The Evolution of Virulence When Parasites Cause Host Castration and Gigantism

Dieter Ebert,[1,2,*] Hans Joachim Carius,[1] Tom Little,[1,3] and Ellen Decaestecker[1,4]

1. Universität Basel, Zoologisches Institut, Vesalgasse 1, 4051 Basel, Switzerland;
2. Université de Fribourg, Département de Biologie, Ecologie et Evolution, Chemin du Musée 10, 1700 Fribourg, Switzerland;
3. Institute for Cell, Animal and Population Biology, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JT, Scotland;
4. Laboratory of Aquatic Ecology, Catholic University of Leuven, Chemin de Bériotstraat 32, 3000 Leuven, Belgium

ABSTRACT: It has been suggested that the harm parasites cause to their hosts is an unavoidable consequence of parasite reproduction with costs not only for the host but also for the parasite. Castrating parasites are thought to minimize their costs by reducing host fecundity, which may minimize the chances of killing both host and parasite prematurely. We conducted a series of experiments to understand the evolution of virulence of a castrating bacterium in the planktonic crustacean *Daphnia magna*. By manipulating food levels during the infection of *D. magna* with the bacterium *Pasteuria ramosa*, we showed that both antagonists are resource-limited and that a negative correlation between host and parasite reproduction exists, indicating resource competition among the antagonists. *Pasteuria ramosa* also induces enhanced growth of its hosts (gigantism), which we found to be negatively correlated with host fecundity but positively correlated with parasite reproduction. Because infected hosts never recovered from infections, we concluded that gigantism is beneficial only for the parasite. Hosts, however, have evolved counteradaptations. We showed that infected hosts have enhanced reproduction before castration. This shift to earlier reproduction increases overall host fecundity and compromises parasite reproduction. Finally, we showed that this resource conflict is subject to genetic variation among host and parasite genotypes within a population and is therefore likely to be an important force in the coevolution of virulence in this system. A verbal model is presented and suggests that the adaptive value of gigantism is to store host resources, which are liberated after parasitic castration for later use by the growing parasite. This hypothesis assumes that infections are long lasting, that is, that they have a high life expectancy.

* Corresponding author; e-mail: dieter.ebert@unibas.ch.

Am. Nat. 2004. Vol. 164, pp. S19–S32. 0003-0147/2004/1640S5-40121$15.00.

Keywords: evolution of virulence, parasitic castration, infectious disease, *Daphnia*, bacteria, *Pasteuria*.

Models on the evolution of the virulence of infectious diseases are largely built on two assumptions: first, that virulence is an unavoidable by-product of parasite reproduction (Bull 1994) and second, that it is in the pathogen's interest to avoid unnecessary host mortality because host death may curtail the parasite's lifetime transmission success. Consequently, pathogens are expected to evolve a balance between their need to reproduce and the costs of harming the host. Parasitic castration, here defined as severe parasite-induced reduction in host fecundity, has been suggested as an alternative strategy for the evolution of virulence. Certain horizontally transmitted parasites (including pathogens) specifically reduce or eliminate host reproductive function. Reproduction draws energy away from survival, so by lessening host reproduction parasites can keep their host alive longer, thereby reducing costs associated with early host death (Baudoin 1975; Obrebski 1975). Further, when parasites consume host reproductive tissue, which is rich in nutrients and energy content, they may directly increase their own fecundity or survival (Jokela et al. 1993).

Models of this process suggest that the optimal degree of virulence is total host castration (Obrebski 1975; Jaenike 1996; O'Keefe and Antonovics 2002). The apparent advantages of castration for the parasite are so strong that imperfect castration has been cited as an example of suboptimal parasite virulence (Jaenike 1996), and it has been asked why all parasites do not castrate their hosts (Ebert and Herre 1996). Models on the evolution of parasitic castration are based on two key assumptions: first, parasite growth and reproduction are limited by host resource, and second, limited resources cause a negative correlation between host fecundity and the production of parasite transmission stages. Testing these assumptions requires a system in which variation in host and pathogen reproductive success can be assessed independently. The first aim of this study was to test these two assumptions, which are key

for the understanding of the evolution of virulence of castrating parasites (Hurd 2001).

Parasite-induced castration is often associated with enhanced body growth of the host (gigantism), a trait that is among the most puzzling parasite-related changes in host life history (Mouritsen and Jensen 1994; Gorbushin 1997; Sorensen and Minchella 1998; Gorbushin and Levakin 1999; Moore 2002). Parasite-induced gigantism has been observed in diverse taxa, including mollusc, crustacean, vertebrate, and plant hosts and bacterial, fungal, and helminth parasites (Ebert et al. 1996; Arnott et al. 2000; Krist 2000; Pan and Clay 2002). Because body size is often correlated with fitness, it has been suggested that gigantism may be a host adaptation (Minchella 1985; Ballabeni 1995). Long-lived hosts that are prevented from reproducing by the parasite transfer the energy normally allocated for reproduction to enhanced growth. Larger hosts may have better survival, be more competitive, and have a higher fecundity if they outlive the infection. This hypothesis requires that infected hosts have a reasonable chance to recover from the infection and resume reproduction. An older and as yet untested hypothesis is that host gigantism is beneficial only for the parasite (Baudoin 1975; Dawkins 1982; Sousa 1983). Under this scenario, the parasite suppresses host reproduction to make resources available for itself that the host would have used for reproduction. Gigantism is then a by-product of more energy being released by castration than the parasite can use at that time. Later in the infection, the parasite may be able to use the resources stored in the host's body tissue. These two hypotheses about gigantism make contrasting predictions: The host-benefit hypothesis predicts that host size will correlate with the lifetime reproductive success of the infected host. In contrast, the parasite-benefit hypothesis predicts a positive correlation between host size and parasite lifetime reproductive success. Another alternative hypothesis is that gigantism is a nonadaptive side effect of parasitic castration (Wright 1971; Minchella et al. 1985; Keas and Esch 1997; Probst and Kube 1999) and that it benefits neither the host nor the parasite. Finally, a parasite-induced shift in a host trait may benefit both antagonists (Karban and English-Loeb 1997), although this idea has not been explored for parasite-induced gigantism. The second aim of this study was therefore to test for correlations between host body size and the reproductive successes of hosts and parasites in order to distinguish among the different hypotheses for gigantism.

We conducted a series of experiments using the planktonic crustacean *Daphnia magna* and the castrating and gigantism-inducing bacterium *Pasteuria ramosa* to test hypotheses on the adaptive significance of castration and gigantism. *Daphnia magna* and *P. ramosa* have been the subject of both laboratory and field studies documenting the potential for reciprocal natural selection (Ebert et al. 1998; Little and Ebert 2000; Carius et al. 2001). Following infection, typically all hosts are castrated by their parasite. This system has a number of features that makes it well suited for studying coevolution and testing assumptions and predictions of mathematical models. First, *Daphnia* can reproduce via apomictic parthenogenesis (sexual reproduction is possible but can be controlled in the laboratory), which permits the separation of genetic from nongenetic effects. Second, host and parasite lifetime reproductive success can be measured independently and can be related to each other and to other traits such as host size or age. Third, fecundity reduction and gigantism due to *P. ramosa* vary across host and parasite genotypes, enabling the covariance of these traits to be measured with other traits of interest, which is important for understanding their evolution. Fourth, host castration by *P. ramosa* is reversible. After treatment with antibiotics, castrated *Daphnia* resume reproduction (Little and Ebert 2000), suggesting that the parasite does not destroy the reproductive machinery of its hosts but may instead use chemical means (e.g., hormonal control) to castrate their hosts. This is important when considering potential benefits to the host because hosts could gain an advantage by resuming reproduction after they outlive the infection.

Material and Methods

The Host

Daphnia magna Straus is a planktonic freshwater crustacean usually found in eutrophic shallow ponds. It is attacked by a variety of bacterial, microsporidial, and fungal parasites (Green 1974; Stirnadel and Ebert 1997; Little and Ebert 1999; Ebert et al. 2001). Prevalence of *Daphnia* parasites can be high (up to 98%), and both field studies and laboratory experiments have demonstrated that these parasites typically have a large impact on *Daphnia* fitness (Green 1974; Stirnadel and Ebert 1997; Little and Ebert 1999; Ebert et al. 2001). *Daphnia* are iteroparous and have indeterminate growth, which is stepwise because a change in body length occurs only when the old carapax is shed at molting. Juveniles go through a series of moltings (instars) before reaching maturity. At 20°C and given 5×10^6 cells green algae (*Scenedesmus* sp.) food per day, the first young are released about 10 days after birth. Juveniles are released with every adult instar, which is about every 3–4 days. The first clutch contains about 10 juveniles, whereas up to 30 juveniles are produced in later clutches. Uninfected hosts live for >60 days under laboratory conditions.

In all experiments described here, *D. magna* were kept

under standardized laboratory conditions with artificial culture medium (Ebert et al. 1998), a temperature of 20°C, and a 16L : 8D cycle. If not mentioned otherwise, individual *Daphnia* were kept in 100 mL medium and were fed daily with 5×10^6 cells of the green algae *Scendesmus* sp. grown in continuous chemostat cultures.

The Parasite

Pasteuria ramosa Metchnikoff 1888 is a bacterial obligate endoparasite of *Daphnia* (Ebert et al. 1996). It has been found in prevalences up to 50% in natural populations (Stirnadel and Ebert 1997; Little and Ebert 1999). Infection takes place when a host comes in contact with waterborne spores or with spores in pond sediments; the likelihood of infection depends on the dose of spores (Regoes et al. 2003). The bacterium grows in the body cavity of its host; in the final state of infection a single host contains several million endospores that fill the entire body cavity. At this point, the infection can be easily recognized by the naked eye. The fitness costs for the host are high because all infections lead to castration. Parasite transmission requires host death because spores are only released from the decaying cadaver. Thus, it is possible to estimate the pathogen's lifetime reproductive success by counting the transmission stages in the dead hosts. The large endospores (diameter about 5 μm) can be easily counted with a hemocytometer using phase contrast microscopy. *Pasteuria ramosa* spores can be stored at −20°C for several years without significant loss in viability.

To infect *Daphnia* we added a suspension of *P. ramosa* spores to the water. The parasite spore suspensions used here were produced by grinding up heavily infected hosts around the time we expected them to die from the infection. Spores were counted and then diluted with a suspension of ground, uninfected hosts such that each suspension contained the same amount of macerated host tissue but different amounts of parasite spores. This was important because ground *Daphnia* tissue might have a nutritional value for the filter feeding hosts. Placebo suspensions contained no spores but contained the same amount of macerated host tissue from uninfected *Daphnia.*

The Two-Food-Level Experiment

This experiment tested for the effect of resource limitation on both antagonists and its consequence for host growth and castration. For this experiment we used one *D. magna* clone isolated from a pond near Gaarzerfeld in North Germany. Two hundred newborn *D. magna*, born within a period of 12 h in four 1.5-L cultures with 20 females each, were placed individually in 100-mL jars under standardized conditions with 2×10^6 cells algae/day. On the third day, they were challenged with 10^5 spores of *P. ramosa* (Gaarzerfeld strain) or with a placebo solution. After five more days each of the two treatment groups was split in half; one portion received a high food level (5×10^6 cells/day), and the other a low food level (10^6 cells/day). We checked all the females every 12 h until they reached maturity to determine age at first reproduction. From each of these four treatment groups, 15 females were checked daily for reproduction and survival until 5 days after the last infected hosts had died. From the other females, on days 24, 30, 36, and 42, we collected three females from each of the four treatment groups (total $n = 48$) and measured their body length. All host females used in this experiment were ground up to assess infection status and, if infected, the number of parasite spores produced. All controls and some of the spore-treated females were uninfected. Obtaining unbiased estimates of host length after day 42 was not possible because of increasing mortality among the infected hosts. We further counted *P. ramosa* spores in all females that died naturally from the infections.

The Four-Clone Experiment

This experiment assessed the covariance among host fecundity, host body size, and age at first reproduction and parasite spore production. In addition to using four different host clones, we used different spore dose treatments to test whether increased spore dose gave the parasite more control over their hosts. The four clones, from *D. magna* populations in southern Finland, North and South Germany, and southern England, were raised in the laboratory. To randomize maternal and grand-maternal environmental effects and to minimize environmental effects, we kept 30 uninfected lines from each clone for three generations under standardized environmental conditions. From each of the $(30 \times 4 =)$ 120 lines, four newborn *Daphnia* from one clutch (second, third, or fourth clutch) were placed individually in 20 mL medium (split-brood design). Each of these four newborn *Daphnia* received a different treatment: 10^5, 10^4, 10^3 or 0 *P. ramosa* spores (Gaarzerfeld strain) per milliliter of medium, administered at day 3 of their life. On day 6, these females were placed in 100 mL medium and checked daily for offspring production and survival. Medium was replaced every adult instar (about 3–4 days). All animals were fed 2×10^6 cells algae/day. On day 32 we measured host body length, tested each female for the presence of *Pasteuria* infection, and counted the number of parasite spores in the ground-up bodies. Relative host body length (length infected/length control) and shift in age at first reproduction (age control − age infected female) were calculated for pairs of females coming from the same clutch.

Genetic Variation among Hosts and Parasite Isolates

Here we report on a further analysis of an experiment published earlier (Carius et al. 2001). *Daphnia magna* individuals that were infected with *P. ramosa* were collected from a pond in Gaarzerfeld, North Germany, in August 1997. They were brought to the lab and placed singly in jars filled with 100 ml medium. Nine infected *D. magna* individuals produced viable offspring before parasitic castration was complete. The offspring, which are genetically identical to their mother but uninfected, were collected and maintained as single female lines in the lab. The nine infected mothers of these clones were the source of the parasite isolates. The females were kept until their death, and the parasite spores were propagated by infecting 30 further hosts from the same host clone. In the final experiment, two spore doses were used (0.2×10^6 and 1×10^6 spores per host), but here only the data from the high dose treatment are presented because the low dose treatment resulted in too few infections. The experiment was a complete cross-infection experiment. With nine *D. magna* clones and nine parasite isolates, there were 81 combinations (plus nine uninfected treatments) with nine replicates each. Replicate lines had been kept under standardized conditions for at least three generations before start of the experiment. For the experiment, juveniles were placed singly in 100-mL jars filled with 20 mL medium. On day 1, the spore solution was added. The daily food supply until day 5 was 2×10^6 algae cells. After 5 days, the individuals were transferred into jars filled with 100 mL 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 30, all infected *Daphnia* were frozen, and later on, 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 $\times 600$ magnification. In total, host fecundity and parasite spore production were assessed in the (9 clones × [9 parasite isolates + 1 control] × 9 replicates =) 810 females. At least one female was infected in 54 of the 81 parasite challenged combinations (in total 309 infected females). Only the infected females were used in the analysis presented here. Further details of this experiment have been published elsewhere (Carius et al. 2001), where the results of two populations and two dose levels are described. Here we analyzed only the high dose treatment from the German population because too few hosts were infected in all other combinations.

Statistical Analysis

For all statistical analysis, the data were tested for departure from normality and transformed if necessary, or nonparametric tests were used. In most cases, the statistical tests are mentioned in "Results" and are not discussed here.

The genetic correlations in the four-clone experiment were calculated with a nested ANCOVA correlation (Falconer and MacKay 1996). The same analysis was used for the test for genetic correlation between host fecundity and spore production from the experiment with nine host clones and nine parasite isolates. However, this correlation is equivalent but not the same as a genetic correlation (Falconer and MacKay 1996) because every host clone was tested in combination with several parasite isolates and vice versa. The correlation is here presented to illustrate that the conflict over resources is also visible using the means of unique genetic combinations of hosts and parasite genotypes.

Results

The Two-Food-Level Experiment

Parasite spore production increased with host age and for a given host age was higher in well-fed hosts than in poorly fed hosts (fig. 1*A*; ANCOVA: food [main effect] $F = 111.64$, df $= 1, 49$, $P < .0001$; age [covariable], $F = 100.42$, df $= 1, 49$, $P < .0001$, $r^2 = 0.82$). Healthy *Daphnia* released their first young at around 10–12 days old and then produced a clutch of eggs every 3–4 days. Infected *Daphnia magna* released their first young about 1.5 days earlier than the controls (fig. 1*B*; Wilcoxon two-sample test (normal approximation): high food, $Z = 2.491$, $P = .012$; low food, $Z = 4.203$, $P < .0001$). Note that to avoid a food effect during the infection procedure, the food treatment was only applied when the hosts were already 8 days old, that is, 5 days after they had been exposed to the parasite spores. Therefore, there was no food effect on age at first reproduction. Infected hosts produced many fewer offspring than controls in both food levels (fig. 1*C*; two-way ANOVA with log-transformed offspring numbers as dependent variable: food, $F = 378.7$, df $= 1, 56$, $P < .0001$; infection, $F = 699.6$, df $= 1, 56$, $P < .0001$; food × parasite interaction, $F = 283.3$, df $= 1, 56$, $P < .0001$; $r^2 = 0.96$). These data showed that high food conditions were beneficial for both hosts and parasites, indicating that both antagonists suffer from resource limitation.

Infected hosts grew to be larger than uninfected hosts (gigantism; fig. 1*D*; two-way ANCOVA: age (covariable), $F = 177.8$, df $= 1, 43$, $P < .0001$; infection, $F = 126.8$, df $= 1, 43$, $P < .0001$; food, $F = 825.3$, df $= 1, 43$, $P <$

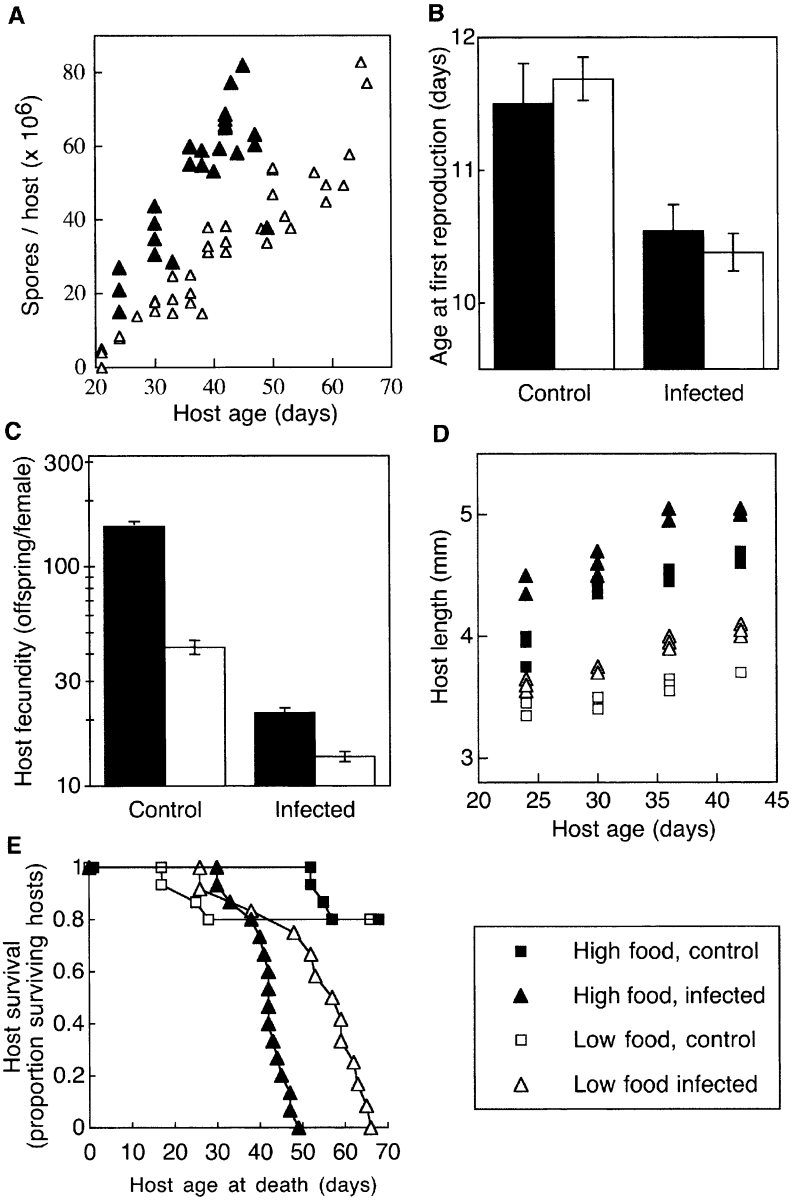

Figure 1: Host and parasite life-history traits in relation to food level (high food = *black symbols*, low food = *white symbols*) and parasitism from the two-food-level experiment. *A*, Parasite spore production plotted against host age (= time past infection + 3 days). *B*, Age at first reproduction in days in control and infected hosts in two food levels. Note that to avoid food effects during the infection procedure, the food treatment was applied only when the hosts were 8 days old. Therefore, there is no food effect on age at first reproduction. *C*, Host fecundity (offspring per female) in control and infected hosts in two food levels. *D*, Host body length (mm) in control and infected hosts in two food levels in relation to host age. *E*, Survival of hosts (proportion of host surviving) in control and infected hosts in two food levels.

.0001; interactions were not significant, $r^2 = 0.96$). The difference in body length between infected and uninfected hosts translated into a difference of about 20%–25% biomass (using a body-length dry-weight conversion for *D. magna* [Yampolsky and Ebert 1994]). Infected hosts died earlier than controls (fig. 1*E*). Under high food conditions, all controls were alive when the last infected host died (Fisher exact test: $P < .0001$), and under low food conditions, 80% of the controls survived the last infected hosts (Fisher exact test: $P < .0001$). None of the infected hosts were able to clear the infection after castration had started, and none were able to reproduce later in life.

The Four-Clone Experiment

The results from this experiment are presented in two parts. Because a substantial number of females became infected only in the highest spore dose treatment, we used only the data from the highest dose treatment in the first part of the analysis (fig. 2 and the corresponding statistical analysis in table 1). In the second part, we specifically address the effect of spore dose; therefore, all data are included.

Among the infected hosts, significant clone effects were present for all traits analyzed (one-way ANOVA with clone as main effect (only highest spore dose): number of parasite spores, $F = 10.36$, df $= 3,53$, $P < .0001$; total host fecundity, $F = 45.79$, df $= 3,54$, $P < .0001$; number of host clutches, $F = 55.56$, df $= 3,54$, $P < .0001$; age at maturity, $F = 24.73$, df $= 3,54$, $P < .0001$; host body length at age 32, $F = 9.75$, df $= 3,53$, $P < .0001$; relative host body length at age 32, $F = 2.83$, df $= 3,53$, $P = .04$). We found evidence for a conflict between host and parasite in the form of a negative correlation between total host fecundity and parasite spore production (fig. 2*A* shows data after correcting for the clone effect, i.e., residuals from one-way ANOVA with clone as main effect; table 1). Further, the quicker the parasite castrated its host (hosts produced fewer clutches), the more spores the parasite produced (fig. 2*B*; table 1), suggesting that host castration is advantageous for the parasite. The negative correlation between parasite spore production and host fecundity remained significant even after correcting for host body length (partial correlation: $r = -0.48$, $P < .001$, $n = 51$). Infected hosts that matured at an earlier age were able to produce more clutches than hosts maturing later (fig. 2*C*; table 1), indicating a benefit to infected hosts for shifting maturation to an earlier age.

During the experiment nearly all infected hosts were found to be larger than the uninfected controls. Larger absolute and relative host body length correlated positively with parasite spore production and negatively with host fecundity (fig. 2*D*–2*G*), suggesting that parasite-induced gigantism in the *D. magna–Pasteuria ramosa* system is beneficial for the parasite and disadvantageous for the host.

The three dose levels used in the four-clone experiment allowed us to test whether parasite manipulation of hosts was dose dependent, that is, whether a higher parasite biomass during early infection gave the parasite a head start in its aim to monopolize host resources, as suggested by Sorensen and Minchella (2001). We found that higher exposure dose resulted in stronger gigantism (fig. 3*A*; two-way ANOVA: relative host length, dose effect, $F = 8.92$, df $= 2,96$, $P = .0003$; relative host length, clone effect, $F = 2.01$ df $= 3,96$, $P = .12$), higher parasite spore counts (fig. 3*B*; two-way ANOVA as before: dose effect, $F = 4.17$, df $= 2,91$, $P = .018$; clone effect, $F = 16.62$, df $= 3,91$, $P < .0001$), and lower host fecundity (fig. 3*C*; two-way ANOVA as before: dose effect, $F = 3.43$, df $= 2,97$, $P = .036$; clone effect, $F = 56.13$, df $= 3,97$, $P < .0001$). Increasing the spore dose to even higher levels will, however, reduce spore counts due to the parasite's density-dependent within-host growth (Ebert et al. 2000*a*). As seen in the two-food-level experiment, infected hosts matured earlier than the uninfected controls in all three exposure regimes of the four-clone experiment, but this effect was not significant (paired *t*-tests in each of the three dose treatments were not significant after Bonferroni correction for three tests; fig. 3*D*). Furthermore, there was no dose and clone effect (difference in age at maturity: dose, $F = 0.09$, df $= 2,88$, $P = .90$; clone, $F = 2.03$, df $= 3,88$, $P = .11$). None of the infected hosts in this experiment were able to clear the infection after castration had started, and none were able to reproduce before the end of the experiment.

Genetic Variation among Hosts and Parasite Isolates

We sought to test for genetic covariation with respect to the conflict over resources between hosts and parasites within a single population. We analyzed data from all combinations of nine *D. magna* clones and nine *P. ramosa* isolates (nine replicates per combination) in which at least one female was infected (54 out of 81 combinations). Parasite spore production and host fecundity were negatively correlated across all data (fig. 4*A*; Pearson's correlation: total phenotypic correlation, $r = -0.455$, df $= 307$, $P < .0001$). A linear regression with all data in fig. 4*A* revealed a slope parameter of -4.66×10^6, meaning that one clutch of host eggs was equivalent to 4.66 million parasite spores. The same correlation but using the unweighted means of each of the 54 host clone-parasite isolate combination revealed a negative covariance as well (fig. 4*B*; Pearson's correlation: $r = -0.366$, df $= 52$, $P = .0065$). Note that the genetic correlation is equivalent but not the same as a genetic correlation as usually discussed in quantitative genetics texts (Falconer and MacKay 1996) because every host clone was tested in combination with several parasite isolates and vice versa. A nested ANCOVA revealed an environmental correlation of $r = -0.487$ (df $= 287$, $P < .001$). None of the infected hosts in this experiment were able to clear the infection after castration had started, and none were able to reproduce before the end of the experiment.

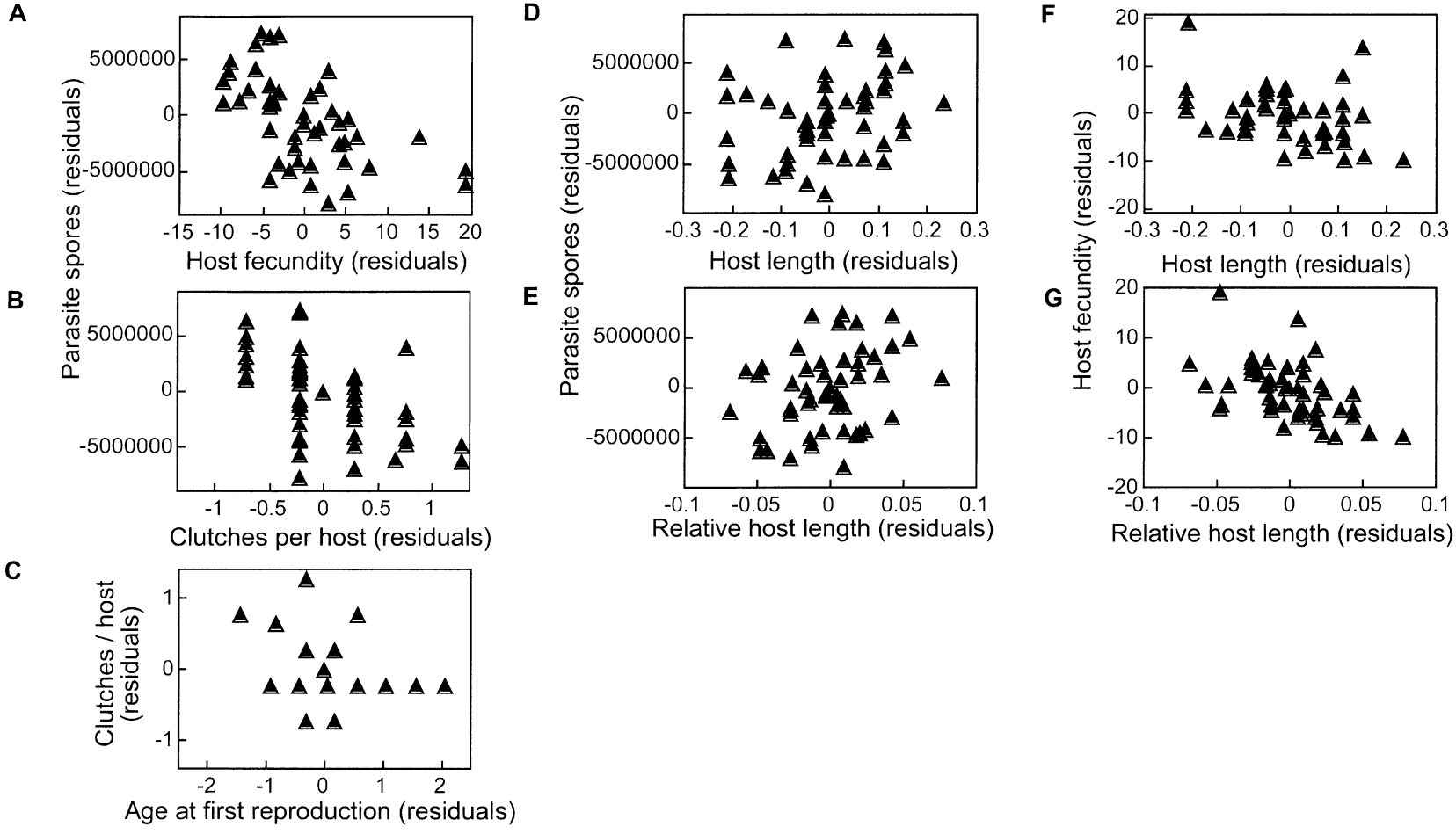

Figure 2: Scatterplot of host and parasite life-history traits in the four-clone experiment after correcting for clone effects (residuals shown). *A*, Number of parasite spores per host plotted against host lifetime fecundity. *B*, Parasite spore numbers per host plotted against number of host clutches. *C*, Number of host clutches plotted against age at first reproduction. Note that some infected females became castrated before maturation and therefore no data for age at first reproduction are available. *D*, Parasite spores plotted against host body length. *E*, Parasite spores plotted against relative host body length (=length infected female/length control female). *F*, Host fecundity (total number of offspring) plotted against host body length. *G*, Host fecundity (total number of offspring) plotted against relative host body length. Only data from the infected females of the highest dose treatment are shown.

Table 1: Phenotypic, genetic, and environmental correlations for host and parasite fitness components from the four-clone experiment

Correlation between traits	Phenotypic	Genetic	Environmental
Parasite spores/host fecundity	−.52***	−.37 NS	−.50***
Parasite spores/number of host clutches	−.52***	−.23 NS	−.49***
Number of host clutches/age at maturity	−.76***	−.96 NS	−.38**
Parasite spores/host body length	.27*	−.12 NS	.29*
Parasite spores/relative host body length	.11 NS	−.54 NS	.32*
Host fecundity/host body length	−.64***	−.87 NS	−.45***
Host fecundity/relative host body length	−.35**	−.48 NS	−.53***

Note: In this analysis, only the data from the highest spore dose are included. Host fecundity is the total number of offspring of an infected female, and parasite spores are the total number of spores per infected host. Degrees of freedom are 56, 3, and 47 for the phenotypic, genetic, and environmental correlations, except for the correlation between number of host clutches and age at maturity (df = 51, 3, and 48, respectively). NS = not significant. Note that a substantial number of females became infected only in the highest dose treatment of the four-clone experiment. Therefore, we used only the data from the infected females of the highest dose treatment in the statistical analysis in this table.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

Discussion

Castration and the Coevolution of Virulence

Adaptive explanations about the evolution of virulence suggest that virulence is an unavoidable side effect of the parasite's attempts to achieve transmission. It has been suggested that host castration is adaptive for pathogens because it allows the pathogen to exploit hosts by minimizing the harmful side effects of killing the host and thus the parasite prematurely. Our study showed that a pathogen directly benefits from castrating its host. This was indicated by two findings. First, because food limitation harmed parasites as well as hosts (fig. 1) by reducing both host and parasite reproductive success, it suggests that the bacterial parasite *Pasteuria ramosa* has high energy requirements. A similar result has been observed for a snail infected with a castrating trematode (Keas and Esch 1997). Second, as a possible consequence of resource competition, host and parasite fecundity were negatively correlated with each other (figs. 2, 4). This has been postulated to be a driving force for the evolution of parasitic castration, especially when castrator biomass represents a substantial portion of host biomass (Baudoin 1975; Sousa 1983), which is typical for castrators (Kuris 1974). The total biomass of *P. ramosa* spores in a host around time of death can make up more than 10% of the host biomass (D. Ebert, unpublished data). To produce such high parasite biomass without killing the host may only be possible because *P. ramosa* can use the resources the host would otherwise invest in reproduction. These resources can be the equivalent of five to 15 clutches of parthenogenetic eggs that a *Daphnia magna* female would produce were it not infected. Consistent with this supposition was the observation that spore yield increased with host age (fig. 1). Killing the host prematurely appears costly for the parasite unless this benefit is discounted by high host adult mortality. Thus, early castration followed by a comparatively long life span of the infected hosts is beneficial for the energy-demanding *P. ramosa*. Other parasites of *Daphnia* do not show such a temporal separation between castrating and killing their host, do not induce gigantism, and appear to have much lower energy demands than *P. ramosa* (Ebert et al. 2000*b*; Bittner et al. 2002).

Our analysis revealed that the negative correlation between parasite and host reproduction is not only visible when correcting for genetic effects (environmental correlation) but also when using the means of the unique genetic combinations of host clone and parasite isolates (fig. 4). This finding goes hand in hand with the earlier demonstration that there are strong host-parasite interactions for host and parasite fitness components in this system (Carius et al. 2001), indicating the possibility for antagonistic coevolution.

Theoretical models about the evolution of parasitic castration agree that from the parasite's perspective, the optimal level of castration is total castration (Obrebski 1975; Jaenike 1996; O'Keefe and Antonovics 2002). The key assumption of these models—the negative correlation between host and parasite reproductive success—was well supported by our study. Nevertheless, castration in the *Daphnia-Pasteuria* system is far from being total. Some hosts produced up to five clutches before being totally castrated (but no hosts escaped castration once they were infected). Our data suggest that one reason *P. ramosa* may not achieve perfect host castration is because infected hosts reproduce earlier than uninfected controls, perhaps to ac-

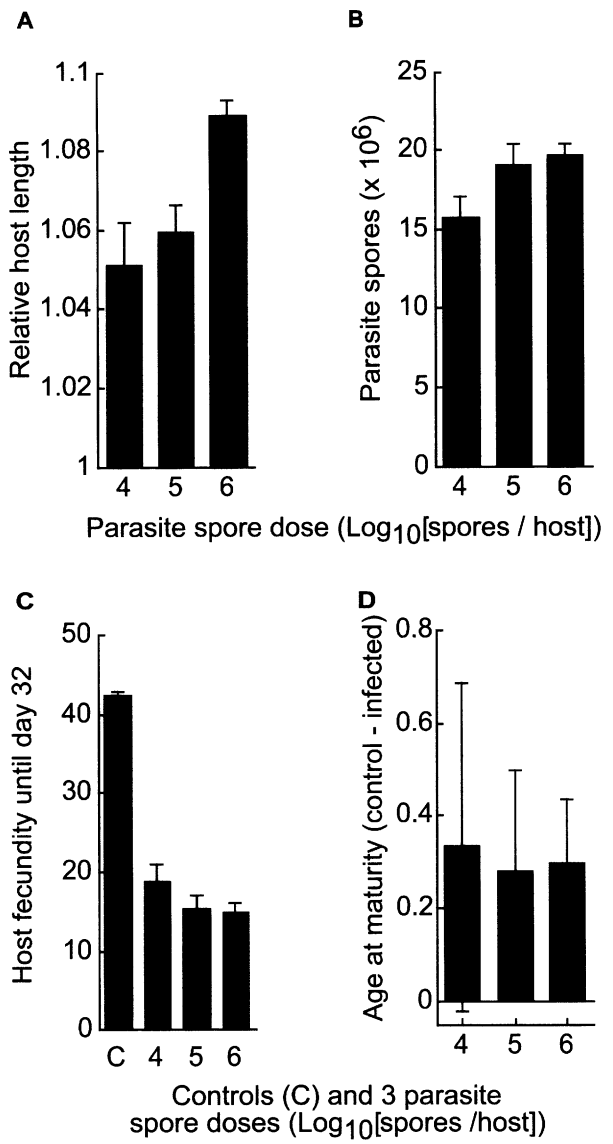

Figure 3: Host and parasite traits in the four-clone experiment in relation to the parasite spore dose to which hosts were exposed. Each bar shows the means across the four host clones used in this experiment. Treated but uninfected females were not included. *A*, Relative host length (=length infected female/length control female). *B*, Parasite spores per host female. *C*, Total host fecundity until the end of the experiment (32 days). At this time all infected hosts were totally castrated, while the controls continued to produce eggs. *D*, Shift in age at maturity (age of controls − age of infected females).

cess resources before the parasite gains control over resource allocation. This earlier host reproduction increases the numbers of clutches relative to those hosts that do not shift age at maturity, indicating that this shift is beneficial for the host and costly for the parasite. On average, one clutch more for the host reduced parasite spore counts by 4.66 million. Thus, it is adaptive for *P. ramosa* to castrate its host early but to kill it late. Future generations of mathematical models may include host evolution to understand and predict the consequences of the conflict over resources.

In summary, the evolution of parasitic castration in the *Daphnia-Pasteuria* system seems to be driven by the parasite's needs for resources. The parasite races against the host's attempts to reproduce before castration is complete, while the host tries to secure at least some resources before its reproductive death. Thus, the evolution of virulence seems to be the result of a tied coevolution between both antagonists, a process often suggested to maintain genetic variation for the traits involved (Clarke 1976; Hamilton 1980; Barrett 1988). Genetic variation for castration and gigantism may have contributed to the variable outcome of studies on host life histories in the presence of parasites (Ballabeni 1995; Sorensen and Minchella 1998; Loot et al. 2002).

The Evolution of Gigantism

In the *Daphnia-Pasteuria* system, gigantism seems not to benefit the host. In our experiments, none of the infected hosts were able to clear the infection or reproduce after castration was complete, despite their long life expectancy after castration (four to five times the age at first reproduction). The host's failure to reproduce is not due to the destruction of the ovaries (castration is reversible in this system through antibiotic treatment [Little and Ebert 2000]); rather, it indicates that the parasite may control its host through chemical manipulation. Thus, although it has been suggested that gigantism is adaptive for the hosts that are able to reproduce later in life (Minchella 1985), this conclusion does not seem to apply to our system. Larger infected hosts had lower fecundity than smaller infected hosts, as has also been observed in a trematode snail system (Gorbushin 1997).

Gigantism appears to benefit the parasite. Our results showed that the degree of gigantism and castration were both positively correlated with parasite lifetime reproductive success. Gigantism probably benefits the parasite because larger hosts are a larger resource and may be used by the parasite for increased spore production. Indeed, in the final stage of infection, *P. ramosa* has such high resource demands that it consumes all available host biomass, filling the entire body cavity of the host with spores. In addition, larger *Daphnia* are more efficient filter feeders (Lampert 1987) and are therefore able to acquire more resources per unit time, which may be ultimately converted into more parasite spores. However, as gigantism occurred even when the absolute amount of food was limited (fig. 1), increasing feeding efficiency alone cannot

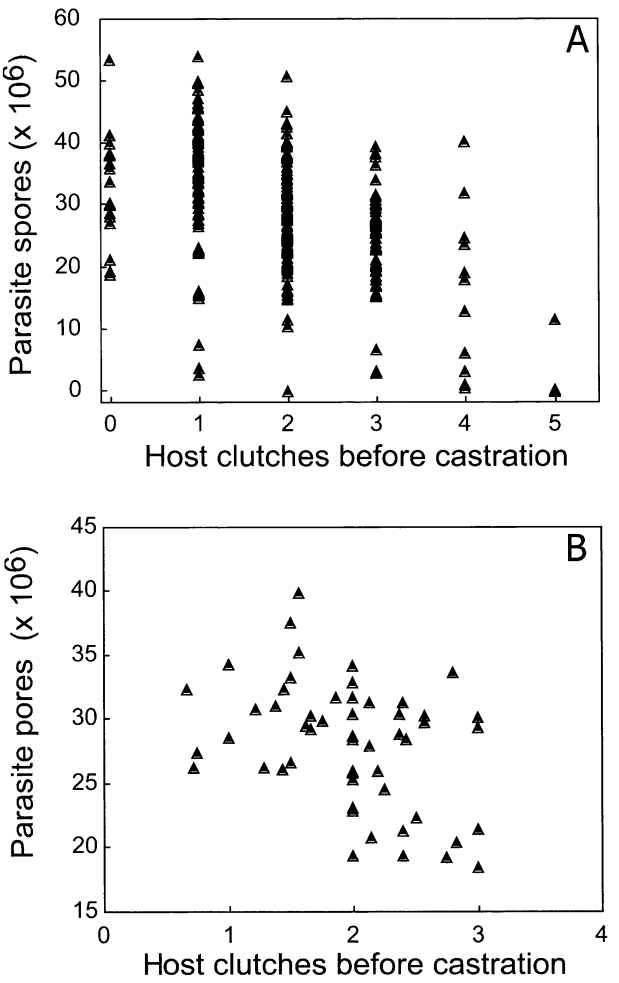

Figure 4: Relationship between parasite spore counts per host female ($\times 10^6$) and the number of clutches an infected host is able to produce before castration (i.e., before reproduction ceased). Parasite spores were counted in all females at age 30 days. All nine host clones and nine parasite isolates used were collected from the same population. *A*, All infected females (Pearson's correlation: $r = -0.455$, $P < .0001$, $n = 309$); *B*, means of each host clone-parasite isolate combination ($r = -0.366$, $P = .0065$, $n = 54$) in which at least one host was infected.

explain gigantism. The hypothesis that larger hosts may provide an advantage to the parasite in form of increased host survival (e.g., due to reduced predation and competition; Baudoin 1975; Dawkins 1982; Arnott et al. 2000) cannot explain the correlation between parasite spore counts and host size in our experiments because all animals were kept separately and without predators. In natural situations, gigantism may, however, provide this additional advantage for the parasite.

The mechanism and evolution of gigantism is poorly understood. We agree with earlier authors (Sousa 1983; Sorensen and Minchella 2001) that it may be a consequence of infection dynamics, that is, a consequence of the growing parasite's changes in energy requirements. We call this the temporal storage hypothesis. It is illustrated schematically in figure 5. To infect a *Daphnia*, only very few *P. ramosa* spores are necessary (as few as 10 spores can lead to infections [Regoes et al. 2003]), suggesting that bacterial biomass is very small during early infections. Thus, in absolute terms the parasite's initial growth requires only very few resources. Although *P. ramosa* may benefit by inducing castration early and preventing the host from using valuable resources for reproduction, its small biomass has no use for the large amounts of resources liberated through castration during the early phase of an infection. Resource allocation studies have shown that the amount of biomass allocated into *Daphnia* reproduction is substantial (>60% of daily biomass production; Lynch et al. 1986), which may be much more than the parasite might need at this stage. Thus, a temporal storage of these resources in form of host body mass could be adaptive. Consistent with the suggestion that liberated resources are allocated into host growth was our finding that the degree of gigantism and the degree of castration were positively correlated with each other. Whether the shift in resource allocation was a parasite strategy or a side product of host physiology of the castrated hosts is not clear. However, even if it were a side product of host physiology, it is beneficial for *P. ramosa*, and because it has no costs for the already castrated (reproductively dead) host, it is not counterselected by the host. The parasite grows, at least initially, with a higher growth rate than its host, thus increasing in resource needs relative to the needs of its host. In later stages of its growth, the parasite (in case of a microparasite, the parasite population) needs, in total, larger amounts of resources that the larger (gigantic) host body may have to offer over a normal-sized host. Thus, the temporal storage hypothesis is both a mechanistic and an evolutionary explanation for the evolution of parasitic castration and gigantism. It is possible to derive a number of testable predictions from this model.

First, temporal storage of resources yields benefits only for long-lasting infections and under conditions of low host (and thus parasite) mortality rates. If the parasite were likely to be cleared by the host immune defense or if the host were to die (parasite induced or due to other reasons), it would not be adaptive for resources to be stored. Thus, castration and gigantism should be found predominantly in systems with long-lasting (chronic) infections (fig. 5) and low host (and parasite) mortality.

Second, gigantism should be induced only when the total energy demands of the growing parasite during the time of castration plus the energy costs of the host's immune defense are substantially lower than the resources liberated by castration. The growing parasite's low resource

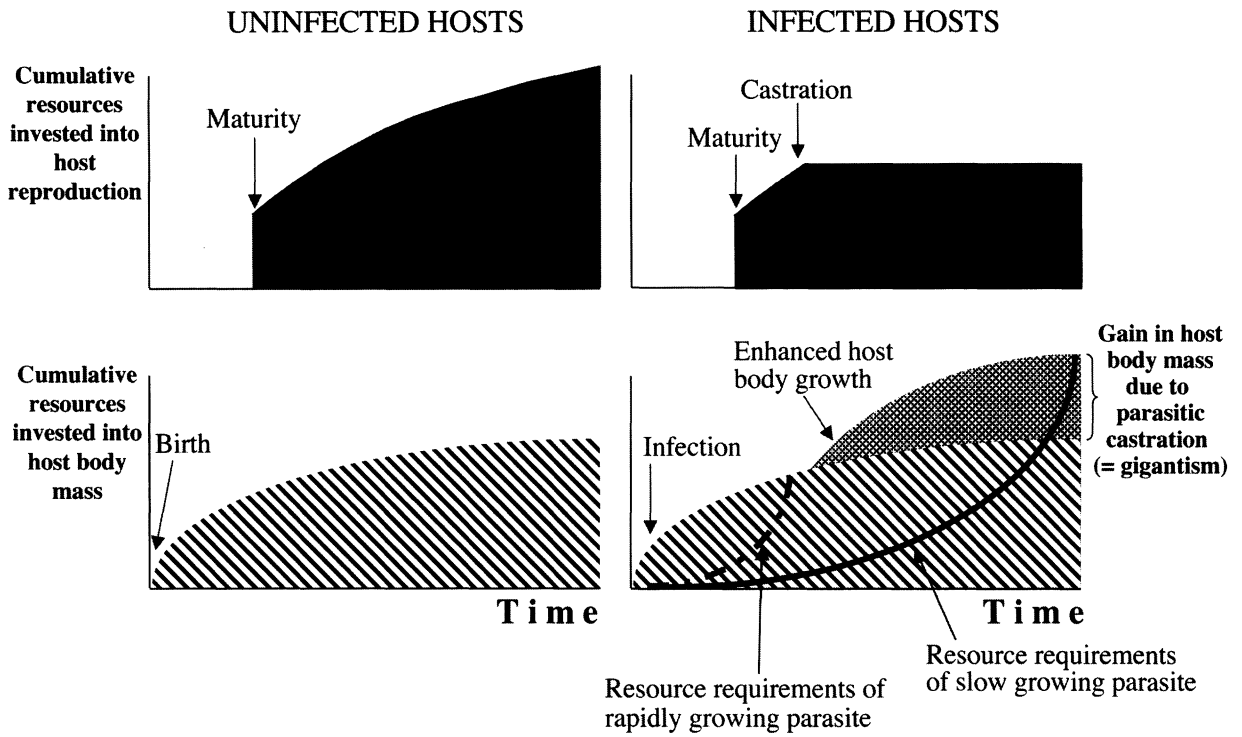

Figure 5: Schematic illustration of the temporal storage hypothesis for the evolution of parasitic castration and gigantism. The two graphs on the left show the cumulative biomass of a healthy host for reproduction (*top*) and growth (=increase in host body mass; *bottom*). The host has indeterminate growth. On the right, the host is infected with a castrating parasite during its juvenile phase. Some time after infection, the parasite castrates the host and thus prevents it from investing more resources into reproduction (*upper right graph*). As the parasite needs little resources during its early growth phase (*solid line in bottom right graph*), the liberated resources are invested into host body growth (see increase in the cumulative resources for host body mass). Later, the now larger parasite can make use of the additional resources present in the larger host body. Early on, a fast-growing parasite (*stippled line in bottom right graph*) would need much of the host's resources to grow and would deplete the host early.

requirements may be a consequence of low parasite biomass during the initial stage of the infection or of slow parasite growth (but see next prediction). The latter may be a strategy to avoid the host immune defense, which would keep the total resource costs (parasite growth plus host defense) of the infection initially low. The second prediction may further narrow down why parasitic castration and gigantism are found only in certain host-parasite systems. Parasites with large total energy demands early during an infection (due to parasite growth or host defense; compare solid and stippled lines for parasite growth in the lower right graph of fig. 5) may not allow excess resources to build up even if the parasite castrates its host. In these cases, resource limitation would occur so early during an infection that the resource-depleted host would also be killed much sooner and the host's fecundity reduction and death would not be separated in time. If, however, castration and host death are clearly separated in time, it is often associated with the finding of gigantism, which is consistent with comparative evidence (Baudoin 1975; Moore 2002).

A third prediction related to the one above is that parasite biomass increases at a higher rate than host biomass until resource depletion slows down parasite growth (fig. 5). For microparasites this can be measured as the population growth rate, while for macroparasites this is individual body increase. It is important here that growth is slowed down by resource depletion and not by the host immune defense. As a consequence, it is expected that parasite biomass reaches a substantial proportion of the host biomass, as has been observed before (Baudoin 1975; Sousa 1983).

Fourth, enhanced host growth relative to uninfected controls should be observed only during early phases of infection, when the parasite biomass is still small. Host growth may be stunted in late phases of infection when the parasite needs more resources. This fourth prediction is consistent with results from earlier studies on gigantism

(Minchella et al. 1985; Gerard and Theron 1997) and may explain some of the variation in host growth across individuals seen in field observations in other castrator systems where the age of the infections was not known (Gorbushin 1997; Sorensen and Minchella 2001).

Fifth, parasites may achieve higher levels of control over the host or gain more rapid control over the host when more individuals enter a host (higher exposure dose). Our results suggest that with higher spore doses, parasites castrate their hosts more effectively, induce stronger gigantism, and produce more spores (fig. 3). Similar results have been reported from other castrator systems (Zakikhani and Rau 1999; Sorensen and Minchella 2001). In an earlier study that included 13 dose levels, we found that *P. ramosa* produced more spores as dose increased. However, beyond a certain dose level, spore production rapidly declined, presumably because within-host competition among parasites (density dependence) became so strong that parasite development within the host was stunted (Ebert et al. 2000*a*). This suggests that higher exposure doses may help the parasite to gain control over the host but at the same time increase density dependence and thus reduce spore yield. We predict that gigantism will also peak at intermediate dose levels. Consistent with this prediction, the highest lifetime cercariae production and largest adult size has been found at intermediate dose levels in a study of a digenean parasite of a snail (Zakikhani and Rau 1999).

Conclusions

Our study suggests that castration and gigantism are adaptive for the parasite *P. ramosa* infecting *D. magna* because they are linked with greater production of parasite spores. More generally, we suggest that castration and gigantism are adaptive for parasites that have a high life expectancy (chronic infections) and that have low resource requirements during the initial stage of an infection but high requirements later on when resources can be mobilized from the enlarged host body. The host body serves as a temporary resource storage unit.

Our study supports the idea that antagonists may compete for resources and that this conflict is subject to genetic variation among both host and parasite genotypes. The traits involved on both sides may be of quantitative genetic nature rather than single gene effects as is often assumed in coevolutionary models (Barrett 1988; Gandon et al. 1996). Whether the parasite or the host is ahead in the "arms race" may depend on the relative evolutionary speed with which the antagonists adapt. This in turn may depend on the rates at which genetic variation is created through mutations, sexual recombination, and migration (Hamilton 1980; Lively 1999). In the *D. magna–P. ramosa* system, neither antagonist appears to have solidly gained the upper hand, indicating that reciprocal selection is ongoing. In such an arms race, adaptations of high value today may be of low value in the future.

Acknowledgments

We thank J. Hottinger for laboratory assistance and K.-H. Jensen for helpful discussions. S. Zweizig improved earlier versions of this manuscript. This study was supported by the Swiss Nationalfonds.

Literature Cited

Arnott, S. A., I. Barber, and F. A. Huntingford. 2000. Parasite-associated growth enhancement in a fish-cestode system. Proceedings of the Royal Society of London B 267:657–663.

Ballabeni, P. 1995. Parasite-induced gigantism in a snail: a host adaptation? Functional Ecology 9:887–893.

Barrett, J. A. 1988. Frequency-dependent selection in plant-fungal interactions. Philosophical Transactions of the Royal Society of London B 319:473–483.

Baudoin, M. 1975. Host castration as a parasitic strategy. Evolution 29:335–352.

Bittner, K., K. O. Rothhaupt, and D. Ebert. 2002. Ecological interactions of the microparasite *Caullerya mesnili* and its host *Daphnia galeata*. Limnology and Oceanography 47:300–305.

Bull, J. J. 1994. Virulence. Evolution 48:1423–1437.

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.

Clarke, B. C. 1976. The ecological relationship of host-parasite relationships. Pages 87–104 *in* A. E. R. Taylor and R. M. Muller, eds. Genetic aspects of host-parasite relationships. Blackwell, Oxford.

Dawkins, R. 1982. The extended phenotype. Oxford University Press, Oxford.

Ebert, D., and E. A. Herre. 1996. The evolution of parasitic diseases. Parasitology Today 12:96–100.

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. Philosophical Transactions of the Royal Society of London B 351:1689–1701.

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*. Proceedings of the Royal Society of London B 265:2127–2134.

———. 2000*a*. Dose effects and density-dependent reg-

ulation of two microparasites of *Daphnia magna*. Oecologia (Berlin) 122:200–209.

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

Ebert, D., J. W. Hottinger, and V. I. Pajunen. 2001. Temporal and spatial dynamics of parasites in a *Daphnia* metapopulation: which factors explain parasite richness? Ecology 82:3417–3434.

Falconer, D. S., and T. MacKay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Harlow.

Gandon, S., Y. Capowiez, Y. Dubois, Y. Michalakis, and I. Olivieri. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. Proceedings of the Royal Society of London B 263:1003–1009.

Gerard, C., and A. Theron. 1997. Age/size- and time-specific effects of *Schistosoma mansoni* on energy allocation patterns of its snail host *Biomphalaria glabrata*. Oecologia (Berlin) 112:447–452.

Gorbushin, A. M. 1997. Field evidence of trematode-induced gigantism in *Hydrobia* spp. (Gastropoda: Prosobranchia). Journal of the Marine Biological Association of the United Kingdom 77:785–800.

Gorbushin, A. M., and I. A. Levakin. 1999. The effect of trematode parthenitae on the growth of *Onoba aculeus*, *Littorina saxatilis* and *L. obtusata* (Gastropoda: Prosobranchia). Journal of the Marine Biological Association of the United Kingdom 79:273–279.

Green, J. 1974. Parasites and epibionts of Cladocera. Transactions of the Zoological Society of London 32:417–515.

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

Hurd, H. 2001. Host fecundity reduction: a strategy for damage limitation? Trends in Parasitology 17:363–368.

Jaenike, J. 1996. Suboptimal virulence of an insect-parasitic nematode. Evolution 50:2241–2247.

Jokela, J., L. Uotila, and J. Taskinen. 1993. Effects of castrating trematode parasite *Rhipidocotyle fennica* on energy allocation of fresh-water clam *Anodonta piscinalis*. Functional Ecology 7:332–338.

Karban, R., and G. English-Loeb. 1997. Tachinid parasitoids affect host plant choice by caterpillars to increase survival. Ecology 78:603–611.

Keas, B. E., and G. W. Esch. 1997. The effect of diet and reproductive maturity on the growth and reproduction of *Helisoma anceps* (Pulmonata) infected by *Halipegus occidualis* (Trematoda). Journal of Parasitology 83:96–104.

Krist, A. C. 2000. Effect of the digenean parasite Proterometra macrostoma on host morphology in the freshwater snail *Elimia livescens*. Journal of Parasitology 86: 262–267.

Kuris, A. M. 1974. Trophic interactions: similarity of parasitic castrators to parasitoids. Quarterly Review of Biology 49:129–148.

Lampert, W. 1987. Feeding and nutrition in *Daphnia*. Memorie dell'Istituto Italiano di Idrobiologia 45:143–192.

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

———. 2000. The cause of parasitic infection in natural populations of *Daphnia* (Crustacea: Cladocera): the role of host genetics. Proceedings of the Royal Society of London B 267:2037–2042.

Lively, C. M. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. American Naturalist 153(suppl.):S34–S47.

Loot, G., R. Poulin, S. Lek, and J. F. Guegan. 2002. The differential effects of *Ligula intestinalis* (L.) plerocercoids on host growth in three natural populations of roach, *Rutilus rutilus* (L.). Ecology of Freshwater Fish 11:168–177.

Lynch, M., L. J. Weider, and W. Lampert. 1986. Measurement of the carbon balance in *Daphnia*. Limnology and Oceanography 31:17–33.

Minchella, D. J. 1985. Host life-history variation in response to parasitism. Parasitology 90:205–216.

Minchella, D. J., B. K. Leathers, K. M. Brown, and J. N. McNair. 1985. Host and parasite counteradaptations: an example from a freshwater snail. American Naturalist 126:843–854.

Moore, J. 2002. Parasites and the behavior of animals. Oxford University Press, Oxford.

Mouritsen, K. N., and K. T. Jensen. 1994. The enigma of gigantism: effect of larval trematodes on growth, fecundity, egestion and locomotion in *Hydrobia ulvae* (Pennant) (Gastropoda, Prosobranchia). Journal of Experimental Marine Biology and Ecology 181:53–66.

Obrebski, S. 1975. Parasite reproductive strategy and evolution of castration of hosts by parasites. Science 188: 1314–1316.

O'Keefe, K. J., and J. Antonovics. 2002. Playing by different rules: the evolution of virulence in sterilizing pathogens. American Naturalist 159:597–605.

Pan, J. J., and K. Clay. 2002. Infection by the systemic fungus *Epichloe glyceriae* and clonal growth of its host grass *Glyceria striata*. Oikos 98:37–46.

Probst, S., and J. Kube. 1999. Histopathological effects of larval trematode infections in mudsnails and their impact on host growth: what causes gigantism in *Hydrobia ventrosa* (Gastropoda: Prosobranchia)? Journal of Experimental Marine Biology and Ecology 238:49–68.

Regoes, R. R., J. W. Hottinger, L. Sygnarski, and D. Ebert. 2003. The infection rate of *Daphnia magna* by *Pasteuria*

ramosa conforms with the mass-action principle. Epidemiology and Infection 131:957–966.

Sorensen, R. E., and D. J. Minchella. 1998. Parasite influences on host life history: *Echinostoma revolutum* parasitism of *Lymnaea elodes* snails. Oecologia (Berlin) 115: 188–195.

———. 2001. Snail-trematode life history interactions: past trends and future directions. Parasitology 123(suppl.):3–18.

Sousa, W. P. 1983. Host life-history and the effect of parasitic castration on growth: a field-study of *Cerithidea californica* Haldeman (Gastropoda, Prosobranchia) and its trematode parasites. Journal of Experimental Marine Biology and Ecology 73:273–296.

Stirnadel, H. A., and D. Ebert. 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology 66:212–222.

Wright, C. A. 1971. Flukes and snails. Allen & Unwin, Plymouth.

Yampolsky, L. Y., and D. Ebert. 1994. Variation and plasticity of biomass allocation in *Daphnia*. Functional Ecology 8:435–440.

Zakikhani, M., and M. E. Rau. 1999. *Plagiorchis elegans* (Digenea: Plagiorchiidae) infections in *Stagnicola elodes* (Pulmonata: Lymnaeidae): host susceptibility, growth, reproduction, mortality, and cercarial production. Journal of Parasitology 85:454–463.