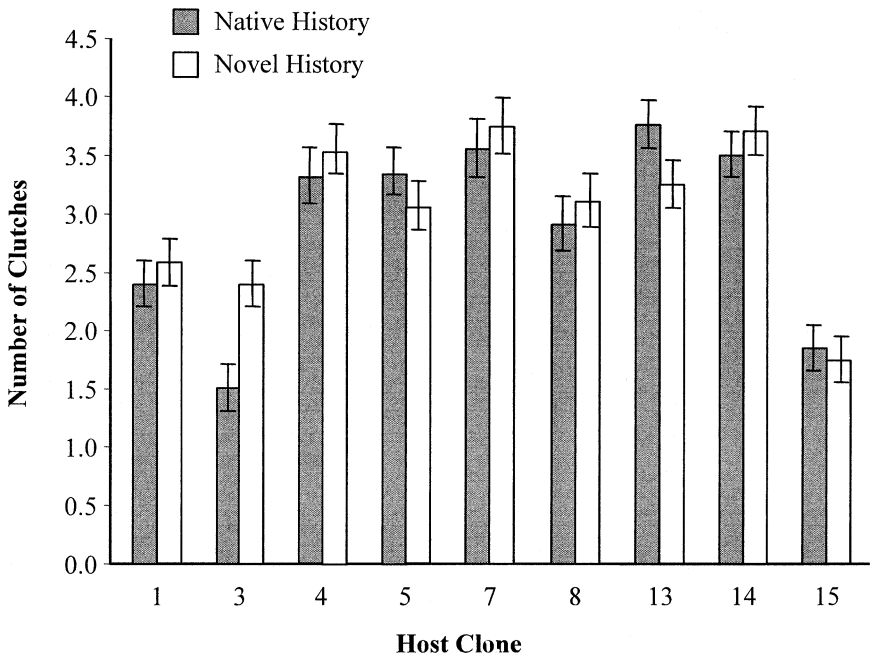

FIG. 3. Results of a test for short-term adaptation of *Pasteuria ramosa* to particular host clones of *Daphnia magna*. Parasite passage history did not influence host fecundity during an infection experiment involving *P. ramosa* and nine genotypes (clones) of *D. magna*. Means and standard errors are given.

that used only a low spore dose (0.2×10^6 spores/*Daphnia*). Levels of infection in this earlier experiment were predictably low, but showed an identical infection pattern. For crosses 1–12, the proportion infected with line P7 was 0.227 ± 0.04 (SE), but no successful infections resulted following exposure to P1. The proportion infected for clones 13–20 was 0.0 for both lines in this earlier experiment.

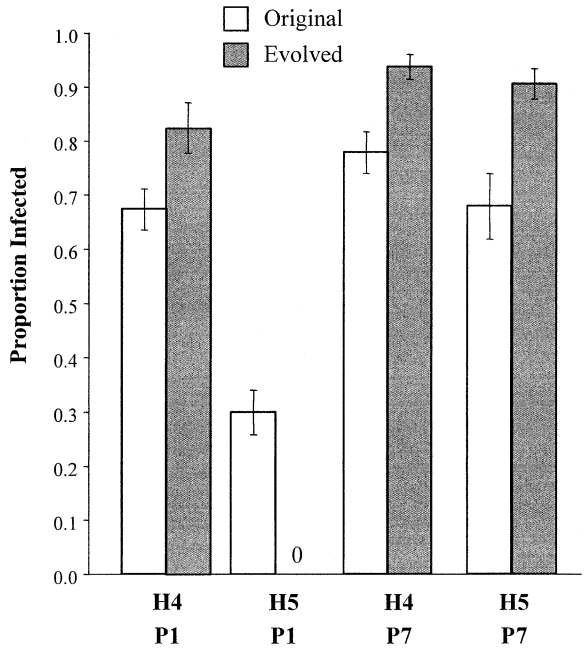

FIG. 4. Results of infectivity assay following five passages of *Pasteuria ramosa* through its host *Daphnia magna*. Line P1 was only passaged on H4, and line P7 was only passaged through H5. The infection assay was a reciprocal cross infection experiment with all four host and parasite combinations. Means and standard errors are given.

DISCUSSION

Parasite Evolution and the Basis of Specificity

Over the course of one passage, *P. ramosa* did not change its infection pattern to a range of host genotypes. Thus, the variation in infection patterns observed here and in earlier experiments with this parasite-host system are unlikely to be the result of a phenotypically plastic response to different host environments. Many other parasites or pathogens undergo rapid adaptive changes in phenotype during infection. For example, species in the genus *Plasmodium* (the cause of malaria) may rapidly switch membrane protein expression to thwart host immunity and enhance parasite survival and growth. Thus, adaptation due to phenotypic plasticity can be extremely rapid, because it involves only changes in expression within a single generation. For *P. ramosa*, however, rapid adaptation seems not to occur as specificity was stable across short time scales and fixed for a given set of genotypes (Figs. 2, 3).

In contrast, across several passages, *P. ramosa* was clearly shown to evolve in the laboratory, although the mechanism(s) (plasticity or genetic change) cannot be known with certainty. Most importantly, this evolution varied substantially depending on host or parasite genetic background (Fig. 4). In particular, one parasite line evolved higher infectivity on both the host clone it had been passaged on and the host clone it had not been passaged on, whereas the other parasite line gained infectivity on the host clone it was grown on but lost infectivity on the other host genotype (i.e., this line evolved

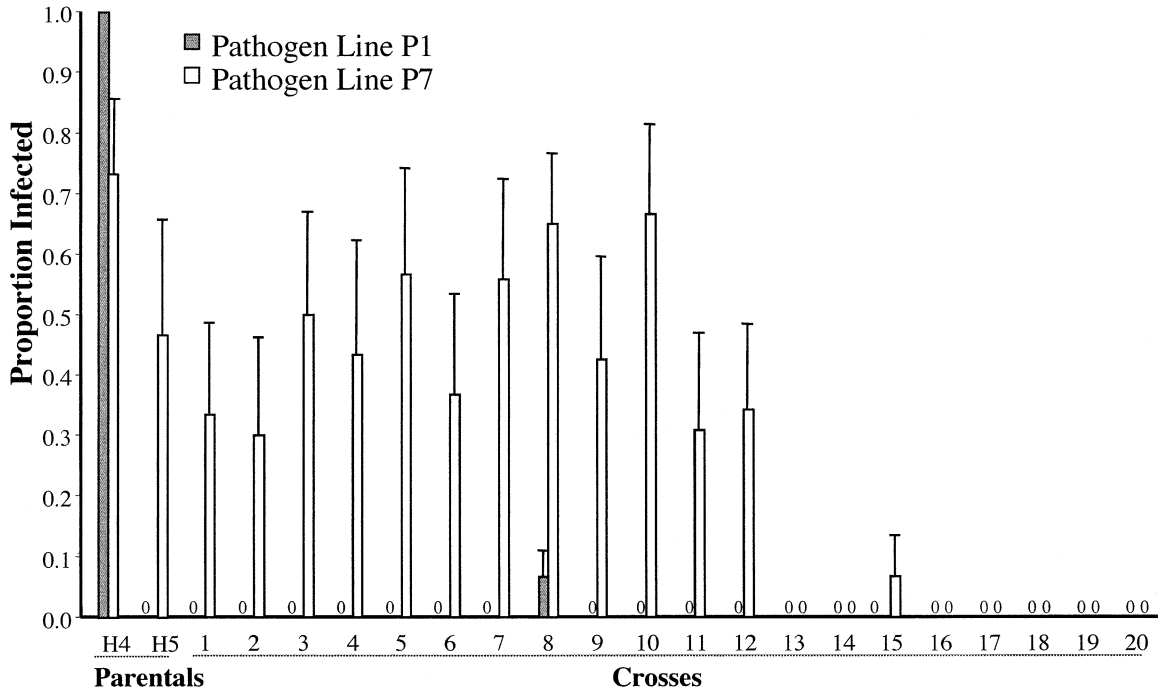

FIG. 5. Approximately half of the F_1 crosses (1–12) produced in matings of two *Daphnia* genotypes (parentals, H4, H5) showed patterns of susceptibility that were identical to one parental type, but the remaining crosses were unlike either parent. Means and standard errors are given.

specificity). Thus, in this system, genetic backgrounds overwhelmingly affect both specificity and the evolution of specificity.

The typical outcome of serial passage experiments is specificity (reviewed in Ebert 1998). However, this trade-off between increased virulence in the host of passage and attenuation of virulence in other hosts has been mostly shown with among-species comparisons. How often this trade-off also occurs at the genotypic level within host species is uncertain, but it is a frequent assumption in models of coevolution, the maintenance of sex and genetic diversity, and the evolution of virulence (Ebert and Hamilton 1996; Woolhouse et al. 2002). Attenuation to former genotypes within a species has been demonstrated in a mouse-nematode model (Dobson and Owen 1977), but opposite patterns have been reported in other rodent models (Mackinnon et al. 2002; McClelland et al. 2004). As our longer-term experimental evolution showed evidence for attenuation in one host genotype-parasite line combination, but not in another, it remains difficult to generalize about precise outcomes aside from noting that the impact of genetic background on virulence evolution is substantial. Our future studies will expand the range of host and parasite genotypes studied with the aim to derive general evolutionary principles regarding specialization. For now, a possible explanation for our findings might be that, with regard to parasite line P7, the two host clones (H4 and H5) are identical at key immune-related loci, while with regard to parasite line P1, H4 and H5 are different.

The Genetic Basis of Resistance

One potential outcome of the evolution of genotype-specific virulence is a frequency-dependent dynamic of host and parasite polymorphism. Frequency-dependent dynamics are most likely when the genetic control of resistance is due to a small number of genes. Infection experiments on F_1 crosses of the hosts on which *P. ramosa* was evolved revealed patterns of susceptibility that were surprisingly discrete: for one set of F_1 offspring, the expression of disease was similar to one parental genotype, while another set resembled neither parental genotype. This observation provides insight into the genetic basis of resistance variation among hosts. In particular, if the genetic control of resistance was attributable to many genes each of small effect (i.e., if resistance was a highly polygenic trait), a continuum of resistance types would have resulted. By contrast, the discrete pattern of susceptibility observed here is compatible with the Mendelian inheritance of a relatively small number of loci.

However, although the genetic control of resistance in the *Daphnia-Pasteuria* system is likely due to a small number of genes of major effect, it is not possible to describe precisely the genotypic arrangement. Backcrosses are required to illuminate the genetic basis of infectivity further, and indeed efforts to obtain these additional crosses have been considerable. Sex in *Daphnia* is environmentally determined, but there are strong genotype-by-environment interactions; some clones are difficult to mate in the laboratory, or diapause is difficult to break (Loaring and Hebert 1981; Ferrari and Hebert 1982). The clones of this study do not mate frequently, and the environmental cues that break diapause have remained elusive.

Using the available data a simple model for inheritance would be the following. If one assumes that each parasite (P1 and P7) interacts with a different host locus, it is possible to construct single-locus models. For P1, host H4 could be

homozygous susceptible, while H5 is homozygous resistant. If resistance is dominant, all F_1 are resistant, which is what we found (see Fig. 5). For P7, both host parent clone might be heterozygous, with resistance being recessive. One-quarter of the F_1 would be homozygotes for the resistant allele, which is roughly consistent with Figure 5 (eight of 20 is not significantly different from one-quarter). This hypothesis is also consistent with the results of the longer-term serial passage experiment, which might be interpreted such that from the perspective of P7 both hosts are equal, whereas they are different from the perspective of P1.

In general, this and other studies indicate a considerable level of specificity in invertebrate host-parasite interactions (e.g., Wedekind and Ruetschi 2000; Carius et al. 2001) that may often be attributable to a small number of genes (Carton et al. 2005). Identifying the genes and molecules involved in highly specific patterns of immunity remains a challenge for the field of invertebrate immunity (Rolff and Siva-Jothy 2003). With simple genetic control of resistance, however, interactions have the potential to undergo negative frequency-dependent genetic oscillations, the occurrence of which is a crucial requirement of the Red Queen hypothesis for the maintenance of sex. Parasitism may therefore be responsible for the widespread occurrence of sexual reproduction.

A further interesting result of the crossing experiment, which is relevant for the adaptive value of sexual recombination, is that, for both parasites, the average infection level of the F_1 clones was lower than the average across the two parents. Thus, it seems that outbreeding not only produced novel genotypes in terms of resistance, but also reduced average infection levels.

Acknowledgments

Thanks to J. Hottinger (Basel), and A. Duncan (Edinburgh), and W. Chadwick (Edinburgh) for assistance in the laboratory. This work was supported by the Swiss-NF grant to DE, a NERC (U.K.) grant GR3/13105 to Andrew Read (Edinburgh), and fellowships from NSERC (Canada) and the School of Biology, University of Edinburgh to TL.

Literature Cited

Akhtar, Y., and M. B. Isman. 2004. Generalization of a habituated feeding deterrent response to unrelated antifeedants following prolonged exposure in a generalist herbivore, *Trichoplusia ni*. J. Chem. Ecol. 30:1349–1362.

Bennett, A. F., and R. E. Lenski. 1997. Evolutionary adaptation to temperature. 6. Phenotypic acclimation and its evolution in *Escherichia coli*. Evolution 51:36–44.

Carius, H.-J., T. J. Little, and D. Ebert. 2001. Genetic variation in a host–parasite association: potential for coevolution and frequency-dependent selection. Evolution 55:1136–1145.

Carton, Y., A. J. Nappi, and M. Poirie. 2005. Genetics of anti-parasite resistance in invertebrates. Dev. Comp. Immunity 29: 9–32.

Clay, K., and P. Kover. 1996. The Red Queen hypothesis and plant/pathogen interactions. Annu. Rev. Phytopathol. 34:29–50.

Dillon, J. G., S. R. Miller, and R. W. Castenholz. 2003. UV-acclimation responses in natural populations of cyanobacteria (*Calothrix* sp.). Environ. Microbiol. 5:473–483.

Dobson, C., and M. E. Owen. 1977. Influence of serial passage on the infectivity and immunogenicity of *Nematospiroides dubius* in mice. Int. J. Parasitol. 7:463–466.

Ebert, D. 1998. Experimental evolution of parasites. Science 282: 1432–1435.

Ebert, D., and W. D. Hamilton. 1996. Sex against virulence: the coevolution of parasitic diseases. Trends Ecol. Evol. 11:79–81.

Ebert, D., H.-J. Carius, T. J. Little, and E. Decaestecker. 2004. The evolution of virulence when parasites cause host castration and gigantism. Am. Nat. 164:S19–S32.

Ebert, D., P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1988: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philos. Trans. R. Soc. B 351:1689–1701.

Ferrari, D. C., and P. D. N. Hebert. 1982. The induction of sexual reproduction in *Daphnia magna*: genetic differences between arctic and temperate populations. Can. J. Zool. 60:2143–2148.

Frank, S. A. 1992. Models in plant-pathogen coevolution. Trends Genet. 8:213–219.

Green, J. 1974. Parasites and epibionts of Cladocera. Trans. Zool. Soc. Lond. 32:417–515.

Haldane, J. B. S. 1949. Disease and evolution. Ric. Sci. Suppl. Ann. 19:68–75.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. Oikos 35:282–290.

Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites. Proc. Nat. Acad. Sci. USA 87:3566–3573.

Hebert, P. D. N., and M. J. Beaton. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, TX.

Jermy, T. 1990. Prospects of antifeedant approach to pest-control: a critical review. J. Chem. Ecol. 16:3151–3166.

Kaltz, O., and J. A. Shykoff. 1998. Local adaptation in host-parasite systems. Heredity 81:361–370.

Klüttgen, B., U. Dülmer, M. Engels, and H. T. Ratte. 1994. ADaM, an artificial freshwater for the culture of zooplankton. Water Res. 28:743–746.

Lajeunesse, M. J., and M. R. Forbes. 2001. Host range and local parasite adaptation. Proc. R. Soc. 269:703–710.

Lambrechts, L., J. Halbert, P. Durand, L. C. Gouagna, and J. C. Koella. 2005. Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitoes to *Plasmodium falciparum*. Malaria J. 4:3–. Available online via http://www.malariajournal.com/content/4/1/3.

Little, T. J., and D. Ebert. 1999. Associations between parasitism and host genotype in natural populations of *Daphnia* (Crustacea: Cladocera). J. Anim. Ecol. 68:134–149.

———. 2000. The cause of parasitic infection in natural populations of *Daphnia*: the role of host genetics. Proc. R. Soc. B. 267: 2037–2042.

Little, T. J., H.-J. Carius, O. Sakwinska, and D. Ebert. 2002. Competitiveness and life-history characteristics of *Daphnia* with respect to susceptibility to a parasite. J. Evol. Biol. 15:796–802.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519–521.

Loaring, J. M., and P. D. N. Hebert. 1981. Ecological differences among clones of *Daphnia pulex* Leydig. Oecologia 51:162–168.

Mackinnon, M. J., D. J. Gaffney, and A. F. Read. 2002. Virulence in rodent malaria: host genotype by parasite genotype interactions. Infect. Genet. Evol. 1:287–296.

McClelland, E. E., F. R. Adler, D. L. Granger, and W. K. Potts. 2004. Major histocompatibility complex controls the trajectory but not host-specific adaptation during virulence evolution of the pathogenic fungus *Cryptococcus neoformans*. Proc. R. Soc. B 271:1557–1564.

Rainey, P. 2004. Bacterial populations adapt—genetically, by natural selection—even in the lab. Microbiol. Today 31:160–162.

Read, A. F., P. Aaby, R. Antia, D. Ebert, P. W. Ewald, S. Gupta, E. C. Holmes, A. Sasaki, D. Shields, F. Taddei, and R. Moxon. 1999. What can evolutionary biology contribute to understanding virulence? Pp. 205–215 *in* S. C. Stearns, ed. Evolution in health and disease. Oxford Univ. Press, Oxford, U.K.

Rolff, J., and M. T. Siva-Jothy. 2003. Invertebrate ecological immunity. Science 301:472–475.

Stirnadel, H. A., and D. Ebert. 1997. The ecology of three *Daphnia* species: their microparasites and epibionts. J. Anim. Ecol. 66: 212–222.
Thompson, J. N., and J. J. Burdon. 1992. Gene-for-gene coevolution between plants and parasites. Nature 360:121–125.
Wedekind, C., and A. Ruetschi. 2000. Parasite heterogeneity affects infection success and the occurrence of within-host competition: an experimental study with a cestode. Evol. Ecol. Res. 2: 1031–1043.
Woolhouse, M. E., J. P. Webster, E. Domingo, B. Charlesworth, and B. R. Levin. 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nat. Genet. 32: 569–577.

Corresponding Editor: J. Koella