New Phytologist

Research

Below-ground abiotic and biotic heterogeneity shapes above-ground infection outcomes and spatial divergence in a host–parasite interaction

Ayco J. M. Tack[1,2], Anna-Liisa Laine[2], Jeremy J. Burdon[3], Andrew Bissett[3] and Peter H. Thrall[3]

[1]Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91 Stockholm, Sweden; [2]Metapopulation Research Group, Department of Biosciences, University of Helsinki, PO Box 65 (Viikinkaari 1), FI-00014 Helsinki, Finland; [3]CSIRO Agriculture Flagship, GPO Box 1600, Canberra, ACT 2601, Australia

Author for correspondence:
Ayco J. M. Tack
Tel: +46(0)8 16 39 59
Email: aycotack@gmail.com

Received: *28 January 2015*
Accepted: *13 March 2015*

New Phytologist (2015) **207:** 1159–1169
doi: 10.1111/nph.13408

Key words: above-ground–below-ground interactions, coevolution, ecotype, environmental heterogeneity, habitat type, host–pathogen, genotype × genotype × environment (G × G × E), tradeoffs.

Summary

- We investigated the impact of below-ground and above-ground environmental heterogeneity on the ecology and evolution of a natural plant–pathogen interaction.
- We combined field measurements and a reciprocal inoculation experiment to investigate the potential for natural variation in abiotic and biotic factors to mediate infection outcomes in the association between the fungal pathogen *Melampsora lini* and its wild flax host, *Linum marginale*, where pathogen strains and plant lines originated from two ecologically distinct habitat types that occur in close proximity ('bog' and 'hill').
- The two habitat types differed strikingly in soil moisture and soil microbiota. Infection outcomes for different host–pathogen combinations were strongly affected by the habitat of origin of the plant lines and pathogen strains, the soil environment and their interactions. Our results suggested that tradeoffs play a key role in explaining the evolutionary divergence in interaction traits among the two habitat types.
- Overall, we demonstrate that soil heterogeneity, by mediating infection outcomes and evolutionary divergence, can contribute to the maintenance of variation in resistance and pathogenicity within a natural host–pathogen metapopulation.

Introduction

Turesson (1922) and Clausen *et al.* (1940) classic transplant experiments inspired a large number of studies that demonstrated plant and animal adaptation to their local abiotic environment (Bradshaw, 1952, 1984; Linhart & Grant, 1996). Such adaptive processes may also have pronounced consequences for ecological dynamics (Ford, 1975), a view that has recently received increasing empirical support (Schoener, 2011). However, despite the realization that ecology and evolution can be strongly intertwined, we still lack fundamental insights into how environmental heterogeneity might affect the ecoevolutionary dynamics between species, especially in natural systems. From an ecological perspective, the outcome of species interactions can be strongly mediated by the abiotic and biotic environment (Chamberlain *et al.*, 2014). Such environmental mediation of interaction outcomes then provides the blueprint for spatial and temporal variation in evolutionary and coevolutionary dynamics (Thompson, 2005), which may feed back to further shape the ecology of species interactions. For example, with respect to host–parasite interactions, theoretical studies have demonstrated that the environment may affect coevolutionary trajectories and promote the long-term maintenance of variation in resistance and infectivity (Gavrilets & Michalakis, 2008; Mostowy & Engelstädter, 2011; Tellier & Brown, 2011; Poisot *et al.*, 2012).

Pathologists have long realized that the environment (Pasteur *et al.*, 1878), host genotype (Biffen, 1905), parasite genotype (Barrus, 1911) and their interactions (Flor, 1956; McNew, 1960; Wolinska & King, 2009) jointly determine the outcome of host–parasite interactions. More recently, there is increasing evidence for geographical variation in coevolutionary dynamics and patterns of local adaptation (Thompson, 2005, 2013). Microcosm (Forde *et al.*, 2004; Vogwill *et al.*, 2009; Lopez Pascua *et al.*, 2012) and field studies (Laine, 2006, 2008) have assessed how a range of ecologically relevant variables such as local encounter rates, productivity and temperature might drive spatial variation in coevolutionary dynamics and strengthen patterns of local adaptation. Nonetheless, several critical gaps in our knowledge remain. First, the majority of studies have focused on above-ground spatial heterogeneity. Given widespread documentation of spatial variation in soil abiotic and biotic conditions (Ettema & Wardle, 2002; Tedersoo *et al.*, 2014), there is a need to investigate how below-ground heterogeneity affects above-ground species interactions (Bonte *et al.*, 2010; Fones *et al.*, 2010). Secondly, identifying the environmental factors shaping species interactions in natural studies remains a challenge, as species exist

within a complex and changing set of environmental conditions. Ultimately, we need to assess spatiotemporal variation in environmental conditions, as well as their direct and interactive effects on the ecology and evolution of species interactions.

Using the flax rust (*Melampsora lini*)–wild flax (*Linum marginale*) system, we have previously demonstrated rapid coevolutionary dynamics (Thrall *et al.*, 2012) and strong pathogen adaptation at the host ecotype level (Laine *et al.*, 2014). In this study, we use an inoculation experiment to assess the contribution of (nonhost) environmental variation in shaping infection outcomes and evolutionary divergence. More specifically, we aim to determine the impact of natural environmental variation on infection outcomes and evolutionary dynamics of *L. marginale* and its specialized rust pathogen *M. lini* across two distinct habitat types ('bogs' and 'hills'), which frequently occur in close proximity within the subalpine region in southeastern Australia. Following an initial assessment of environmental factors, we identified that soil moisture and soil microbial community structure showed the most pronounced differences among the habitat types. We then used these key factors in a multifactorial cross-inoculation experiment to investigate how environmental differentiation among habitat types might affect infection outcomes and coevolutionary trajectories. Our findings demonstrate the key role of the environment in the outcome of ecological interactions and the maintenance of variation in resistance and pathogenicity in host and parasite associations.

Materials and Methods

Study system

The native flax *Linum marginale* Cunn. is a perennial herbaceous plant growing throughout southern Australia. Within the Kosciuszko National Park in southern New South Wales, the plant mainly grows in montane areas and subalpine frost hollows. In these areas, flax grows as two distinct ecotypes: a hill ecotype that is found on well-drained hill sites and a bog ecotype that occurs in boggy areas and along stream courses (Carlsson-Granér *et al.*, 1999). Morphologically, plants from the two habitat types can be distinguished by their overall size, the number of basal shoots produced, and the length and adherence of sepals to buds and capsules (Carlsson-Granér *et al.*, 1999).

The flax rust *Melampsora lini* (Ehrenb.) Lév. is an obligate fungal pathogen that, in Australia, is a specialist on *L. marginale* (Lawrence, 1989; Lawrence & Burdon, 1989). Spores are wind-dispersed, and when infection is successful, the first symptoms can be detected by highly localized light-green flecks on the leaves, which turn into orange-coloured lesions (uredinia or pustules) in which urediniospores are produced asexually. This part of the life cycle takes *c.* 12–14 d during the peak growing season, and multiple pathogen generations take place during a season. In contrast to the lowlands, the sexual cycle has not been observed in montane areas like Kosciuszko National Park. Hence, the pathogen overwinters on sporadic green leaves on which a few uredinia may survive during winter when the majority of plants die back to their root stocks (Jarosz & Burdon, 1992).

Significant genetic divergence and fixed allelic differences have been documented between the host plants originating from the bog and hill habitat types (Thrall *et al.*, 2001). It has also been shown that there is extensive among-habitat phenotypic (Carlsson-Granér *et al.*, 1999; Thrall *et al.*, 2001) and genetic differentiation (Laine *et al.*, 2014) for the pathogen. We therefore refer to plant lines and pathogen strains from the two habitat types as 'ecotypes'. Previous studies on the wild flax–flax rust system have documented considerable variability in the incidence and severity of epidemics across populations and years, with high amounts of disease reducing host survival (Jarosz & Burdon, 1992). Furthermore, marked differences within and among populations within and among years in pathogen infectivity (Burdon & Jarosz, 1992) and host resistance (Burdon & Thompson, 1995; Thrall *et al.*, 2001) are associated with strong local adaptation of the pathogen to its local host ecotype (Laine *et al.*, 2014) and rapid coevolutionary dynamics (Thrall *et al.*, 2012).

Study sites

For this study, we focused on the same eight populations of *L. marginale* used by Laine *et al.* (2014; Fig. 1). Four of these populations (CBL_H, CEM, G3 and SH2) occurred in characteristic hill habitat, whereas the other four (CBL_B, G2, P1, PS) occurred in typical bog habitats. To account for spatial variation, each habitat type was represented within distinct areas (separated by > 10 km) within the Kosciuszko region (Fig. 1).

Identification of key environmental differences among the habitat types

We first identified the key environmental factors that differed among the habitat types. For this, we assessed soil moisture, ambient temperature, soil chemistry, and soil microbial biota.

Soil moisture In each of the eight plant populations, we randomly collected five 10-cm-deep soil cores with field weights between 30 and 80 g into plastic bags, which were then sealed. Collections were made at two time points: at the onset of epidemics on 14 December 2009 and again during the peak of epidemics on 11 January 2010. Percentage soil moisture was determined by weighing the soil core mass before and after drying in an oven at 65°C for 24 h.

Ambient temperature Temperature loggers (HOBO Pendant® Temperature/Alarm Data Logger 8K – UA-001-08; Onset Computer Corp., Bourne, MA, USA) were placed at the study sites between 12 January and 23 March 2010. The loggers measured temperature every 30 min and were placed 25 cm above the ground to measure temperature similar to that experienced by *Linum* plants and developing rust infections. At site CBL-B the logger was stolen halfway through the growing season, and hence temperature data are available for the remaining seven sites only.

Soil chemistry To test for differences in soil abiotic conditions between the habitat types, we analysed a single pooled soil sample

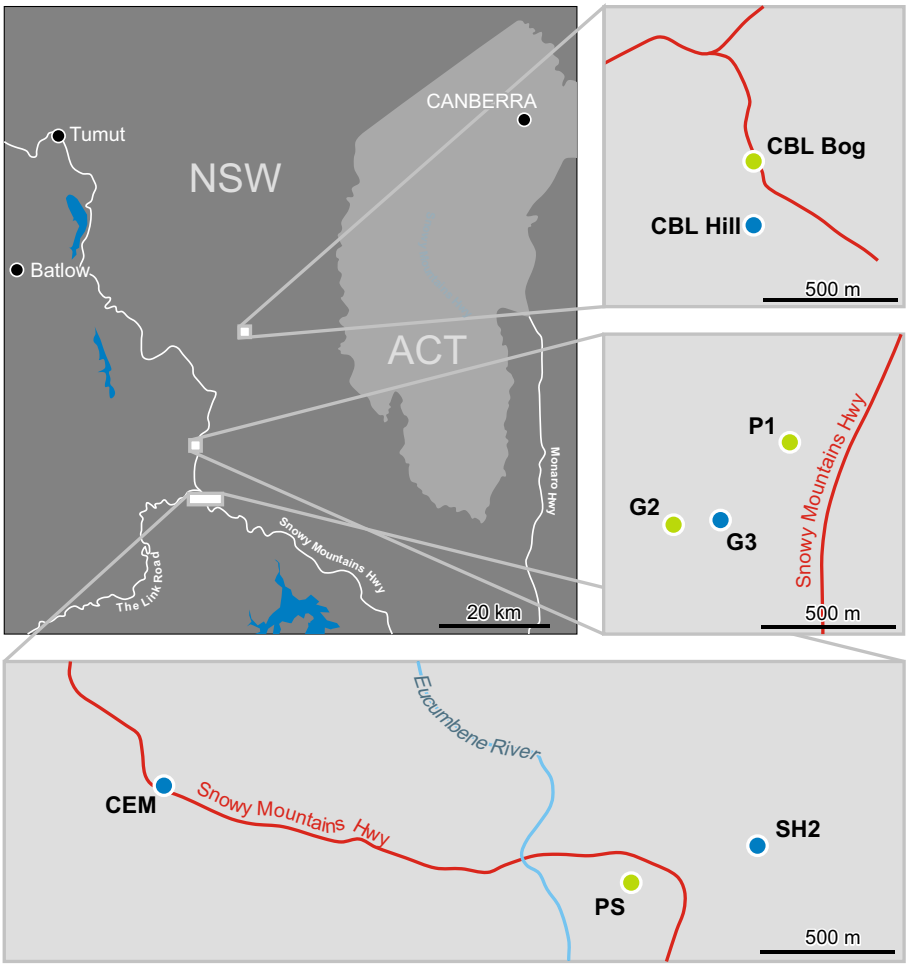

Fig. 1 Map of the study area. The large map illustrates New South Wales (NSW) and the Australian Capital Territory (ACT) in southeast Australia. Within NSW, three areas were selected with at least one bog and one hill population. Green circles, bog populations; blue circles, hill populations.

for each of the eight populations. Each pooled soil sample consisted of three 10-cm-deep soil cores taken from random locations within each of the respective populations. Samples were collected on 25 February 2010, and following air-drying and homogenization of the cores from each population, they were analysed by Incitec Pivot Ltd, Southbank, Australia (based on 500 g samples) for pH, electric conductivity, organic carbon, soil texture, nitrate, phosphorus, potassium, calcium, magnesium, sodium, aluminium, chloride, copper, iron, manganese, zinc and sulphate (Supporting information Table S1).

Biotic component of the soil Soil community composition (bacteria and fungi) at the bog and hill sites was assessed using terminal restriction fragment length polymorphism (T-RFLP). T-RFLP is a polymerase chain reaction-based community fingerprinting method that is commonly used for comparative microbial community analyses (Van Dorst *et al.*, 2014). The method assumes that individual terminal restriction fragment lengths are representative of unique operational taxonomic units (OTUs) present within the sampled community.

DNA was extracted in duplicate from 0.25 g aliquots of soil and pooled, using a Power Soil DNA Isolation kit (Mo Bio Laboratories Inc., Solana Beach, CA, USA) according to the manufacturer's instructions and quantified spectrophotometrically (NanoDrop ND-1000; Thermo Scientific, Wilmington, DE, USA). Multiplex T-RFLP targeting bacteria and fungi was performed using primers 27f–519r (bacteria) and ITS1f–ITS4r (fungi) as described in Singh *et al.* (2006). Each 50 µl PCR reaction contained *c.* 10 ng template, and was completed with Platinum Taq DNA polymerase (Invitrogen) according to the manufacturer's instructions: 1.5 mM $MgCl_2$, 0.2 mM each high-performance liquid chromatography-purified primer, 1× PCR buffer, 1 U of Taq DNA polymerase, and 50 µM of each dNTP (Promega). PCR comprised one cycle of 94°C for 3 min; 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s; and a final extension step of 72°C for 10 min. Triplicate PCRs were pooled, isopropanol-precipitated, quantified as described, and 70 ng digested independently with the restriction endonucleases *Msp*I, *Ali*I and *Hinf*I. Digests were isopropanol-precipitated, resuspended in 10 µl formamide containing LIZ600 size standard (Applied Biosystems, Foster City, CA, USA), denatured at 95°C (3 min) and separated by capillary electrophoresis (Abi Prism 3130xl Genetic 110 Analyzer; Applied Biosystems). T-RFLP profiles were first checked for stable baselines, voltage and calibration, and peaks in the range 50–500 bp. Absolute peak areas were initially determined with GeneMapper software v4.0 (Applied Biosystems) using a minimum peak height of five fluorescence units. Final minimum peak height threshold was determined

with the T-REX T-RFLP online analysis tool (http://trex.biohpc.org/; Culman *et al.*, 2009), removing peaks with heights less than twice the SD computed over all peaks. Data comprising the area of these 'true' peaks were exported for conversion to sample-by-fragment tables and subsequently to sample-by-binned-OTU tables by the custom R script 'interactive binner' (Ramette, 2009) using a relative fluorescence intensity (RFI) cutoff of 0.09%, a window size of 1 and a shift size of 0.1. T-RFLP profiles were finally analysed as relative abundance (RFI) after being fourth-root-transformed.

Statistical analyses We first investigated differences in abiotic conditions among the two habitat types. We modelled log-transformed soil moisture as a function of the fixed variables 'habitat type' and 'date' (including the interaction), and specified a compound symmetric covariance structure for the subject 'population' (nested under 'habitat type') to account for sampling the same populations twice. To analyse differences in ambient temperature among the two habitat types, we first calculated the average, minimum and maximum daily temperatures for each population ($n = 71$ d). We then modelled each response variable as a function of the fixed factors 'habitat type' and 'day' (including the interaction), and specified the first-order autoregressive covariance structure for the subject 'population' (nested under 'habitat type') to account for repeatedly sampling the same populations through time. To derive the degrees of freedom we used the Kenward–Roger adjustment (Littell *et al.*, 2006). Repeated-measures analyses were implemented in SAS 9.3. Soil abiotic characteristics were analysed using linear discriminant analysis (function lda in package vegan in R 2.15.1) (R Core Team, 2012; Oksanen *et al.*, 2013), where we reduced the initial set of soil variables using variance inflation factors following Neter *et al.* (1996) with a threshold of five. Significance was evaluated using a chi-squared test on a contingency table with the number of correct and incorrect predictions for each habitat type using jackknife-based classification (Borcard *et al.*, 2011). Multivariate analyses of the microbial soil community were conducted on Bray–Curtis similarities calculated from relative abundance data. The multivariate approach used in this study was similar to that advocated by Clarke & Warwick (2001), namely the following steps: a visual representation of the community, for which we employed nonmetric multidimensional scaling (nMDS) (Clarke, 1993) on three axes; and discrimination of samples using significance testing. The effect of 'treatment' (bog or hill) on community structure was tested with one-way analysis of similarity (ANOSIM) (Clarke, 1993) using PRIMER v6 (http://www.primer-e.com/).

Environmental differentiation among habitat types Soil moisture was significantly higher in bog than in hill populations (Fig. 2a; $F_{1,6} = 15.59$, $P = 0.008$). While we detected no difference in soil moisture content between the sample dates ($F_{1,70} = 0.16$, $P = 0.89$), there was some evidence of an interaction between sampling date and habitat type (Fig. 2a; $F_{1,70} = 8.78$, $P = 0.004$). In contrast to soil moisture, the mean, minimum and maximum daily temperatures varied strongly

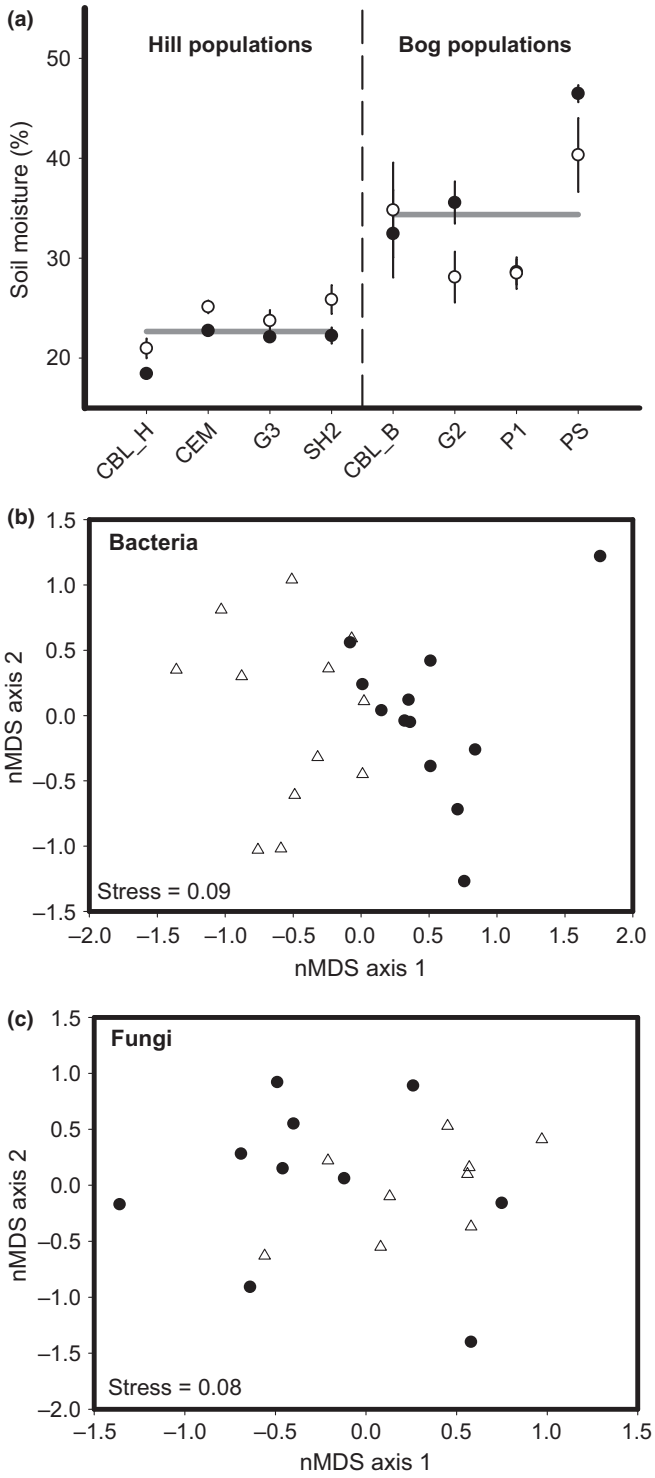

Fig. 2 Variation among bog and hill sites in the abiotic and biotic environment. (a) Differences in soil moisture among habitat types. Closed symbols depict soil samples collected in December at the start of epidemics and open symbols depict samples collected in January at the peak of epidemics, where vertical lines reflect ± SE of the mean and horizontal grey lines represent ecotypic averages of soil moisture in bog and hill populations. (b, c) Nonmetric multidimensional (nMDS) plot of the bacterial (b) and fungal (c) soil communities (triangles, samples from bog populations; circles, samples from hill populations).

among days ($P < 0.001$ for all three response variables), but did not vary among habitat types ($P > 0.05$ for all three response variables). Likewise, temporal changes in temperature did not differ among the habitat types (interaction among 'habitat type' and 'day'; $P > 0.8$ for all three response variables).

The structure of the abiotic soil environment did not differ significantly between bog and hill sites ($X_1^2 = 0.5$, $P = 0.48$). When testing differences in individual compounds, potassium (hill > bog), and to a lesser extent sodium (hill < bog), differed significantly among the two habitat types ($F_{1,6} = 17.68$, $P = 0.006$ and $F_{1,6} = 7.74$, $P = 0.03$, respectively). The bog and hill sites contained distinct bacterial communities (Fig. 2b, ANOSIM Rho = 0.26, $P < 0.001$), but such a clear distinction was not found for soil fungal communities (Fig. 2c, ANOSIM Rho = 0.09, $P = 0.10$).

Large-scale inoculation experiment

Origin of host plant lines and pathogen isolates and experimental design For the *L. marginale–M. lini* study system we have recently shown strong patterns of pathogen local adaptation (measured as infectivity) among the ecotypes (Laine *et al.*, 2014). However, how abiotic and biotic nonhost variables might affect infection outcomes and evolutionary dynamics has not yet been investigated. We therefore investigated the potential for the host–pathogen interaction to be modified by the environmental variables that were most clearly differentiated among bog and hill sites. To do this, we selected plant lines and rust pathogen isolates from three bog and four hill populations (Fig. 1; see also Laine *et al.*, 2014). Seed was collected directly from individual plants in the field, noting that the low amount of outcrossing in mountain populations of *L. marginale* (≤ 3%; Burdon *et al.*, 1999) ensured relative within-line uniformity. Owing to limited seed set in some plant lines, we deviated from a fully reciprocal cross-inoculation design. Instead, we reciprocally inoculated seven plant lines with seven pathogen isolates (one randomly selected plant line and pathogen isolate from each of three bog and four hill populations) and repeated this for three sets of plant lines and pathogen isolates (Table S2). This design amounted to 147 plant line × pathogen strain combinations. Each combination of plant lines and pathogen strains was tested in four experimental treatments (see the following section), resulting in a total of 588 pairwise inoculations. Seeds were missing or did not germinate for 44 plants, and an additional three plants died during the experiment (see Table S2).

Experimental treatments The key environmental differences among the bog and hill habitat types were soil moisture and soil bacterial community structure (Fig. 2), and we therefore manipulated the watering regime and the soil community in a multifactorial design.

Soil was collected from each of the four bog and four hill sites. Soils from each ecotype were then bulked and homogenized to form a single representative bog or hill soil. Seeds from each plant line were grown in cylindrical tubes (diameter = 8 cm, depth = 15 cm; *c.* 700 ml of soil). For the glasshouse experiment, pots were two-thirds filled with a 1 : 1 sterilized vermiculite : sand mixture. For each pot, 50 g of either a bog or hill soil was layered over this mixture and then covered with the vermiculite : sand mixture to within 1 cm from the top. The multifactorial design consisted of four experimental treatments: high moisture and soil biota from the bog habitat type; high moisture and soil biota from the hill habitat type; low moisture and bog soil biota; and low moisture and hill soil biota. To mimic natural variation in moisture between bog and hill soils, we established different watering regimes: in the high-moisture treatment (representing the bog sites) plants were watered daily with 100 ml, whereas plants in the dry treatment were watered twice a wk with 20 ml.

Eight weeks after planting, plants were inoculated with isolates of *M. lini* in a settling tower (10 plants per tower; 10 mg of rust spores mixed with 10 parts of talc per inoculation) according to the inoculation matrix (Table S2).

Measured responses variables As life-history stages may be differentially affected by environmental conditions, we measured multiple plant and pathogen life-history traits. From day 6 (post-inoculation) onwards we checked plants daily for the first appearance of flecking (representing the first visible stage in pustule formation and henceforth referred to as the 'incubation period') and the appearance of orange-coloured pustules (representing the first possible time point at which pathogen transmission could occur, and henceforth referred to as the 'latent period'). At 12 d postinoculation we scored pathogen infection type using a categorical scoring system with five classes: (1) fully susceptible [S]: large full-sized sporulating pustules (uredinia) on all leaves; (2) partial resistance [P_2]: large full-sized sporulating pustules on the younger leaves, grading down to no pustules on the oldest leaves; (3) partial resistance [P_3]: large full-sized pustules on only the youngest one or two leaves; (4) partial resistance [P_4]: no sporulation, but with necrotic flecks on older leaves; (5) fully resistant [R]: no macroscopic evidence of infection. A frequently observed phenomenon was leaf drop, particularly after the initiation of disease (leaf drop occurred in roughly half of the inoculations). This was recorded as a binary variable (0, no leaf abscission; 1, leaf abscission).

Analysis We used the framework of generalized linear mixed models (GLMMs) to analyse the data in SAS 9.3 (Littell *et al.*, 2006). To investigate the role of genotype and environment in pathogen performance and plant resistance, we analysed each of the response variables as a function of 'host ecotype', 'pathogen ecotype', 'soil humidity', 'soil biota' and their two-, three- and four-way interactions. To account for variation among host and pathogen populations, we included the random factors 'host population' and 'pathogen population' (nested within host and pathogen ecotype, respectively). Likewise, we included the random factors 'plant line' and 'pathogen genotype' (nested within host and pathogen population, respectively) to account for variation among plant lines and pathogen strains from the same population.

To determine the factors with a significant impact on the observed response, we reduced each maximal GLMM to a minimum adequate GLMM by sequentially removing nonsignificant

fixed factors ($P > 0.10$) from the model using backward selection (Crawley, 2012).

Results

The impact of plant and pathogen ecotype and environmental variation on host resistance and pathogen performance

The expression of resistance strongly differed among plant ecotypes, with the majority of hill plants expressing partial resistance (Figs 3a, 4a; Tables 1, S3). By contrast, the bog plants were fully susceptible (S) to roughly half of the pathogen strains (Figs 3a, 4a). When accounting for the habitat of origin of both the plant and the pathogen, it further becomes apparent that bog pathogens induce higher partial resistance in hill plants (mostly P_3), whereas hill pathogens induce relatively low partial resistance in hill plants (mostly P_2; Fig. 3b). As a result, the average resistance score was affected by the interaction between plant and pathogen genotype (Table 1). Notably, full resistance (R) was nearly absent and occurred in only a few nonnative host–pathogen interactions (Fig. 3b). As shown in earlier studies (Laine *et al.*, 2014), hill pathogens induced the least resistance in their local plant ecotype (Fig. 4b). However, bog pathogens and hill pathogens performed equally well on plants from the bog ecotype (Fig. 4b).

Pathogens from the hill ecotype induced higher resistance responses when plants were grown in low soil moisture (Fig. 4c; Table 1). Leaf abscission in response to pathogen infection took place in roughly half of the inoculations and occurred much more frequently on hill ecotype plants (Fig. 4d; Table 1). Leaf abscission was closely related to the degree of plant resistance: leaf abscission was nearly absent when plants were either fully susceptible or resistant (< 1%), but increased with partial resistance of the plant (P_2, 60.2%; P_3, 69.8%; P_4, 87.5%). The incidence of leaf abscission was also strongly affected by the interaction between plant and pathogen ecotype; leaf abscission occurred in > 90% of the encounters between bog pathogens and hill plants, whereas hill pathogens induced leaf abscission on < 60% of the hill plants (Fig. 4d; Table 1). Leaf abscission was somewhat lower when plants were grown in soil inoculated with soil biota from the hill ecotype (Fig. 4e; Table 1).

The incubation period (time to appearance of first disease symptoms) was affected by the interaction between plant and pathogen ecotype. The incubation period was shortest for bog pathogens infecting bog plants, whereas hill pathogens developed at the same pace regardless of plant ecotype (Fig. 4f; Table 1). Incubation period was also affected by the interaction between pathogen ecotype and soil moisture, with hill pathogens developing relatively slowly on plants growing in low soil moisture (Fig. 4g; Table 1). Latent period (time to when disease transmission could first occur) was weakly affected by the interaction between plant and pathogen genotype (Fig. 4h; Table 1) and further affected by a three-way interaction among pathogen ecotype, soil biota and soil moisture (Fig. 4i; Table 1). Notably, the latent period was relatively long for bog pathogens in their sympatric (local) environmental conditions (Fig. 4i). Finally, there was a trend for pathogen ecotype to affect the number of days between the first flecking and pustule eruption (Fig. 4j; Table 1). Overall, the majority of quantitative life-history traits were affected by the interaction between host and pathogen ecotype. Moreover, several of the traits were affected by the interaction between pathogen ecotype and the environment; by contrast, host ecotype did not interact with the environment to shape infection outcomes.

Discussion

Previous work on the *Linum–Melampsora* interaction has shown strong local adaptation between adjacent habitat types (bogs and hills) occurring within a natural metapopulation (Laine *et al.*, 2014). These findings beg the question of what factors might maintain such strong differentiation at these local spatial scales. Here we used an experimental approach to explicitly investigate the potential for environmental heterogeneity to impact on the ecology of the interaction. We first showed that bog and hill habitat types were clearly differentiated with respect to soil moisture and soil biota. We then manipulated these belowground environmental factors in a multifactorial cross-inoculation experiment. Our findings highlight that environmental heterogeneity – here in the form of two distinct habitat types frequently occurring in close proximity – can drive ecological interactions and spatial divergence in resistance and aggressiveness, thereby maintaining genetic variation in interaction traits within a natural host–pathogen metapopulation.

The impact of genetic differentiation and environmental variation on infection outcomes

While interactions between plant and pathogen genotypes have been a major focus of research since the seminal studies of Flor

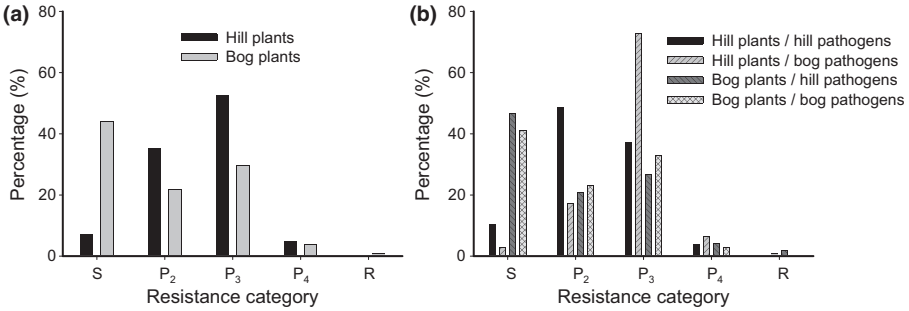

Fig. 3 Histogram showing the resistance type expressed by the bog and hill plants (*Linum marginale*) in response to either the full set of pathogens (*Melampsora lini*) (a) or separately for pathogens originating from the bog and hill habitat type (b).

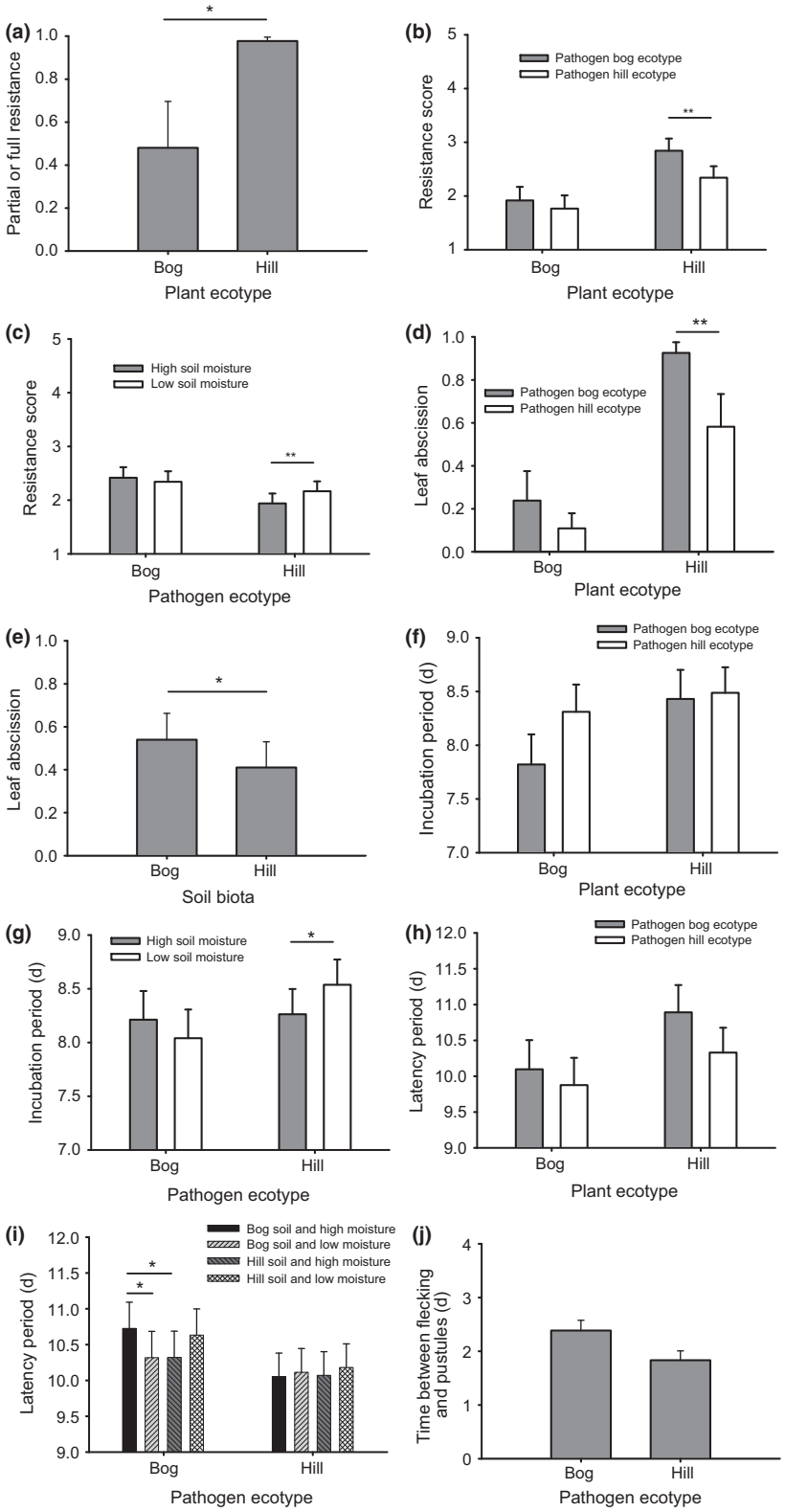

Fig. 4 The impact of host ecotype, pathogen ecotype, environmental factors and their interactions on host resistance and pathogen performance of flax rust *Melampsora lini* on the wild flax *Linum marginale*. (a) Host resistance is affected by plant ecotype. (b, c) The resistance level is affected by the interaction between plant and pathogen ecotype (b) and the interaction among pathogen ecotype and soil moisture (c). (d, e) Leaf abscission in response to pathogen infection is affected by the interaction among plant and pathogen ecotype (d) and the soil biota (e). (f, g) The incubation period is affected by the interactions between plant and pathogen ecotype (f) and pathogen ecotype and soil moisture (g). (h, i) Latent period is affected by the interaction between plant and pathogen ecotype (h) and the three-way interaction among pathogen ecotype, soil biota and soil moisture (i). (j) The time between the first appearance of flecking and pustules is weakly affected by pathogen ecotype. The bold outline of vertical bars indicates (where relevant) the sympatric (local) combinations of plants, parasites and environmental variables. Shown are (back-transformed) least-squares means and + SE; significant pairwise differences within each ecotype (as indicated on the *x*-axis) or among main factors (a, e, j) are indicated: *, $P < 0.05$; **, $P < 0.01$.

(1956; as reviewed in Thompson & Burdon, 1992), our results indicate that the direct impact of the environment and genotype × environment (G × E) interactions are also important predictors of infection outcomes. Moreover, the existence of E × E and G × E × E interactions illustrate the need for multifactorial designs to understand variation in pathogen performance and how it might relate to patterns of local adaptation. Interestingly, while G_{path} × E interactions were common, the host genotype did not interact with the environment; however, future research in this and other pathosystems involving a

Table 1 The effect of host ecotype, pathogen ecotype, soil humidity, soil biota and their interactions on host resistance and pathogen performance for the interaction between flax rust (*Melampsora lini*) and wild flax (*Linum marginale*). Shown are *P*-values of the fixed effects as estimated with generalized linear mixed models, with significant values in bold

Response variable	Host ecotype	Pathogen ecotype	Soil moisture	Soil biota	Host ecotype × pathogen ecotype	Host ecotype × soil humidity	Host ecotype × soil biota	Pathogen ecotype × soil humidity	Pathogen ecotype × soil biota	Soil humidity × soil biota	Host ecotype × pathogen ecotype × soil humidity	Host ecotype × soil humidity × soil biota	Pathogen ecotype × soil humidity × soil biota	Host ecotype × pathogen ecotype × soil humidity × soil biota
Infectivity ($n = 541$)	**0.019**		–	–	–	–	–	–	–	–	–	–	–	–
Resistance level ($n = 541$)	**0.045**	0.120	0.188	–	**0.004**	–	–	**0.009**	–	–	–	–	–	–
Leaf abscission ($n = 541$)	**0.009**	0.067	–	**0.024**	**0.021**	–	–	–	–	–	–	–	–	–
Incubation period ($n = 531$)	0.061	0.447	0.532		**0.010**	–	–	**0.007**	–	–	–	–	–	–
Latent period ($n = 517$)	0.141	0.373	0.814	0.988	**0.035**	–	–	0.394	0.572	**0.015**	–	–	**0.032**	–
Time between flecking and pustules ($n = 517$)	–	0.052	–	–	–	–	–	–	–	–	–	–	–	–

range of environmental factors is needed to assess the generality of this phenomenon. Overall, these results provide evidence that G × E interactions are not merely an interesting laboratory phenomenon, but have high ecological relevance in natural systems. By contrast, we did not detect G × G × E interactions, even though our design had enough resolution to detect those. Interestingly, the low amount of variation explained by G × G × E interactions is supported by the few studies currently available in wild host–parasite systems (Laine, 2007; Hall & Ebert, 2012). If this turns out to be a general rule, it has pronounced implications for theoretical modelling, which increasingly assumes the existence of spatially or temporally variable G × G interactions (e.g. Gavrilets & Michalakis, 2008; Mostowy & Engelstädter, 2011).

While many studies only measure a single pathogen trait, our data highlight that the impact of environmental and genetic variation differs strikingly among plant and pathogen life-history traits. In particular, infectivity was not affected by the environment. This mirrors comparable findings in both plant and animal pathosystems that infectivity may be relatively insensitive to G × E interactions (Laine, 2007; Duneau *et al.*, 2011). The traditional focus on this qualitative trait (infectivity) could then have relegated G × E interactions as minor drivers of host–parasite interactions. By contrast, all quantitative traits were affected by the environment, even though the relevant environmental factor(s) varied among the life-history stages.

The impact of environmental heterogeneity on (co)evolutionary dynamics

Despite the potential for strong gene flow to occur among populations belonging to different habitat types, especially for an aerially dispersed rust pathogen such as *M. lini*, we found clear environmentally driven differentiation in interaction traits among plant and pathogen populations from the two habitat types. Some traits were clearly adaptive for the pathogen, where hill pathogens were more infective and induced less leaf abscission on the hill plants than the bog pathogens. The adaptation of hill pathogens to their local plants may be driven by strong selection pressures as a result of higher average plant resistance in hill populations; by contrast, the low resistance of bog pathogens may preclude strong natural selection. The high leaf drop of the hill plants may have evolved as a means of getting rid of infection that disrupts the plant cuticle and makes plants more vulnerable to water loss, which is probably more of a problem in the drier hill habitat. However, despite spatial consistency in the impact of the environment on the host–parasite interaction, the adaptive nature of specific plant and pathogen responses to local environmental conditions was not always apparent: why would plants in the bog environment not evolve higher resistance? Why do hill pathogens induce higher resistance and develop more slowly in their native (low) moisture conditions? Why do bog pathogens develop relatively slowly in their local environmental conditions? These results illustrate the notion that coevolutionary dynamics may result in adaptive mismatches, or natural selection – in this case, in response to the below-ground abiotic and biotic environment – can be evolutionarily constrained (Dargent *et al.*, 2013; Garland, 2014).

Based on previous studies within the same and related pathosystems, we postulate that tradeoffs and limited gene flow play a key role in explaining the distinct coevolutionary dynamics among the two habitat types. Carlsson-Granér *et al.* (1999) demonstrated very low survival of hill plants in the bog habitat in a 2 yr transplant experiment and that reciprocal crosses between bog and hill host lines were far less successful when the bog plant was the maternal parent. This supports the idea that a tradeoff between high amounts of resistance and survival in the bog habitat, as well as reduced offspring survival, may select against the introgression of resistance genes from hill populations into those growing in bogs and supports the role of the environment as a potential driving force for the large spatial and temporal variation in resistance within and among host species (Laine *et al.*, 2011; Tack *et al.*, 2012). Equally, the high amount of resistance and leaf abscission of the hill plants in response to the bog pathogen will reduce pathogen gene flow from the bog to the hill habitat, whereas a tradeoff between high infectivity and spore production (Thrall & Burdon, 2003) could prevent pathogen migration from the hill to the bog habitat. The rapid purging of immigrant pathogen genotypes is further supported by the absence of full resistance in local host–parasite interactions, whereas full resistance was detected in a few cross-ecotype inoculations. Overall, these results shed light on how spatial divergence in host resistance and pathogen aggressiveness can persist in the face of high dispersal rates among habitat types that can be as close as several hundred metres. Further, a positive relationship between developmental time and pustule spore load may explain the slower development of pathogen strains in their local environment. Given the omnipresence of tradeoffs and evolutionary constraints in host–parasite systems (Laine & Barrès, 2013; Susi & Laine, 2013; Bruns *et al.*, 2014), we argue that the apparent inconsistency in adaptive signals across qualitative and quantitative infection traits will be mirrored in the majority of host–parasite systems. This is evidenced by an emerging number of local adaptation studies measuring a range of life-history traits (Lemoine *et al.*, 2012; Tack *et al.*, 2014).

Providing a feedback between ecology and evolution, environmentally mediated spatial evolutionary divergence in resistance could, in turn, affect epidemiological dynamics: thus susceptible bog populations sustain higher amounts of infection than the more resistant hill populations (Laine *et al.*, 2014).

Below-ground drivers of host–parasite dynamics

Studies of evolutionary responses of single species to below-ground heterogeneity – in particular of plants to serpentine and heavy-metal soils – have provided some of the most convincing examples of rapid evolution and local adaptation (Bradshaw, 1952, 1984; Brady *et al.*, 2005). Our study demonstrates that below-ground abiotic and biotic heterogeneity may also drive the ecological outcome of above-ground species interactions and thereby exert selection pressures on those interactions. Illustrating the evolutionary potential of pathogens in response to the soil

environment, Fones *et al.* (2010) showed that naturally colonizing bacteria growing on hyperaccumulating *Noccea caerulans* had a higher zinc tolerance than bacteria isolated from nonaccumulating plants, suggesting local adaptation of the bacteria to high metal concentrations. As another example, a recent artificial selection experiment by Bonte *et al.* (2010) demonstrated that spider mites may adapt to plants interacting with either mycorrhizal fungi or nematodes (as compared with control plants) within the relatively short time span of 15 spider mite generations.

Conclusion

Overall, our study highlights the fact that environmental heterogeneity has the potential to shape the outcome of species interactions and spatial divergence in interaction traits despite the potential for frequent dispersal among neighbouring populations. While environmental heterogeneity has previously been identified as a key determinant of species diversity (Stein *et al.*, 2014), we show here that environmental heterogeneity can also maintain variation at the genetic level relevant for species interactions. The high degree of environmental heterogeneity in natural settings – as compared with agricultural fields – might then provide at least a partial explanation for the classic observation that genetic variation in natural pathosystems is higher in natural than in agricultural systems (Burdon, 1987; Tack *et al.*, 2012). Although we did not detect G × G × E interactions, the general importance of a complex set of G × E interactions matches the framework of the geographic mosaic of coevolution, which emphasizes that environmental heterogeneity plays a major role in the ecology and evolution of species interactions across spatial scales (Thompson, 2005). Importantly, below-ground variation in the abiotic and biotic environment is likely to represent a major but unrecognized ecological and evolutionary force in natural communities and contribute to the long-term maintenance of variation in resistance and pathogenicity.

Acknowledgements

We thank Caritta Eliasson for help with the glasshouse experiment and Guillaume Blanchet for advice on the multivariate analysis of the abiotic soil environment. A-L.L. and A.J.M.T. were supported by funding from the Academy of Finland (grants 250444 and 136393 to A-L.L.). This research was supported by the National Institutes of Health (NIH grant 5RO1 GM074265-01A2).

References

Barrus MF. 1911. Variation of varieties of beans in their susceptibility to anthracnose. *Phytopathology* **1**: 190–195.

Biffen RH. 1905. Mendel's laws of inheritance and wheat breeding. *Journal of Agricultural Science* **1**: 4–48.

Bonte D, De Roissart A, Vandegehuchte ML, Ballhorn DJ, Van Leeuwen T, de la Peña E. 2010. Local adaptation of aboveground herbivores towards plant phenotypes induced by soil biota. *PLoS ONE* **5**: e11174.

Borcard D, Gillet F, Legendre P. 2011. *Numerical ecology with R.* New York, NY, USA: Springer.

Bradshaw AD. 1952. Populations of *Agrostis tenuis* resistant to lead and zinc poisoning. *Nature* **169**: 1098.

Bradshaw AD. 1984. Ecological significance of genetic variation between populations. In: Dirzo R, Sarukhán J, eds. *Perspectives in plant population ecology*. Sunderland, MA, USA: Sinauer, 213–228.

Brady KU, Kruckeberg AR, Bradshaw HD Jr. 2005. Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics* **36**: 243–266.

Bruns E, Carson ML, May G. 2014. The jack of all trades is master of none: a pathogen's ability to infect a greater number of host genotypes comes at a cost of delayed reproduction. *Evolution* **68**: 2453–2466.

Burdon JJ. 1987. *Diseases and plant population biology.* Cambridge, UK: Cambridge University Press.

Burdon JJ, Jarosz AM. 1992. Temporal variation in the racial structure of flax rust (*Melampsora lini*) populations growing on natural stands of wild flax (*Linum marginale*): local versus metapopulation dynamics. *Plant Pathology* **41**: 165–179.

Burdon JJ, Thompson JN. 1995. Changed patterns of resistance in a population of *Linum marginale* attacked by the rust pathogen *Melampsora Lini. Journal of Ecology* **83**: 199–206.

Burdon JJ, Thrall PH, Brown AHD. 1999. Resistance and virulence structure in two *Linum marginale–Melampsora lini* host–pathogen metapopulations with different mating systems. *Evolution* **53**: 704–716.

Carlsson-Granér U, Burdon JJ, Thrall PH. 1999. Host resistance and pathogen virulence across a plant hybrid zone. *Oecologia* **121**: 339–347.

Chamberlain SA, Bronstein JL, Rudgers JA. 2014. How context dependent are species interactions? *Ecology Letters* **17**: 881–890.

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**: 117–143.

Clarke KR, Warwick RM. 2001. *Change in marine communities: an approach to statistical analysis and interpretation.* Plymouth, UK: PRIMER-E.

Clausen J, Keck DD, Hiesey WM. 1940. *Experimental studies on the nature of species. I. Effect of varied environments on western North American plants.* Washington, DC, USA: Carnegie Institution of Washington Publication.

Crawley MJ. 2012. *The R Book.* Chichester, UK: John Wiley & Sons Ltd.

Culman SW, Bukowski R, Gauch HG, Cadillo-Quiroz H, Buckley DH. 2009. T-REX: software for the processing and analysis of T-RFLP data. *BMC Bioinformatics* **10**: 171.

Dargent F, Scott ME, Hendry AP, Fussmann GF. 2013. Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proceedings of the Royal Society B: Biological Sciences* **280**: 20132371.

Duneau D, Luijckx P, Ben-Ami F, Laforsch C, Ebert D. 2011. Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host–parasite interactions. *BMC Biology* **9**: 11.

Ettema CH, Wardle DA. 2002. Spatial soil ecology. *Trends in Ecology & Evolution* **17**: 177–183.

Flor HH. 1956. The complementary genic systems in flax and flax rust. *Advances in Genetics* **8**: 29–54.

Fones H, Davis CAR, Rico A, Fang F, Smith JAC, Preston GM. 2010. Metal hyperaccumulation armors plants against disease. *PLoS Pathogens* **6**: e1001093.

Ford EB. 1975. *Ecological genetics.* London, UK: Chapman & Hall.

Forde SE, Thompson JN, Bohannan BJM. 2004. Adaptation varies through space and time in a coevolving host–parasitoid interaction. *Nature* **431**: 841–844.

Garland T Jr. 2014. Trade-offs. *Current Biology* **24**: R60–R61.

Gavrilets S, Michalakis Y. 2008. Effects of environmental heterogeneity on victim–exploiter coevolution. *Evolution* **62**: 3100–3116.

Hall MD, Ebert D. 2012. Disentangling the influence of parasite genotype, host genotype and maternal environment on different stages of bacterial infection in *Daphnia magna. Proceedings of the Royal Society B: Biological Sciences* **279**: 3176–3183.

Jarosz AM, Burdon JJ. 1992. Host–pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini. Oecologia* **89**: 53–61.

Laine A-L. 2006. Evolution of host resistance: looking for coevolutionary hotspots at small spatial scales. *Proceedings of the Royal Society B: Biological Sciences* **273**: 267–273.

Laine A-L. 2007. Pathogen fitness components and genotypes differ in their sensitivity to nutrient and temperature variation in a wild plant–pathogen association. *Journal of Evolutionary Biology* **20**: 2371–2378.
Laine A-L. 2008. Temperature-mediated patterns of local adaptation in a natural plant–pathogen metapopulation. *Ecology Letters* **11**: 327–337.
Laine AL, Barrès B. 2013. Epidemiological and evolutionary consequences of life-history trade-offs in pathogens. *Plant Pathology* **62**: 96–105.
Laine A-L, Burdon JJ, Dodds PN, Thrall PH. 2011. Spatial variation in disease resistance: from molecules to metapopulations. *Journal of Ecology* **99**: 96–112.
Laine A-L, Burdon JJ, Nemri A, Thrall PH. 2014. Host ecotype generates evolutionary and epidemiological divergence across a pathogen metapopulation. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20140522.
Lawrence GJ. 1989. Flax rust from *Linum marginale*: pathogenicity reactions on the *Linum usitatissimum* set of differential varieties. *Canadian Journal of Botany* **67**: 3187–3191.
Lawrence GJ, Burdon JJ. 1989. Flax rust from *Linum marginale*: variation in a natural host–pathogen interaction. *Canadian Journal of Botany* **67**: 3192–3198.
Lemoine M, Doligez B, Richner H. 2012. On the equivalence of host local adaptation and parasite maladaptation: an experimental test. *American Naturalist* **179**: 270–281.
Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* **27**: 237–277.
Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. 2006. *SAS® for mixed models*. Cary, NC, USA: SAS Institute Inc.
Lopez Pascua L, Gandon S, Buckling A. 2012. Abiotic heterogeneity drives parasite local adaptation in coevolving bacteria and phages. *Journal of Evolutionary Biology* **25**: 187–195.
McNew GL. 1960. The nature, origin, and evolution of parasitism. In: Horsfall JG, Dimond AE, eds. *Plant pathology: an advanced treatise*. New York, NY, USA: Academic Press, 19–69.
Mostowy R, Engelstädter J. 2011. The impact of environmental change on host–parasite coevolutionary dynamics. *Proceedings of the Royal Society B-Biological Sciences* **278**: 2283–2292.
Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. 1996. *Applied linear statistical models*. Irwin, IL, USA: McGraw–Hill Higher Education.
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. *vegan: community ecology package*. R package version 2.0-10. [WWW document] URL http://CRAN.R-project.org/package=vegan [accessed 6 April 2015].
Pasteur L, Joubert JF, Chamberland C. 1878. Sur le charbon des poules. *Comptes Rendus des seances De L'Academie Des Sciences* **87**: 47–48.
Poisot T, Thrall PH, Hochberg ME. 2012. Trophic network structure emerges through antagonistic coevolution in temporally varying environments. *Proceedings of the Royal Society B: Biological Sciences* **279**: 299–308.
R Core Team. 2012. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
Ramette A. 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. *Applied and Environmental Microbiology* **75**: 2495–2505.
Schoener TW. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* **331**: 426–429.
Singh BK, Nazaries L, Munro S, Anderson IC, Campbell CD. 2006. Use of multiplex terminal restriction fragment length polymorphism for rapid and simultaneous analysis of dfifferent components of the soil microbial community? *Applied and Environmental Microbiology* **72**: 7278–7285.
Stein A, Gerstner K, Kreft H. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters* **17**: 866–880.
Susi H, Laine A-L. 2013. Pathogen life-history trade-offs revealed in allopatry. *Evolution* **67**: 3362–3370.
Tack AJM, Horns F, Laine A-L. 2014. The impact of spatial scale and habitat configuration on patterns of trait variation and local adaptation in a wild plant parasite. *Evolution* **68**: 176–189.
Tack AJM, Thrall PH, Barrett LG, Burdon JJ, Laine A-L. 2012. Variation in infectivity and aggressiveness in space and time in wild host–pathogen systems: causes and consequences. *Journal of Evolutionary Biology* **25**: 1918–1936.
Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A *et al.* 2014. Global diversity and geography of soil fungi. *Science* **346**: 1256688.
Tellier A, Brown JKM. 2011. Spatial heterogeneity, frequency-dependent selection and polymorphism in host–parasite interactions. *BMC Evolutionary Biology* **11**: 319.
Thompson JN. 2005. *The geographic mosaic of coevolution*. Chicago, IL, USA: University of Chicago Press.
Thompson JN. 2013. *Relentless evolution*. Chicago, IL, USA: University Of Chicago Press.
Thompson JN, Burdon JJ. 1992. Gene-for-gene coevolution between plants and parasites. *Nature* **360**: 121–125.
Thrall PH, Burdon JJ. 2003. Evolution of virulence in a plant host–pathogen metapopulation. *Science* **299**: 1735–1737.
Thrall PH, Burdon JJ, Young A. 2001. Variation in resistance and virulence among demes of a plant host–pathogen metapopulation. *Journal of Ecology* **89**: 736–748.
Thrall PH, Laine A-L, Ravensdale M, Nemri A, Dodds PN, Barrett LG, Burdon JJ. 2012. Rapid genetic change underpins antagonistic coevolution in a natural host–pathogen metapopulation. *Ecology Letters* **15**: 425–435.
Turesson G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* **3**: 211–350.
Van Dorst J, Bissett A, Palmer AS, Brown M, Snape I, Stark JS, Raymond B, McKinlay J, Ji M, Winsley T *et al.* 2014. Community fingerprinting in a sequencing world. *FEMS Microbiology Ecology* **89**: 316–330.
Vogwill T, Fenton A, Buckling A, Hochberg ME, Brockhurst MA. 2009. Source populations act as coevolutionary pacemakers in experimental selection mosaics containing hotspots and coldspots. *American Naturalist* **173**: E171–E176.
Wolinska J, King KC. 2009. Environment can alter selection in host–parasite interactions. *Trends in Parasitology* **25**: 236–244.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Methods and units for quantification of the soil structure and chemistry

Table S2 Experimental inoculation matrix

Table S3 *F*-values and associated degrees of freedom for the statistical results reported in Table 1

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

Supporting Information Tables S1-S3

New Phytologist Supporting Information

Article title: Belowground abiotic and biotic heterogeneity shapes aboveground infection outcomes and spatial divergence in a host-parasite interaction
Authors: Tack AJM, Laine A-LL, Burdon JJ, Bissett A, Thrall PH

The following Supporting Information is available for this article:

Table S1. Methods and units for quantification of the soil structure and chemistry. Samples were analysed by Incitec Pivot Ltd, Southbank, Australia (http://www.incitecpivotfertilisers.com.au/Soil%20Plant%20Tests/Nutrient%20Advantage)

Measurement	Method	Unit
pH	1:5 $CaCl_2$	
Electric conductivity (EC)	Sat Ext	dS/m
Organic carbon (OC)		%
Soil texture		
Nitrate nitrogen	NO_3	mg/kg
Phosphorus	Colwell	mg/kg
Potassium	Amm-acet	Meq/100g
Calcium	Amm-acet	Meq/100g
Magnesium	Amm-acet	Meq/100g
Sodium	Amm-acet	Meq/100g
Aluminium	KCl	mg/kg
Chloride		mg/kg
Copper	DTPA	mg/kg
Iron	DTPA	mg/kg
Manganese	DTPA	mg/kg
Zinc	DTPA	mg/kg
Sulfate	KCl40	mg/kg

Table S2. Experimental inoculation matrix. Shown are the number of inoculations for combinations of plant lines (rows) and pathogen strains (columns). Due to restricted seed availability, we subdivided plant lines and pathogen strains in three blocks (which can be identified by background colour). Selected plant-pathogen combinations were tested in four environmental conditions based on moisture ('low', 'high') and soil biota ('bog type', 'hill type'). Seeds were missing or did not germinate for 44 plants, and an additional three plants died during the experiment; further deviations from the original design are due to seed limitation. Plant lines and pathogen strains from the hill ecotype are shown in italic font.

	CBL HILL-3	G2-6	*G3-9*	P1-6	*SH2-4*	CBL BOG-2	*CEM-3*	CBL BOG-1	*CBL HILL-10*	G2-8	P1-9	*CEM-5*	*G3-4*	*SH2-7*	CBL BOG-6	G2-2	*G3-8*	P1-3	*SH2-10*	*CBL HILL-4*	*CEM-7*
CBL BOG-3	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CBL HILL-4	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CEM-5	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G2-3	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P1-7	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SH2-5	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G3-2	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G2-5	0	0	0	0	0	0	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CBL BOG-4	0	0	0	0	0	0	0	4	4	4	4	4	4	4	0	0	0	0	0	0	0
CBL HILL-10	0	0	0	0	0	0	0	4	4	4	4	4	4	4	0	0	0	0	0	0	0
CEM-10	0	0	0	0	0	0	0	4	4	4	4	4	4	4	0	0	0	0	0	0	0
G3-5	0	0	0	0	0	0	0	4	4	4	4	4	4	4	0	0	0	0	0	0	0
SH2-9	0	0	0	0	0	0	0	4	4	4	4	4	4	4	0	0	0	0	0	0	0
P1-2	0	0	0	0	0	0	0	4	4	4	4	0	0	0	0	0	0	0	0	0	0
CBL BOG-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4
CBL HILL-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4
CEM-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4
G3-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	3
SH2-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	3	3
P1-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0

Table S3. F-values and associated degrees of freedom for the statistical results reported in Table 1 in the main manuscript. Analysed are the effect of host ecotype, pathogen ecotype, soil humidity, soil biota and their interactions on host resistance and pathogen performance for the interaction between flax rust (*Melampsora lini*) and wild flax (*Linum marginale*).

Response variable	Host ecotype	Pathogen ecotype	Soil moisture	Soil biota	Host ecotype x Pathogen ecotype	Host ecotype x Soil humidity	Host ecotype x Soil biota	Pathogen ecotype x Soil humidity	Pathogen ecotype x Soil biota	Soil humidity x Soil biota	Host ecotype x Pathogen ecotype x Soil humidity	Host ecotype x Soil humidity x Soil biota	Pathogen ecotype x Soil humidity x Soil biota	Host ecotype x Pathogen ecotype x Soil humidity x Soil biota
Infectivity (n = 541)	**$F_{1,5} = 11.81$**		-	-	-	-	-	-	-	-	-	-	-	-
Resistance level (n = 541)	**$F_{1,5} = 7.06$**	$F_{1,5} = 3.52$	$F_{1,499} = 1.74$	-	**$F_{1,499} = 8.61$**	-	-	**$F_{1,499} = 6.86$**	-	-	-	-	-	-
Leaf abscission (n = 541)	**$F_{1,5} = 16.89$**	$F_{1,5} = 5.42$	-	**$F_{1,500} = 5.14$**	**$F_{1,500} = 5.39$**	-	-	-	-	-	-	-	-	-
Incubation period (n = 531)	$F_{1,5} = 5.80$	$F_{1,5} = 0.68$	$F_{1,489} = 0.39$		**$F_{1,489} = 6.66$**	-	-	**$F_{1,489} = 7.47$**	-	-	-	-	-	-
Latent period (n = 517)	$F_{1,5} = 3.06$	$F_{1,5} = 0.96$	$F_{1,471} = 0.06$	$F_{1,471} = 0.00$	**$F_{1,471} = 4.50$**	-	-	$F_{1,471} = 0.73$	$F_{1,471} = 0.32$	**$F_{1,471} = 6.01$**	-	-	**$F_{1,471} = 4.62$**	-
Time between flecking and pustules (n = 517)	-	$F_{1,5} = 6.46$	-	-	-	-	-	-	-	-	-	-	-	-