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Research

Genetic variation changes the interactions between the parasitic plant-ecosystem engineer *Rhinanthus* and its hosts

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Within-species genetic variation is a potent factor influencing between-species interactions and community-level structure. Species of the hemi-parasitic plant genus *Rhinanthus* act as ecosystem engineers, significantly altering above- and below-ground community structure in grasslands. Here, we show the importance of genotypic variation within a single host species (barley—*Hordeum vulgare*), and population-level variation among two species of parasite (*Rhinanthus minor* and *Rhinanthus angustifolius*) on the outcome of parasite infection for both partners. We measured host fitness (number of seeds) and calculated parasite virulence as the difference in seed set between infected and uninfected hosts (the inverse of host tolerance). Virulence was determined by genetic variation within the host species and among the parasite species, but *R. angustifolius* was consistently more virulent than *R. minor*. The most tolerant host had the lowest inherent fitness and did not gain a fitness advantage over other infected hosts. We measured parasite size as a proxy for transmission ability (ability to infect further hosts) and host resistance. Parasite size depended on the specific combination of host genotype, parasite species and parasite population, and no species was consistently larger. We demonstrate that the outcome of infection by *Rhinanthus* depends not only on the host species, but also on the underlying genetics of both host and parasite. Thus, genetic variations within host and parasite are probably essential components of the ecosystem-altering effects of *Rhinanthus*.

Keywords: host–parasite interactions; community genetics; genetic variation; ecosystem engineer; *Rhinanthus*; barley

1. INTRODUCTION

For over 30 years, within-species genetic variation has been considered a key factor in influencing the structure of ecological communities [1]. Empirical evidence is now mounting that highlights the importance of genetic diversity, genotypic identity, genotype × genotype and genotype × environment interactions in organizing ecological communities.

Focusing on interactions among individuals, recent evidence from grassland studies demonstrates that higher within-species genetic diversity promotes the maintenance of species diversity over time, as well as the increased survival of individual species [2,3]. Reusch *et al.* [4] show that increased within-species genetic diversity in the eel grass, *Zostera marina*, enhances community recovery following a climatic perturbation event. Extensive studies of a North American cottonwood system indicate that genetically determined levels of condensed tannins within a hybridizing complex of two tree species (*Populus fremontii* and *Populus angustifolia*) are associated with different arthropod communities [5], and that tree chemistry and genetics have wide-ranging effects on whole community composition and dynamics [6–8]. Furthermore, arthropod communities on the evening primrose (*Oenothera biennis*) are found to assemble according to plant genotype as well as micro-environment [9], and host plant genetic diversity determines the abundance of an ecosystem engineer, the goldenrod bunch gall midge, on *Solidago altissima* [10]. In addition, work by Tétard-Jones *et al.* [11] shows that the performance of plants and aphids in an experimental system depends on the interaction between plant and aphid genotype and the community changes of rhizobacteria in the soil.

At the population scale, the geographical mosaic theory provides evidence of the importance of genetic variation in ecological communities, where variation among populations can determine the strength of selection and evolutionary trajectories between two coevolving species [12]. Alternatively, Palkovacs &

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Post [13] show that differential selection for foraging traits in landlocked and anadromous populations of alewives (the fish *Alosa pseudoharengus*) can cause cascading community-level effects, changing zooplankton community composition. Finally, Bangert *et al.* [14,15] show that the effects observed in North American cottonwood communities can be seen both within and across populations at the regional scale.

(a) Rhinanthus—*a parasitic plant and ecosystem engineer*

Plants in the genus *Rhinanthus* are facultative hemiparasitic plants, distributed throughout grasslands in Europe and North America [16,17]. They are generalist parasites, and one species (*Rhinanthus minor*) has over 50 recorded host plants [18]. *Rhinanthus* are root parasites, attaching to their hosts via specialized structures called haustoria and extracting nutrients from the host xylem [16].

Parasitic plants, in general, and *Rhinanthus* in particular, can have an enormous impact on the structure and function of the natural communities in which they grow [19–21]. However, the effect of genetic variability in *Rhinanthus* systems on host response to infection and parasite attachment to hosts has rarely been considered (but see [22]). *Rhinanthus* plants act as ecosystem engineers, dramatically altering the diversity and productivity of the grasslands they inhabit [23,24]. Changes in community diversity are, at least in part, the result of differential resistance to infection between potential host plants [25,26], causing a shift in the competitive balance between species within the community [27].

The parasites generally act to suppress grasses, thereby facilitating forb proliferation and potentially enhancing biodiversity [19,27]. This has led to the parasite's use as a management tool for the restoration of degraded grasslands [28–31]. While the directionality of this trend (suppression of grasses and promotion of forbs) is conserved across a number of studies, the magnitude of the effect is highly variable, with grass biomass suppressed by 8–84% and forb abundance promoted by 5–57% [21]. Such unpredictability currently limits the use of *Rhinanthus* in restoration ecology.

In addition to the effects of *Rhinanthus* on associated plant communities, presence of the parasitic plant is associated with changes in soil microbial communities [32], long-term nutrient availability [33] and the outcome of host–mycorrhizal interactions [34,35]. *Rhinanthus* also influences host interactions with aphid herbivores [36] and above-ground arthropod community structure (S. Hartley 2010, personal communication).

(b) *Host–parasite interactions*

Interactions between hosts and parasites are often characterized by host resistance and tolerance, and parasite virulence and transmission, as these traits affect the fitness, and therefore evolutionary potential, of one or both partners. Host-resistance traits typically act to prevent either infection by the parasite (qualitative resistance) [37], as demonstrated for *Rhinanthus*–host interactions by Cameron *et al.* [25], or reduce the fitness of the parasite (quantitative resistance) [37]. Host-tolerance traits act to mitigate the negative impact of the parasite on host fitness and are measured as the fitness of an infected individual compared with an uninfected individual [38]. Tolerance traits may not affect parasite fitness directly, but can still have important evolutionary effects on parasites as well as hosts [39]. Parasite virulence is defined as (i) the rate at which a host becomes infected, and (ii) the damage inflicted to the host by infection with the parasite [40]. The latter definition is measured as the difference in host fitness between an infected and uninfected individual and is essentially the inverse of host tolerance, where the least-tolerant host supports the most virulent parasite. Virulence, and therefore tolerance, can change with the density of infection, and the relationship between density of infection and host fitness may not be linear. This means that depending on the density of infection, relative tolerance and virulence of infected hosts can change [39]. Finally, the transmission ability of the parasite defines how well a parasite can infect further host individuals [41], which in the case of *Rhinanthus*, is linked to the production of seed. All these traits are inextricably linked together, as host traits influence parasite traits and vice versa [39].

Recent efforts have advanced our understanding of local adaptation and coevolution between hosts and parasites, and much host–parasite work is set within this context (e.g. [42–47]). However, local adaptation and coevolutionary dynamics are difficult to detect [22] and understand for generalist parasites with multiple [48], and in the case of *Rhinanthus*, simultaneous hosts, as there are complex interactions among hosts as well as between the parasite and the hosts. Community genetics offers an alternative structure in which to consider complex host–parasite interactions, where simple coevolution between two species does not exist. In his seminal paper, Antonovics [49] introduced community genetics as a means to free us 'from the overly restrictive frame of reference, the reciprocality, that coevolutionists would choose for their own discipline…' thus enabling us to '…generalize community processes in terms of interactions that occur among genotypes as individuals…' From this point of view, in combination with the fact that *Rhinanthus* can have far-reaching effects on ecological communities, the interactions among *Rhinanthus* and its hosts are an excellent model with which to investigate community genetic effects.

(c) *Aims*

Here, we investigate the role that genetic variation within host and parasitic plants might play in determining the outcome of the interaction between the two and determine the value of such a system in answering community genetics questions. We use an established barley (*Hordeum vulgare*)–*Rhinanthus* model system [50–52] to investigate the effects of within and between species diversity using four genotypes of the barley host, and individuals from two populations of two *Rhinanthus* species (*R. minor* L. and *Rhinanthus angustifolius* C.C.Gmel.). The system we used was not natural, but was chosen as a functional *Rhinanthus*–host system that had genetic

tools available for the hosts (all host genotypes are doubled haploid parental genotypes for quantitative trait loci (QTL) mapping lines [53,54]). While not directly applicable for this study, these tools facilitate future mechanistic work on resistance, tolerance and virulence of host–parasitic plant systems. It is also worth noting that many of the recent theoretical advances in evolutionary biology have been based on non-natural laboratory-based systems (e.g. [55,56]), which have enabled the development of hypotheses that can then be tested in more complex field environments.

We used a common garden experimental design, where differences in response among populations of the same species indicate an underlying genetic difference. We determined the effect of parasite infection on host plants by counting the number of seed (a measure of fitness) produced by infected and uninfected barley. We calculated parasite virulence as the difference in seed set between infected and uninfected hosts (*sensu* [41]) in order to investigate whether host genotype, parasite species and parasite population affected host response to infection. We also measured the height of *Rhinanthus* plants as a proxy for parasite size and fecundity (as size and fecundity are closely positively related factors; [57]) to determine if host genotype, parasite species and parasite population affected parasite response to host attachment.

2. MATERIAL AND METHODS

We obtained seeds of four barley (*H. vulgare* L.) doubled haploid genotypes (Morex, Steptoe, Oregon Wolfe Dominant and Oregon Wolfe Recessive) from P. Hayes (Oregon State University, OR, USA). Genotypes Morex and Steptoe are barley cultivars and the Oregon Wolfe barleys originate from multiple marker stock [53,54]. We obtained seeds of *R. angustifolius* C.C.Gmel from two Dutch populations (Doode Bemde and Wageningen) from R. Wesselingh and V. Ducarme (Université Catholique de Louvain, Belgium) and seeds of *R. minor* L. from two UK populations (Wiltshire and Somerset) from Emorsgate Seeds (Kings Lynn, Norfolk, UK).

We surface-sterilized the *Rhinanthus* seeds (3% v/v sodium hypochlorite solution, 2–3 min) and germinated them in the dark at 4°C over a three- to four-month period in sealed Petri dishes (9 cm diameter) containing moist, sterile filter paper and capillary matting. We planted and germinated barley seeds in soil (John Innes no. 1) in the dark at 20°C one week before they were required and then moved the seedlings into the light (20°C, 16 L : 8 D) 2 days before transplanting them into the experimental pots.

We transplanted single barley seedlings at the one or two fully expanded leaf-stage, into the centre of large plastic pots (15 cm diameter) filled with horticultural sand. Then, we planted *Rhinanthus* seedlings (2–4 per pot) with approximately 1 cm radicles at a distance of 2–3 cm from the barley near the surface of the sand. We lightly covered *Rhinanthus* seedlings with sand and sprayed with water. We planted more than one *Rhinanthus* seedling per pot, to ensure attachment of at least one parasite to the host plant, as not all seedlings successfully attach to the host plant. We also prepared uninfected controls of each barley genotype. We placed pots on upturned saucers in a greenhouse with supplementary lighting to provide a 16 L : 8 D photoperiod and watered them every day with 100 ml of 1/4 strength Hoaglands solution [58] for the duration of the experiment.

Two weeks after planting, we scored the *Rhinanthus* plants for morphological characteristics associated with attachment and continued monitoring levels of attachment for two weeks more. Attached *Rhinanthus* plants show inflated leaves and rapid growth when compared with unattached plants. Leaves also change colour upon attachment from dark green to yellowish-green [59]. We reduced *Rhinanthus* density to a single plant per pot at four weeks post-planting or when 80 per cent of the pots in a treatment group contained a minimum of one attached plant, whichever occurred sooner. Additional *Rhinanthus* plants were removed as soon as practicable at the start of the experiment to reduce the impact of multiple attachments to a single host plant. The experimental pots contained either a single barley plant and a single *Rhinanthus* plant (treatments) or single barley plants without *Rhinanthus* (negative controls). After four months, when the barley plants had set seed, we collected plants from all pots (shoots and roots) and left them to air-dry in paper bags. Once dry, we separated the *Rhinanthus* and barley plants from each other. We determined the total above-ground dry weight (shoot and fruit) for the barley, counted the number of barley seeds and recorded the height of the *Rhinanthus*. As density of the parasite infection was held constant at a single plant per host, we calculated parasite virulence as the difference in individual host plant fitness from the mean of the appropriate control group using the following equation:

$$\overline{V_{\mathrm{b,r}}} = \frac{\sum(w_{\mathrm{b,r}} - \overline{w_{\mathrm{bc}}})}{n_{\mathrm{b,r}}},$$

where $\overline{V_{\mathrm{b,r}}}$ is the mean virulence of *Rhinanthus* from population (r) growing on barley genotype (b), $w_{\mathrm{b,r}}$ is the seed set of an individual of barley genotype (b) infected by a plant from *Rhinanthus* population (r), $\overline{w_{\mathrm{bc}}}$ the average seed set of the uninfected control of barley genotype (b) and $n_{\mathrm{b,r}}$ the number of replicates per treatment.

We used a fully factorial experimental design with four barley genotypes, five *Rhinanthus* treatments (two populations per species and an uninfected control) and eight replicates, giving an initial total of 160 experimental pots (128 host–parasite pairs and 32 controls). Pots were fully randomized on a single bench within the greenhouse. *Rhinanthus* plants from all populations failed to grow in 15 pots across all host genotypes and we removed these from the analyses, giving a final total of $n = 145$ (113 host–parasite pairs and 32 controls).

In order to determine the effect of infection by *Rhinanthus* on the barley genotypes, we analysed barley fitness (number of seeds) using a partially nested three-way analysis of variance (ANOVA) with barley genotype, *Rhinanthus* treatment (both species and uninfected controls) and *Rhinanthus* population nested within *Rhinanthus* treatment as fixed effects ($n = 145$). We used Bonferroni-corrected multiple

contrast tests to compare the effect of *Rhinanthus* infection with the uninfected controls.

In order to determine if there was an interaction between *Rhinanthus* population and barley genotype on either the host or parasite, we analysed parasite virulence and *Rhinanthus* height using two separate three-way partially nested ANCOVAs with barley genotype, *Rhinanthus* species and *Rhinanthus* population nested within-species as fixed factors ($n = 113$). We included *Rhinanthus* height as a covariate in the analysis of parasite virulence and the total above-ground barley dry weight as a covariate in the analysis of *Rhinanthus* height. The covariates were included as an indicator of either parasite or host plant size to determine if larger parasites were more virulent and larger hosts supported larger parasites. We used post hoc Tukey–Kramer tests to analyse main effects further. We performed all statistical analyses in JMP v. 8. The following statistical models were used:

— Barley fitness = barley genotype + *Rhinanthus* treatment + *Rhinanthus* population (*Rhinanthus* treatment) + barley genotype × *Rhinanthus* treatment + barley genotype × *Rhinanthus* population (*Rhinanthus* treatment).
— Parasite virulence = *Rhinanthus* height + barley genotype + *Rhinanthus* species + *Rhinanthus* population (*Rhinanthus* species) + barley genotype × *Rhinanthus* species + barley genotype × *Rhinanthus* population (*Rhinanthus* species).
— *Rhinanthus* height = barley total above-ground dry weight + barley genotype + *Rhinanthus* species + *Rhinanthus* population (*Rhinanthus* species) + barley genotype × *Rhinanthus* species + barley genotype × *Rhinanthus* population (*Rhinanthus* species).

3. RESULTS

Four weeks after planting, attachment levels for all *R. angustifolius* treatments were over 80 per cent. Most *R. minor* treatments were also over 80 per cent attachment, except for two *R. minor* treatments from the Wiltshire population (Oregon Wolfe Recessive (75%), Morex (63%)) and two from the Somerset population (Steptoe (75%), Oregon Wolfe Dominant (75%)).

(a) *The effect of* Rhinanthus *infection on barley fitness*

Barley fitness was significantly affected by barley genotype (three-way partially nested ANOVA: $F_{3,125} = 95.27$, $p < 0.0001$), *Rhinanthus* treatment ($F_{2,125} = 54.06$, $p < 0.0001$) and an interaction between barley genotype and *Rhinanthus* treatment ($F_{6,125} = 6.15$, $p < 0.0001$). There was neither a significant effect of *Rhinanthus* population nested within treatment ($F_{2,125} = 1.76$, $p = 0.18$), nor an interaction between population nested within treatment and barley genotype ($F_{6,125} = 1.35$, $p = 0.24$). Barley fitness is therefore influenced by a combination of barley genotype and *Rhinanthus* treatment. Of the uninfected controls, genotype Steptoe was the most fecund (145 seeds ± 16; mean ± s.d.) and genotype Oregon Wolfe Dominant the least fecund (31 seeds ± 20; mean ± s.d.). When compared with uninfected control plants, both species of *Rhinanthus* reduced host fitness for barley genotypes Steptoe, Morex and Oregon Wolfe Recessive ($p < 0.001$). However, infection with either species of *Rhinanthus* did not have an impact on the fitness of barley genotype Oregon Wolfe Dominant ($p = 0.63$). Despite this, fitness of infected barley genotype Oregon Wolfe Dominant remained low in comparison with the majority (but not all) of the other infected barley genotypes (figure 1).

(b) *Effect of barley genotype,* Rhinanthus *species and population on parasite virulence*

Parasite virulence (measured as the difference in individual host plant fitness from the mean of the appropriate control group) was significantly affected by barley genotype (three-way partially nested ANCOVA: $F_{3,96} = 46.38$, $p < 0.0001$) and *Rhinanthus* species ($F_{1,96} = 20.30$, $p < 0.0001$). There was no significant effect of *Rhinanthus* population nested within-species ($F_{2,96} = 1.18$, $p = 0.31$) and there were no significant interactions between barley genotype and either *Rhinanthus* species ($F_{3,96} = 1.24$, $p = 0.30$) or population nested within-species ($F_{6,96} = 1.47$, $p = 0.20$). *Rhinanthus* height was not a significant covariate in the analysis ($F_{1,96} = 0.94$, $p = 0.33$), indicating that parasite size does not influence the virulence of the respective parasites. Instead, virulence is influenced by barley genotype and *Rhinanthus* species. Of the two parasite species, *R. angustifolius* was significantly more virulent than *R. minor* (Tukey–Kramer test: $p < 0.0001$) and all parasites infecting Oregon Wolfe Dominant barley were significantly less virulent than the parasites infecting the other three barley genotypes (Tukey–Kramer test: all $p < 0.05$; figure 2). As parasite virulence can be seen as the inverse of host tolerance, under the circumstances herein, barley genotype Oregon Wolfe Dominant can also be described as the most tolerant host.

(c) *Effect of barley genotype,* Rhinanthus *species and population on parasite size*

Parasite size (measured as height) was marginally significantly affected by barley genotype (three-way partially nested ANCOVA: $F_{3,96} = 2.77$, $p = 0.046$), significantly affected by *Rhinanthus* species ($F_{1,96} = 10.28$, $p = 0.0018$), *Rhinanthus* population nested within-species ($F_{2,96} = 4.36$, $p = 0.016$) and an interaction between barley genotype and *Rhinanthus* population nested within-species ($F_{6,96} = 2.89$, $p = 0.012$). Total above-ground barley dry weight was not a significant covariate in the analysis ($F_{1,96} = 3.38$, $p = 0.069$), indicating that the size of barley plants does not influence the size of the parasite. Rather, parasite size is determined by the specific combination of host genotype, *Rhinanthus* species and population (figure 3). As parasite size and fecundity are closely related, the larger parasites should also be the most fecund. The largest parasite was *R. angustifolius* from population Doode Bemde growing on Oregon Wolfe Recessive (height = 54.4 cm ± 8.6; mean ± s.d.) and the smallest was *R. minor* from Somerset growing on Oregon Wolfe Dominant (height = 31.6 cm ± 10.5;

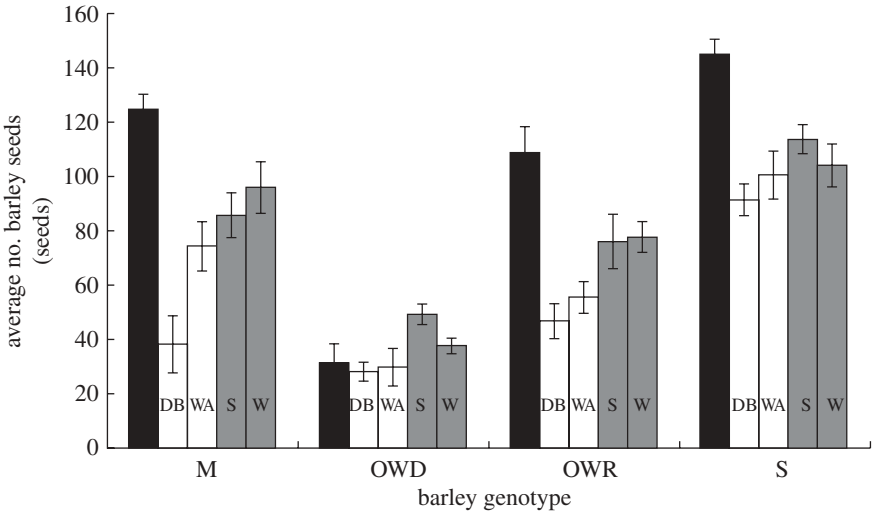

Figure 1. Average number of barley seeds for four genotypes of barley (Morex (M), Oregon Wolfe Dominant (OWD), Oregon Wolfe Recessive (OWR) and Steptoe (S) infected with *Rhinanthus angustifolius* (white bars) from two Dutch populations (Doode Bemde (DB) and Wageningen (WA)) and *Rhinanthus minor* (grey bars) from two UK populations (Somerset (S) and Wiltshire (W)). Black bars are uninfected barley controls. Error bars are ± 1 s.e.

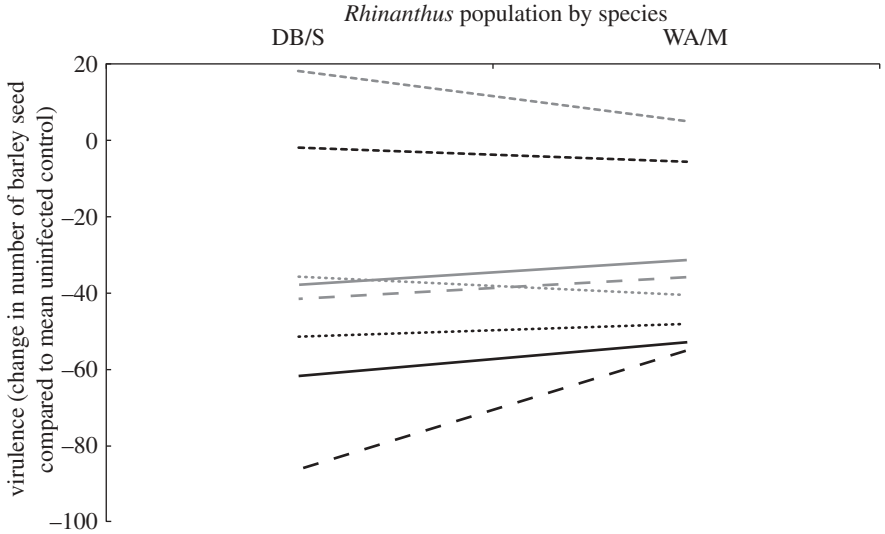

Figure 2. Reaction norms of parasite virulence (number of barley seeds normalized by controls) for four genotypes of barley (Morex, Oregon Wolfe Dominant (OWD), Oregon Wolfe Recessive (OWR) and Steptoe) infected with *Rhinanthus angustifolius* (RA, black lines) and *Rhinanthus minor* (RM, grey lines). Each line represents the mean reaction norm for a single genotype infected by two populations of a single species of *Rhinanthus*. *Rhinanthus angustifolius* originates from two Dutch populations (Doode Bemde (DB) and Wageningen (WA)) and *R. minor* from two UK populations (Somerset (S) and Wiltshire (W)). OWR, solid lines; Morex, long dashed lines; OWD, short dashed lines; Steptoe, dotted lines.

mean $\pm$ s.d.), suggesting that these two parasites should also have the highest and lowest seed set and therefore transmission ability, respectively, of the combinations tested. As quantitative resistance can also be defined as a reduction in host fitness, Oregon Wolfe Dominant has the highest resistance to *R. minor* from Somerset, and Oregon Wolfe Recessive has the lowest resistance to *R. angustifolius* from Doode Bemde of all the combinations tested.

4. DISCUSSION

Here we demonstrate, for the first time in a *Rhinanthus*–barley system, that the outcome of infection for both host and parasite, in terms of host fitness, host tolerance, parasite virulence and transmission ability, depends on genetic variation within both partners.

(a) *Ecosystem effects*

We know from previous studies that *Rhinanthus* can have cascading effects on grassland communities that reach far beyond its immediate impact on an individual host plant. Presence of *Rhinanthus* can influence plant [60,61], soil [32] and arthropod [36] community structure as well as the cycling of nutrients within the system [33]. From other research we know that genetic variation within a focal plant can change the outcome of interactions with arthropods, soil microbes [6,11] and the effects of an associated ecosystem engineer

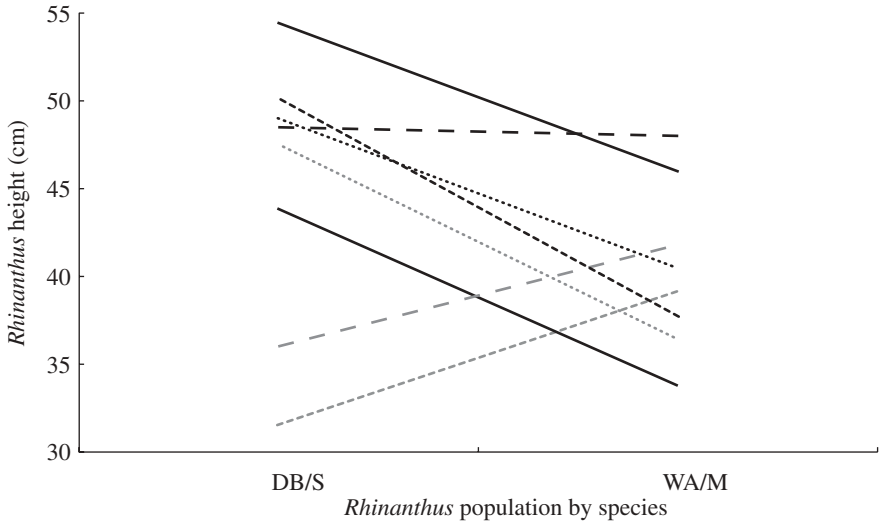

Figure 3. Reaction norms of *Rhinanthus* height (cm) for four genotypes of barley (Morex, Oregon Wolfe Dominant (OWD), Oregon Wolfe Recessive (OWR) and Steptoe) infected with *Rhinanthus angustifolius* (RA, black lines) and *Rhinanthus minor* (RM, grey lines). Each line represents the mean reaction norm between two populations of the same species of *Rhinanthus* growing on a single genotype of barley. *Rhinanthus angustifolius* originates from two Dutch populations (Doode Bemde (DB) and Wageningen (WA)) and *R. minor* from two UK populations (Somerset (S) and Wiltshire (W)). OWR, solid lines; Morex, long dashed lines; OWD, short dashed lines; Steptoe, dotted lines.

[10]. Finally, we know genetic variation to be an important factor driving competition among, and long-term survival of, grassland plant species [2,3,62]. A proportion of the effects of *Rhinanthus* on plant community species composition is attributed to the differences in resistance to, and tolerance of, parasite infection by potential host species [25]. Here, we show that genetic variation in the host can change its tolerance to infection and the virulence of the parasite. We also show that an interaction between genetic variation in the host and parasite changes the size, potential seed set and hence transmission ability of *Rhinanthus* and resistance of the host. Therefore, it follows that the effects of *Rhinanthus* on community composition probably depend on genetic variation within host and parasitic plants. Some variation in the plant community diversity response to *Rhinanthus* has been noted [21] and we propose that this is due, at least in part, to the genetic variation within host and parasite species.

We used a non-natural host for this study, so caution needs to be exercised when extrapolating these results to natural host–*Rhinanthus* communities. However, in an earlier study on local adaptation in *R. angustifolius* (syn. *serotinus*), Mutikainen *et al.* [22] showed similar patterns of variation in the response of different populations of the natural grass host *Agrostis capillaris* to infection as we found among the genotypes of barley. Similarly, in an alternative natural host–parasitic plant system (*Urtica dioica–Cuscuta europaea*), Koskela *et al.* [37] demonstrated genetic variation in host resistance and tolerance traits. Thus, it seems likely that the genetic variation we observed in response to infection between *Rhinanthus* and barley should translate to a more natural community.

We examined variation among two hybridizing species of *Rhinanthus* and two populations of each species growing on distinct barley genotypes. Ideally, we would have used distinct genotypes of *Rhinanthus* as well, but these are not available currently for either parasite species. Hybrid complexes have been used previously in similar studies to good effect where the two parental species represent morphological and genetic extremes of types [6]. The pure species of *Rhinanthus* we used fall into two distinct morphological groups based on their floral characteristics. However, when hybrid and backcross individuals are included in the analysis, phenotypic variation across species and hybrids is continuous [63,64]. The two *Rhinanthus* species are genetically distinct and genetic differences between the parent and the hybrid groups can also be detected [64]. Analysis using recently developed microsatellite markers for *R. minor* indicates that there is genetic variation among populations [65]. Therefore, it is reasonable to assume that phenotypic differences among populations are due, in part, to genetic differences.

Common garden experiments are an established technique for separating the effect of within-species genetic variation from environmental-based variation [66]. However, the phenotypic variation observed among populations in a common garden also includes maternal effects, for which we did not control. While maternal effects are an important factor controlling the traits of seeds and young plants, these effects generally decrease with age [67]. Maternal effects are, therefore, not likely to be a major cause of variation in final adult height of *Rhinanthus* (as measured here). However, we cannot completely rule out the possibility that variation among populations of *Rhinanthus* in our experiment is caused by a mixture of genetic variation and maternal effects.

(b) *Host–parasite interactions*

Previous studies concentrate on the reduction of host biomass following infection by *Rhinanthus* species

(see [19] for review). Here, we show that infection also reduced host fitness in terms of seed production to three of the four host genotypes tested. Unsurprisingly, different host genotypes vary in their fecundity, with Oregon Wolfe Dominant producing substantially fewer seeds overall than Oregon Wolfe Recessive, Morex or Steptoe. Seed production was not reduced in infected Oregon Wolfe Dominant hosts when compared with uninfected controls, which showed that the least virulent *Rhinanthus* infections occurred on this genotype. This suggests that Oregon Wolfe Dominant barley is highly tolerant to infection by *Rhinanthus*, while the other genotypes are less so. All barley genotypes are doubled haploid lines. The two Oregon Wolfe barleys were constructed to form one strain with multiple dominant traits (Oregon Wolfe Dominant) and another strain with multiple recessive traits (Oregon Wolfe Recessive) from an original population of barley [68]. Some of the dominant traits can be attributed to wild barley (*H. vulgare* L. subsp. *spontaneum* (K. Koch) Thell.), while most of the recessive traits originate from agricultural barley strains [68]. Both the genotypes Steptoe and Morex are strains of barley that were developed to have a number of good agronomic traits [53]. The high tolerance of infection but low fitness of Oregon Wolfe Dominant suggests that there is a trade-off between fitness and tolerance of parasite attack, and that the agricultural strains, bred to maximize fitness, may have lost tolerance to parasite infection.

In contrast to resistant host genotypes, tolerant hosts may not reduce the fitness of an infecting parasite [39]. This defence strategy allows the parasites to persist and spread in a population. For tolerance to be an evolutionarily stable strategy, there must be some fitness advantage to tolerant hosts, i.e. an infected tolerant host should have a higher fitness than an infected non-tolerant host. The most tolerant host in our study, Oregon Wolfe Dominant, also had the lowest overall fitness. This was the case both before and after infection, although its relative fitness when compared with the other barley genotypes changed with infection status. We kept infection density constant at a single parasite per host, but other studies have shown that host tolerance can change with parasite density, with more tolerant hosts at one parasite density becoming relatively less tolerant at another [39]. The effect on host plant tolerance of multiple infections with *Rhinanthus* is unknown. However, in natural populations, multiple *Rhinanthus* infections of a single host are possible and differential tolerance gradients with changing infection density would probably also influence host community dynamics.

In terms of the parasites, *R. angustifolius* has a larger impact on host fitness than *R. minor* for all barley genotypes and is thus the more virulent parasite species under our experimental conditions. We hypothesized that this might be the result of *R. angustifolius* being a more robust plant than *R. minor* [69], better able to abstract host resources. However, we found that although *R. angustifolius* is generally larger than *R. minor*, this is not always the case. For example, *R. minor* from the Somerset population, growing on barley genotype Steptoe, is a similar size to many *R. angustifolius*–host genotype combinations, while *R. angustifolius* from the Wageningen population growing on barley genotypes Oregon Wolfe Dominant and Steptoe is similar size to many of the *R. minor*–host genotype combinations. In addition, when included as a covariate in the analysis, parasite size had no significant effect on virulence, suggesting the virulence was independent of parasite size.

Parasite size gives an indication of potential seed set [25] or transmission ability of the parasites [41] and also host resistance to infection [37]. In our study, parasite size depended on the specific combination of parasite population and host genotype, suggesting that genetic variation within both host and parasite affects parasite fitness. Tolerance and resistance have been proposed as complementary plant-defence strategies, where a fully tolerant genotype has no need of resistance and vice versa. While in extreme cases this may be so, evidence for a clear negative relationship between tolerance and resistance traits in plants is conflicting [38,70]. In our study, the largest parasite was *R. angustifolius* from Doode Bemde growing on Oregon Wolfe Recessive barley and the smallest *R. minor* from Somerset growing on Oregon Wolfe Dominant. These represent the least and most resistant host–parasite combinations, respectively. Virulence of *R. angustifolius* from Doode Bemde growing on Oregon Wolfe Recessive was relatively high, suggesting that tolerance of this combination was low. Virulence of *R. minor* from Somerset growing on Oregon Wolfe Dominant was low, suggesting that tolerance of this combination was high. These results indicate a positive, rather than negative relationship between tolerance and resistance in barley. This corroborates evidence from a meta-analysis by Leimu & Koricheva [70], who found positive correlations between tolerance and resistance traits in crop plants but negative correlations in wild plants.

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