Evolutionary Ecology Research, 2007, **9**: 459–469

Parasite transgenerational effects on infection

Tom Little,[1]* Jane Birch,[1] Pedro Vale[1] and Michelle Tseng[2]

[1]*Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, UK and* [2]*Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada*

ABSTRACT

Question: Do past conditions experienced by parasites mediate current levels of infectivity and virulence in the host–parasite combination of *Daphnia magna* and *Pasteuria ramosa*?

Methods: We varied either temperature (three levels: 15, 20 or 25°C) or food supplied to the host (two levels) during a primary infection event, and then harvested parasites and measured their infectivity during a secondary infection event that was subject to the same environmental variation.

Result: Past temperatures did not influence any of the infection-related traits measured. By contrast, past food conditions appeared to impact infection, with parasite spores originating from well-fed hosts generally being more harmful. There was no indication that parasites had become specialized to their past environment. Four host genotypes were included in the experiment, and there was evidence that one of four was more sensitive to the environmental history of parasites than were the other hosts, i.e. there was an interaction between host genotype and parasite treatment effects.

Conclusion: Overall, parasite transgenerational effects appear to influence the level of harm parasites cause.

Keywords: *Daphnia*, genetic variation, genotype × environment interaction, maternal effect, parasitism, *Pasteuria*, pathogen, virulence.

INTRODUCTION

Parasites are ubiquitous and are important factors in host population dynamics and community structure (e.g. Anderson and May, 1982; Hudson *et al.*, 1998; Boots and Sasaki, 2003) and may even be responsible for the maintenance of genetic diversity and sexual reproduction in their hosts (e.g. Hamilton, 1980; Lively, 1987). Infection outcomes are, like all traits, dependent on the genotype, the environment they experience, and genotype × environment interactions. An additional aspect of environmental variation that is increasingly recognized to have a profound impact on the expression of disease, among other traits (see Mousseau and Fox, 1998), is the condition experienced by the parental or previous generation.

* Author to whom all correspondence should be addressed. e-mail: tom.little@ed.ac.uk

Transgenerational effects on resistance have been demonstrated in a wide range of vertebrate and invertebrate taxa. For example, in mammals, nutrient deficiencies in mothers limit the development of the offspring immune system (Gershwin *et al.*, 1985), while food stress in the crustacean *Daphnia* appears to enhance parasite resistance in their offspring (Mitchell and Read, 2005). Comparable cross-generational effects in parasites have received less attention. Certainly, the evolution of parasites/pathogens has been widely studied (Ebert, 1998, 1999; Mackinnon and Read, 2004; McClelland *et al.*, 2004; Read *et al.*, 2004), and there is ample evidence for environmentally dependent expression of parasite traits, such as life history (Buckling *et al.*, 1997; Gemmill *et al.*, 1997; Kaltz and Koella, 2003; Poulin, 2003; Schjorring, 2004) and virulence (Wedekind *et al.*, 1998; Bedhomme *et al.*, 2004). However, limited data are available regarding one clearly important circumstance: how the environmental conditions parasites experience at one point in time determines their subsequent infectivity or virulence. There are two such studies we are aware of: Little *et al.* (2006) conditioned a bacterial parasite of *Daphnia* in a range of different host genotypes and showed that this experience did not influence the outcome of later exposures, while Tseng (2006) reared a protozoan mosquito parasite in hosts fed either high or low food to show that parasites originating from well-fed hosts were more virulent to subsequent hosts.

In the present study, we examined the infectivity and virulence of parasites that were conditioned in a range of host environments (high or low food, or under a range of temperatures). We specifically sought to determine whether the condition of the host populated by one generation of parasites could mediate susceptibility and infection outcomes in the next, which would shed light on the speed with which parasites can become specialized to their environments in the short term. The parasite we studied is a bacterium and as such has the potential to mutate and evolve rapidly. Thus, it is not possible to resolve whether any conditioning effects were due to genetic change or changes in expression patterns (plasticity), although over the short time-frames we studied plasticity was expected to dominate. Nevertheless, we were motivated to determine if environmental variation impacted, whatever the mechanism, transmissibility and virulence across a transmission event.

METHODS

Study system

Daphnia magna is a planktonic crustacean found in still freshwater bodies and is host to many bacterial, microsporidian, and fungal parasites (Green, 1974; Stirnadel and Ebert, 1997; Little and Ebert, 1999). *Pasteuria ramosa*, the best studied of the *Daphnia* parasites, is a bacterial, spore-forming, obligate endoparasite of *D. magna* that greatly reduces host fecundity. Transmission is horizontal, achieved by the release of spores from the decomposing cadavers of previously infected hosts (Ebert *et al.*, 1996).

The *D. magna* and *P. ramosa* used in the present study were collected from a farm pond at Leitholm, Scottish Borders (2°20.410′W, 55°42.131′N) in the summer of 2003 and maintained in the laboratory in a state of clonal reproduction. Both experiments presented here used the same four host clones, which were originally isolated as isofemale lines and subsequently shown to differ at allozyme loci. Initial infections in the F_0 generation were performed using a diverse mixture of parasite spores (see Mitchell and Read, 2005; Mitchell *et al.*, 2005).

Parasite conditioning in the F_0 generation

For the temperature studies, parasites were passaged through hosts at 15, 20 and 25°C and harvested from infected hosts sacrificed on degree day 600 as described in a previous study (Mitchell *et al.*, 2005) and which is long enough for the development of transmission spores (Ebert *et al.*, 1996). Degree day is simply the actual number of days multiplied by the appropriate temperature and is used to standardize comparison of organisms studied at different temperatures. Mitchell *et al.* (2005) have shown that degree days accurately represent physiological time in *Daphnia*. For the food level studies, we grew parasites on hosts kept at 20°C and fed them either high food (3.5×10^6 algal cells per *Daphnia*) or low food (1.5×10^6 algal cells per *Daphnia*), and harvested parasite spores on day 30 for subsequent use in the F_1 generation.

Infection assays in the F_1 generation

Host standardization

The food and temperature experiments were conducted and analysed separately, although every effort was made to make them comparable by keeping methods similar. Before the experiments, replicates of each of the four *D. magna* clones were acclimated under standardized conditions at a 14 : 10 hour light : dark cycle in controlled climate chambers at 20°C. Regarding temperature, prior acclimation conditions have been shown to have little impact, at least compared with current conditions, on *Daphnia* life history and susceptibility (Mitchell *et al.*, 2005). Prior food conditions can affect susceptibility, but interactions between prior conditions and current conditions are not evident for either food (Guinnee *et al.*, 2007) or temperature (Mitchell *et al.*, 2005). *Daphnia* were kept in synthetic pond medium (Klüttgen *et al.*, 1994), and were fed exclusively on *Scenedesmus obliquus*, a green algae cultured in chemostats with Chu B medium. During acclimation, clonal replicates were fed 3.5×10^6 algal cells per *Daphnia* per day and maintained as 5 females in 200 ml (temperature experiment) or 20 females in 2000 ml (food experiment) that was changed three times a week. Host acclimation lasted for three generations, where each generation was started using second or third clutch neonates. Acclimating all replicates for three generations is a process designed to ensure that each replicate is independent, enabling a split-brood experimental design (see Ebert *et al.*, 1998), where replicate need not be entered into statistical models.

Temperature

For the temperature experiment, there were five replicates of each clone. From each replicate, groups of five female offspring less than 24 hours old were assigned to a treatment and placed in a jar containing 60 ml of *Daphnia* medium. Each jar contained a teaspoon of purified sand at the bottom, which tends to reduce variation in infection levels and increases the incidence of infection (Mitchell *et al.*, 2004). To each jar, we added 1×10^5 *P. ramosa* transmission spores. These spores were from the F_0 generation of parasites, and thus there were three types of 'parasite history': previously grown at 15, 20 or 25°C. The infection period was 150 degree days (i.e. 6 days at 25°C, 7.5 days at 20°C, and 10 days at 15°C). Every day, until degree day 150, each jar was stirred to increase chances of contact with parasite spores. One degree day after degree day 150, each group of five *Daphnia* were transferred to a jar containing 200 ml of *Daphnia* medium. Each jar was checked for newborns daily. When

newborns were present, the adult females were moved to a new jar and the offspring counted. In the absence of any clutches, *Daphnia* were transferred to a new jar with fresh medium every 60 degree days. The experiment finished on degree day 600. During the infection period, *Daphnia* were fed 3.5×10^6 algal cells per *Daphnia* every other day, but this was increased to 3.5×10^6 algal cells per *Daphnia* per day afterwards until the end of the experiment. The comparatively low level of food during the infection period encourages the *Daphnia* to graze the sand, increasing contact with the parasite.

Food

For the food experiment, there were six replicates of each clone. From each replicate, groups of five newborns less than 24 hours old were assigned to a treatment and placed in a jar containing sand and parasite spores. These spores were from the F_0 generation of parasites, and thus represented two types of 'parasite history': they were parasites previously propagated through hosts fed either low food (3.5×10^6 algal cells per *Daphnia* every other day) or high food (3.5×10^6 algal cells per *Daphnia* every day). The infection period was 7 days and each jar was stirred daily. On day 8, each group of five *Daphnia* was transferred to a jar containing 200 ml of *Daphnia* medium. Each jar was checked for newborns daily, and when present the adult females were moved to a new jar and the offspring counted. In the absence of any clutches, *Daphnia* were transferred to a new jar with fresh medium every 3 days. The experiment finished on day 30. During the infection period, all *Daphnia* were fed 3.5×10^6 algal cells per *Daphnia* every other day. The low and high food treatments, which were identical to those used in the F_0 generation, were realized during the post-infection period: *Daphnia* assigned to the low current food treatment were fed 3.5×10^6 algal cells per *Daphnia* every other day, while *Daphnia* assigned to the high current food treatment saw their food levels double to 3.5×10^6 algal cells per *Daphnia* per day. The food experiment was conducted at 20°C.

Analysis

We used general linear models as implemented in SAS procedure GENMOD to determine how the response variables infectivity (the proportion of hosts infected), host offspring production per host, and survivorship (the proportion of hosts surviving in each jar at the end of the experiment) were affected by the 'parasite history', 'current conditions', and 'host clone' in a fully factorial model. Offspring production largely mirrors infectivity (the main effect of infection is a loss of reproduction) but may reflect additional information, for example if the length of the pre-patent period varies among treatments. For the two response variables based on proportions (infectivity and survivorship), we defined the error distribution to be binomial (DIST = BIN, LINK = LOGIT options in GENMOD), while offspring production was analysed using basic analysis of variance (ANOVA). The response variable age at first reproduction (which is the first day that offspring were observed in a jar) was analysed using proportional hazards analysis (age at first reproduction is essentially a 'time to event' variable). For the temperature experiment, 'parasite history' and 'current conditions' were entered as continuous variables, while for the food experiment the two food levels were fixed factors. Host clone was a fixed factor. Two manipulations of the data were required. First, the offspring counts were square root transformed to meet the assumptions of ANOVA. Second, for the temperature experiment, we converted the time-scale from real time to degree days to enable direct comparison among temperatures. Although our three

generations of host acclimation were designed to make replicates truly independent, we nevertheless repeated the analysis with replicate (nested with host clone and treated as a random effect) as a further explanatory variable, but this had no effect on the outcome of the statistical analyses (data not shown).

RESULTS

Temperature

Forty-five percent of hosts became infected in the temperature experiment. 'Current temperature' had a strong impact on infectivity and host reproduction in the face of parasitism (Table 1, Fig. 1). However, 'parasite history' did not have a significant influence on these response variables either as a main effect or through an interaction with current conditions. Host clone effects were highly significant, although they showed no interaction with either 'current temperature' or with 'parasite history' (Table 1). Age at first reproduction was not affected by 'parasite history', 'current conditions', clone effects or any interactions between them. Survivorship was significantly influenced by 'current temperature' and also differed among clones (Table 1).

Food

Thirty-two percent of hosts became infected in the food experiment. 'Current food' had a strong impact on both the proportion of hosts infected and host reproduction in the face of parasitism (Table 1 Fig. 2). 'Parasite history' had no effect on infectivity or offspring production, and there was no interaction between 'parasite history' and 'current conditions' (Table 1, Fig. 2). Host clone effects were significant for both the proportion of hosts infected and offspring production (Table 1). For offspring production, interactions between host clone and both 'current food' and 'parasite history' were evident (Table 1, Fig. 3). Age at first reproduction was influenced by current food conditions, but not affected by 'parasite history', clone effects or any interactions between them. Survivorship was influenced by 'current food', clone effects and, notably, 'parasite history' where hosts exposed to parasites previously grown on poorly fed hosts survived longer (proportion surviving = 0.79, standard error = 0.031) than those exposed to parasites previously grown on well-fed hosts (proportion surviving = 0.66, standard error = 0.038).

DISCUSSION

This study propagated parasites for one host generation in one of a range of environments, and then for a second generation propagated them in either the same or a different environment. These experiments were designed to study how conditions experienced by the previous generation of parasites might mediate current levels of infectivity and virulence. Present temperature clearly influences infection outcomes in our study system (see also Mitchell *et al.*, 2005), but past temperatures experienced by parasites appeared not to be important for current infection. This pattern is similar to that in a previous study that showed that past exposure to different host genotypes did not affect current patterns of infectivity (Little *et al.*, 2006). In the present study, the amount of food currently available to hosts had a strong impact on infection outcomes, and, in contrast to past temperatures, past food conditions

Table 1. Results of statistical analyses relating four host traits to the effect of current environment and the environment parasites experienced one generation previously

	Test statistic	d.f., error d.f.	*P*
TEMPERATURE			
Infectivity			
Current temperature	6.90	1, 161	0.009
Parasite history	0.11	1, 161	N.S.
Host clone	40.80	3, 161	<0.0001
Offspring			
Current temperature	8.60	1, 158	0.004
Parasite history	1.10	1, 158	N.S.
Host clone	19.10	3, 158	<0.0001
Age at first reproduction			
Current temperature	1.80	1, 158	N.S.
Parasite history	1.10	1, 158	N.S.
Host clone	4.90	3, 158	N.S.
Survivorsip			
Current temperature	17.10	1, 174	<0.0001
Parasite history	0.12	1, 174	N.S.
Host clone	12.23	3, 174	0.007
FOOD			
Infectivity			
Current food	7.58	1, 74	0.006
Parasite history	2.62	1, 74	N.S.
Host clone	25.79	1, 74	<0.0001
Offspring			
Current food	42.54	1, 71	<0.0001
Parasite history	0.98	1, 71	N.S.
Host clone	3.89	2, 71	0.012
Current food*host clone	5.46	3, 71	0.002
Parasite history*host clone	3.05	3, 71	0.034
Age at first reproduction			
Current food	5.31	1, 70	0.021
Parasite history	0.16	1, 70	N.S.
Host clone	5.02	3, 70	N.S.
Survivorsip			
Current food	13.77	1, 75	0.034
Parasite history	5.35	1, 75	0.021
Host clone	13.77	3, 75	0.003

Note: We ran two experiments, one involving temperature variation, the other involving variation in the amount of food supplied to hosts. Each experiment used multiple host clones that were also tested for differences in the response variables. Test statistics are *F*-ratios for offspring counts, but a likelihood ratio χ^2 for the other response variables.

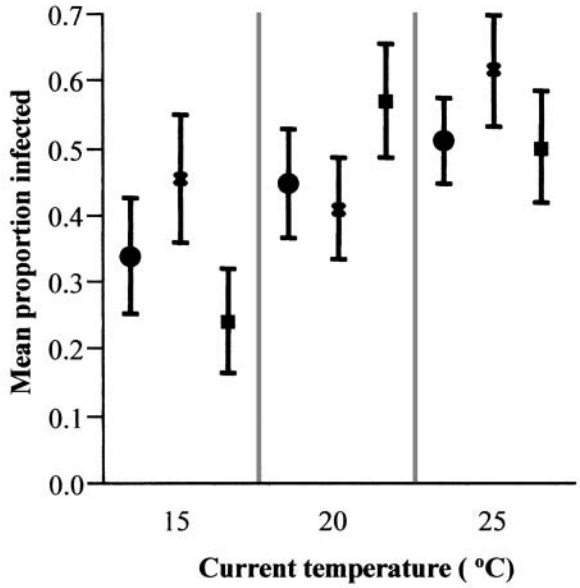

Fig. 1. Infection levels for *Daphnia* hosts reared at three temperatures when parasites had, one generation previously, been propagated at three temperatures (●, 15°C; × , 20°C; ■, 25°C). Bars are standard errors.

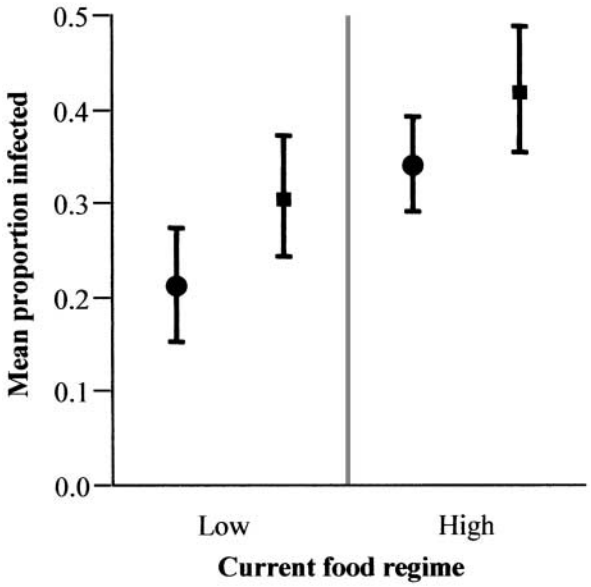

Fig. 2. Infection levels for *Daphnia* hosts reared at two food levels when parasites had, one generation previously, been propagated at different food levels (●, low food; ■, high food). Bars are standard errors.

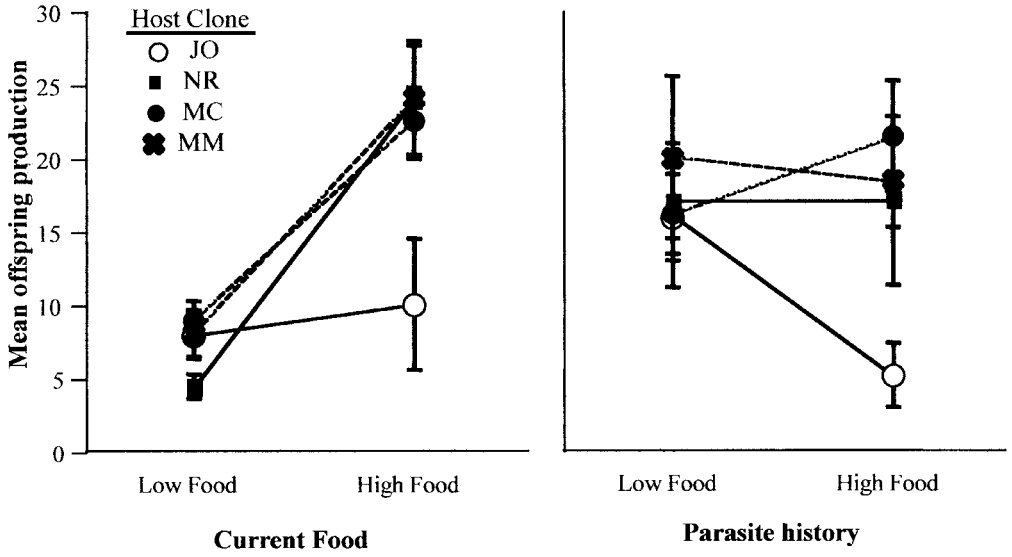

Fig. 3. Left panel shows a genotype × environment (G × E) interaction regarding offspring production under parasitism in a low or high current food environment. Right panel shows a G × E interaction where E is the environmental history (food level) of the parasite. JO, NR, MC, and MM are different host clones. Bars are standard errors

experienced by parasites did impact current infections. In particular, parasites propagated on relatively poorly fed hosts harmed (in terms of host survival) new hosts less than did parasites previously propagated on well-fed hosts. Additionally, parasite history impacted the level of reproduction hosts attained, although the direction of this effect was variable and dependent on host genotype (Fig. 3). Thus, our data indicate that, while not a pervasive factor, past environments experienced by parasites can influence the harm that parasites cause.

Although we do recommend caution in interpreting these results (this study incorporated a large number of statistical tests and significance levels for tests involving parasite history were not overwhelmingly strong), the potential effects of parasite conditioning raise some interesting points for discussion. In particular, the 'parasite history' × host clone interaction we observed potentially adds a complex context to the host–parasite interaction. This pattern implies that the conditions experienced by the previous generation of parasites will impact some host clones to a greater degree than others, raising the possibility that past parasite environments impact parasite-mediated selection. This situation is analogous to that found in a study of maternal effects on host resistance: the conditions experienced by the maternal host generation determined host resistance in the current generation, but some host genotypes appeared to be more sensitive than others (Mitchell and Read, 2005). In general, such transgenerational effects are likely to exhibit unique evolutionary features (Mousseau and Fox, 1998). For example, a time lag between previous selection, acting on the previous generation, and the evolutionary response, which occurs in the current generation, may be counterproductive if current conditions are markedly different from maternal conditions (Kirkpatrick and Lande, 1989). Adapting this thinking to co-evolutionary interactions will require

the incorporation of a complex array of host and parasite factors and their interaction with a developing environment.

That parasites previously propagated on poorly fed hosts caused relatively little mortality compared with parasites previously propagated on well-fed hosts could indicate that low resource environments affect the quality of parasite transmission spores. Previous studies have clearly established that poor environments impact the quantity of spores produced (Ebert *et al.*, 2004). We carefully controlled for this effect in the second round (the F_1 exposure) of infections by standardizing spore doses to the same level regardless of parasite history, and thus the present data, in principle, show that the cost of inhabiting poorly fed hosts is not only the production of a small number of transmission spores but also their relative lack of effectiveness. The issue of spore quality could, however, be confounded by incomplete knowledge regarding when a spore is mature and capable of transmission. We diagnose the life stage of spores based on their morphology, and although this has been studied in depth (Ebert *et al.*, 1996), we cannot be certain that what we identify as a mature parasite spore is indeed yet capable of initiating infection. Our results could therefore reflect slow parasite growth, and especially slow maturation rate, in a low food environment. This hypothesis should be testable by harvesting spores over time and comparing infectivity of standardized doses.

Effects of host genetic background (host clone) were ubiquitous in this and other studies of this host–parasite system (Little and Ebert, 2000; Carius *et al.*, 2001; Duncan *et al.*, 2006). Here, genetic background (clone) also showed strong interactions with current food levels (i.e. a standard genotype × environment interaction) but somewhat surprisingly, host clone did not interact with current temperature. In a previous study (Mitchell *et al.*, 2005) on host and parasites from the same source populations, such a genotype × (temperature) environment interaction was clearly evident. However, Mitchell *et al.* (2005) studied a relatively large number of host clones (eight vs. four in the present study), which offered substantial additional power for the detection of genotype-specific effects.

We might also have expected significant 'parasite history' × 'current environment' interactions if parasites had become adapted, or acclimated, to their previous environment. In this case, we would have observed, for example, that parasites previously propagated at high temperatures would perform well at high current temperatures. Acclimation has been demonstrated, for example, in studies of other microorganisms (Bennett and Lenski, 1997; Dillon *et al.*, 2003; Rainey, 2004) or in plant–herbivore interactions where insect herbivores acclimate to the chemical environment of their hosts (Jermy, 1990; Akhtar and Isman, 2004). Acclimation, being based on phenotypic plasticity, can be expected to occur quite rapidly, and it thus was not unreasonable to expect a bacterium such as *P. ramosa* to have done so over the time-frames provided by this study.

Given longer time-frames, evolutionary events could drive adaptations and specialization, as they probably have in other systems (Mackinnon and Read, 2004; McClelland *et al.*, 2004) and indeed in other studies on the *Daphnia–Pasteuria* system (Little *et al.*, 2006). However, even in these cases, it remains difficult to determine when specialization is due to genetic differences (evolution) or differential patterns of expression (acclimation/phenotypic plasticity). In general, the process of specialization is of considerable interest to the field of parasitology and evolutionary thinking on disease. For example, an understanding of specialization can shed light on what limits the host range of certain diseases or help to explain why the spread of disease is sometimes associated with increasing virulence (Read *et al.*, 1999). We speculated that parasite specialization via acclimation to host environments could contribute to this

process, but our data indicate that it does not, at least over ecological time-scales. Perhaps the significant interactions we observed with host clone indicate that longer-term evolutionary interactions are of greater importance.

ACKNOWLEDGEMENTS

This work was supported by a NERC (UK) grant GR3/13105 to Andrew Read (Edinburgh), and fellowships from NSERC (Canada) and the School of Biology, University of Edinburgh to T.L.

REFERENCES

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

Anderson. R.M. and May, R.M. 1982. Coevolution of hosts and parasites. *Parasitology*, **85**: 411–426.

Bedhomme, S., Agnew, P., Sidobre, C. and Michalakis, Y. 2004. Virulence reaction norms across a food gradient. *Proc. R. Soc. Lond. B*, **271**: 739–744.

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

Boots, M. and Sasaki, A. 2003. Parasite evolution and extinctions. *Ecol. Lett.*, **6**: 176–182.

Buckling, A.G.J., Taylor, L.H., Carlton, J.M.R. and Read, A.F. 1997. Adaptive changes in *Plasmodium* transmission strategies following chloroquine chemotherapy. *Proc. R. Soc. Lond. B*, **264**: 553–559.

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

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

Duncan, A., Mitchell, S.E. and Little, T.J. 2006. Parasite-mediated selection in *Daphnia*: the role of sex and diapause. *J. Evol. Biol.*, **19**: 1183–1189.

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

Ebert, D. 1999. The evolution and expression of parasite virulence. In *Evolution in Health and Disease* (S.C. Stearns, ed.), pp. 161–172. Oxford: Oxford University Press.

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

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

Ebert, D., Carius, H.-J., Little, T.J. and Decaestecker, E. 2004. The evolution of virulence when parasites cause host castration and gigantism. *Am. Nat.*, **164**: s19–s32.

Gemmill, A.W., Viney, M.E. and Read, A.F. 1997. Host immune status determines sexuality in a parasitic nematode. *Evolution*, **51**: 393–401.

Gershwin, M.E., Beach, R.S. and Hurley, L.S. 1985. *Nutrition and Immunity*. Orlando, FL: Academic Press.

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

Guinnee, M.A., Gardener, A., Howard, A.E., West, S. and Little, T.J. 2007. The causes and consequences of variation in offspring size: a case study using *Daphnia. J. Evol. Biol.*, **20**: 577–587.

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

Hudson, P.J., Dobson, A.P. and Newborn, D. 1998. Prevention of population cycles by parasite removal. *Science*, **282**: 2256–2258.

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

Kaltz, O. and Koella, J.C. 2003. Host growth conditions regulate the plasticity of horizontal and vertical transmission in *Holospora undulata*, a bacterial parasite of the protozoan *Paramecium caudatum. Evolution*, **57**: 1535–1542.

Kirkpatrick, M. and Lande, R. 1989. The evolution of maternal characters. *Evolution*, **43**: 485–503.

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

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

Little, T.J. and Ebert, D. 2000. The cause of parasitic infection in natural populations of *Daphnia:* the role of host genetics. *Proc. R. Soc. Lond. B*, **267**: 2037–2042.

Little, T.J., Watt, K. and Ebert, D. 2006. Parasite–host specificity: experimental studies on the basis of parasite adaptation. *Evolution*, **60**: 31–38.

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

Mackinnon, M.J. and Read, A.F. 2004. Immunity promotes virulence evolution in a malaria model. *PLoS Biol.*, **2**: 1286–1292.

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

Mitchell, S.E. and Read, A.F. 2005. Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proc. R. Soc. Lond. B*, **272**: 2601–2607.

Mitchell, S.E., Read, A.F. and Little, T.J. 2004. The effect of a pathogen epidemic on the susceptibility to infection, reproductive investment and genetic structure of the cyclical parthenogen *Daphnia magna. Ecol. Lett.*, **7**: 848–858.

Mitchell, S.E., Rogers, E.S., Little, T.J. and Read, A.F. 2005. Host–parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, **59**: 70–80.

Mousseau, T.A. and Fox, C.W. 1998. The adaptive significance of maternal effects. *Trends Ecol. Evol.*, **13**: 472–474.

Poulin, R. 2003. Information about transmission opportunities triggers a life-history switch in a parasite. *Evolution*, **57**: 2899–2903.

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

Read, A.F., Aaby, P., Antia, K., Ebert, D., Ewald, P.W., Gupta, S. *et al.* 1999. What can evolutionary biology contribute to understanding virulence? In *Evolution in Health and Disease* (S.C. Stearns, ed.), pp. 205–215. Oxford: Oxford University Press.

Read, A.F., Gandon, S., Nee, S. and Mackinnon, M.J. 2004. The evolution of pathogen virulence in response to animal and public health interventions. In *Infectious Disease and Host–Pathogen Evolution* (K.R. Dronamraju, ed.), pp. 265–292. Cambridge: Cambridge University Press.

Schjorring, S. 2004. Delayed selfing in relation to the availability of a mating partner in the cestode *Schistocephalus solidus. Evolution*, **58**: 2591–2596.

Stirnadel, H.A. and Ebert, D. 1997. The ecology of three *Daphnia* species – their microparasites and epibionts. *J. Anim. Ecol.*, **66**: 212–222.

Tseng, M. 2006. Interactions between the parasite's previous and current environment mediate the outcome of parasite infection. *Am. Nat.*, **168**: 565–571.

Wedekind, C., Strahm, D. and Scharer, L. 1998. Evidence for strategic egg production in a hermaphroditic cestode. *Parasitology*, **117**: 373–382.