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LETTER

Temperature-mediated patterns of local adaptation in a natural plant–pathogen metapopulation

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Abstract

There have been numerous investigations of parasite local adaptation, a phenomenon important from the perspectives of both basic and applied evolutionary ecology. Recent work has demonstrated that temperature has striking effects on parasite performance by mediating trade-offs in parasite life history and through genotype × environment interactions. To test whether parasite local adaptation is mediated by temperature, I measured the performance of sympatric populations against allopatric populations of a fungal pathogen, *Podosphaera plantaginis*, on its host *Plantago lanceolata*, across a temperature gradient. I used data on parasite life history and epidemiology to derive fitness estimates to measure local adaptation. The results demonstrate unambiguously that trajectories of host–parasite co-evolution are tightly coupled with parasite adaptation to the abiotic habitat, as the strength, and even direction, of local adaptation varied with temperature. Patterns of local adaptation further depended on how parasite fitness was estimated, highlighting the importance of choosing relevant fitness measures in studies of local adaptation.

Keywords

Co-evolution, fitness components, genotype × environment interactions, host–parasite interaction, infection, local adaptation.

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INTRODUCTION

The astounding levels of biodiversity accounted for by parasites (Thompson 1994), their prevalence in ecosystems (Lively 2001) and the threats imposed by disease to humans, livestock and crops (Anderson & May 1991; Woolhouse *et al.* 2001; Gilligan 2002) have motivated a multitude of studies striving to understand how parasite infectivity and transmission evolve. In spatially structured systems, most studies have looked at parasite local adaptation which is indicated by a higher mean fitness of parasites on local vs. foreign hosts or by a higher mean fitness of local parasites than foreign parasites on local hosts (Gandon *et al.* 1998). The 'evolutionary snapshots' provided by local adaptation studies have become a powerful alternative to expensive and time consuming studies documenting reciprocal evolutionary change in both host and parasite populations. Indeed, many studies covering a wide range of biological interactions have reported parasite local adaptation, while there is also conflicting evidence (for reviews see Kaltz & Shykoff 1998; Dybdahl & Storfer 2003; Greischar & Koskella 2007; Hoeksema & Forde 2008).

What serves as a good measure of fitness for local parasite populations is a non-trivial question, and one that has received far too little attention in studies of local adaptation. The fitness of individual parasite genotypes is often primarily determined by two distinct features of their performance affecting transmission: infectivity, which describes the ability to successfully attack particular host genotypes, and a fitness measure describing the extent of parasite growth on infected hosts. The intensity of infection, measured as the number of transmission propagules is relatively straightforward to measure and for most interactions its relation to transmission seems plausible (cf. Ebert 1994; Hochberg 1998; MacKinnon & Read 1999). However, infectivity and transmission potential may be negatively correlated (Brodny *et al.* 1988; Thrall & Burdon 2003; Lambrechts *et al.* 2006), the development of an initially successful infection may be arrested

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(Fels & Kaltz 2006; Laine 2007b), and life-history stages leading to the production of transmission propagules may be negatively correlated (Thrall *et al.* 2005; Fels & Kaltz 2006). Hence, using a single fitness measure without accounting for variation in pathogen life-history traits and their relationships may provide a poor estimate of true fitness of parasite populations.

Parasites are not only expected to adapt to their hosts but also to their abiotic habitat (Kawecki & Ebert 2004). Variation in temperature represents one of the most ubiquitous sources of environmental variation, and parasites with a free transmission stage are considered particularly vulnerable to variation in temperature (Truscott & Gilligan 2003). Temperature has been shown to affect parasite ability to establish or maintain infection, its latency as well as its severity (e.g. Burdon 1987; Thomas & Blanford 2003; Fels & Kaltz 2006). There is increasing evidence that the effect of temperature on parasite fitness may be mediated through genotype × environment (G × E) interactions, suggesting that adaptation to biotic and abiotic habitat may be strongly linked (Ferguson & Read 2002; Price *et al.* 2004; Mitchell *et al.* 2005; Fels & Kaltz 2006). Yet, to date, no study has estimated how temperature may mediate adaptation of parasites to their hosts.

Here, using the interaction between *Plantago lanceolata* and its obligate fungal pathogen, *Podosphaera plantaginis*, I test how patterns of local adaptation are affected by variation in temperature. *Plantago lanceolata* and *Po. plantaginis* interact naturally southwest of mainland Finland in the Åland archipelago where the host populations are abundant and highly fragmented. Regionally the pathogen persists as a metapopulation with frequent extinctions and colonizations (Laine & Hanski 2006). The work was carried out in the laboratory with every attempt taken to ensure that parasite performance was measured in a manner that is relevant to the mean fitness of parasite populations in the field. In particular, the following aspects were accounted for:

Experimental design

The study was carried out by testing the performance of local pathogen populations against pathogen populations originating from other parts of the Åland Islands. Such 'local vs. foreign' designs have been proposed sufficient to infer local adaptation (Kawecki & Ebert 2004). 'Local vs. foreign' design was chosen because although fully reciprocal experimental designs are ideal for studies of local adaptation (Thrall *et al.* 2002), they require large spore stocks. Strains of *Po. plantaginis* are known to vary in their sporulation ability (e.g. Laine 2005), and choosing highly sporulating pathogen strains required of a fully reciprocal design would give a biased estimate of the local parasite population.

Genotype × temperature effects

The performance of local and foreign pathogen strains on three host populations was tested across a temperature gradient. In the interaction between *Po. plantaginis* and *Pl. lanceolata* the initial infectivity/resistance has been shown to remain stable over a temperature range (Laine 2007b). However, further parasite development is mediated by temperature often through genotype-specific interactions with the environment (Laine 2007b). The temperature range chosen for the study represents typical temperatures of the growing season in Åland.

Pathogen fitness

A fitness estimate based on several life-history stages accounts for possible negative trade-offs in the life cycle of the pathogen. *Podosphaera plantaginis* genotypes should infect as many hosts as possible within the limited season of spread to enhance their probability of overwinter survival, a critical stage in the life cycle of the pathogen. However, rapid growth may be penalized if it results in exhausting the source of nutrients for the pathogen (i.e. the trade-off hypothesis; Bull 1994; Kirchner & Roy 2002; Dybdahl & Storfer 2003). I explored both scenarios and the resulting patterns of local adaptation by calculating two different fitness estimates.

Pathogen performance in the field

Finally, to obtain an estimate of how well laboratory estimated pathogen fitness corresponds with infection prevalence in the field, the three host populations were surveyed for powdery mildew infection in the summer of 2006.

MATERIALS AND METHODS

Study system

The host plant *Plantago lanceolata* L. (Plantaginaceae) is a perennial plant that is considered an obligate outcrosser, a trait maintained both by protogyny and by an S-RNase driven self-incompatibility system (Ross 1973). The seeds of *Pl. lanceolata* have no special dispersal mechanisms; as they ripen, they are simply dropped to the ground close to the mother plant (Bos 1992). Clonally produced side rosettes are a common means of reproducing for *Pl. lanceolata* (Mook *et al.* 1992).

Podosphaera plantaginis (Castagne; U. Braun and S. Takamatsu) is an obligate powdery mildew fungus in the order Erysiphales within the Ascomycota (Yarwood 1978), which in Finland is restricted to *Pl. lanceolata* (A.-L. Laine,

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unpublished data). During the growing season the epidemic builds up following repeated asexual cycles of reproduction, as the mildew is wind-transmitted within (Ovaskainen & Laine 2006) and among (Laine & Hanski 2006) host populations. At the end of each growing season, the pathogen populations crash as most host individuals die back to root stock. The overwintering success of the powdery mildew on host plants is a highly stochastic process and hence a given pathogen strain will maximize its probability of survival by infecting as many hosts as possible during the growing season (Ovaskainen & Laine 2006). The interaction between *Po. plantaginis* and *Pl. lanceolata* is characterized by strain-specific resistance as the same host individual is resistant to some strains of the pathogen while susceptible to others (Laine 2004).

Laboratory inoculation experiment

To test whether populations of *Po. plantaginis* are locally adapted to their hosts, and whether adaptation depends on temperature, I conducted a laboratory inoculation study. Three host populations (population IDs 609, 1915 and 3350) were chosen for the experiment from different parts of the Åland islands (Fig. 1). Data from the regional surveys showed the mildew had persisted in each of the populations for three years. Furthermore, the pathogen had persisted since year 2001 in the local metapopulations each of the three host populations belonged to (classification of local metapopulations has been carried out by using a hierarchic clustering algorithm; for details see Laine & Hanski 2006).

Sampling of host and pathogen lines for the experiment

Seeds from 20 haphazardly chosen plants were collected into paper envelopes in each of the three *Pl. lanceolata* populations (IDs 609, 1915 and 3350) between 10 and 14 August in 2006. On August 17, the seeds were sown into 0.8 l pots of 50–50 potting soil-vermiculite mixture and they were grown under greenhouse conditions of 16L/8D photoperiod and + 20 ± 2 °C. From each of the three host populations I chose 10 plants, each representing a different maternal line, for the experiment.

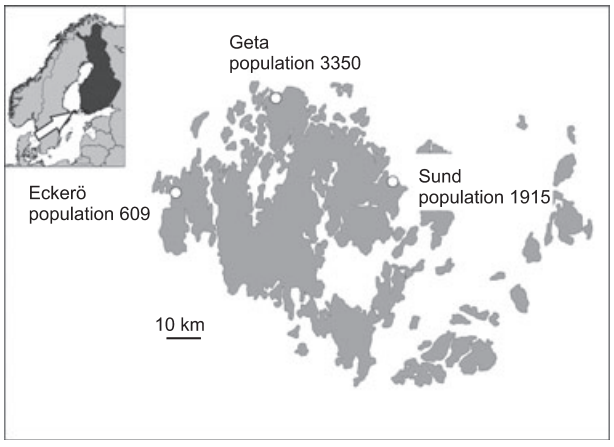

Figure 1 Locations of the three sympatric combinations of host–pathogen populations sampled for the laboratory cross inoculation experiment in the Åland Islands in Finland.

From each of the focal *Po. plantaginis* populations (IDs 609, 1915 and 3350), 20 infected plants were sampled as detached infected leaves onto Petri dishes containing moist filter paper on 25–26 of August 2006. Additional 5–10 bulk samples of the powdery mildew were collected as infected leaves from each of the *Po. plantaginis* populations for the allopatric pairings in the study (IDs 877, 2719 and 3484). The infectivity/sporulation profiles of the collected samples were scored on a set of eight host plants known to vary in their resistance to *Po. plantaginis*. For populations 609 and 1915, three strains that were determined to be genetically diverse based on their infection scores, were chosen for the experiment and pathogen population 3350, which was larger than the other two (see Results), was represented by four fungal strains. In addition to the sympatric strains, each of the three host populations was also challenged by strains of three different allopatric populations, each represented by two strains. The allopatric populations chosen for the study differed for the host populations and they were always located 10 km or further away from the host populations (the pathogen population IDs used for each pairing are presented in Table S4).

Inoculation experiment

In the laboratory, the mildew isolates were purified and propagated according to methods described in Laine 2007b. In the experiment a detached leaf from each host plant was exposed to a single pathogen strain. Infection outcome and its timing on detached leaves are similar to leaves still intact with the plant (Laine, unpublished data). The leaf was placed on moist filter paper in a 9-cm Petri dish, and spores from an infected leaf were gently brushed with a fine paint brush over the entire surface of the healthy leaf. Colonies of similar age and size (*c.* 1.0 cm Ø) were used for the inoculations in order to obtain as similar spore densities as possible. Inoculated dishes were placed in three growth chambers at +17, +20 and +23 °C temperatures with a 16L/8D photoperiod. Each host–pathogen combination was repeated for each of the three temperatures. The experiment was carried out in two parts. The second trial was initiated one week after the first one had ended, and the growth chambers were assigned different temperatures from the first trial to minimize any possible effect of the growth chamber. The subset of inoculations (4%) not producing a response (fungal growth or leaf necrosis) was repeated.

The dishes were checked daily and watered if necessary. The following characteristics of the pathogen life cycle were

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recorded under a dissecting microscope daily starting on the fourth day after inoculation until the twelfth day: Whether or not the pathogen successfully infected its host, the number of days it took for the pathogen to form hyphae, the number of days required for spore production to take place, and finally the overall spore production level (i.e. transmission potential) at day 12. Spore production was measured as a categorical variable ranging from mycelial growth to heavy spore production. To help identify the resistant responses, leaf necrosis was also recorded as a categorical variable. Both keys are described in detail in Laine 2007b. A resistant response was identified when no fungal growth could be observed or when fungal development was arrested at an early mycelial stage with clear necrotic coloration.

Estimating fitness for *Po. plantaginis*

I combined laboratory measured sporulation times (l) and rates of spore production (m) to calculate the basic reproductive capacity of *Po. plantaginis* strains over the growing season. Hence, the fitness estimate accounts not only for the maximum spore production level of the infection but also for the time in which it was attained (i.e. both germination and sporulation times). For the estimation I assumed that a single plant was infected in the beginning of the growing season, and calculated the cumulative number of plants that would become infected over the entire growing season. I assumed that the density of infected plants remained low enough for the effect of saturation to be neglected, and hence each infectious plant was always assumed to cause m new infections each day. This assumption is supported by the results of a study modelling the dynamics of local epidemics in this system which demonstrated the importance of seasonality in this system – conditions become unsuitable for infection development before all available hosts are infected (Ovaskainen & Laine 2006). I considered two alternatives with respect to the duration of the infectious period. Fitness estimate f_1 assumes that the mildew infection on a given plant does not exhaust its nutrients and is able to maintain the attained spore production level throughout the growing season. Fitness estimate f_2 assumes that nutrient availability in the infected plant is limited and that spore production will dampen off as the source is depleted; in other words, there is a penalty for exploiting the host too efficiently. In this case I assumed that spore production lasts for d = 36, 18, 9, 6 days for strains with spore production level m = 1, 2, 4, 6, respectively.

The fitness calculation was implemented through a simple algorithm that kept track of the amount and age of infections throughout the growing season, which was estimated to be 60 days. Denoting by $s_i(t)$ the number of plants that were infected i days before the current day t (in the sense of a spore landing on the plant i days ago), the initial condition is given by $s_0(1) = 1$ and $s_i(1) = 0$ for $i > 0$, and the aging of the existing infections is described by $s_i(t + 1) = s_{i-1}(t)$. New infections are initiated due to the existing infections that are currently producing spores, so that $s_0(t+1) = m\sum_{i=l}^{u} s_i(t)$ where the upper limit of the summation is $u = 60$ in case of f_1 and $u = \min(l + d - 1,60)$ in case f_2. In both cases, the fitness estimate was calculated as $f = \sum_{i=0}^{60} s_i(60)$.

Data analysis

I analysed patterns of local adaptation of *Po. plantaginis* in the three host populations at three temperatures for infectivity, ability to sporulate and spore production rate as well as the two fitness estimates, f_1 and f_2, with generalized linear mixed models (GLMM) as implemented in SAS 9.1 (SAS Institute 1999). For the analyses the fitness estimates were log-transformed to meet the assumption of normally distributed errors. However, even after the transformation the data were bimodally distributed with a peak for the infections with zero fitness (non-compatible or compatible infections failing to sporulate) while for compatible, sporulating infections the data were normally distributed following the transformation. Hence, I analysed separately whether the fitness of inoculations was zero or not assuming a binomial distribution of errors and a logit link function. For compatible infections, I analysed whether or not they sporulated assuming a binomial distribution of errors and a logit link function. The analyses of the two fitness estimates, f_1 and f_2, were carried out only for the compatible sporulating infections (fitness > 0). In all models host population, pathogen population, and temperature were defined as fixed factors. In an experiment such as the one here with three fixed levels of temperature, it is more appropriate to treat it as a class variable rather than a continuous one. To account for the fact that sympatry/allopatry depends on the host population (i.e. population 1915 was sympatric when infecting hosts from population 1915 but allopatric on hosts from the other populations), sympatry/allopatry was nested within host population and defined as a fixed term in the models. Host and pathogen genotypes, nested within their respective source populations, were defined as random variables. The host genotype × pathogen genotype combination that was replicated across the three temperatures was accounted for as a repeated measures response complying a compound symmetry structure (Littell *et al.* 1996). I analysed spore production (infection categories ranging between 1 and 4; see 'Laboratory inoculation experiment') using an ordinal regression to account for the categorical nature of the response variable and the ordered structure of the categories. For all models, the timing of the inoculations was

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included in the model but as it had no statistically significant effect on the results, it was not included in the final analysis. I started out with full models and dropped non-significant interactions from the models in a backward stepwise manner. The initial full model was the same for all response variables, including a full design of interactions among variables as well as the timing of the inoculation.

To test how the different fitness estimates were associated, and whether the associations were mediated by temperature, I used ANCOVA where the mean performance of pathogen strains was associated in all possible combinations with the other components of pathogen performance (infectivity, fitness > 0, and ability to sporulate for compatible infections, spore production, f_1 and f_2). Temperature was included as a covariate in the model and when significant, interactions were included in the model. The models were weighted by 1 − SE of the means.

Surveying pathogen prevalence and weather data

On 18–24 of August in 2006, infection prevalence in the three study populations (population IDs 609, 1915 and 3350) was estimated by using both transects and quadrats. In each population three transects, evenly spaced, were run through the population to encompass as much of the host population as possible. The position of each *Pl. lanceolata* individual in contact with transects was mapped and its infection status was recorded. Additional data were gathered within ten haphazardly placed 0.5 × 0.5 m quadrats where the number of *Pl. lanceolata* individuals was counted and their infection status was recorded. The data from the transects and quadrats were used to provide estimates of host population size and infection prevalence.

Data on temperature at 70 cm above ground level for the three study populations were provided by the Finnish Meteorological Institute. The temperature estimates for the months of June, July and August were produced by the AROME weather predictor (Fig. 3). Two estimates were obtained for each day; one for 12:00 o'clock noon and one for 24:00 midnight. Differences in temperature among the populations were analysed as ANCOVA in the PROC MIXED procedure in SAS 9.1 (SAS Institute 1999). Population and time of day were explanatory variables in the model and the Julian date was included as a covariate. Significant interactions were included in the model.

RESULTS

Patterns of local adaptation of *Po. plantaginis*

Infectivity, the proportion of genotypes a given pathogen strain is capable of infecting, was not affected by temperature (Table 1). There was, however, a significant difference in the proportion of genotypes the sympatric strains were capable of infecting compared with the allopatric pathogen strains (Table 1). The pattern of local adaptation varied for the pathogen populations when performance was measured as infectivity: When the effect of sympatry–allopatry was analysed separately with a GLMM for each population, only pathogen population 1915 showed signs of local adaptation by infecting a higher proportion of the hosts than the allopatric strains ($F_{1,197} = 10.97$, $P = 0.001$). Pathogen population 609 appeared to be locally maladapted, but this trend was not statistically significant ($F_{1,164} = 10.97$, $P = 0.074$). Infectivity of pathogen population 3350 did not differ from the allopatric pathogen populations ($F_{1,189} = 10.97$, $P = 0.934$).

When the ability of pathogen populations to cause an infection that leads to sporulation was analysed as a binary fitness function, this was most strongly affected by temperature and there was only a slight, statistically non-significant trend for differences among sympatric and allopatric pathogen populations to successfully sporulate on their hosts ($P = 0.093$; Table S1). When the ability to sporulate was analysed only for the compatible responses, temperature was the only variable that significantly had an effect on whether or not a given infection successfully sporulated (Table S2). The measurements of spore production did not produce evidence of local adaptation (Table S3). Spore production was significantly affected by temperature and both the host and the pathogen populations differed significantly in how they responded to variation in temperature (Table S3).

The first fitness estimate, f_1, accounting for overall spore production level of pathogen strains, and how fast it was achieved, was extremely sensitive to temperature (Fig. 2; Table 2). While in part the effect of temperature was direct, it was also mediated by how differently pathogen populations responded to differences in temperature. This effect was so strong that the strength and direction of local adaptation varied with temperature in the three host populations (Fig. 2; Table 2). While pathogen population 609 outperformed the allopatric populations at +17 °C, it appeared maladapted at +20 °C and +23 °C (Fig. 2). Fitness of pathogen population 1915 was higher than that of the allopatric populations at +20 °C and +23 °C, yet it was outperformed by only one allopatric population at +17 °C. The trend of local adaptation for pathogen population 3350 was similar to that of pathogen population 1915 (Fig. 2).

The second fitness estimate, f_2, that included a penalty for exploiting the host too efficiently, yielded patterns very similar as those produced by f_1 but the differences among the pathogen populations were reduced (Fig. 2). Here temperature was the only variable that had an effect on the fitness of pathogen populations and there were no

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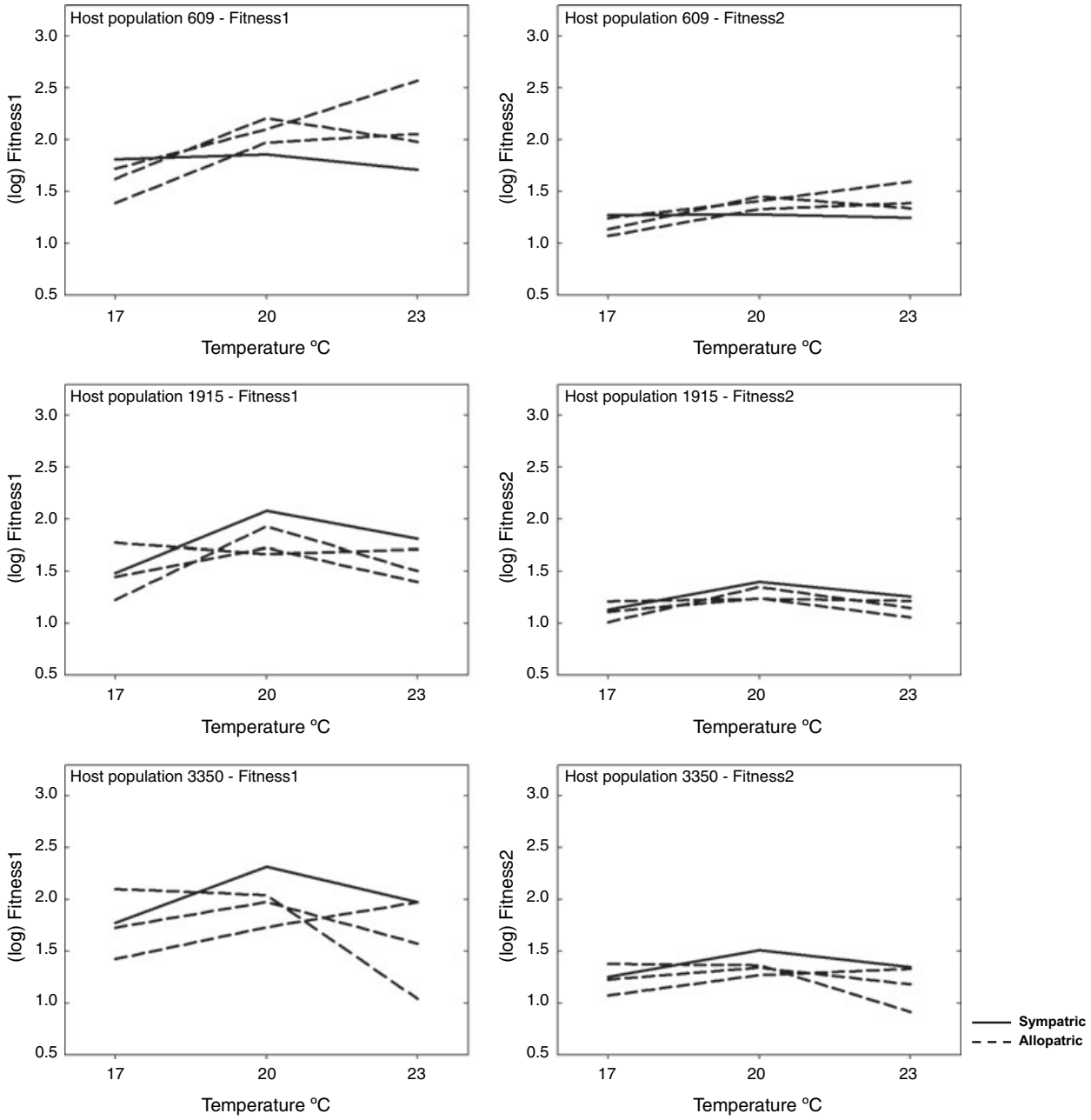

Figure 2 Reaction norms for the means of the two fitness estimates, f_1 and f_2, of the sympatric (solid line) and allopatric pathogen populations (dashed lines) infecting *Plantago lanceolata* across a temperature gradient. Means and their standard errors are presented in Table S4.

statistically significant differences in performance among the sympatric and allopatric pathogen strains (Fig. 2; Table 2) For averages and their standard errors of the two fitness estimates, see Table S4.

In general, no strong positive or negative associations emerged between the different components of pathogen performance (Fig. S1). Only spore production and the fitness estimates were strongly positively associated, as would be expected given that the fitness estimates are in part based on the spore production ability of the pathogen strains. Also, the two estimates of fitness, f_1 and f_2, were strongly positively correlated (Fig. S1).

Pathogen prevalence within host populations and temperature variation

Both the transects and quadrats indicated that the prevalence of *Po. plantaginis* was highest in population 3350

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Table 1 Results of a GLMM analysing the infectivity of sympatric and allopatric mildew populations on three host populations

Source	Estimate (± SE) for random effects	*Z*/*F*	*P*-value
Host strain	1.76 ± 0.80	2.18	0.01
Pathogen strain	1.00 ± 0.90	1.11	0.134
Compound symmetry	0.63 ± 0.08	8.36	< 0.0001
Residual	0.01 ± 0.01	13.82	< 0.0001
Sympatry–allopatry (host population)$_{2,533}$		4.65	0.01
Host population$_{2,27}$		0.04	0.965
Pathogen population$_{4,7}$		0.47	0.756
Temperature$_{2,533}$		2.03	0.132

Wald's *Z*-statistic is given for random effects and the *F*-statistic is given for fixed effects.

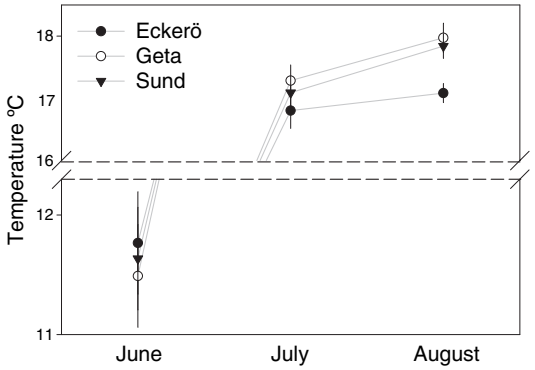

Figure 3 Temperature (measured at 12:00 o'clock) during June–August in the three study areas: Eckerö, Geta and Sund.

(Table 3). The quadrats may give a more realistic picture of infection prevalence because the distribution of the mildew is highly aggregated within the host populations (Ovaskainen & Laine 2006). When percentage of infected plants was averaged over all quadrats within each of the host populations, infection prevalence was highest in population 3350 (Table 3). The number hosts counted in the quadrats in populations 609, 1915 and 3350 were 363, 148 and, 492 respectively, with population 3350 supporting the highest host density. The patterns of infection prevalence did not seem to correspond very tightly with laboratory estimated degree of local adaptation. In other words, the most locally adapted pathogen population was not the one that infected the most hosts in its sympatric population in the wild.

Ambient temperature measured at 70 cm above ground varied significantly between the populations ($F_{2,543}$ = 3.2, P = 0.041). Furthermore, the day and night time temperatures varied among the study sites as indicated by the significant interaction 'population × time' ($F_{2,543}$ = 3.77, P = 0.024). Also, the rankings of the populations changed with season, with Eckerö (Population ID 609) being the warmest site in June, but having the coolest temperatures in July and August (marginally significant interaction 'population × Julian date', $F_{2,543}$ = 2.92, P = 0.054; Fig. 3).

Table 2 Results of GLMMs analysing the fitness estimates (f_1 and f_2) of sympatric and allopatric mildew populations on three host populations

Source	Estimate (± SE) for random effects	*Z*/*F*	*P*-value
Fitness$_1$			
Host strain	0.05 ± 0.03	1.79	0.037
Pathogen strain	0.003 ± 0.01	0.35	0.364
Compound symmetry	−0.03 ± 0.04	−0.84	0.403
Residual	0.74 ± 0.06	11.89	< 0.0001
Sympatry–allopatry (host population)$_{3,392}$		1.38	0.25
Host population$_{2,27}$		1.79	0.186
Pathogen population$_{5,7}$		1.11	0.434
Temperature$_{2,392}$		13.9	< 0.0001
Temperature × sympatry–allopatry$_{10,392}$		2.09	0.0245
Fitness$_2$			
Host strain	0.02 ± 0.01	1.64	0.05
Pathogen strain	0.00 ± 0.003	0.12	0.45
Compound symmetry	−0.01 ± 0.01	−1.00	0.317
Residual	0.29 ± 0.02	12.31	< 0.0001
Sympatry–allopatry (host population)$_{3,412}$		1.31	0.27
Host population$_{2,27}$		1.93	0.164
Pathogen population$_{5,7}$		1.08	0.445
Temperature$_{2,412}$		34.04	< 0.0001

Wald's *Z*-statistic is given for random effects and the *F*-statistic is given for fixed effects.

Table 3 Prevalence of *Podosphaera plantaginis* in the three host populations estimated as the averaged percentage of infected plants along the transects and within quadrats

Population	Transect (%)	Quadrat (%)	Quadrats with mildew (%)
609	8.8	10.8	40
1915	6.7	5.0	40
3350	33	18.3	70

DISCUSSION

The three populations of *Po. plantaginis* differed in their pattern of local adaptation. Furthermore, for individual pathogen populations, patterns of local adaptation varied

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with the fitness estimate that was used as well as incubation temperature. While the potential of parasites' fitness to be mediated by their adaptation to their abiotic habitat through G × E interactions has been previously recognized (Kawecki & Ebert 2004; Ridenhour & Nuismer 2007), this is the first study to experimentally demonstrate that parasite local adaptation to their hosts is tightly coupled with their adaptation to temperature. While fine spatio-temporal temperature variation may be very important for pathogens locally, the mean temperatures of the study sites were reflected in the patterns of local adaptation obtained for the three pathogen populations.

The differences among pathogen populations in their degree of local adaptation observed here is in accordance with a previous study of local adaptation of *Po. plantaginis* (Laine 2005). Detecting variation and even parasite maladaptation in itself is not surprising given the cyclical nature of co-evolution and the potential of gene flow to keep local populations in a maladapted state (Kaltz & Shykoff 1998; Thompson *et al.* 2002; Forde *et al.* 2004; Morgan *et al.* 2005; Thompson 2005). However, interestingly here I found that detecting local adaptation depends on how pathogen performance is measured even for individual pathogen populations, which highlights the importance of what we use as an estimate of pathogen performance in studies of local adaptation. Fitness estimate f_1 accounted for the maximum spore production of infections and how fast it is achieved. This should be a relevant measure for *Po. plantaginis* given the limited growing season followed by highly stochastic probability to successfully overwinter. Furthermore, it accounts for the negative association of spore production ability and sporulation time across a temperature gradient (Laine 2007b). Estimating parasite performance with f_1 provided evidence of local adaptation through an interaction with temperature so that the magnitude and even direction of the difference between sympatric and allopatric pathogen strains varied with temperature. The pathogen population (ID 609) from Eckerö, where coolest temperatures were measured in July and August, also reached its highest fitness at the lowest temperature on its sympatric hosts. This suggests that the local pathogen population had adapted to slightly cooler climate in sympatry. While it should be noted that the recent temperatures may not be representative of the historical selective regime that produced the current distribution of genotypes present in the populations, this result does demonstrate the ability of these genotypes to maximize their fitness according to temperatures of the growing season during which they were sampled. Also, given that the turnover rate of local populations is very high (Laine & Hanski 2006), they will need to adapt to local climatic conditions relatively fast. The current results demonstrate that pathogen populations are capable of maximizing their mean performance in some environments and that by accounting for variation in temperature in the laboratory makes it possible to find evidence of co-evolution that could be missed under constant conditions.

However, whether the ability to produce as many transmission propagules as quickly as possible is the true estimate of fitness for *Po. plantaginis* is difficult to say. Pathogen growth and transmission potential are assumed to be under conflicting evolutionary selection pressures, as locally adapted parasites should infect a maximum number of hosts but not necessarily cause greatest damage (Kirchner & Roy 2002; Dybdahl & Storfer 2003). Although mildew infection alone is unlikely to cause host mortality during the summer, infected plants will have less nutrients to store in the underground root stocks at the end of the growing season, which may have negative effects on establishment, growth and reproduction in the following summers (Bushnell 2002). The second fitness estimate, f_2, that penalized highly sporulating infections, produced patterns of local adaptation similar to those by f_1 but no statistically significant evidence for local adaptation emerged. The difference in the obtained patterns of local adaptation by the two different fitness estimates could mean two things: First, local pathogen populations are not adapted in the sense that they exploit their hosts at a too fast rate to support their own growth and sporulation in the long term, or second, even the fastest growing strains do not cause damage that would result in a negative feedback to the pathogen through reduced host density. When infection coincides with severe drought it may induce host mortality (Laine 2004), yet under normal conditions there are no data to support the first conclusion. Hence, it is plausible that the optimal pathogen strategy varies though space and time with changing environmental conditions.

What is an appropriate fitness estimate may depend very much on the characteristics of the interaction (Refardt & Ebert 2007; Sicard *et al.* 2007). Infectivity has been the most common estimate of parasite performance in studies of local adaptation (Greischar & Koskella 2007). However, as infectivity may be undermined by poor growth or at worst, no reproduction (Fels & Kaltz 2006; Laine 2007b), it may not be the best possible estimate of parasite fitness, or at least not the only one that should be used.

The ability to efficiently infect sympatric host genotypes may reflect selective establishment that in itself should not be interpreted as reciprocal co-evolution between the parasite and its host. Arriving genotypes unable to infect the local host genotypes will be filtered out and the established parasites will be adapted, to at least some degree, to their local host population (Burdon 1993). Here, the three pathogen populations showed different patterns of local adaptation when their performance was measured as infectivity. In accordance with earlier results, infectivity

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remained robust over the temperature gradient (Laine 2007b). This suggests that there is little potential for temperature to influence evolutionary trajectories of infectivity. The weak associations between infectivity and parasite growth observed here, and in other studies (Laine 2005; Sicard *et al.* 2007), strongly suggests that the genetic basis and the effects of selection may be quite different for the different traits of parasite performance.

Adapting to their hosts is a prerequisite for obligate parasites for their survival and reproduction. Adapting to the physical environment may be of nearly equal importance and we should expect parasites to achieve their fitness optimum in their native environment (Kawecki & Ebert 2004). It has been suggested that studies of local adaptation should be conducted in the field as reciprocal transplant experiments rather than in the laboratory where individuals are removed from their natural environment where their fitness evolved (Ridenhour & Nuismer 2007). Two experiments with an identical sampling scheme carried out as a field transplant experiment and laboratory cross-infection study with *Pl. lanceolata* and *Po. plantaginis* demonstrated that indeed the two approaches are in discordance in the measures of local adaptation they provide (Laine 2007a). However, for this interaction it was concluded that field studies may fail to detect mean population local adaptation of *Po. plantaginis* because relevant life-history stages of the pathogen are difficult to measure in the field and furthermore, field infections may be attributed to a single highly infective clone, which gives a poor estimate of population level fitness (Laine2007a). Also in this study, there was no tight correspondence between field estimated pathogen prevalence and degree of local adaptation measured in the laboratory suggesting that only a few highly infective strains may cause a large proportion of infections in the field. Hence, for many interactions a good alternative to field transplant experiments may be to incorporate relevant environmental variation into the laboratory experiment.

It is now becoming increasingly clear that parasite evolution is not independent of their natural abiotic habitat. Hence, changes in the physical environment, e.g. due to climate change or increased fragmentation, may have far reaching consequences given that co-evolution generates biological diversity, molds the ecological structure of communities and drives tight co-adaptation between species (Thompson 2005). The sensitivity of the evolutionary trajectories of parasite transmission potential to variation in temperature highlights the potential of ongoing climate change to impact the risks imposed by disease.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Figure S1 Associations between the different estimates of pathogen fitness.

Table S1 Results of a GLMM analysing whether the fitness of allopatric and sympatric populations of *Podosphaera plantaginis was* zero (both incompatible responses and compatible responses failing to sporulate) or greater than zero (compatible responses producing spores).

Table S2 Results of a GLMM analysing whether the compatible responses of allopatric and sympatric populations of *Podosphaera plantaginis* infecting *Plantagolanceolata* sporulated or not.

Table S3 Results of mixed effects ordinal regression analysing the spore production of allopatric and sympatric populations of *Podosphaera plantaginis* infecting *Plantago lanceolata*.

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Table S4 The averages and standard errors of means of estimates f_1 and f_2 of the pathogen populations on three host populations.

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