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# TEMPORAL PATTERNS OF GENETIC VARIATION FOR RESISTANCE AND INFECTIVITY IN A *DAPHNIA*-MICROPARASITE SYSTEM

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*Abstract.*—Theoretical studies have indicated that the population genetics of host-parasite interactions may be highly dynamic, with parasites perpetually adapting to common host genotypes and hosts evolving resistance to common parasite genotypes. The present study examined temporal variation in resistance of hosts and infectivity of parasites within three populations of *Daphnia magna* infected with the sterilizing bacterium *Pasteuria ramosa.* Parasite isolates and host clones were collected in each of two years (1997, 1998) from one population; in two other populations, hosts were collected from both years, but parasites from only the first year. We then performed infection experiments (separately for each population) that exposed hosts to parasites from the same year or made combinations involving hosts and parasites from different years. In two populations, patterns were consistent with the evolution of host resistance: either infectivity or the speed with which parasites sterilized hosts declined from 1997 to 1998. In another population, infectivity, virulence, and parasite spore production did not vary among host-year or parasite-year. For this population, we also detected strong within-population genetic variation for resistance. Thus, in this case, genetic variability for fitness-related traits apparently did not translate into evolutionary change. We discuss a number of reasons why genetic change may not occur as expected in parasite-host systems, including negative correlations between resistance and other traits, gene flow, or that the dynamic process itself may obscure the detection of gene frequency changes.

*Key words.*—Coevolution, genetic variation, parasite, resistance, selection.

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Parasitism (including parasites and pathogens) is a powerful determinant of host survival and fitness, as evidenced by its often dramatic influence on host population sizes (e.g., Fenner and Ratcliffe 1965; Van Alfen et al. 1975; Hudson et al. 1998). Although the impact of infectious disease on host abundance seems certain, the genetic interaction between hosts and their biological enemies is not so well understood. And yet, the evolutionary significance of parasitism is of great interest, as biologists have surmised that parasite-host interactions may play a key role in some vexing phenomena. In particular, dynamic genetic interactions, or arms races, are a common outcome of theoretical models, and this dynamic aspect of the parasite-host interaction could promote the maintenance of sexual reproduction and genetic polymorphism (Haldane 1949; Levin 1975; Jaenike 1978; Hamilton 1980).

The potential for coevolutionary change within populations has been established by studies showing genetic variation for host resistance and parasite infectivity (Hill et al. 1991; Thompson and Burdon 1992; Roy and Bierzychudek 1993; Grosholz 1994; Ebert et al. 1998; Webster and Woolhouse 1998; Little and Ebert 1999), and the past occurrence of coevolution has been inferred by the geographic distributions of virulence and resistance variants (Lively 1989; Ebert 1994; Berenbaum and Zangerl 1998; Gilbert et al. 1998). Other single-time-point studies have sought to infer that coevolutionary change involves frequency-dependent dynamics by determining whether the level of infection experienced by a particular host genotype is dependent on the frequency of that genotype within its population (Chaboudez and Burdon 1995; Dybdahl and Lively 1995; Little and Ebert 1999). This approach has provided mixed results, possibly because, as pointed out by Frank (1991) and Dybdahl and Lively (1995), many spatial patterns may be consistent with the expectations of frequency dependence, depending on the time lag between the evolution of resistance in hosts and counteradaptation in parasites. Thus, direct evidence for dynamics will probably have to come from time series studies.

Efforts to directly observe short-term (one or a few generations) parasite-mediated gene frequency changes in nature are rare and have sometimes yielded results that are contrary to predictions based on patterns of genetic variation for resistance (Fuxa et al. 1988; Burdon and Jarosz 1991; Burdon and Thompson 1995; Henter 1995; Henter and Via 1995; Little and Ebert 1999). A key factor mediating longer-term dynamics in parasite-host models is the difference in evolutionary rate, which depends on generation time, population size, and mutation rates. Parasites are thought to have higher evolutionary rates due to their shorter generation times and larger populations sizes, and therefore ought to adapt relatively rapidly to overcome host defenses. Parasite adaptation has been observed in studies of agricultural systems, which show that pests adapt to overcome resistance in crops (e.g., Maxwell and Jennings 1983), and in serial passage experiments showing that within-host parasite growth rate increases with time (reviewed in Ebert 1998). In one of the only studies of adaptation and dynamics within a natural population, Dybdahl and Lively (1998) showed that recently common snail clones have comparatively high levels of infection with a trematode, as would be expected if parasites rapidly adapt to whichever host genotype is temporarily common.

*Daphnia* (Crustacea: Cladocera) and their microparasites are ideal for coevolutionary time studies. First, *Daphnia* can reproduce clonally via apomictic parthenogenesis (sexual reproduction is possible, but can be controlled so that it does

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not occur in the laboratory). Second, transmission spores of many horizontally transmitted parasites can be stored in the freezer until needed for infection experiments. Thus, hosts and parasites can be collected and their genotypes frozen until used in an experiment. Here we report on studies of genetic change in resistance and infectivity in *D. magna* and its bacterial parasite *Pasteuria ramosa*. This system has been the target of both laboratory and field studies that have documented considerable potential for reciprocal natural selection (Ebert et al. 1998; Little and Ebert 2000a). To test for evolutionary change, we sampled hosts and parasites from each of two years to test if the average genetic-based resistance differed between years and if parasites fitness (as estimated by transmission spore production) depended on whether they infected contemporary hosts or hosts from another time period.

## Materials and Methods

### *Collections and Experiments*

Hosts and parasites were collected from three ponds (Gaarzerfeld, Kremsdorf, and Kniphagen, each pond named after the closest village or estate) in northern Germany in September 1997 and again in August 1998. Such a period represents 10 to 15 *Daphnia* generations. For Gaarzerfeld, hosts and parasites were present in both years, whereas for Kremsdorf and Kniphagen hosts and parasites were present in 1997, but only uninfected hosts were found in 1998 collections. From each pond and each sampling date a random sample of 31–35 female hosts were collected. Females found to be uninfected at the time of collection were placed singly in 100-ml jars containing synthetic pond medium (Klüttgen et al. 1994) and maintained as uniclonal lines until used in experiments. To ensure that experiments were performed on a random population sample, we sought to include both females that were healthy at the time of collection and females that were infected in field collections, in proportions that reflected natural parasite prevalences (7–11%, depending on the population). Because *P. ramosa* sterilizes its host, to establish clonal lines of *Daphnia* infected at the time of collection, we first treated infected individuals with tetracycline (0.25 mg/ml water), after which they resumed clonal reproduction and were maintained as were healthy females (Little and Ebert 2000a). Studies on the effect of antibiotics on *Daphnia* resistance two generations after tetracycline treatment revealed no lasting effects of the drug (Little and Ebert 2000a).

An additional 35 to 60 females found to be infected at each time of collection were placed in groups of 10 in 1l jars and fed well until they died. Solutions of parasite transmission spores were obtained by grinding these females in a small amount of water and then stored at −20°C until needed. Spore concentrations were determined by counting spores in a bacterial counting chamber and diluting accordingly so that for each spore dose used in the infection experiments 20 μl of spore solution was added.

To equilibrate maternal and environmental effects among hosts prior to experiments, all isolates were maintained singly in 100-ml jars and fed a constant amount of food ($3.5 \times 10^6$ cells/day of chemostat grown green algae, *Scenedesmus* sp.) for at least three generations. Water changes occurred five days after birth, and then each time a female had a clutch. Each new generation was seeded with a single newborn from the third clutch, and newborns from the third clutch of the third generation were used in infection experiments. Each population was studied separately, that is, hosts were exposed only to parasites from their own pond. Different populations were experimented upon at different times, but different time points of the same population were always studied simultaneously. For the Kremsdorf and Kniphagen populations, infection experiments exposed hosts from 1997 or 1998 to a parasite spore solution from 1997. For Gaarzerfeld, a reciprocal cross experiment was performed including hosts and parasites from both years.

Infection experiments followed a split-brood design. Individual offspring from each female isolate were placed singly into plastic test tubes containing 5 ml synthetic pond water (control) or 5 ml water and 20 μl of parasite spores (treatments). There were three different spore-dose treatments for each population. Infection with *P. ramosa* is spore-dose dependent (Ebert et al. 1998), and this can vary among populations. A range of spore-dose treatments were used to ensure moderate infection levels amenable to statistical analyses. Pilot studies on each population were used to determine an appropriate range of spore doses. For Gaarzerfeld, the pilot study showed that a spore dose of $2.5 \times 10^4$ spores/ml gave infection levels of about 50%, and it was thus estimated that doses of $2 \times 10^3$, $2 \times 10^4$, and $1 \times 10^5$ spores/ml ought to give a reasonable range of infection levels in the main experiment. A sufficient quantity of spores were available to make these spore solutions for the 1997 parasites of Gaarzerfeld, but an insufficient quantity were available from the 1998 collections. Thus, for infections with the 1998 parasites of Gaarzerfeld, lower spore doses of $1 \times 10^3$, $1 \times 10^4$, and $5 \times 10^4$ were used. For Kremsdorf, spore doses of $1 \times 10^3$, $1 \times 10^4$, and $5 \times 10^4$ were used, and for Kniphagen, spore doses of $1 \times 10^2$, $1 \times 10^3$, and $2 \times 10^4$ were used.

After a five-day infection period, *Daphnia* were transferred to 100 ml of water. For all populations and treatments, *Daphnia* were fed $3.5 \times 10^6$ algae cells/day. Each experiment was run until no further infections were recorded, a period of 22 days (the prepatent period for infection with *P. ramosa* ranges from five days to about 20 days) during which the *Daphnia* were transferred to fresh water each time they had a clutch. For those *Daphnia* that became infected, and thus produced no clutches, transfers occurred every three to four days.

### *Analysis*

For each population and each time point, the aim was to collect a sufficient number of host individuals to represent the population, that is, at least 30 individuals. Thus, host isolate is the appropriate unit of replication for these experiments. From Gaarzerfeld there were 31 host isolates from each year, and four within-isolate replicates per dose treatment. For both Kremsdorf and Kniphagen, the experiments included host 35 isolates from each year, but no within-isolate replication.

Infectivity, a binary trait, was analyzed with host year and spore-dose as main effects in a logistic regression (DIST = BIN, LINK = LOGIT, Type III analysis, SAS procedure

TABLE 1. Statistics for genetic differences between *Daphnia magna* collected in 1997 and those collected in 1998 when exposed to transmission spores from sympatric populations of the parasite *Pasteuria ramosa* from 1997 or 1998. Fecundity is the number of clutches prior to sterilization of infected hosts. Infectivity statistics are Type III likelihood-ratio chi-square values, and those for parasite spore production and host fecundity are *F*-values. Parasite spore production was compared for only one spore-dose.

| | | Infectivity | | Host fecundity | | Parasite spore production |
|---|---|---|---|---|---|---|
| Population | Parasite year | Host year (df = 1) | Spore dose (df = 2) | Host year (df = 1) | Spore dose (df = 2) | Host year (df = 1) |
| Gaarzerfeld | 1997 | 0.17 | 102.5*** | 4.48 | 0.80 | 0.68 |
| | 1998 | 0.02 | 122.3*** | 2.30 | 0.15 | 0.10 |
| Kremsdorf | 1997 | 0.01 | 101.5*** | 19.25* | 15.71 | 0.46 |
| Kniphagen | 1997 | 7.50** | 68.46*** | 4.21 | 5.24 | 0.37 |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

GENMOD; SAS Institute 1990). We inspected the deviance divided by the degrees of freedom to assess over/underdispersion. Analyses showed that this value was always within the range 0.77–0.97, indicating good fit of the model. The infective dose necessary to infect 50% of hosts (termed ID50) was calculated by logistic regression of experimental infectivity on spore dose (SAS Procedure PROBIT). Fecundity of infected females (the number of clutches prior to sterilization) was analyzed by first calculating the average number of clutches produced for each treatment and host year and then performing a two-way analysis of variance (SAS procedure GLM). To estimate parasite fitness, the number of transmission spores produced by infected females at day 30 postinfection was determined. For this, infected females (high spore-dose treatment only) were ground singly in 300 μl of water and the number of spores in the resulting suspension were counted in a hemocytometer. Spore counts were analyzed with ANOVA (main factor, host year, SAS procedure GLM). For Gaarzerfeld, where parasites from two years were used, analyses were carried out separately for each parasite year (1997 and 1998). For the Gaarzerfeld population, because there were within-isolate replicates of hosts, analyses of infectivity were extended to consider differences among individual host isolates within a given year. This is a test for within-population genetic variation for resistance.

For all analyses of this study, it is not known with certainty whether the same clone was represented more than once among the isolates, which could lead to a form of pseudoreplication (although this would reduce the likelihood of detecting, for example, variation among isolates). However, allozyme electrophoresis indicated the population samples were polymorphic and about as diverse as would be expected under sexual reproduction (i.e., the samples showed no deviations from genetic equilibria). The Gaarzerfeld population was particularly genetically diverse (three polymorphic loci, 2.67 alleles/locus) with almost all of the isolates being unique allozymic clones.

## RESULTS

For the Gaarzerfeld and Kremsdorf populations, no evidence that the average infectivity differed between 1997 and 1998 was found; parasites from a given year were equally infective regardless of which hosts they contacted (Table 1; Fig. 1). For the Kniphagen population, however, a significant effect of host year was found, with hosts from 1997 being significantly more susceptible than hosts from 1998 (Table 1; Fig. 1, top panel). For Gaarzerfeld, Kremsdorf, and Kniphagen, respectively, uninfected hosts in this study produced 3.5, 3.7, or 3.7 clutches on average, whereas those that became infected produced an average of 1.0, 1.4, or 1.2 clutches. For Gaarzerfeld and Kniphagen, fecundity of infected females was the same regardless of whether parasites infected hosts from 1997 or 1998 (Table 1; Fig. 1, middle panel). For Kremsdorf, infected hosts from 1998 produced more clutches ($P = 0.048$) than those from 1997 (Table 1; Fig. 1, middle panel). Parasite spore production averaged over infected hosts did not significantly differ in any of the populations (Table 1; Fig. 1, bottom panel). Spore-dose effects were invariably significant for infectivity, with higher spore doses leading to higher infectivity, but did not significantly affect the fecundity of infected females (Table 1).

Variation for infectivity among individual host isolates in Gaarzerfeld was pronounced within both years for infectivity (1997 hosts exposed to 1997 parasites $\chi^2 = 139.7$, $P < 0.001$; 1998 hosts exposed to 1998 parasites $\chi^2 = 146.9$, $P < 0.0001$). To visualize variation among hosts for susceptibility within years, the proportion (to a maximum of 12) of infected isolate replicates across all three dose levels is shown in Figures 2A (1997 hosts and parasites) and 2B (1998 hosts and parasites).

For Gaarzerfeld, the one population for which parasites were collected from both years, changes in the parasite population were not detected: Infectivity, as measured by the number of parasites transmission spores required to infect 50% of hosts (ID50), was similar for all parasite-host year combinations (Fig. 1). Comparison of parasites from different years also suggested that individual host isolates reacted similarly to parasites regardless of which year the parasite spores were isolated, that is, if a particular host isolate was highly susceptible to parasites from 1997, it also tended to be highly susceptible to spores from 1998. We ranked clones within a year and compared their susceptibility to parasites from the two years using a Spearman rank-order correlation. For both years the correlation was highly significant (1997: $r^2 = 0.77$, $P < 0.001$; 1998: $r^2 = 0.79$, $P < 0.001$). Figure 3 shows infectivities in a scattergram to illustrate this consistent reaction of individual host isolates.

## DISCUSSION

This study tested for genetic change in host resistance and parasite infectivity within three natural populations of *D. magna* infected with *P. ramosa.* In the Kniphagen population,

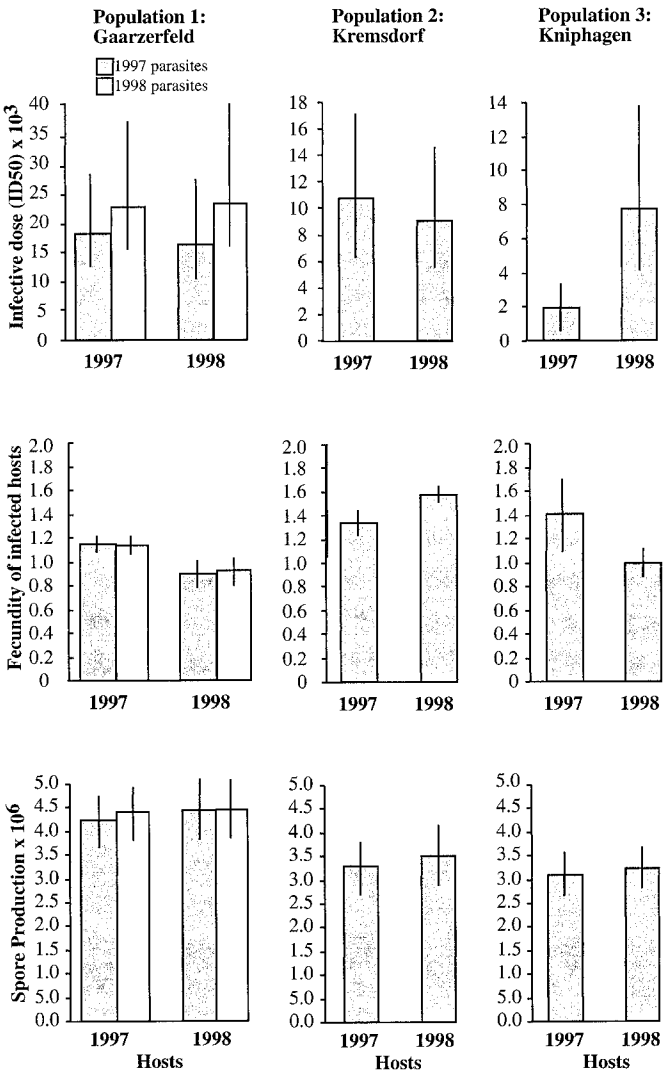


FIG. 1. Infective dose (ID50, the number of spores required to infect 50% of hosts), fecundity (number of clutches prior to sterilization) of infected hosts, and parasite spore counts resulting from infection experiments with the cladoceran *Daphnia magna* and its bacterial parasite *Pasteuria ramosa.* For the Gaarzerfeld population, experiments exposed hosts collected in 1997 or 1998 to parasites collected in 1997 or 1998. For Kremsdorf and Kniphagen, hosts from 1997 or 1998 were exposed only to parasites from 1997. Significant difference were detected between hosts from different years for infectivity in the Kniphagen population and fecundity of infected hosts in Kremsdorf (see Table 1). Bars around the ID50 are 95% confidence limits; bars for other measures are standard errors.

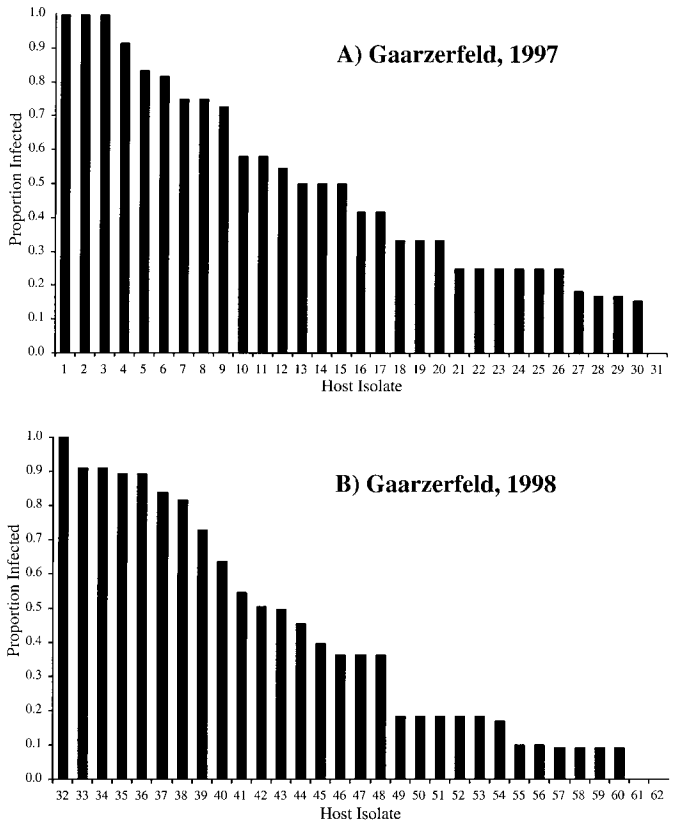


FIG. 2. Variation for susceptibility among 31 host isolates of *Daphnia magna* collected in 1997 (A) and 31 isolates collected in 1998 (B) and exposed to the parasite *P. ramosa* collected at the same time from the same pond (Gaarzerfeld). There were four replicates and three spore-dose treatments per host isolate, and therefore 12 observations per isolate. Susceptibility represents the number of infections observed across all spore-doses, as a proportion of the total number of possible observations (ranging from 0/12 to a maximum of 12/12).

parasites from 1997, showed greater infectivity on hosts collected in 1997 and significantly lower infectivity on hosts collected the following year. In the Kremsdorf population, when infected with 1997 parasites, host collected in 1997 had lower fecundity than hosts from 1998. Thus, in both these populations, evolutionary change was detected, and it appeared that hosts evolved greater resistance. In Gaarzerfeld, the most intensively studied population, infectivity, virulence, and parasite spore production (an estimate of parasite fitness) did not vary among years. The lack of change in Gaarzerfeld is evident at both the population (Fig. 1) and the individual (Fig. 3) level. This result for Gaarzerfeld is surprising, because significant genetic variation for infectivity was present in both years (Figs. 2A, B). Previous studies on the Gaarzerfeld population also revealed marked differences in resistance among clones and a range of parasite strains that differed in their infectivity (Carius et al. 2001). It is also clear that *P. ramosa* has substantial fitness effects, with infected hosts having about one-third the fecundity of healthy hosts in this study. Thus, the requirements for coevolutionary change, differential reproductive success and heritable variation for fitness-related traits, were met in Gaarzerfeld. Apparently, however, this did not lead to a directional evolutionary change in resistance.

A lack of selective change in the face of substantial heritability has been encountered in other studies of natural, unmanipulated populations. A common explanation for this has been that selection is too weak or heritabilities too low to be detected against a background of environmental noise (Endler 1986; Williams 1992). A relevant example involving parasitism comes from a study of mouse resistance to a nematode, where it was observed that heritabilities measured under laboratory conditions may vanish behind environmental

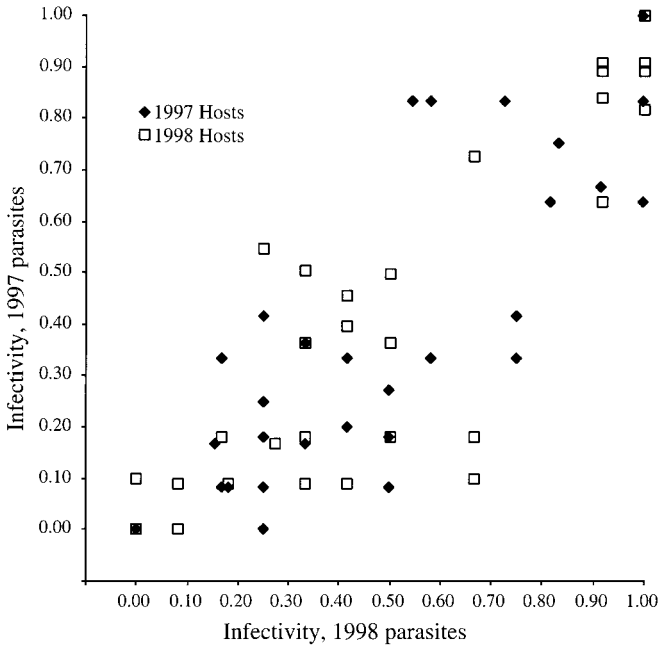


FIG. 3. Scattergram depicting susceptibility of individual Gaarzerfeld host clones collected in either 1997 (squares) or 1998 (diamonds) and exposed to parasites from either 1997 (y-axis) or 1998 (x-axis). Each symbol represents a single host clone and is the number of observed infections (as a proportion of the total number of possible observations; see Fig. 2) when exposed to parasites from either 1997 or 1998.

noise in a natural arena (Scott 1991). This indicates the need for caution when drawing conclusions about selection when heriatabilities are measured during controlled exposure. However, in another experiment (Little and Ebert 2000a), using the same 1997 samples of the present study, we showed that genetic variation for resistance explained natural patterns of infection in Gaarzerfeld. From this result, we estimated that selection on resistance in Gaarzerfeld could result in a 12% increase in mean resistance in one host generation. In the same study, however, estimates of the strength of selection in Kniphagen (for which change was detected in the present study) were essentially zero. Thus, based on patterns of genetic diversity and estimates of selection differentials, the present study found change where it was least expected and stasis was found where change was most expected. We now consider factors that may have obscured expected patterns.

Genetic change may be constrained by trade-offs. Indeed, it has been widely discussed and investigated whether resistance to parasites might be costly and traded off against other fitness components (Levin and Lenski 1983; May and Anderson 1983; Simms and Rausher 1987; Kraaijeveld and Godfray 1997). Depending on the strength of selection and the size of the trade-off, a highly susceptible genotype may change little in frequency despite having a higher parasite load because it has high fitness when it has by chance avoided infection (or produces clutch[s] prior to parasite-induced sterilization). To interpret responses to selection in our system, it may be necessary to obtain more accurate estimates of host genotype-specific fitness costs of infection with *P. ramosa.* This would require considering the probability of infection and the speed of sterlization once infected, weighted by the average lifetime reproductive success of uninfected females.

Other forms of constraint are also possible. The selective effects of parasitism might be obscured given some linkage disequilibrium between resistance genes and genes that respond to other agents of selection (see Parker 1991). For example, seasonal changes in temperature have been shown to be a determinant of the genetic structure of *D. magna* populations (Carvalho 1987; Carvalho and Crisp 1987). Given the appropriate linkage, if seasonal selective forces are strong relative to parasite-mediated selection, sampling populations at about the same time each year, as was done in this study, might reveal little apparent change because similar (temperature-adapted) genotypes are present at both times. *Daphnia* are cyclical parthenogens and their populations are often in linkage disequilibrium (e.g., Hebert 1974; Little and Ebert 1999; Little and Ebert 2000b), although allozyme analyses of the populations of this study showed little evidence for linkage. Linkage generated through apomixis may accelerate natural selection because favored gene complexes remain intact. Mean fitness of the population may then decline when recombination destroys selected linkage groups (genetic slippage; see Lynch and Deng 1994). The timing of sexual recruitment in our study populations is not known precisely, but the occurrence of genetic slippage does not change our central expectation that *P. ramosa* could drive genetic change. The expectation of genetic change is reasonable whether our populations are obligately parthenogenetic or entirely amphimictic.

The prevalence of disease in *Daphnia* populations is known to be highly variable (Stirnadel and Ebert 1997), and this study confirms this as *P. ramosa* had disappeared from two of the three populations by the second sampling date. The cause of these fluctuations are not clear, and there is evidence that both genetic and environmental factors influence the occurrence of *P. ramosa* (Little and Ebert 2000a). In regards to genetic aspects, although theoretical work has clearly indicated that interactions between hosts and parasites promotes polymorphism, this polymorphism may be steady, cyclic, or chaotic. If polymorphisms are cyclic, where genotypes oscillate between high and low frequency due to an advantage while rare (see Dybdahl and Lively 1995, 1998), this could obscure the detection of genetic change. For example, the time separating two sampling points may be roughly one cycle, and thus many of the same host and parasite genotypes are present at both times. Different populations are not likely to cycle synchronously (see Burdon and Thrall 1999; Lively 1999), so the detection of genetic change in one population, but not others, might be expected.

A few final points about the detection of genetic change in parasite-host systems merit discussion. First, genetically determined host behaviors could play a role in determining infection in the field, but perhaps not in the laboratory if behavior there is somehow constrained. In this regard, any genotype $\times$ environment interaction could result in laboratory infection patterns that do not reflect natural patterns. Second, *Daphnia* and *P. ramosa* both produce resting stages, which may be dispersed passively or lay dormant for a period. Gene flow from either neighboring populations or from the

seed bank in the pond bottom may contribute significantly to the genetic composition of the population. If genes enter a population faster than selection can mold their frequencies, significant adaptive change is not possible. The importance of migration and spatial structuring for parasite-host coevolution has recently been reviewed by Burdon and Thrall (1999). Finally, spore solutions were derived from more than 30 infected hosts and thus contained a mixture of parasite strains. It was assumed that a mixture of parasite strains has properties that reflect the average parasite present. However, it might be uncommon for a host to encounter such a range of parasite diversity in high concentrations. Interactions between parasite strains, as occurs in malarial infections (Taylor et al. 1997), may have given our spore solutions infective properties that do not reflect the natural situation. It is also conceivable that infectivity is largely determined by the most virulent strain present, with other strains having little effect regardless of their frequency. Future studies might gain better resolution by exposing populations to a series of separate parasite strains, as has been done in studies of plant-pathogen systems (e.g., Burdon and Jarosz 1991; Burdon and Thompson 1995).

### *Conclusions*

For two populations (Kniphagen and Kremsdorf), we found evidence of genetic change for traits relevant to parasitic infection, but owing to the Gaarzerfeld population, this study also offers an example where genetic variation for resistance apparently did not translate into evolutionary change. Furthermore, based on the predicted response to selection (Little and Ebert 2000a), change in the Kniphagen population was relatively unexpected, whereas change seemed likely for Gaarzerfeld. Our results indicate that it can be difficult to use observations of genetic variation for resistance to predict the outcome of interactions. Other empirical studies of parasitism (Parker 1991; Scott 1991; Chan et al. 1994; Henter and Via 1995; Little and Ebert 1999) have drawn similar conclusions, suggesting that the interaction between genetic, environmental, and ecological factors may greatly complicate the relationship between resistance and the response to selection. In the longer term, such factors may have a decisive impact on whether coevolution will produce the oscillations that favor the maintenance of sexuality.

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