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Trade-off between latent period and transmission success of a plant pathogen revealed by phenotypic correlations

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ABSTRACT

Questions: Can trade-offs and genotype–environment interactions maintain variability for fitness-related life-history traits?

Hypothesis: Transmission success, the equivalent of fecundity, is traded off against minimizing the latent period, the equivalent of age at maturity.

Organisms: The non-lethal parasite *Hyaloperonospora arabidopsis* (= *Hyaloperonospora parasitica*) and its host plant *Arabidopsis thaliana*.

Methods: We measured the latent period, transmission success, and host seed production of all combinations of infections between three parasite strains and three host lines, allowing us to calculate phenotypic correlations between these parasite traits and determine the relationship between host and parasite traits.

Conclusions: Infected plants that sporulated more rapidly (short latent period) transmitted their parasites less well, revealing a phenotypic trade-off between these important parasite life-history traits. This phenotypic trade-off may help to explain why the latent period remains variable in nature and has not achieved a uniformly minimal value.

Keywords: *Arabidopsis thaliana*, genotype–environment interactions, host–parasite interactions, *Hyaloperonospora arabidopsis*, *Hyaloperonospora parasitica*, *Peronospora*, phenotypic trade-off.

INTRODUCTION

Many studies on infectious diseases have focused on symptom management and disease progress in host populations. One question of practical importance is whether the appearance of symptoms could be delayed if not perhaps completely avoided. What would be the consequences of delaying the appearance of symptoms on host fitness and the progress of disease in a host population? The appearance of symptoms is often followed by unavoidable parasite damage to its host (e.g. reduction in host fitness due to infection, also known as virulence), caused when a parasite appropriates from the host the resources and

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habitat necessary for its development and maturity (Ebert, 1999). The parasite thereby can begin a new cycle of transmission with production of new dispersal propagules and then infection of new hosts. The latent period is the time from infection until the production of the first dispersal propagules, which usually coincides with first symptoms, in particular in plant foliar diseases. The latent period determines the minimum time between subsequent generations and should be considered an important life-history trait of parasites, analogous to 'age at maturity' in non-parasitic organisms.

Studies on the evolution of life-history traits consider a trade-off between age or size at reproductive maturity and overall lifetime fecundity [parasitic nematodes (Gemmil *et al.*, 1999); filamentous fungi (Pringle and Taylor, 2002)]. In fact, age and size at maturity reflect the same composite life-history trait because organisms mature along an age–size trajectory (Stearns and Koella, 1986), particularly if they have a continuous and indeterminate growth. Earlier maturity permits higher survival to maturity and shorter generations (Agnew and Koella, 1999; Agnew *et al.*, 1999). Delayed maturity, with a longer period of growth, leads to larger size accompanied by higher initial fecundity at maturity (Stearns, 1992). Some empirical studies, however, reveal plastic responses in the opposite direction, with earlier maturity at large size in good environments but late maturity at small size in poor ones (Ford and Seigel, 1994; Agnew and Koella, 1999; Abedon *et al.*, 2001, 2003; Weetman and Atkinson, 2002).

A parasite that establishes quickly in an empty habitat will outperform other slower parasites, even if all of them have an equivalent propagation rate (Pringle and Taylor, 2002). Experimental studies and epidemiological simulations with the wheat rust fungus (*Puccinia* spp.) have shown that the strain with the shortest latent period dominates disease progress in the field (Lehman and Shaner, 1996). In mid-season, however, when infections of a single host by two different strains are frequent, a rust strain with a longer latent period may be able to out-compete the more rapid one if it tolerates higher population densities of parasites in the same host (Newton *et al.*, 1999). This suggests a trade-off between rapid maturity and competitive ability within the host and such a trade-off could be instrumental in maintaining genetic variation for the latent period itself, which otherwise should be selected to be as short as possible.

We carried out an experimental study with the host plant *Arabidopsis thaliana* and its natural non-lethal oomycete parasite *Hyaloperonospora arabidopsis* [= *Hyaloperonospora parasitica* (Göker *et al.*, 2004)]. *Hyaloperonospora arabidopsis* is a common pathogen in natural populations of *A. thaliana* in the spring in temperate climates (Holub *et al.*, 1994). The disease is called 'downy mildew' because of its production of downy spore-bearing bodies on infected leaves. We inoculated plants of three host genotypes (lines) with each of three parasite genotypes (strains) and measured the latent period – that is, the time until first sporulation (= age at maturity) – and transmission success during the early stages of infection in all combinations. Plants were allowed to complete their life cycle, permitting us to measure host fitness and parasite virulence.

We assessed whether the latent period varied across parasite strains, among host types or in interaction between the two players, and examined the relationships between the latent period and two other important life-history traits of the parasite – transmission success and virulence, the latter estimated as change in host fitness, respectively parasite and host fitness components. We asked the following questions: (1) Does the latent period vary among parasite strains or host lines or as a result of their interaction? (2) Does parasite transmission covary with the latent period? A positive relationship between these two life-history traits could explain the maintenance of variation in the latent period. (3) Do these

life-history traits of the parasite covary differently when it infects different host lines? Such strain- or line-specific interactions could explain the maintenance of variation for these traits. (4) Does host reproductive success when parasitized by different parasite strains covary with parasite transmission, parasite spore production or the latent period of the infection?

MATERIALS AND METHODS

Parasite and host material and parasite maintenance

The oomycete parasite *Hyaloperonospora arabidopsis* produces two types of infection: that initiated by infection of the root system of a seedling by a sexual oospore that leads to immediate systemic infection with hyphae that penetrate and spread throughout the plant, and that initiated by asexual conidiospores that are locally dispersed in the host population onto the same or different host individuals and expressed as foci of infection on the leaves that received them. After a latent period, hyphae can differentiate to produce asexual spores on the surface of the infected leaves or mate and produce sexual spores inside the infected leaves. Here we concentrate only on the asexual cycle of infection. Each cycle requires only a few days and asexual spores are considered the major cause of foliar disease epidemics (McCartney and Fitt, 1998). Sexual spores, on the other hand, remain in the soil and infect the roots of subsequent generations of young plants (Slusarenko and Schlaich, 2003).

We used three strains of *H. arabidopsis* that we had isolated directly from plants found naturally infected in the field. Two strains were from the campus of Université Paris-Sud in Orsay but collected from sites that were about 100 m apart in two different years (Ors3 was collected in April 2004 and S18 in April 2005). The third strain Fri3 was collected from the campus of the University of Fribourg, Switzerland in May 2004. These strains differed in virulence factors as revealed by different infection profiles when inoculated onto a set of host plants. After isolation, the strains were maintained as asexual cultures in the greenhouse on seedlings of susceptible *A. thaliana* lines. For strain maintenance by asexual propagation, seeds were sown in $5 \times 5 \times 5$ cm pots. When seedlings were 14 days old they were inoculated with a suspension of spores harvested from plants of the same line that had shown symptoms for about 7 days. To inoculate new seedlings, we trimmed sporulating plants with scissors and centrifuged the collected leaves in tap water to generate a suspension of on average 50 spores per microlitre that we sprayed onto the new seedlings. Fungal isolates were re-inoculated every 15 days to maintain sporulating cultures. After inoculation, plants were kept under a plastic dome to prevent cross-contamination.

For the experiment we chose three host lines that were susceptible to all three parasite strains. These host lines were obtained from at least two generations of selfing of plants grown from seed from a collection of *A. thaliana* plants from different sites in Europe. The line Gb was issued from a plant collected in Great Britain, the line Pyr was issued from a plant collected in the Pyrenees, and the line P10 was issued from a plant collected in Loiret, in central France. Line Gb was used for asexual parasite propagation and maintenance of the parasite strain Ors3, and Pyr for asexual parasite propagation and maintenance of the parasite strains Fri3 and S18.

Experimental protocol and measurements

Experiments were started in June 2005 and plants were harvested in November 2005. All seeds of the three lines were sown the same day in $5 \times 5 \times 5$ cm pots, and then placed in the dark at 5–6°C for 5 days to synchronize germination. Pots were then randomized in the greenhouse at 25°C during the day and 16°C at night. Plants were inoculated when 3 weeks old. All plants receiving the same treatment were grouped in one container the day of inoculation and re-randomized the day after. Plants were inoculated with a single spore suspension containing one of the three strains Ors3, Fri3 or S18 (15 replicates per host line) or a water control (7 replicates per host line). The spore suspensions contained a mean of 52 ± 3 spores per mictolitre. A drop (4 ± 1 µl) of the spore suspension was placed on each of six leaves using a micropipette. Controls received a drop (4 ± 1 µl) of water on each of six leaves. The containers were covered with transparent plastic film to avoid contamination and to maintain high humidity (95–100%) and then transferred to a climate chamber at 19°C during the day and 12°C at night (14 h light/10 h dark). The day after, covers were removed and plants were re-randomized. Each plant was put inside an individual covered transparent plastic cylinder to isolate it from the others and to maintain high humidity (95–100%). Inoculated plants were examined daily for visible signs of sporulation. Downy mildew spores are released in the early morning only, following a strict circadian rhythm mainly determined by photoperiod (Su *et al.*, 2000). We counted the number of leaves bearing spores and carried out parasite transmission trials from the fifth day to the ninth day after inoculation using all plants as spore sources regardless of whether they had already sporulated or not. The transmission trials employed the method described in Salvaudon *et al.* (2005), spraying each inoculated plant with water and allowing the droplets to fall on healthy young plants placed below them. This 5-day period was chosen to ensure that only transmission from primary infections was considered – that is, before symptoms of additional secondary infections on the inoculated plants could appear.

The latent period was measured as the number of days from inoculation to either the appearance of the first spores on infected leaves or successful transmission. This second measure accounted for 24 of the 134 inoculated plants (one plant was lost) where transmission occurred before we observed sporulation, though clearly the spores were there. We measured daily success of transmission by counting the number of leaves that bore conidiophores on the new plants 8 days after they were sprayed. Plants from each daily transmission trial were placed together in a tray and covered with a plastic dome to maintain high humidity. They were kept in the same climate chamber at 19°C during the day and 12°C at night. Because the latent period varied, we did not have data on a full 5 days of transmission for all spore sources. However, data for at least 3 days were available for all but two plants that sporulated. These last two sporulated 8 days after inoculation so only 2 days of transmission were available and they were therefore excluded from the analysis of cumulative transmission success. Hence we considered parasite transmission success for primary focus of infection calculated as the cumulative first 3 days of transmission. The inoculated spore sources were kept in the same climate chamber for 28 days – that is, 19 days following the transmission experiments – to let the disease become established. They were then all moved to the greenhouse in mid-August where they were kept at approximately 25°C during the day and 16°C at night and with a natural photoperiod to complete their life cycle. *Arabidopsis thaliana* does not have synchronous maturation of fruits and seeds, so we examined plants regularly and collected fruits as they matured. All fruits and seeds

produced by a single plant were collected into a paper bag and, when dry, seeds were separated and bulk weighed per plant. We used seed weight as our estimate of plant fitness (1/1000 g precision balance, Sartorius Osi, France). Parasite virulence was estimated for each combination of parasite strain and host line as the difference between average seed weight of the control plants of that same line and the inoculated plants. Because of an aphid infestation at the end of the experiment, we were unable to harvest the plants of the late-maturing host line Pyr.

Statistical analyses

Statistical analyses were performed with JMP version 5.1.2 (SAS Institute, Cary, NC). We examined whether latent period varied among the different combinations of parasite strains and host lines with a two-way factorial analysis of variance (ANOVA) followed by Tukey multiple comparison tests. Subsequently, we analysed variation in cumulative parasite transmission over the first 3 days of infection using a two-way factorial analysis of covariance (ANCOVA) that included host line and parasite strain as classification variables and latent period and cumulative sporulation on the inoculated source plant for the same 3 days as covariates. The model tested all interactions between classification variables and with a single covariate at a time.

We compared host seed production among host lines, inoculation treatments (parasite strains and water control), and their interaction with a two-way ANOVA that included greenhouse tray as a blocking factor. To construct the best model for explaining variation in host fitness for only the inoculated plants, we performed a stepwise full factorial ANCOVA with parasite strain and host line as classification variables, greenhouse tray as a blocking factor, and latent period, cumulative sporulation, and cumulative transmission over the first 3 days of the infection as covariates. The model tested all interactions between classification variables and with a single covariate at a time. Explanatory variables and combinations were entered or removed by the stepwise procedure at a critical value of $P = 0.25$ to construct the minimal explanatory model, which included only the blocking factor greenhouse tray, host line, and the covariate latent period.

RESULTS

Variability in latent period

Of the 134 plants inoculated 133 sporulated successfully, whereas none of the water-inoculated controls sporulated, indicating that contaminations between inoculated plants were unlikely. The latent period lasted on average 6.08 ± 0.12 days. The latent period varied by parasite strain and host line but not by their interaction (Fig. 1). Strain S18 had a significantly shorter latent period than strain Ors3, and strain Fri3 had a latent period intermediate between these two. Symptoms appeared later on the host line Pyr than on the other two lines.

Cumulative transmission increased with latent period and cumulative sporulation

Cumulative transmission differed among host lines, parasite strains, and their interaction (Table 1, see Fig. 2) and increased with increasing latent period and with increasing number

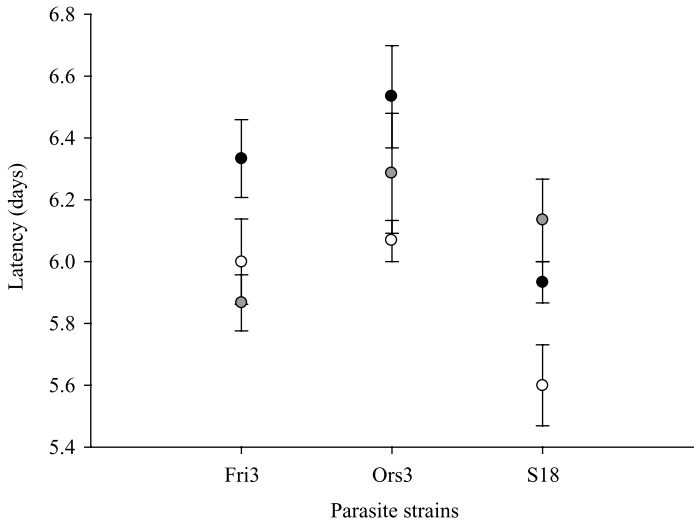

Fig. 1. The latent period of inoculated plants (mean ± S.E.) for the nine combinations of host lines and parasite strains. Each symbol represents a unique combination of host and parasite lines. Black symbols represent combinations with the parasite strain Fri3, grey symbols combinations with the parasite strain Ors3, and white symbols combinations with the parasite strain S18. A two-way factorial ANOVA revealed significant differences among parasite strains ($F_{2,125} = 7.47$; $P = 0.0009$) – with S18 having a significantly shorter latent period than Ors3, and Fri3 having a latent period not significantly different from the other two (Tukey multiple comparison test) – and among host lines ($F_{2,125} = 6.52$; $P = 0.002$) but no significant effect of their interaction ($F_{4,125} = 2.35$; $P = 0.0573$). The model R^2 was 0.23.

Table 1. Results of analysis of covariance of cumulative disease transmission (measured as number of leaves infected on new plants) testing the effects of parasite strain, host lines, latent period, and cumulative sporulation

Traits	d.f.	MS	*F*-value	Slope ± S.E.
Strain	2	14.63	0.31	
Host line	2	1735.24	37.41***	
Strain × host line	4	40.89	0.88	
Latent period	1	319.75	6.89*	4.61 ± 1.75
Strain × latent period	2	118.69	2.56	
Host line × latent period	2	36.31	0.78	
Strain × host line × latent period	4	156.81	3.38*	
Cumulative sporulation	1	351.85	7.58*	1.24 ± 0.45
Strain × cumulative sporulation	2	23.88	0.51	
Host line × cumulative sporulation	2	35.39	0.76	
Strain × host line × cumulative sporulation	4	72.98	1.57	
Error	105	46.38		

Note: The model tested all interactions between factors and with a single covariate at a time. The model R^2 was 0.78.

***$P < 0.0001$; **$P < 0.001$; *$P < 0.05$.

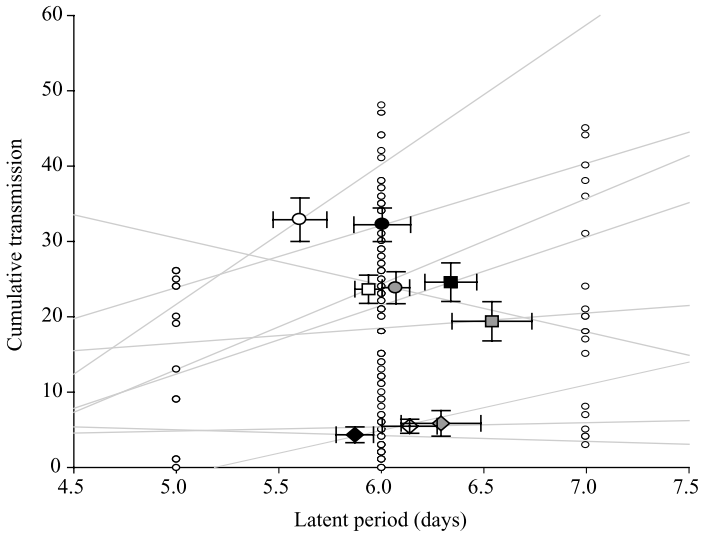

Fig. 2. Cumulative disease transmission (measured as number of leaves infected on new plants) for each infected plant (○ – not identified by host line or parasite strain) and the relationship between transmission and latent period (regression lines for each combination, also not identified) over the first 3 days of spore release. The mean of the nine combinations of host lines and parasite strains are superimposed to illustrate the genotype patterns. Black symbols represent the parasite strain Fri3, grey symbols the parasite strain Ors3, and white symbols the parasite strain S18. Diamonds represent the host line P10, squares the host line Pyr, and circles the host line Gb.

of sporulating leaves on the source plant (Table 1). The different parasites strains had different transmission success as a function of latent period (strain × latent period interaction) and we found a three-way interaction between host line, parasite strain, and latent period such that transmission of certain combinations of host and parasite responded differently to variation in the latent period (Fig. 2).

Host fitness varied with latent period

The host line P10 was more fecund than Gb, seed production varied among greenhouse trays, and no parasite strain diminished host fitness on either host line for which seeds could be harvested (Fig. 3). Of the infected plants, those whose infections had a longer latent period produced more seed (slope ± S.E. = 128.45 ± 62.5, $F_{1,84} = 4.22$, $P = 0.04$; from stepwise minimal explanatory model ANCOVA that retained ecotype, tray effect, and the covariate latent period).

DISCUSSION

Variation for life-history traits

Host lines and parasite strains differed in their latent periods, implying that genetic variation for this character persists in nature in both protagonists. Moreover, traits usually regarded as traits only of the parasite were controlled by both protagonists (for latent

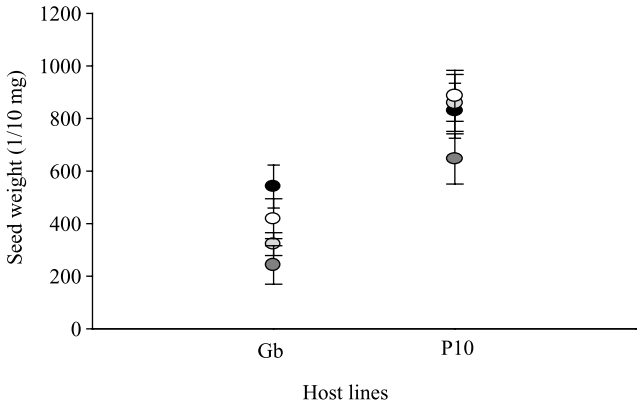

Fig. 3. Seed weight (mean ± S.E.) of the two host lines that reached maturity inoculated with the three parasite strains and control. Each symbol represents a unique combination of host and parasite lines. Black symbols represent combinations with the parasite strain Fri3, light grey symbols combinations with the parasite strain Ors3, and white symbols combinations with the parasite strain S18; dark grey symbols represent controls. A two-way factorial ANOVA on variation in seed production revealed a significant difference between the host lines ($F_{1,93} = 42.29$; $P < 0.0001$) and among greenhouse trays ($F_{3,93} = 7.17$; $P = 0.0002$) but no significant effect of inoculation treatment (three parasite strains and one water control; $F_{3,93} = 1.02$; $P = 0.38$) or of the interaction between inoculation treatment and host line ($F_{3,93} = 0.4$; $P = 0.7528$). The model R^2 was 0.47.

period) or only by the host (for cumulative transmission over the first 3 days). The important role played by host identity in the expression of quantitative traits of disease (Salvaudon *et al.*, 2005), in addition to its more widely acknowledged qualitative ability to cause infections (Holub *et al.*, 1994), has already been recognized in this plant–parasite system as well as in host and parasite systems more generally (Lambrechts *et al.*, 2006). Such maintenance of genetic variation for life-history traits despite directional selection remains a paradox for evolutionary biology and several hypotheses have been proposed that may explain it, including mutation–selection balance, frequency-dependent selection, genotype–environment interactions, and genetic constraints due to genes with pleiotropic effects that are under conflicting selection pressures (Barton and Turelli, 1989). Even without genetic pleiotropic effects, characters may be involved in functional trade-offs such that variation in one leads to variation in the other. For such characters, correlational selection for a number of trait combinations with equivalent fitnesses can retard the erosion of genetic variation for the traits themselves (Roff and Fairbairn, 2007). Here we discuss the type of genotype–environment interactions that we observed, with parasite fitness varying as a function of the latent period of the different host and parasite combinations, and how possible trade-offs between the latent period and other parasite fitness traits may contribute to the maintenance of variation for these important life-history traits.

Genotype–environment interactions

A strain with a short latent period should have a selective advantage because it begins to transmit sooner, but if the strain with the shortest latent period is not the same in all environments or hosts, a different genotype will be selected in different contexts. We found that the latent period depended on both host and parasite identity. Phenotypic plasticity for

age at maturity of hosts in response to parasites is well known (e.g. Agnew and Koella, 1999; Lass and Bittner, 2002; Korves and Bergelson, 2003; Vizoso and Ebert, 2005). Prey may even have specific plastic responses to different types of predators (Riessen, 1999). Parasites may also respond plastically to different environmental conditions of infection (Vizoso and Ebert, 2005), though not in all cases (Agnew and Koella, 1999). Nonetheless, one of our strains was globally faster on the three host lines tested. This does not rule out, of course, that this strain would be slower on some other untested line, nor do we know the characteristics of the natural host population in which this parasite was found. Our experiment confronted parasite strains with hosts that they have probably never encountered in nature. Therefore, the details of which strain would perform better in which type of host population are not especially relevant. Here we found a significant interaction between host and parasite identity for the phenotypic expression of cumulative transmission in relation to the latent period (Table 1, Fig. 2). Indeed, the simple finding of a significant interaction suggests the possibility for the maintenance of variation in both of these important life-history traits. We therefore discuss the consequences of this interaction for the possible existence of a trade-off between latent period and cumulative transmission.

Trade-off between latent period and cumulative transmission

In our experiment, cumulative transmission over the first 3 days of spore release increased with increasing latent period (Table 1), although there was a genotype–environment interaction (Fig. 2). Genetic variation for characters under strong directional selection can be maintained if the character is involved in a trade-off with other fitness components (Roff and Fairbairn, 2007). Furthermore, if there are pleiotropic effects among many characters, variation in any one trait might be a side-effect of polymorphisms maintained by forces independent of the observed character (Barton and Turelli, 1989). Pleoitropic effects or linkage between the genes controlling the latent period and spore production were used to explain how selection for a shorter latent period reduces spore production in a fungal wheat pathogen (Lehman and Shaner, 1996). We found a phenotypic trade-off between the latent period and cumulative transmission, with increased transmission associated with a longer latent period. Taking longer to sporulate in the first place, therefore, led to more successful transmission for the primary disease foci, in accordance with theoretical expectations for trade-offs between parasite fitness traits (Ford and Seigel, 1994; Agnew *et al.*, 1999; Riessen, 1999) and with empirical investigations on physiological trade-offs between age at first reproduction and investment in that first reproduction (Stearns, 1992). Genetic correlations between these characters should be calculated on the genotype means. Here we have only three host and parasite lines respectively, giving nine combinations, too few to investigate properly genetic correlations. We note, however, that the sign of the relationship between traits for the means of the genotypic combinations did not always reflect that of the phenotypic correlations (Fig. 2), as is known in other systems (Travis, 1984; Stearns, 1992; Blanckenhorn and Heyland, 2004).

Infected hosts do not produce less seed

Seed production increased with longer latent period

Infections with longer latent periods led to more parasite transmission but also higher host seed production than did those with shorter latent periods. The experimental conditions, chosen initially to favour asexual parasite transmission and subsequently to maximize host

seed production, may have been so benign that infection had no negative effects on seed production. Nonetheless, leaves bearing spores are yellow and die earlier than non-sporulating ones (personal observation), suggesting a direct cost in lost photosynthetic biomass of infection. Since all plants were inoculated 14 days after germination, a time when they are producing new leaves that are expanding rapidly, even small differences in the timing of leaf loss at this stage may have major effects for the plants, so parasite-induced damage that happened earlier would lead to greater relative loss of photosynthetic capacity. This could explain our finding that plants suffering from infections with longer latent periods produced more seeds than those with shorter latent periods. To test this, one could experimentally remove leaves at different times to simulate the different timing of parasite-induced leaf senescence. However, asexual spore release lasted a short time only compared with the total life span of the plants, so infected plants may have compensated for the detrimental effects of the infection generally, but even better for infections with a long latent period where sporulation initiated later, explaining the lack of clear negative effects of infection under our experimental conditions.

Lack of parasite virulence

Nevertheless, the presence in *A. thaliana* of multiple specific resistance genes against this pathogen points to the parasitic nature of *H. arabidopsis* (Slusarenko and Schlaich, 2003). A lack of significant virulence, and the positive effects of infection of *A. thaliana* under experimental conditions, have already been observed for this (Salvaudon *et al.*, 2005) and other parasites (Kover and Schaal, 2002; Goss and Bergelson, 2007). Parasites can stimulate host fitness in a number of ways, including altering plant architecture and increasing branching (see de Mazancourt *et al.*, 2005) or by inducing generalized defence reactions that protect the post-infection plants from other stresses (Korves and Bergelson, 2003).

CONCLUSION

This experimental study revealed unexpected relationships between a non-lethal parasite and its host. Host identity influenced the expression of quantitative traits of the infection such as latent period and transmission and the parasite caused no detectable virulence. Moreover, we found a phenotypic trade-off between the timing and amount of transmission but genetic and phenotypic relationships revealed different relationships between these parasite traits. Trade-offs are notoriously difficult to reveal, changing with the quality of host environment and parasite genotype–environment interactions. However, the detected phenotypic trade-off may contribute to the maintenance of genetic variation in the latent period, a life-history trait important for both host and parasite fitness.

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