Evolution, 55(6), 2001, pp. 1136–1145

GENETIC VARIATION IN A HOST-PARASITE ASSOCIATION: POTENTIAL FOR COEVOLUTION AND FREQUENCY-DEPENDENT SELECTION

HANS JOACHIM CARIUS,[1,2] TOM J. LITTLE,[1,3] AND DIETER EBERT[1,4]
[1]*Zoologisches Institut, Universität Basel, Rheinsprung 9, CH-4051 Basel, Switzerland*
[2]*E-mail: achim.carius@epost.de*
[4]*E-mail: d.ebert@unibas.ch*

Abstract.—Models of host-parasite coevolution assume the presence of genetic variation for host resistance and parasite infectivity, as well as genotype-specific interactions. We used the freshwater crustacean *Daphnia magna* and its bacterial microparasite *Pasteuria ramosa* to study genetic variation for host susceptibility and parasite infectivity within each of two populations. We sought to answer the following questions: Do host clones differ in their susceptibility to parasite isolates? Do parasite isolates differ in their ability to infect different host clones? Are there host clone-parasite isolate interactions? The analysis revealed considerable variation in both host resistance and parasite infectivity. There were significant host clone-parasite isolate interactions, such that there was no single host clone that was superior to all other clones in the resistance to every parasite isolate. Likewise, there was no parasite isolate that was superior to all other isolates in infectivity to every host clone. This form of host clone-parasite isolate interaction indicates the potential for coevolution based on frequency-dependent selection. Infection success of original host clone-parasite isolate combinations (i.e., those combinations that were isolated together) was significantly higher than infection success of novel host clone-parasite isolate combinations (i.e., those combinations that were created in the laboratory). This finding is consistent with the idea that parasites track specific host genotypes under natural conditions. In addition, correspondence analysis revealed that some host clones, although distinguishable with neutral genetic markers, were susceptible to the same set of parasite isolates and thus probably shared resistance genes.

Key words.—Arms race, *Daphnia magna*, frequency-dependent selection, host-parasite coevolution, infectivity, *Pasteuria ramosa*, Red Queen hypothesis, resistance.

Received March 20, 2000. Accepted January 20, 2001.

Host-parasite coevolution is reciprocal natural selection on host resistance and parasite infectivity (Thompson 1994). Models suggest that this form of reciprocal selection may be frequency dependent (Clarke 1976; Hamilton 1980; Bell and Maynard Smith 1987; Hamilton et al. 1990), that is, parasites are selected to overcome the resistance of common hosts and consequently hosts with rare resistance genes have a selective advantage. It has been suggested that the selective advantage of being rare can favor sexual reproduction as means of producing diverse offspring, with rare resistance genes (Red Queen hypothesis, Jaenike 1978; Hamilton 1980). Two main assumptions underlie these models: First, that there is genetic variation in both host resistance (prevention of parasite invasion and development) and parasite infectivity (ability to infect and proliferate in a host, associated with host fitness reduction), otherwise evolutionary change is not possible. Second, for frequency-dependent selection to operate, genotype-specific host-parasite interactions must be present, whereby parasites show infectivity specific to particular host genotypes, and hosts show resistance specific to particular parasite genotypes.

Empirical studies documenting the presence of genetic variation for resistance against plant pathogens are numerous (e.g., Dinoor 1970, 1977; Burdon 1987; de Nooij and van der Aa 1987; Burdon and Jarosz 1991; Chaboudez and Burdon 1995), whereas studies of the genetic variation for resistance against animal parasites are relatively scarce (Sorci et al. 1997). Well-documented cases include mollusk-trematode (Lively 1989; Grosholz 1994; Dybdahl and Lively 1998; Webster and Woolhouse 1998), bird-mite (Møller 1990; Boulinier et al. 1997), insect-parasitoid/parasite (Henter and Via 1995; Kraaijeveld and Van Alphen 1995; Baer and Schmid-Hempel 1999; Hufbauer and Via 1999), *Daphnia*-parasite (Ebert 1994; Ebert et al. 1998; Little and Ebert 1999), and mammal-parasite associations (Smith et al. 1999). Genetic variation for infectivity among pathogens has been detected in both plant pathogens (Oates et al. 1983; de Nooij and van Damme 1988; Jarosz and Burdon 1991) and animal parasites (Lively 1989; Carton and Nappi 1991; Ebert 1994; Kraaijeveld and Van Alphen 1994; Henter 1995).

It has been predicted that dynamic host-parasite arms races are most likely if the interactions within populations are characterized by genotype-specific resistance and infectivity whereby the outcome (resistant or susceptible) of host-parasite encounters depend on the genotype of both opponents (Hamilton 1980). In addition, these interactions should be such that no single host genotype is more resistant to all parasite genotypes than any other host genotype and no single parasite genotype has the highest fitness in association with all host genotypes. Also, the parasite should have strong fitness-reducing effects, such that the selective impact is high (Howard and Lively 1994; Clay and Kover 1996). With these requirements in place, negative frequency-dependent selection can operate without fixation of a single host or parasite genotype (Clarke 1976; Barrett 1988).

To date, we know very little about genotype-specific interactions and even less about frequency-dependent selection. Current evidence is limited to a few plant-pathogen (Burdon and Jarosz 1991, 1992; Jarosz and Burdon 1991; Bevan et al. 1993a,b; Burdon 1994; Chaboudez and Burdon 1995; Espiau et al. 1998) and animal-parasite associations (Dybdahl

[3] Present address: Institute for Cell, Animal and Population Biology, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JT, Scotland; E-mail: little@holyrood.ed.ac.uk.

and Lively 1998; Lively and Dybdahl 2000). More studies on the variation within populations for resistance and infectivity are needed to determine how widespread the potential for frequency-dependent selection is (Clay and Kover 1996). Here we present the results of two experiments that tested for both the presence of genetic variation in resistance and infectivity and for the interaction between parasite isolates and host clones in two populations of the freshwater crustacean *Daphnia magna* and its bacterial microparasite *Pasteuria ramosa.*

Materials and Methods

The Study Organisms

The host.—*Daphnia magna* Straus is a planktonic freshwater crustacean usually found in eutrophic shallow ponds. It is known to be attacked by a variety of bacterial, microsporidial, and fungal parasites (Green 1974; Stirnadel and Ebert 1997; Little and Ebert 1999; Ebert et al. 2000). Prevalence of *Daphnia* parasites can be high (up to 98%) and field studies and laboratory experiments have demonstrated that these parasites typically have an large impact on *Daphnia* fitness (Ebert 1995; Stirnadel and Ebert 1997; Little and Ebert 1999). *Daphnia magna* reproduces by cyclical parthenogenesis and can be kept in the laboratory in the state of clonal reproduction. *Daphnia* have an undetermined, stepwise growth pattern—a change in body length occurs only when the old carapax is shed at molting. Juveniles go through a series of molts (instars) before reaching maturity. At 20°C and 5×10^6 algae per day, maturity is reached about 10 days after birth. Juveniles are released with every adult instar, which is about every three days. The first clutch is about 10 juveniles, and up to 30 juveniles are produced in later clutches.

The parasite.—*Pasteuria ramosa* Metchnikoff 1888 is a bacterial obligate endoparasite of *Daphnia* (Ebert et al. 1996). It has been found in prevalence up to 50% (Stirnadel and Ebert 1997; Little 1999). Infection takes place through ingestion of waterborne spores by the filter-feeding host. The bacterium grows in the body cavity of its host, and in the final state of infection a single host contains several million endospores, which fill the entire body cavity. The infection can then be easily recognized by the naked eye. The fitness costs for the host are high because an infection leads to sterilization. Parasite transmission requires host death, because spores are only released from the decaying cadaver.

Experimental Design

To test for the presence of genetic variation for resistance and infectivity in the *D. magna–P. ramosa* system, we separately examined two populations where *D. magna* was infected with *P. ramosa.* The experiments were essentially identical and were performed consecutively. The first experiment assayed *D. magna* clones and *P. ramosa* isolates from a population in Russia, the second experiment assayed *D. magna* clones and *P. ramosa* isolates from a population in Germany. In both experiments, nine clones of *D. magna* and nine isolates of *P. ramosa* were used. The experiments differed only in the procedure for collecting host clones and parasite isolates, and in some details of the assay (see below).

Culture conditions.—*Daphnia magna* clones were kept under standardized conditions for at least three generations before the start of the experiment. Twenty-four single female lines were established per clone. An artificial culture medium was used (Klüttgen et al. 1994; medium composition modified after Ebert et al. 1998). Females were kept singly in 100-ml jars and fed daily with 5×10^6 cells of the algae *Scendesmus gracilis.* The temperature was 20°C and the light: dark cycle was 16:8 h.

Allozyme electrophoresis.—Allozyme phenotypes of all *Daphnia* clones were distinguished using cellulose acetate electrophoresis (Hebert and Beaton 1993). Five polymorphic enzyme loci were analyzed: aspartate amino transferase (*Aat,* EC 2.6.1.1), glucose-6-phosphate isomerase (*Gpi,* EC 5.3.1.9), mannose-6-phosphate isomerase (*Mpi,* EC 5.3.1.8), malate dehydrogenase (*Mdh,* EC 1.1.1.37), and phosphoglucomutase (*Pgm,* EC 5.4.2.2).

Distinction of parasite isolates.—We were not able to distinguish parasite genotypes with genetic markers. Each *Daphnia* from which we obtained the parasite could be multiply infected, and thus each parasite isolate might have consisted of different parasite genotypes. However, there are reasons to assume that the parasite isolates were predominantly single genotypes (see Discussion).

First Experiment

Collection of host clones.—*Daphnia magna* were hatched from resting eggs obtained from a mud sample collected in 1995 from a pond in the Moscow Zoo, Moscow, Russia. Only mud near the surface was taken (an approximately 3-cm layer). Hatching was induced under continuous light and a temperature of about 20°C. All hatching females were genotyped using allozyme electrophoresis (see above). Nine unique multilocus genotypes were identified, and we randomly picked one clone from each multilocus genotype to be included in the experiment. Each clone was designated with a number.

Collection and propagation of parasite isolates.—It has been hypothesized (Green 1974) and demonstrated (Ebert 1995) that the sediment of ponds functions as a spore bank for *Daphnia* parasites. We used the same sediment sample (from which the resting eggs of the *D. magna* clones originated) to obtain the isolates of *P. ramosa.* About 50 g of sediment was put into each of nine 400-ml jars; these jars were filled up with artificial culture medium and stirred well to promote the suspension of parasite spores. Thirty juveniles from each of the nine *D. magna* clones were added to the jars, so that each jar contained juveniles of a single clone. After 10 days, the *Daphnia* were transferred singly into 100-ml jars and fed daily. After 20 days, uninfected individuals were discarded. Dead infected *Daphnia* were placed singly into Eppendorf tubes and frozen (−20°C). From this pool of parasite isolates, one infected individual per *Daphnia* clone was randomly chosen to be the source of parasite spores. The parasite isolate was designated with the same number as the *Daphnia* clone that was used to isolate it. To obtain a sufficient number of spores, each of the nine parasite isolates was propagated once in the same host clone genotype from

which it was isolated. For this purpose, a spore solution was prepared by grinding up the cadaver in 1 ml of medium and then added to a jar containing 30 *Daphnia* juveniles. The individuals were then screened daily, and dead infected *Daphnia* were placed into single Eppendorf tubes and frozen (−20°C). For the preparation of the nine spore solutions to be used in the experiment, dead infected *Daphnia* where pooled according to the spore isolate they were infected with. From each batch of infected *Daphnia*, spore solutions of two concentrations (doses) were prepared. Because *P. ramosa* is known to show a strong dose effect (Ebert et al. 1998), two spore doses were used to achieve a infection level where statistical analysis is reliable. The high dose was 5×10^5 spores and the low dose 0.2×10^5 spores per jar. Spore solutions were frozen until the start of the experiment.

The assay.—The experiment was a complete cross-infection experiment. With nine *D. magna* clones and nine parasite isolates there were 81 combinations and two dose levels. Each host clone-parasite isolate combination included nine replicates. A split brood design was used. The third clutch of a single mother was split over the 19 different treatments ([9 parasite isolates × 2 doses] + 1 control = 19). In a few cases, the clutch contained less then 19 juveniles. The missing juveniles were then taken from another mother of the same clone, which had released offspring the same time. For the start of the assay (day 0), the juveniles were placed singly in 100-ml jars filled with 20 ml of medium. On two consecutive days (day 1 and 2) 0.5 ml of spore solution was added, resulting in a total spore dose of 5×10^5 and 0.2×10^5 spores per jar in the high- and low-dose treatments, respectively. The daily food supply until day 5 was 2×10^6 algae cells. After day 5, the individuals were transferred into jars filled with 100 ml of medium and fed daily 5×10^6 algae cells. The medium was changed with every clutch, and *Daphnia* that stopped reproduction due to parasite infection received fresh medium every third day. Infections and the number of clutches were recorded. On day 20, all individuals that appeared to be uninfected were dissected and checked for signs of infection under the microscope. On day 30, the remaining infected *Daphnia* were singly placed in Eppendorf tubes with 0.3 ml of medium and frozen. For every infected *Daphnia* the number of mature parasite spores was counted; cadavers were ground up and the spore counts were determined in a bacteria counting chamber (0.1 mm depth, Neubauer ruling) under a light microscope with 600× magnification.

Second Experiment

Collection of host clones.—*Daphnia magna* individuals that were infected with P. ramosa were collected from a pond in Gaarzerfeld, Germany, in August 1997. They were brought in to the laboratory and placed singly in jars filled with 100 ml of medium. Nine infected *D. magna* individuals produced viable offspring before parasitic sterilization was complete. The offspring, which are genetically identical to their mother but uninfected, were collected and maintained as single female lines in the laboratory. Allozyme electrophoresis of the offspring revealed that all nine clones had a unique multilocus genotype and each was designated with a number. The nine infected mothers of these clones were the source of the parasite isolates, which were designated with the same number.

Collection and propagation of parasite isolates.—The nine infected *Daphnia* (see above) were kept until their death and the parasite isolates were then treated like the isolates from the first experiment to obtain a sufficient number of parasite spores. This design allowed us to use nine *D. magna* clones and the nine parasite isolates that had infected these clones in their natural environment.

The assay.—Most aspects of the experiment were those described for the first experiment (see above), except for the following. The two spore doses were 0.2×10^6 and 1×10^6 spores per jar filled with 20 ml of medium, respectively, and were administered at day 1. Uninfected individuals were discarded at day 25.

Statistical Analysis

Using the Procedure GENMOD of the SAS statistic package (SAS Institute 1992), a binary logistic regression was performed to analyze the effect of host clone and parasite isolate on the proportion of infected *Daphnia.* Significance of the main effects was assessed by pseudo-*F*-tests based on the mean deviance (Schmid and Dolt 1994; Kaltz et al. 1999). Mean deviance (MD) is the deviance divided by the degrees of freedom (analogous to mean squares obtained from least-square methods). All effects were considered random and the analysis was performed with Type 3 and Dscale option of Procedure GENMOD (SAS Institute 1992). Only the deviance of the host clone-parasite isolates interaction was tested directly against the χ^2-distribution. In the first experiment, due to the very low infection rates in the low-dose treatment, a reliable statistical analysis could be performed only for the high-dose treatment. In the second experiment, the two doses were analyzed separately.

For the second experiment, a correspondence analysis was performed to visualize both the similarity among different parasite isolates with respect to their infection profile and among different host clones with respect to their susceptibility profile, and also to visualize the interaction between parasite isolates and host clones. The correspondence analysis describes similarity as the presence or absence of a susceptible reaction and also takes the infection frequency (number of replicates that became infected) into account. Correspondence analysis was done using Procedure CORRESP in SAS (SAS Institute 1992). For this analysis the infection data of the high- and low-dose treatment were pooled. Pooling of data increases the sample size, but is conservative, because it tends to decrease the differences in infection frequencies.

For the second experiment, we also compared the compatibility of original host clone-parasite isolate combinations, that is, those isolated together, to novel combinations, that is, those created in the laboratory. Following the approach of Kaltz et al. (1999), we partitioned the variation in the proportion of infected *Daphnia* explained by the host clone-parasite isolate interaction (64 df) deviance into an original-versus-novel effect (1 df) and a residual term (63 df). The residual term was used as error term to test for a significant difference in infection success of original (diagonal in Figs. 1C, D) and novel combinations (off-diagonal in Figs. 1C, D).

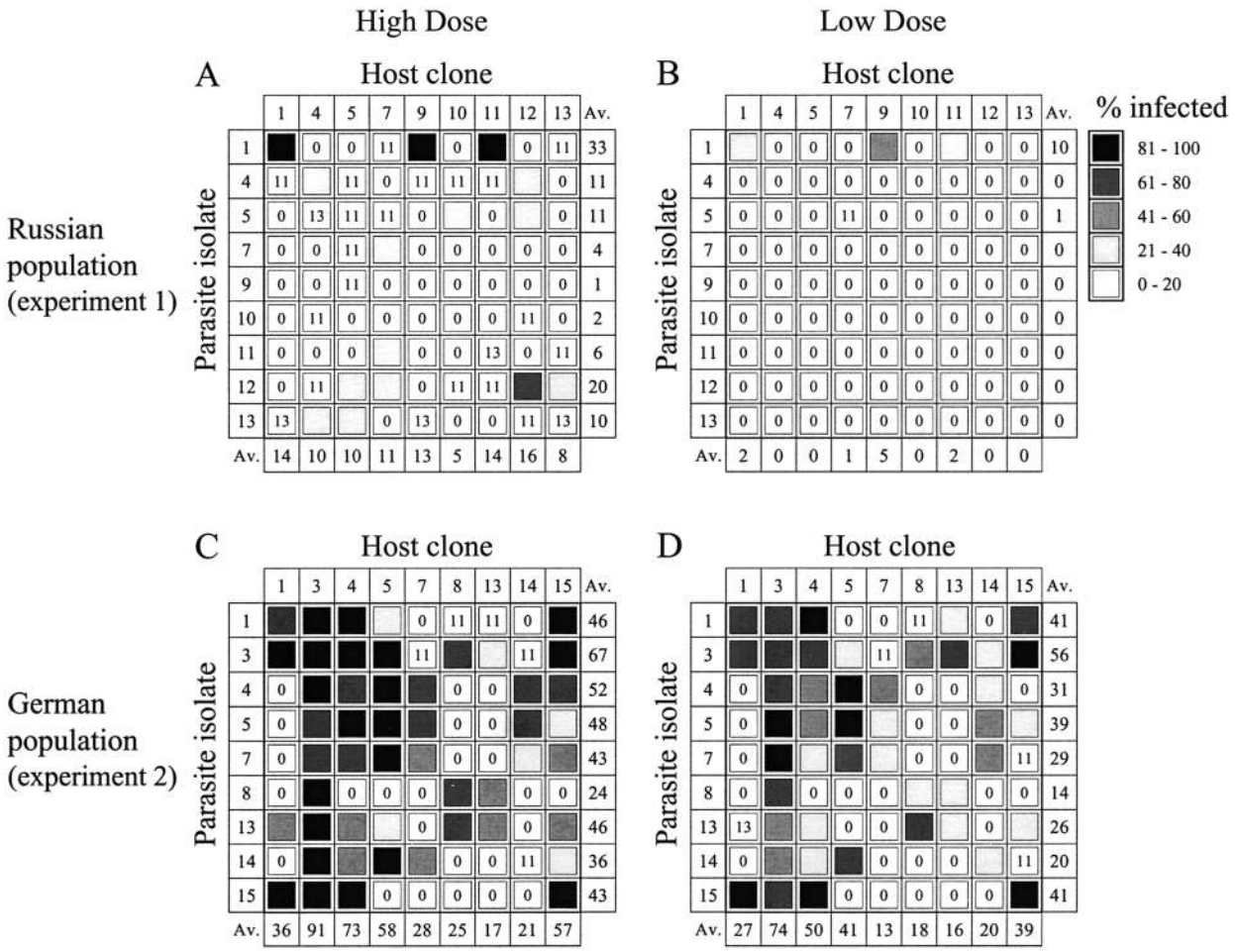

FIG. 1. Percentage infected hosts in all 81 host clone-parasite isolate combinations. Percentage of hosts infected (usually of nine replicates) are indicated by the gray shading. For the lowest infection range (0–20%), the actual infection percentage is given to distinguish between zero and low infection rates. The bottom row and most right column (labeled Av.) of each table shows the average percentage of infected hosts. Russian population: high-dose treatment (A), low-dose treatment (B); German population: high-dose treatment (C), low-dose treatment (D).

Proportions from the high-dose and low-dose treatment were pooled before analysis.

Host clone fitness was determined by the number of clutches produced until sterilization or the end of the experiment. The effect of host clone, parasite isolate, and host clone-parasite isolate interaction on host fecundity were analyzed with a Poisson regression using Procedure GENMOD in SAS (SAS Institute 1993). Pseudo-*F*-tests were carried out based on the MD. All effects were considered random. The MD of the host clone-parasite isolate interaction was used as error term for the main effects host clone and parasite isolate. The MD of the residual was used as error term for the host clone-parasite isolate interaction. For this analysis, the number of clutches of infected and uninfected *Daphnia* of each host clone-parasite isolate combination was included. By including fecundity data of both infected and uninfected *Daphnia* into the analysis, the effects of differential susceptibility and variation in the timing of sterilization were accounted for. Prior to this analysis, we tested whether uninfected host clones differed in fecundity by analyzing the control treatment with a Poisson regression.

RESULTS

First Experiment

The overall infection level in the low-dose treatment was 1.3% (n = 713) and in the high-dose treatment 11.3% (n = 708) indicating a strong dose effect (Fisher exact test, $P < 0.0001$). Due to the low number of infections in the low-dose treatment, which were restricted to only four parasite isolate-host clone combinations, a statistical analysis for this treatment could not be performed. The absence of infection in *Daphnia,* which appeared uninfected during the experiment, was confirmed by dissecting individuals under the microscope.

Infection pattern.—Figures 1A and B show the outcome of all parasite isolate-host clone combinations as infection rates (proportion of replicates that became infected) for the high- and low-dose treatments, respectively. Clones were susceptible to from three to six different parasite isolates. Analysis of the high-dose treatment shows that the nine host clones did not differ in their overall average susceptibility (binary logistic regression: clone: df = 8, MD = 1.14, F = 0.49, P

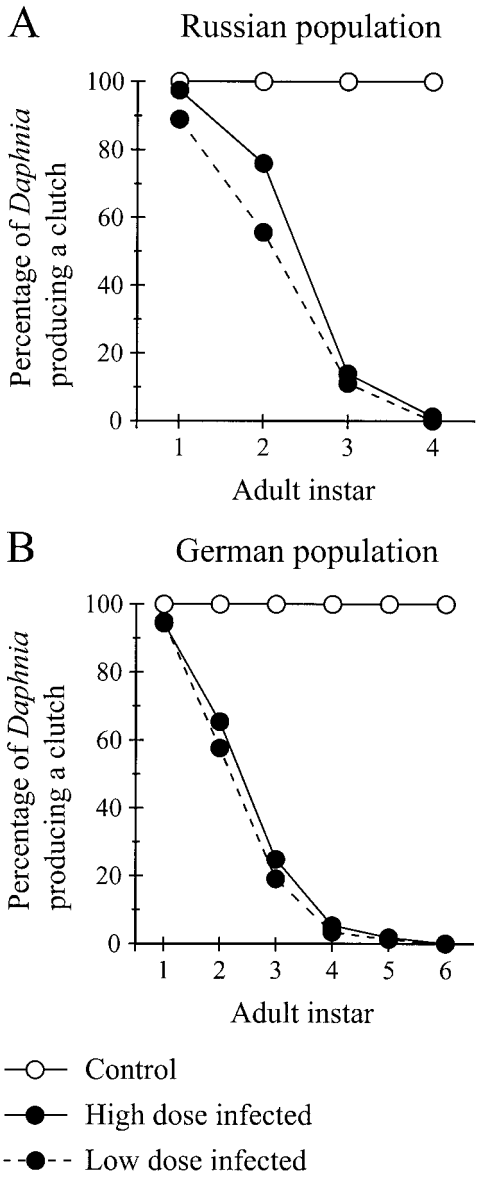

FIG. 2. Percentage of *Daphnia* of the three treatments (control and infected *Daphnia* of the high- and low-dose treatments) producing a clutch in a given adult instar. (A) Russian population; (B) German population.

= 0.86). However, within parasite isolates, host clones differed in their susceptibility. This can be seen, for example, within parasite isolate 1 in both the high- and low-dose treatment, where host clones 1, 9, and 11 had a high susceptibility, whereas the other host clones were resistant. In the high-dose treatment all parasite isolates achieved at least one infection. The number of different host clones that could be attacked by a parasite isolate ranged from one to seven. Parasite isolates differed in their average infectivity (isolate: df = 8, MD = 7.87, $F = 3.38$, $P < 0.01$) . There were also significant parasite isolate-host clone interactions, that is, parasite isolates showed infectivity specific to certain host clones and host clones showed resistance specific to certain parasite isolates (clone × isolate: df = 64, MD = 2.33, $P < 0.0001$).

TABLE 1. For the second experiment, statistics of a binary logistic regression model testing the effects of host clone, parasite isolate, and host clone-parasite isolate interaction on the proportion of infected *Daphnia magna*. Significance of the main effects was assessed by pseudo-*F*-tests based on the mean deviance (MD), the deviance divided by the degrees of freedom (analogous to mean squares obtained from least-square methods). All effects were considered random. Only the deviance of the host clone-parasite isolates interaction was tested directly against the χ^2 distribution.

	Source	df	MD	*F*	*P*
Low dose	clone	8	15.38	3.26	<0.01
	isolate	8	6.94	1.47	0.19
	clone × isolate	64	4.72		<0.0001
High dose	clone	8	25.77	4.64	<0.001
	isolate	8	6.40	1.15	0.34
	clone × isolate	64	5.55		<0.0001

Fitness effects.—The determination of the spore counts revealed that in all cases parasite infection resulted in the production of mature spores (average number of spores = 7.2 × 10^6, SE = 0.2 × 10^6). Host clone fitness, measured as number of clutches produced, was lowered by parasite infection. Although the majority of infected *Daphnia* were sterilized after the second adult instar, the *Daphnia* of the control produced clutches throughout the experiment (Fig. 2A). Host clones of the control treatment did not differ in fecundity (control: df = 8, MD = 0.18, $F = 1.2$, $P = 0.31$; residual: df = 72, MD = 0.15).

The analysis of fecundity data of the high-dose treatment showed that host clones differed in the overall average number of clutches produced. Parasite isolates differed in the overall virulence and there were significant host clone-parasite isolate interactions (Poisson regression: clone: df = 8, MD = 3.335, $F = 8.66$, $P < 0.0001$; isolate: df = 8, MD = 1.2, $F = 3.12$, $P < 0.01$; clone × isolate: df = 64, MD = 0.385, $F = 1.35$, $P < 0.05$; residual: df = 648, MD = 0.286).

Second Experiment

The overall infection level in the low-dose treatment was 32.9% ($n = 718$) and in the high-dose treatment 45.1% ($n = 725$). The infection levels of the low- and high-dose treatment differed significantly (Fisher exact test, $P < 0.0001$).

Infection pattern.—Figures 1C and D show the outcome of all parasite isolate-host clone combinations as infection rates for the high- and low-dose treatment, respectively. Clones were susceptible to four to nine different parasite isolates. Analysis of the high-dose treatment showed that host clones differed in their average susceptibility, but parasite isolates did not differ in their average infectivity (Table 1). However, there were significant host clone-parasite isolate interactions, reflected in the specific infectivity of parasite isolates and specific resistance of host clones. The results of the low-dose treatment reflect the high-dose treatment: The effects of host clones and parasite isolate-host clones interactions were significant, whereas the effect of parasite isolates was not (see Table 1) and the distribution of infections over the 9 × 9 matrix was very similar (Figs. 1C, D). The correspondence analysis (see below) revealed that parasite isolate 4, 5, 7, and 14 had almost identical infection profiles. We could not ex-

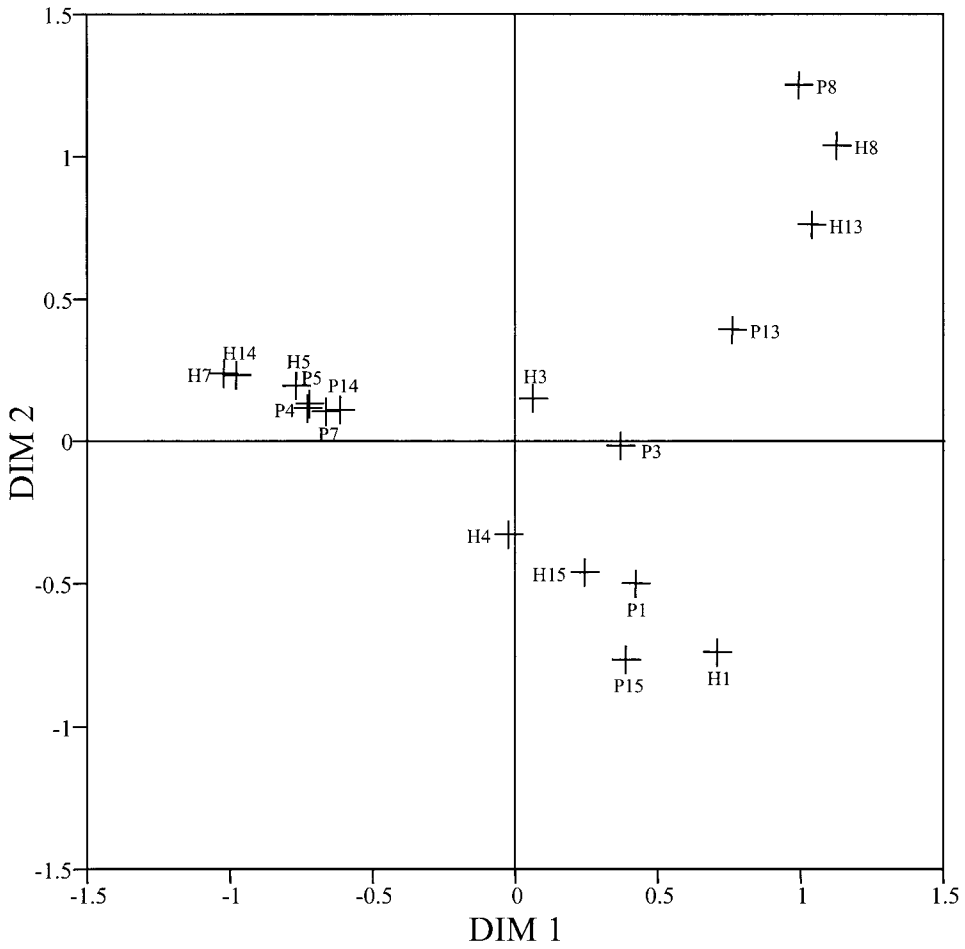

FIG. 3. The first and second dimension of a correspondence analysis based on the infection frequencies (pooled across dose levels) of the second experiment (German population). Host clones are labeled with H and the respective number. Parasite isolates are labeled with P and the respective number.

clude the possibility that these parasite isolates represent the same parasite genotype. Therefore we pooled the data of these four isolates and repeated the analysis (clone with 8 df, isolate with 5 df, and interaction with 40 df). The significance of the factors host clone, parasite isolate, and the interaction was not changed compared to the analysis with nine parasite isolates except for the clone effect in the low-dose treatment, which went from significant to marginally significant (high-dose treatment: clone: df = 8, MD = 25.44, $F = 2.94$, $P < 0.05$; isolate: df = 5, MD = 8.42, $F = 0.97$, $P = 0.45$; clone × isolate: df = 40, MD = 8.65, $P < 0.0001$; low-dose treatment: clone: df = 8, MD = 15.14, $F = 2.17$, $P = 0.0515$; isolate: df = 5, MD = 9.41, $F = 1.35$, $P = 0.27$; clone × isolate: df = 40, MD = 6.99, $P < 0.0001$).

Because of the way hosts and parasites were isolated from this population, we were able to compare original parasite isolate-host clone combinations to novel parasite isolate-host clone combinations, that is, those combinations that were created in the experiment. The infectivity of original host clone-parasite isolate combinations (proportion infected *Daphnia* = 0.642; 0.604, 0.680, 95% confidence interval) was higher than the infectivity of novel combinations (proportion infected *Daphnia* = 0.358; 0.345, 0.371). This difference was significant (binary logistic regression: original-versus-novel: df = 1, MD = 70.85, $F = 8.61$, $P < 0.01$; residual: df = 63, MD = 8.23)

Correspondence analysis.—Some host clones were susceptible to the same set of parasite isolates. This can be seen in both the low- and high-dose treatment (see Figs. 1C and D). Furthermore, different parasite isolates infected the same set of host clones. A correspondence analysis based on the pooled infection data was performed to visualize these similarities (Fig. 3). The first two dimensions of the correspondence analysis explained 94.8% of the variation (first dimension: 61.4%, second dimension: 33.4%). The quality criteria of datapoints, that is, how good the first two dimensions describe the infection or susceptibility profile of a parasite isolate or host clone, is above 0.9 for most of the points. This indicates a good representation of most host clones and parasite isolates.

Closeness of points representing host clones indicates that host clones have a similar profile of parasite isolates to which they are susceptible (Fig. 3). The graph shows that there are three more-or-less clearly defined groups of host clones (group 1: host clones 8 and 13; group 2: host clone 5, 7, and 14; group 3: host clone 15, 4, and 1). Host clone 3 is close

to the origin, indicating the small difference it has to the average susceptibility profile. This clone is about equally susceptible to all parasite isolates (Figs. 1C, D)

Nearness of points representing parasite isolates means that parasite isolates have a similar profile of host clones they can infect (Fig. 3). There are three defined groups of parasite isolates (group 1: isolate 8 and 13; group 2: isolate 4, 5, 7, and 14; group 3: isolate 1 and 15). Isolate 3 is situated on the border of the first and the fourth quadrant and does not clearly belong to one of the parasite isolate groups. However, in contrast to host clone 3, parasite isolate 3 shows a clear pattern of specificity (Figs. 1C, D).

Considering parasite isolates and host clones together illustrates associations among parasite isolates and host clones (Fig. 3). In this case, the angle between vectors of parasite isolates and host clones, with the origin as the starting point, indicates the association (Benzécri 1992; Micheloud 1997). Groups of parasite isolates and host clones that lay in approximately the same direction (acute angle) from the origin are in positive association, indicating a higher-than-average susceptibility of this group of host clones to this group of parasite isolates. Groups of parasite isolates and host clones that lay in obtuse angle to each other are in negative association, indicating that these groups of host clones have a lower than average susceptibility to these parasite isolates. Finally, groups of parasite isolates and host clones that lay in right angle to each other show neither a positive nor negative association, indicating that these groups of host clones have a susceptibility similar to the average susceptibility to these parasite isolates.

In most cases, parasite isolates tend to lay in the same direction as the host clone they were originated from. Seven of the nine parasite isolates show a positive association with the host clone they were originated from indicated by very small angles (range: 1–9°). The two parasite isolates that do not show positive associations are isolates 3 (70°) and 4 (95°). In both pairs at least one opponent is close to the origin, indicating a low specificity.

Fitness effects.—The determination of spore counts for the high-dose treatment revealed that in all cases parasite infection resulted in the production of mature spores (average number of spores = 28.4×10^6, SE = 0.6×10^6). Spore counts for the low-dose treatment were not determined. Host clone fitness in the high- and low-dose treatment was reduced by parasite infection. Although the majority of infected *Daphnia* were sterilized after the second adult instar, the *Daphnia* of the control produced clutches throughout the experiment (Fig. 2B). Host clones of the control treatment did not differ in fecundity (Poisson regression: control: df = 8, MD = 0.045, $F_{8,72} = 1.58$, $P = 0.15$; residual: df = 72, MD = 0.0285). The analysis of the fecundity data of the high- and low-dose treatment showed that host clones differed in the overall average number of clutches produced. Parasite isolates differed significantly in the overall virulence in the low-dose treatment. The differences in overall virulence in the high-dose treatment were marginally significant. There were significant host clone-parasite isolate interactions in both treatments (Table 2).

TABLE 2. For the second experiment, statistics of a Poisson regression testing the effects of host clone, parasite isolate, and host clone-parasite isolate interaction on host fecundity (number of clutches). The fecundity data include infected and uninfected *Daphnia* from each host clone-parasite isolate combination. Pseudo-*F*-tests were carried out based on the mean deviance (MD), the deviance divided by the degrees of freedom (analogous to mean squares obtained from least-square methods). All effects were considered random. The mean deviance of the host clone-parasite isolate interaction was used as error term for the main effects host clone and parasite isolate. The mean deviance of the residual was used as error term for the host clone-parasite isolate interaction.

	Source	df	MD	*F*	*P*
Low dose	clone	8	14.254	4.82	<0.001
	isolate	8	7.658	2.59	<0.05
	clone × isolate	64	2.958	4.97	<0.0001
	residual	648	0.5955		
High dose	clone	8	21.298	5.83	<0.0001
	isolate	8	7.423	2.03	0.057
	clone × isolate	64	3.653	6.99	<0.0001
	residual	648	0.5229		

DISCUSSION

We found significant genetic variation among clones of the freshwater crustacean *D. magna* for susceptibility to its parasite *P. ramosa* and significant genetic variation among isolates of the bacterial parasite *P. ramosa* for infectivity to its host *D. magna*. Reciprocal selective pressure acting in this association is likely to be strong, because infected *Daphnia* are sterilized and parasites that cannot successfully invade the host *Daphnia* produce no transmission stages at all. The potentially large fitness costs on both sides, together with the genetic variation detected in this study, suggest considerable potential for coevolution in this association.

Furthermore, we found significant interactions between *D. magna* clones and *P. ramosa* isolates; *D. magna* clones possessed resistance specific to certain parasite isolates and *P. ramosa* isolates possessed host-clone-specific infectivity. The rank order for resistance of host clones changes with the infecting parasite isolate and the rank order for infectivity of parasite isolates changes with the attacked host clone. In addition, there was no host clone that was the most resistant clone to all nine parasite isolates and no parasite isolate that was the most infective isolate on all host clones tested. This was true for both populations used in our study. Under such conditions it is easy to envision that host clone and parasite gene frequencies in a population are critical for the selective outcome. Selection favors those parasites able to infect common host clones, with these host clones being subsequently disfavored. Thus, our findings indicate that frequency-dependent selection can operate in this system and the coevolution may be oscillatory, as envisioned under the Red Queen hypothesis.

The potential for frequency-dependent selection in the *D. magna–P. ramosa* association is reinforced by another finding of our study. Due to the sampling procedure of the parasite isolates in the second experiment, we are able to compare original parasite isolate-host clone combinations, that is, those combinations that were isolated together (diagonal in Figs. 1C, D), to novel parasite isolate-host clone combinations, that is, those combinations that were created in the

laboratory (off-diagonal in Figs. 1C, D). The infection success of original host clone-parasite isolate combinations was higher than the infection success of novel combinations. It seems unlikely that the difference between original and novel combinations is due to a physiological adaptation of the parasite (each parasite experienced its original host clone for two generations before the experimental assay). Experimental investigations (H. J. Carius and T. Little, unpubl. data) give no support for such short-term physiological adaptation, although we cannot exclude this possibility with absolute certainty. The above-average compatibility of original combinations is reflected in the results of the correspondence analysis, which showed a highly positive association between the original combinations of host clones and parasite isolates in seven of nine combinations. This finding is consistent with the idea that parasites track specific host genotypes under natural conditions.

The two experiments are marked by a different procedure for obtaining host clones and parasite isolates. In the first experiment, the host clones and parasite isolates were obtained from a mud sample. The isolation procedure somewhat mimics the situation in a *Daphnia* pond in spring. *Daphnia* hatch from resting eggs and parasite spores are taken up by filter-feeding hosts, either in the free water or feeding on the suspensions stirred up from the pond sediment (hungry *D. magna* browse the sediment surface; Ebert 1995; Ebert et al. 1997). We had no knowledge regarding resistance of these clones prior to the isolation of the parasites. A potential artifact of this sampling design, which would increase the genetic variation within the sample, is that *Daphnia* resting eggs and parasite spores from different years might have been included because the mud sample could have contained material that settled over the course of a few years. We reduced this effect by collecting only the uppermost layer of mud, but we cannot exclude the possibility that older resting eggs and parasite spores were present. However, due to the feeding activity of animals (e.g., ground-feeding ducks, zoo-benthos) this upper mud layer can get disrupted, and hatching of ephippia from different years might be common. Genetic variation in the sample is also increased because we subsampled from the isolated clones those with unique multilocus genotypes. In the second experiment, we obtained host clones and parasite isolates by collecting infected *D. magna* individuals from the wild and therefore host clones and parasite isolates represent naturally occurring infections. Thus, the sample from the German population is biased in that we did not include uninfected host clones. We did not subsample the clones from this population to obtain unique multilocus genotypes; all nine isolated clones were distinct as collected. Therefore, in contrast to the Russian sample, our estimate of genetic variation for resistance in the German population is conservative. Despite these differences, both experiments similarly indicated the potential for frequency-dependent selection.

All host clones in our populations were distinct based on allozyme phenotype, yet some were similar in respect to their susceptibility profile, suggesting that they shared resistance genes. Similarly, we found that different parasite isolates had the same infection profile. The correspondence analysis revealed that isolate 4, 5, 7, and 14 were almost identical in their infection profile. However, because we were not able to distinguish parasite isolates with genetic markers, they might represent either different parasite genotypes that share virulence genes or identical parasite genotypes. Because we could not exclude the later explanation, we pooled the infection data of similar parasite isolates and repeated the analysis of the infection data to reevaluate the effect of host clone, parasite isolate and host clone-parasite isolate interactions, but our conclusion did not change. Alternatively each *Daphnia* could be multiply infected and thus each parasite isolate might have consisted of different parasite genotypes. However, due to the parasite propagation process prior to the assay, the parasite likely went through a genetic bottleneck. Furthermore, one might expect that differences among parasite isolates in the infectivity profile and host clone-parasite isolate interactions are less likely to be detected, if the parasite isolates were mixtures of genotypes. Thus, although we cannot rule out polymorphism of our isolates, we feel it is not unreasonable to assume that the observed interaction pattern is dominated by single parasite genotype-host genotype interactions.

The form of genetic control underlying infections has important implications for the operation of frequency-dependent selection (Parker 1994, 1996; Frank 1996a,b). The two most often discussed models are the gene-for-gene and matching-alleles models. Matching-alleles models have demonstrated the potential for frequency-dependent selection and selection for sex (Hamilton et al. 1990; for a review, see Otto and Michalakis 1998; Lively 1999). In contrast, gene-for-gene interactions seem not to favor frequency-dependent selection and selection for sex, because single overall superior genotypes can go to fixation (Parker 1994; Clay and Kover 1996). Among the 18 host clones and the putative 18 parasite isolates, we did not detect an overall superior genotype.

Local species interactions are part of the raw material for coevolutionary change (Thompson 1999). The interaction within local host-parasite populations is not independent of neighboring populations because populations can differ in their resistance and virulence genes and, through gene flow, new resistance and virulence genes can enter local populations (Gandon et al. 1996, 1998). Genetic drift, founder effects, and local extinctions can also shape the interaction and contribute to the large-scale picture (Thrall and Burdon 1997; Burdon and Thrall 1999; Thompson 1999). The present study gives insight only into the local interaction of *D. magna* with *P. ramosa*, but studies of among-population variation in the *D. magna–P. ramosa* association showed a low level of between-population variation relative to the strong genetic variation for resistance present within populations, suggesting that local interactions are dominant (Ebert et al. 1998). Traits under strong frequency-dependent selection are expected to show such patterns according to models by Schierup et al. (2000a,b). However, a comprehensive investigation, including several populations surveyed over time, is needed to appraise the extent to which this host-parasite association is influenced by temporal and spatial fluctuations in a metapopulation context.

Acknowledgments

We thank J. Hottinger and M. Capaul for assistance in the laboratory. We thank P. Jarne and two anonymous reviewers

for helpful comments on earlier versions of the manuscript. This work was supported by Swiss Nationalfond grants 3100-043093.95 and 31-53595.98.

Literature Cited

Baer, B., and P. Schmid-Hempel. 1999. Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151–154.

Barrett, J. A. 1988. Frequency dependent selection in plant fungal interactions. Philos. Trans. R. Soc. Lond. B Biol. Sci. 319: 473–483.

Bell, G., and J. Maynard Smith. 1987. Short-term selection for recombination among mutually antagonistic species. Nature 328: 66–68.

Benzécri, J. P. 1992. Correspondence analysis handbook. Dekker, New York.

Bevan, J. R., D. D. Clarke, and I. R. Crute. 1993a. Resistance to *Erysiphe fischeri* in two populations of *Senecio vulgaris.* Plant Pathol. (Oxf) 42:636–643.

———. 1993b. Variation for virulence in *Erysiphe fischeri* from *Senecio vulgaris.* Plant Pathol. (Oxf) 42:622–635.

Boulinier, T., G. Sorci, J.-Y. Monnat, and E. Danchin. 1997. Parent-offspring regression suggests heritable susceptibility to ecto-parasites in a natural population of kittiwake *Rissa tridactyla.* J. Evol. Biol. 10:77–85.

Burdon, J. J. 1987. Phenotypic and genetic patterns of resistance to the pathogen *Phakopsora pachyrhizi* in populations of *Glycine canescens.* Oecologia 73:257–267.

———. 1994. The distribution and origin of genes for race-specific resistance to *Melamspora lini* in *Linum marginale.* Evolution 48: 1564–1575.

Burdon, J. J., and A. M. Jarosz. 1991. Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*. I. Patterns of resistance and racial variation in a large host population. Evolution 45:205–217.

———. 1992. Temporal variation in the racial structure of flax rust (*Melampsora lini*) populations growing on natural stands of wild flax (*Linum marginale*): local versus metapopulation dynamics. Plant Pathol. (Oxf) 41:165–179.

Burdon, J. J., and P. H. Thrall. 1999. Spatial and temporal patterns in coevolving plant and pathogen associations. Am. Nat. 153: S13–S33.

Carton, Y., and A. Nappi. 1991. The *Drosophila* immune-reaction and the parasitoid capacity to evade it: genetic and coevolutionary aspects. Acta Oecol. 12:89–104.

Chaboudez, P., and J. J. Burdon. 1995. Frequency-dependent selection in a wild plant-pathogen system. Oecologia 102:490–493.

Clarke, B. 1976. The ecological genetics of host-parasite relationships. Pp. 87–103 *in* A. E. R. Taylor and R. Muller, eds. Genetic aspects of host-parasite relationships. Blackwell, London.

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

de Nooij, M. P., and J. M. M. van Damme. 1988. Variation in host susceptibility among and within populations of *Plantago lanceolata* L. infected by the fungus *Phomopsis subordinaria* (Desm.) Trav. Oecologia 75:535–538.

de Nooij, M. P., and H. A. van der Aa. 1987. *Phomopsis subordinaria* and associated stalk disease in natural populations of *Plantago lanceolata.* Can. J. Bot. 65:2318–2325.

Dinoor, A. 1970. Sources of oat crown rust resistance in hexaploid and tetraploid wild oats in Israel. Can. J. Bot. 48:153–161.

———. 1977. Oat crown resistance in Israel. Ann N Y Acad Sci 267:356–366.

Dybdahl, M. F., and C. M. Lively. 1998. Host-parasite coevolution: evidence for rare advantage and time-lagged selection in a natural population. Evolution 52:1057–1066.

Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. Science 265:1084–1086.

———. 1995. The ecological interactions between a microsporidian parasite and its host *Daphnia magna.* J. Anim. Ecol. 64:361–369.

Ebert, D., P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philos. Trans. R. Soc. Lond. B Biol. Sci. 351:1689–1701.

Ebert, D., R. J. H. Payne, and W. W. Weisser. 1997. The epidemiology of pasrasitic diseases in *Daphnia.* Pp. 91–111 *in* K. Dettber, G. Bauer, and W. Völkl, eds. Vertical food web interactions: evolutionary patterns and driving forces. Springer, Heidelberg.

Ebert, D., C. D. Zschokke-Rohringer, and H. J. Carius. 1998. Within- and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa.* Proc. R. Soc. Lond. B Biol. Sci. 265:2127–2134.

Ebert, D., M. Lipsitch, and K. L. Mangin. 2000. The effect of parasites on host population density and extinction: experimental epidemiology with *Daphnia* and six microparasites. Am. Nat. 156:459–477.

Espiau, C., D. Riviere, J. J. Burdon, S. Gartner, B. Daclinat, S. Hasan, and P. Chaboudez. 1998. Host-pathogen diversity in a wild system: *Chondrilla juncea–Puccinia chondrillina.* Oecologia 113:133–139.

Frank, S. A. 1996a. Problems inferring the specificity of plant-pathogen genetics. Evol. Ecol. 10:323–325.

———. 1996b. Statistical properties of polymorphism in host-parasite genetics. Evol. Ecol. 10:307–317.

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

Gandon, S., D. Ebert, I. Olivieri, and Y. Michalakis. 1998. Differential adaptation in spatially heterogeneous environments and host-parasite coevolution. Pp. 325–342 *in* S. Mopper and S. Y. Strauss, eds. Genetic structure and local adaptation in natural insect populations: effects of ecology, life history, and behavior. Chapman and Hall, New York.

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

Grosholz, E. D. 1994. The effects of host genotype and spatial distribution on trematode parasitism in a bivalve population. Evolution 48:1514–1524.

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. Natl. 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.

Henter, H. J. 1995. The potential for coevolution in a host-parasitoid system. II. Genetic variation within a wasp population in the ability to parasitize an aphid host. Evolution 49:439–445.

Henter, H. J., and S. Via. 1995. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. Evolution 49: 427–438.

Howard, R. S., and C. M. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature 367:554–557.

Hufbauer, R. A., and S. Via. 1999. Evolution of an aphid-parasitoid interaction: variation in resistance to parasitism among aphid populations specialized on different plants. Evolution 53: 1435–1445.

Jaenike, J. 1978. A hypothesis to account for the maintenance of sex within populations. Evol. Theory. 3:191–194.

Jarosz, A. M., and J. J. Burdon. 1991. Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*. II. Local and regional variation in patterns of resistance and racial structure. Evolution 45:1618–1627.

Kaltz, O., S. Gandon, Y. Michalakis, and J. A. Shykoff. 1999. Local maladaptation in the anther-smut fungus *Microbotryum violaceum* to its host plant *Silene latifolia*: evidence from a cross-inoculation experiment. Evolution 53:395–407.

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.
Kraaijeveld, A. R., and J. J. M. Van Alphen. 1994. Geographical variation on resistance of the parasitoid *Ascobara tabia* against encapsulation by *Drosophila melanogaster* larvae: the mechanism explored. Physiol. Entomol. 19:9–14.
———. 1995. Geographical variation in encapsulation ability of *Drosophila melanogaster* larvae and evidence for parasitoid-specific components. Evol. Ecol. 9:10–17.
Little, T. J. 1999. Parasite mediated selection in *Daphnia.* Ph.D. diss., Zoologisches Institut, University of Basel, Switzerland.
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.
Lively, C. M. 1989. Adaptation by a parasitic trematode to local populations of its snail host. Evolution 43:1663–1671.
———. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. Am. Nat. 153:S34–S47.
Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature 405:679–681.
Micheloud, F. X. 1997. Correspondence analysis. Available via http://www.micheloud.com/FXM/COR/e/inter2.html.
Møller, A. P. 1990. Effects of haematophagous mite on the barn swallow (*Hirundo rustica*): a test of the Hamilton and Zuk hypothesis. Evolution 44:771–784.
Oates, J. D., J. J. Burdon, and J. B. Brouwer. 1983. Interactions between *Avena* and *Puccinia* species. II. The pathogens *Puccinia coronata* Cda and *Puccinia graminis* f. sp. avenae Eriks. and Henn. J. Appl. Ecol. 20:585–596.
Otto, P. O., and Y. Michalakis. 1998. The evolution of recombination in changing environments. Trends Ecol. Evol. 13: 145–151.
Parker, M. A. 1994. Pathogens and sex in plants. Evol. Ecol. 8: 560–584.
———. 1996. The nature of plant-parasite specificity. Evol. Ecol. 10:319–322.
SAS Institute. 1992. SAS/STAT. Ver. 6.08. SAS Institute, Inc., Cary, NC.
———. 1993. SAS/STAT software: the GENMOD Procedure. Rel. 6.09. SAS Institute, Inc., Cary, NC.
Schierup, M. H., D. Charlesworth, and X. Vekemans. 2000a. The effect of subdivision on variation at multi-allelic loci under balancing selection. Genet. Res. 76:51–62.
———. 2000b. The effect of hitch-hiking on genes linked to a balanced polymorphism in a subdivided population. Genet. Res. 76:63–73.
Schmid, B., and C. Dolt. 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. Evolution 48:83:1525–1549.
Smith, J. A., K. Wilson, J. G. Pilkington, and J. M. Pemberton. 1999. Heritable variation in resistance to gastro-intestinal nematodes in an unmanaged mammal population. Proc. R. Soc. Lond. B Biol. Sci. 266:1283–1290.
Sorci, G., A. P. Møller, and T. Boulinier. 1997. Genetics of host-parasite interactions. Trends Ecol. Evol. 12:196–200.
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. 1994. The coevolutionary process. Univ. of Chicago Press, Chicago.
———. 1999. Specific hypotheses on the geographic mosaic of coevolution. Am. Nat. 153:S1–S14.
Thrall, P. H., and J. J. Burdon. 1997. Host-pathogen dynamics in a metapopulation context: the ecological and evolutionary consequences of being spatial. J. Ecol. 85:743–753.
Webster, J. P., and M. E. J. Woolhouse. 1998. Selection and strain specificity between snail intermediate hosts and their parasite schistosomes. Evolution 52:1627–1634.

Corresponding Editor: P. Jarne