

Research article

Local adaptation of a generalist hemiparasitic plant to one of its potential host plants

Tobias M. Sandner¹✉, Milan J. Brand¹, Manuel Hickler¹, Lotte Korell^{1,2,3} and Diethart Matthies¹

¹Department of Biology, Plant Ecology, Philipps-Universität Marburg, Marburg, Germany

²Department of Community Ecology, Helmholtz Centre for Environmental Research (UFZ), Halle, Germany

³German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Correspondence: Tobias M. Sandner (tobias.sandner@biologie.uni-marburg.de)

Oikos

2024: e10330

doi: [10.1111/oik.10330](https://doi.org/10.1111/oik.10330)

Subject Editor: Simon Peter Hart

Editor-in-Chief:

Gerlinde B. De Deyn

Accepted 31 March 2024



Coevolution is often found in parasite–host interactions, but has not yet been described for hemiparasitic plants and their hosts. Root hemiparasites like *Rhinanthus alectorolophus* perform photosynthesis but also parasitize other plant species, some of which (e.g. *Plantago lanceolata*) may defend themselves against parasite attack by blocking the haustoria of the parasites. We grew seedlings of the hemiparasite *R. alectorolophus* and the potential host *P. lanceolata* from seven grassland sites in a factorial design. To detect differences in host defence, we also included hosts from two ‘naïve’ populations from regions where the parasite does not occur. *R. alectorolophus* grew consistently larger and had higher fitness with sympatric than with allopatric hosts, suggesting parasite adaptation to local host populations. Moreover, *R. alectorolophus* remained smallest with allopatric hosts from the same region and reached intermediate sizes with allopatric hosts from other regions or naïve hosts, suggesting host adaptation to parasites at the regional scale. Parasite presence reduced the size of the host plants already after four weeks, but only that of hosts with ‘experience’ of the parasite, suggesting an early host response. Follow-up experiments confirmed that parasites attach to hosts already after four weeks and hosts respond by changing belowground allocation patterns. However, parasite roots did not preferentially grow towards sympatric hosts. Our results suggest that local adaptation to hosts can occur even in generalist parasites and does not require specialization on individual hosts. We discuss the role of potential mechanisms, including variation in chemical signalling (early) and in host defence (late effects).

Keywords: arms race, coevolution, hemiparasite, host choice, keystone species, *Plantago lanceolata*, reciprocal adaptation, *Rhinanthus alectorolophus*

Introduction

Hemiparasitic plants are capable of photosynthesis, but depend to different degrees on the water, nutrients and additional carbohydrates taken up from their host plants (Těšitel 2016). They connect to the stems (stem parasites) or roots (root parasites) of



www.oikosjournal.org

their hosts with specialized organs called haustoria (Heide-Jørgensen 2013). While some parasitic plants are weeds causing substantial damage to agriculture (Parker 2012, Gressel and Joel 2013), most root-hemiparasites are grassland plants, some of which (including *Rhinanthus alectorolophus*) are regarded as keystone species that suppress dominant grass species and increase biodiversity across trophic levels (Pywell et al. 2004, Hartley et al. 2015, Korell et al. 2020). Most root hemiparasitic plants, including the European genus *Rhinanthus* (Orobanchaceae), are generalists using a wide range of host species. For example, excavation of root systems has identified haustorial connections of *Rhinanthus minor* to 50 plant species (Gibson and Watkinson 1989) and functional haustoria of *Melampyrum* and *Rhinanthus* species with 14–17 host species (Holá et al. 2017). More than 20 species have been experimentally confirmed as hosts for *Melampyrum arvense* and *R. alectorolophus*, including grasses, legumes and forbs (Matthies 2017, 2021). Nevertheless, plant species differ strongly in their quality as hosts for hemiparasites (Hautier et al. 2010, Brown et al. 2021, Matthies 2021), which is due to differences in the quality and quantity of resources supplied (Govier et al. 1967, Seel and Jeschke 1999) and due to differences in host defence, as some species are capable of specific mechanisms to block the formation of haustoria or their functioning (Rümer et al. 2007, Timko and Scholes 2013). Even within parasite species there is variation among populations (Jonstrup et al. 2016, Sandner and Matthies 2017) and among seed families within populations (Moncalvillo and Matthies 2023) in the quality of individual host species for the parasite. The amount and importance of this variability is not yet understood.

Variation in the quality of hosts for different parasite populations or genotypes may lead to adaptation of parasites to the best hosts. However, hosts can also adapt to avoid damage by the parasites. For example, many *Sorghum bicolor* genotypes resistant to the functional holoparasite *Striga hermonthica* are known from Africa, and they are particularly common where the parasite occurs (Rich et al. 2004, Bellis et al. 2020). *Rhinanthus* performance has been shown to depend on interactions between host and parasite genotypes (Mutikainen et al. 2000, Rowntree et al. 2011), which is a prerequisite for reciprocal adaptation. The selection for increasing host resistance and increasing parasite virulence can lead to coevolutionary dynamics, resulting in increased performance of either parasites or hosts with sympatric compared to allopatric partners (Kaltz and Shykoff 1998, Greischar and Koskella 2007). However, until now there is no evidence for local adaptation of hemiparasites to their hosts, because in the few experimental studies hemiparasites did not grow better with sympatric than allopatric hosts (Lammi et al. 1999, Koskella et al. 2000, Mutikainen et al. 2000 for the generalist holoparasite *Cuscuta*).

Different reasons have been proposed to explain the lack of adaptation of hemiparasites to their local hosts. For example, hemiparasites and their host plants have relatively similar generation times, which is in strong contrast to microparasites, which evolve much faster than their hosts (Mutikainen et al.

2000). Moreover, the very restricted ability to forage for specific hosts in diverse grassland habitats may prevent generalist parasites from specializing on specific hosts (Mutikainen et al. 2000, Moncalvillo and Matthies 2023). However, the apparent lack of local adaptation of hemiparasites could also be due to differences in the defence mechanisms of host plants. All prior studies have focused on interactions of hemiparasites with a dominant host species in their communities from which defence mechanisms are not known (Lammi et al. 1999, Mutikainen et al. 2000, Ahonen et al. 2006). In contrast, coevolution is more likely when a host may evolve an effective defence mechanism which has to be overcome by the parasite.

It is difficult to detect coevolution between parasites and their hosts. When both species reciprocally adapt to each other, patterns of local adaptation may not be visible (Brockhurst and Koskella 2013). The dynamic nature of coevolution may lead to a geographic mosaic where local adaptation is detected only in some years and at relatively local spatial scales (Lively 1999). To increase the probability to detect local adaptation it is thus useful to study it at different spatial scales (Kaltz and Shykoff 1998, Greischar and Koskella 2007, Adiba et al. 2010). Here, we suggest to also study ‘naïve’ host populations which have not been in contact with the parasite and should thus be less resistant against parasite attack than ‘experienced’ host populations. In such comparisons, increased parasite performance with naïve vs experienced hosts would indicate adaptation of host populations to the parasite, which in classical tests for local adaptation (parasites grown with sympatric vs allopatric hosts) may be masked by simultaneous local adaptation of the parasite (Fig. 1c).

We studied coevolution in the interactions between the hemiparasite *R. alectorolophus* and its host *Plantago lanceolata*. This species has been shown to be able to completely block the haustoria of *R. minor* (Rümer et al. 2007). However, a recent study found that *P. lanceolata* from a commercial seed supplier was a suitable host for *R. alectorolophus* from one population, while it was a very poor host for parasites from another population (Sandner and Matthies 2017), which may suggest the evolution of adaptation to *P. lanceolata* in some populations of *R. alectorolophus*. We collected seeds of hosts and parasites in three regions in central Europe from grassland sites where both occur, but also included seeds of *P. lanceolata* from sites where *R. alectorolophus* does not occur. By growing pairs of hosts and parasites from different populations in a reciprocal design, we addressed the following questions: 1) does *R. alectorolophus* grow better with hosts from sympatric than from allopatric populations, suggesting adaptation of the parasite to local host populations (Fig. 1a)? 2) Does *P. lanceolata* grow better with parasites from sympatric than allopatric populations, suggesting adaptation of the host to the local parasite populations (Fig. 1b)? 3) Does *R. alectorolophus* perform better with naïve hosts than with experienced hosts that have been in contact with the parasite, suggesting either adaptation of the host (Fig. 1b) or even coevolution of both species (Fig. 1c)?

Because first results suggested a stronger growth of parasites with sympatric hosts already at a time when they had

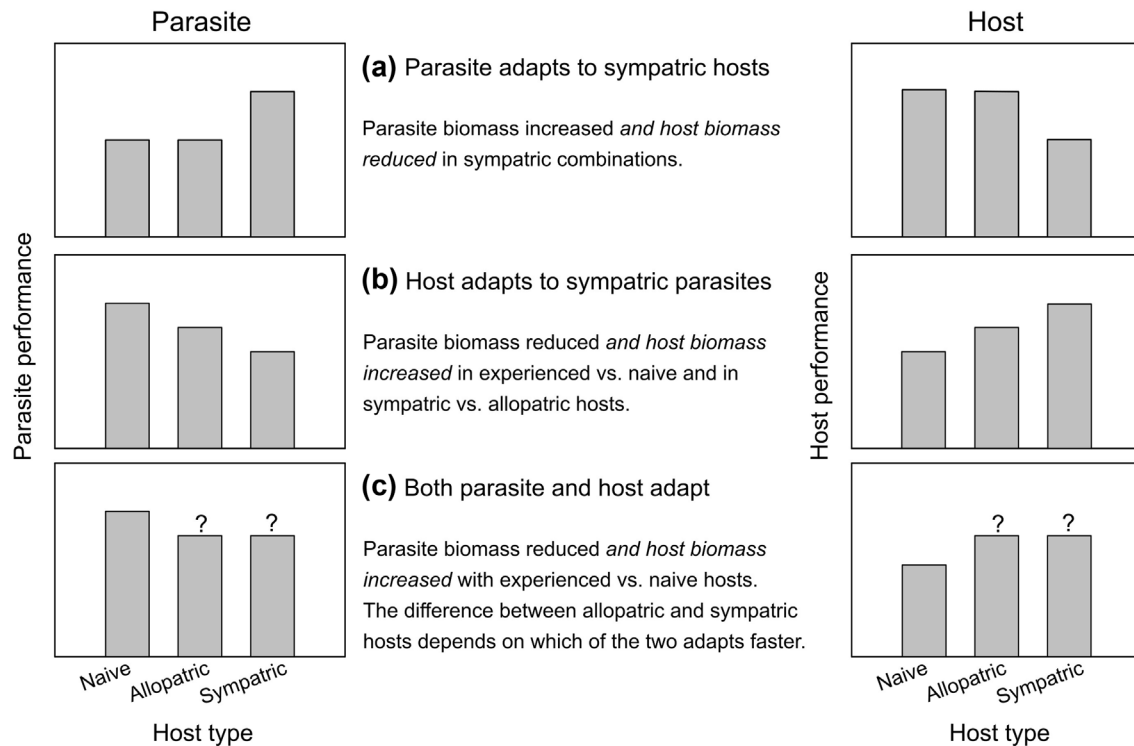


Figure 1. Schematic illustration of the expected outcomes of parasite–host coevolution. Bars show the expected differences in parasite (left) and host performance (right) when grown with naïve or experienced (allopatric or sympatric) hosts. Note in case (c) when both parasite and host adapt to each other that the relative performance of allopatric or sympatric hosts (or parasites) may differ depending on whether the parasite or the host adapts faster; the absence of differences between these two groups does thus not necessarily indicate the absence of coevolution.

not necessarily attached to a host, we performed a follow-up experiment to study the effect of early connections between *R. alectorolophus* and *P. lanceolata* on parasite and host root traits. Moreover, we exposed parasite seedlings to sympatric and allopatric host seedlings in agar plates and asked: 4) do parasite seedlings recognize the roots of sympatric host seedlings and grow preferably in their direction?

Material and methods

Study species

Rhinanthus alectorolophus is an annual hemiparasite from the Orobanchaceae family. It grows in mesic or dry and nutrient-poor grasslands of central Europe and reaches its northern distribution limit in Germany. Seeds of *Rhinanthus* spp. germinate in the absence of hosts after cold stratification and seedlings then form haustorial connections to roots of their hosts. *Rhinanthus alectorolophus* uses a broad range of species as hosts, but shows preferences for some species. Although on average the species grows better with grasses and legumes than with forbs, good and poor host species are found within each of these functional groups (Sandner and Matthies 2018, Matthies 2021). The quality of a species as a host is also known to differ among seed families (i.e. genetic lineages) of *R. alectorolophus* (Moncalvillo and Matthies 2023) and populations of the parasite (Sandner and Matthies 2017). The yellow flowers of *R. alectorolophus* are pollinated by bumblebees,

but self-fertilization is possible (Sandner and Matthies 2017). Seeds are relatively large (4.8 mg, Sandner and Matthies 2017) and have a winged margin. In closely related *R. minor*, the terminal velocity of the seeds was found to be relatively low ($2\text{--}3\text{ m s}^{-1}$) compared with non-winged seeds of similar size of other grassland species (Bullock et al. 2003, Zhu et al. 2022), which allows for some not very efficient wind dispersal, but also aids dispersal by mowing machinery (Bullock et al. 2003). The seed bank is assumed to be short lived (3–5 years, Oberdorfer 2001).

Plantago lanceolata (Plantaginaceae) is a common perennial grassland plant often cooccurring with *R. alectorolophus*. Its distribution is much larger than that of the parasite, and it occurs throughout Germany, also in regions where *R. alectorolophus* is not present. *Plantago lanceolata* is predominantly wind-pollinated (Clifford 1962, Oberdorfer 2001), but in contrast to the parasite is a perennial with an average lifespan of nearly three years (max = 8 years, Roeder et al. 2017, Steiner et al. 2021). The conditional life span of the closely related *P. media* has even been estimated as 28 years (Ehrlén and Lehtilä 2002). The soil seed bank is reported to be long-lived (> 15 years, Oberdorfer 2001).

Because *Rhinanthus* is insect-pollinated and the seeds are large and only dispersed over short distances (Bullock et al. 2003), gene flow may be very limited in *Rhinanthus* species in comparison to wind-pollinated *P. lanceolata*. If one calculates the mean F_{ST} for populations at a distance of less than 100 km from each other using data for *Rhinanthus angustifolius*

by Jonstrup et al. (2018) from Sweden and by Smith et al. (2020) for *P. lanceolata*, one obtains an F_{ST} value for *P. lanceolata* of 0.026 and for *R. angustifolius* of 0.176, indicating stronger genetic differentiation among populations of *R. angustifolius* than among those of *P. lanceolata*. Moreover, the increase in genetic distance between pairs of populations with geographical distance is much stronger for *R. angustifolius* than for *P. lanceolata* (Supporting information).

Study sites

We collected seeds of both species at 3–6 sites in each of three regions in Germany and Austria (Supporting information). In addition, we collected seeds of *Plantago* at three sites where *Rhinanthus* does not occur (Fig. 2a). However, as seeds from some populations did not germinate at the same time as those from the other populations, the experiment finally contained plants from eight sites with joint occurrence of host and parasite and two host-only sites (Table 1). The distance between populations within a region ranged from 400 m to 20 km, but was < 2 km in most cases. The distance between regions was 80 km (NH-RH), 350 km (RH-KW) and 430 km (NH-KW). The two 'naïve' populations of *Plantago* were separated by at least 15 km (Mar) or 250 km (Lun) from the nearest *R. alectorolophus* population ever reported by floristic records (BfN 2022), and more than 80 / 350 km from the nearest *Rhinanthus* population used in this study. The floristic survey in Germany includes training of volunteers and control of data quality to guarantee a comprehensive coverage of the

country (Bettinger 2013) – species absences in these records thus indicate true absences. Although this was excluded for the year in which seeds were collected, in previous years other parasite species may have occurred in both naïve populations, potentially including hemiparasites *R. minor*, *Odontites ver-nus* or pathogenic fungi. As there are hardly any regions in Germany where no parasitic plant species occur, we decided to include one close and one distant host population that had no contact with the studied parasite species during the last 100 years. At each site, we collected seeds from 20 individuals per species and pooled them per population.

Experimental set-up

For both *Rhinanthus* and *Plantago*, 50 seeds from each population were placed on several petri dishes on moist filter paper and stratified at 4°C for two weeks (*Plantago*) or four months (*Rhinanthus*). When the seeds of *Rhinanthus* started to produce radicles, they were transferred to conditions of fluctuating temperature and light (12 h light, 15 / 8°C) to produce cotyledons.

One seedling each of host and parasite were planted close to each other in 0.5-l pots filled with a 1:1 mixture of sand and commercial potting soil and placed in a climate chamber (20°C, 14 h lighting by LEDs). Depending on the number of seedlings available, 3–6 replicates were set up for each combination of host and parasite population (333 pots). As controls, 3–6 seedlings from each parasite population were also grown without a host, and three host seedlings from each

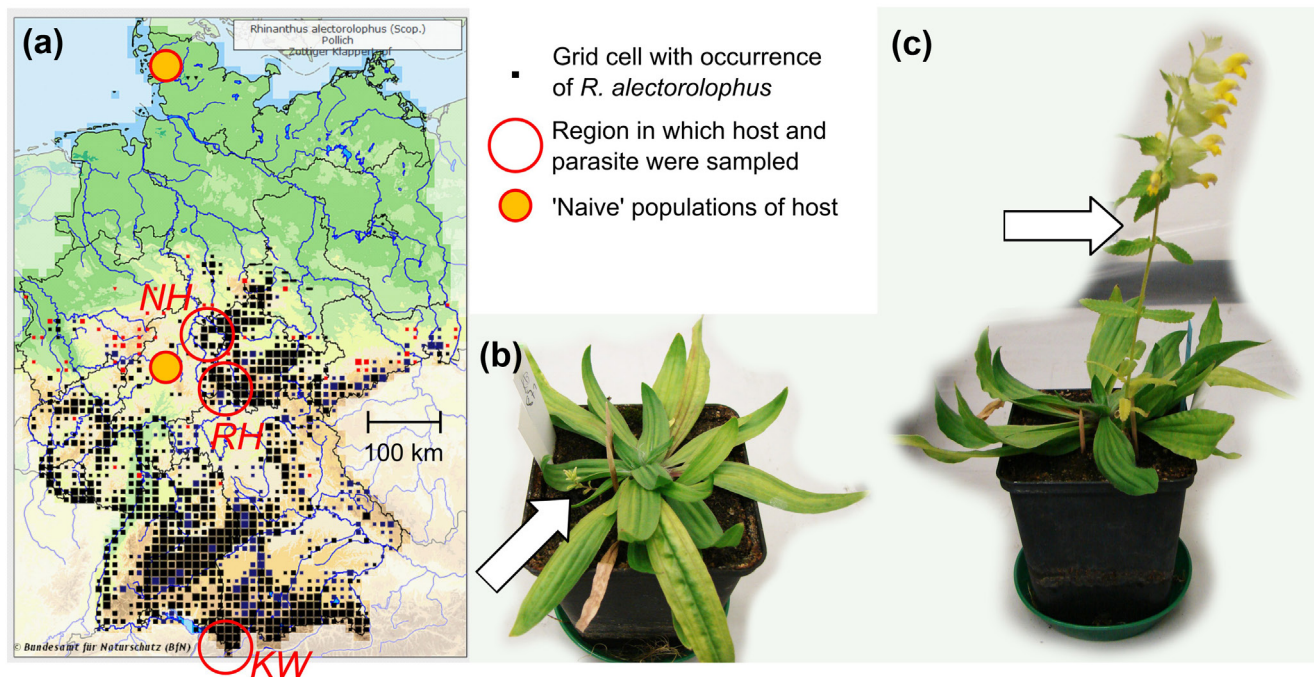


Figure 2. The study system. (a) Map showing the distribution of the hemiparasite *Rhinanthus alectorolophus* in Germany (black squares, printed with permission from BfN 2022) and the location of the regions where seeds of both host and parasite (red circles) or only host seeds (filled smaller circles) were collected, see Table 1. *Plantago lanceolata* occurs in nearly all German grid cells. (b–c) Images of plants from the experiment shortly before harvest showing rosettes of *P. lanceolata* together with (b) a very small parasite (left of the host), and (c) a large and flowering parasite. Parasites are indicated by arrows.

Table 1. Design of the experiment: Number of replicate pots planted for each combination of host and parasite population. Colours of the cells denote sympatric (green), near allopatric (yellow), far allopatric (red) combinations of host and parasite population and naïve host populations (pink). Hi2 was not included as a host population due to poor germination of host seeds.

			Parasite population								
	Host region	Pop.	Eis	Rös	Dün	Kle	Mil	Rie	Hi1	Hi2	No parasite
Host population	Northern Hesse (NH)	Eis	5	5	3	5	3	5	5	6	3
		Rös	5	5	3	5	3	5	5	6	3
	Rhön (RH)	Dün	5	5	3	5	3	5	5	6	3
		Kle	5	5	3	5	3	5	5	6	3
		Mil	5	5	3	5	3	5	5	6	3
	Kleinwalsertal (KW)	Rie	5	5	3	5	3	5	5	6	3
		Hi1	5	5	3	5	3	5	5	6	3
		Hi2	—	—	—	—	—	—	—	—	—
	Naïve range of host	Lun	5	5	3	5	3	5	5	6	3
		Mar	5	5	3	5	3	5	5	6	3
No host			5	5	3	5	3	5	5	6	

population without a parasite (397 pots in total, Table 1). Plants received every two weeks 20 ml of a fertilizer solution (5 g l⁻¹, N:P:K = 14:7:14%, Hakaphos Gartenprofi, Compo, Münster, Germany). Of the parasite seedlings, 69% died in the first week after planting, but mortality was not related to host origin ($\chi^2 = 1.0$, df = 3, $p = 0.80$). Instead, mortality differed between parasite populations ($\chi^2 = 45.9$, df = 7, $p < 0.001$), likely due to genetic differences and differences in the age of seedlings at the time of starting the experiments. Dead seedlings were replaced over a period of two weeks, except for populations with a shortage of germinated seedlings. The replacement of seedlings did not influence the final performance of the parasites (Supporting information).

After four weeks of growth, the number of leaves of each *Plantago* plant was counted and the length of the longest leaf was measured for all hosts and parasites. Six weeks later, it was recorded which plants had survived and which were flowering, and the above-ground biomass of both species was harvested separately, dried for 48 h at 80°C and weighed. The pots with the roots were frozen at -20°C. To study the connection between host and parasite, the root systems of one of the largest parasites and its host were carefully washed and microscopic images were taken of serial sections of a large haustorium (Supporting information).

Root allocation experiment

To study potential early effects of hemiparasite presence on early root growth of the hosts, we grew in a greenhouse 36 *Plantago* seedlings with and 36 without a *Rhinanthus* seedling in 0.5-l pots that were filled with sand to facilitate washing of the roots (14 h light, SON-T Agro 400W). We used host seeds from two naïve and two experienced populations and parasite seedlings from three different populations, none of which was sympatric to any of the host populations used (Supporting information). Parasite and host populations were combined in a full-factorial design with three replicates for each combination. Plants received every week 20 ml of a fertilizer solution (5 g l⁻¹, N:P:K = 14:7:14%, Hakaphos Gartenprofi). After four weeks of growth the length of the longest leaf of each *Rhinanthus* and the length and width of the longest leaf and the number of leaves of each *Plantago* was

measured. The plants were then harvested above ground, the roots of both species carefully washed free of soil, checked for haustorial connections and separated. It should be noted that we could only tell which parasites had attached to host roots, but not whether these connections were functional or not. Roots of *Plantago* floating in water were scanned at 600 dpi using a flatbed scanner with a transparency unit. All plant material was dried for 48 h at 80°C and weighed.

Host choice experiment

To test if the increased early size of parasite seedlings with sympatric hosts might have been due to the early recognition of roots from sympatric host populations, we performed a host choice experiment in agar following the methods described in Sandner et al. (2022), except that closed petri dishes (without holes) were used to reduce algal contamination. We used parasite and host seeds collected in four populations in northern Hesse (Supporting information). Seeds were surface sterilized in commercial bleach (2.6% sodium hypochlorite) for 3 min and washed thoroughly with purified water before vernalization at 4°C (*Rhinanthus* only) and germination. Square petri dishes (12 × 12 cm²) were filled with 100 ml agar (0.5%), in which three cuts were made to place the roots of a parasite seedling (in the centre) and two host seedlings (at 1.5 cm distance at either side). Parasite seedlings from each of the four populations were given the choice between *Plantago* seedlings from the same population (sympatric, n = 15) or one of the three other populations (allopatric, each n = 5), resulting in 15 replicates × 4 populations = 60 petri dishes. To avoid directional effects, the sympatric hosts were planted on the right or left side of the parasite in equal numbers. Petri dishes were placed at an angle of 45° in a growth chamber (19°C, 12 h LED light). After seven days, we took orthogonal photos of the petri dishes and measured the maximum lateral extent of the parasite root system in the direction of the two host seedlings. As there was no indication of host choice, which could have been due to the removal of beneficial microbes by seed surface sterilization, we repeated the experiment with host seedlings grown from unsterilized seeds from the same populations. The number of plates was slightly lower in the second experimental

run, because we were limited by the number of parasite seedlings available (9–11 replicates $\times$ 4 populations = 40 petri dishes). Differences between the two experimental runs must be interpreted with caution and may also have been due to factors other than sterilization. However, we tried to keep all other experimental conditions as similar as possible, except for the surface sterilization of the host seeds.

Data analysis

We differentiated between four types of hosts for each parasite population: 1) host originating from the same site (= sympatric) as the parasite, 2) host originating from a different site in the same region where the parasite occurs (= near allopatric), 3) host originating from a different region than the parasite, but from sites where the parasite occurs (= far allopatric) and 4) host originating from a site where the parasite does not occur (= naïve hosts). We tested the effect of host type on the performance of the parasite (leaf length after four weeks of growth, biomass at harvest) using linear mixed models including parasite population as a random factor using the R-package ‘lme4’ (Bates et al. 2015). The length of the longest leaf of *Rhinanthus* is known to be a good measure of size as it correlates strongly with parasite biomass (Supporting information). Adjusted denominator degrees of freedom, F- and p-values of the fixed effects were determined using Satterthwaite’s approximation using the R-package ‘lmerTest’ (Kuznetsova et al. 2017). Pairwise differences between different host types were tested with a Tukey test using the R-package ‘emmeans’ (Lenth 2023). An alternative analysis using a hierarchical ANOVA yielded similar results (Supporting information). Parasite biomass was log-transformed prior to analyses to meet the assumptions of homoskedasticity and normally distributed residuals. Survival of the plants was analysed with generalized linear mixed models with a logit link and a binomial error distribution including host type as a fixed factor and parasite population as a random factor. For the binomial models, p-values were determined by using Wald tests with the R-package ‘car’ (Fox and Weisberg 2019).

A multiplicative fitness function was created by calculating for each combination of host $\times$ parasite population the mean survival of parasites until harvest and the geometric mean of the biomass of surviving parasites. Both values were multiplied to represent the mean biomass produced by each parasite seedling planted. We used biomass and not flower number, because this variable shows a more continuous distribution and is more useful for the small sample size of 154 surviving plants, of which only 20 flowered. In annual plants (including *Rhinanthus*), fitness is closely related to plant biomass. Although in the current experiment many medium-sized parasites did not produce any flowers, this could be different under different growing conditions (e.g. more light). The effect of host type on the parasite fitness function was analyzed by a general linear mixed model including parasite population as a random factor. Moreover, we used linear regression with a quadratic term to test the effect of the (log) geographic distance between the sites of origin of host and

parasite on parasite fitness. For this analysis, distance was set to 10 m for sympatric combinations.

As a measure of early host size we used the cumulative leaf length after four weeks (number of leaves $\times$ length of the longest leaf), because it better represented variation in plant size than leaf length alone (Supporting information). We used general linear mixed models to analyse effects on host performance (early leaf size, final biomass). These models included parasite presence (yes or no), host naïvety (host population with or without the parasite) and their interaction as fixed factors. Host population was included as a random factor, but it was removed from the analysis of final host biomass, because it did not significantly improve the result (likelihood ratio test). After restricting the dataset to only experienced host populations we used a general linear model with type I sums of squares including the contrasts ‘parasite presence’, ‘sympatric (yes or no)’ and ‘near versus far allopatric’ to test if suppression was stronger by sympatric, near or far allopatric parasites. Host leaf length was log-transformed prior to analysis to meet the assumptions of homoskedasticity and normally distributed residuals.

To test if parasite effects on host size were related to parasite size, we performed linear regressions of cumulative host leaf length on parasite leaf length after four weeks of growth and of final log host biomass on final log parasite biomass.

In the root allocation experiment, the scans of the roots of *Plantago* were analyzed using Root Graph (Cai et al. 2015) to determine average root thickness and total root length of lateral roots. We analyzed the effect of attachment (unattached / attached parasite) on leaf length and biomass of the parasite using ANOVAs. We analyzed host traits (shoot and root dry mass, root thickness) using ANOVAs with type I sums of squares with the factors parasite presence (yes / no) and attachment (unattached / attached parasite). Host or parasite population were not included as random factors, because they did not significantly improve the result (likelihood ratio test). Similarly, whether a host population was naïve or experienced did not influence any response variable except host shoot mass – for this trait we present results of a model including parasite attachment $\times$ host naïvety. We used general linear models to analyze the effects of parasite presence on allometric relationships between log shoot mass and log root mass and between lateral root length and total root biomass. Similarly, for the subset of plants with a parasite, we used general linear models to analyze the effects of attachment of the parasite on allometric relationships.

We analysed the host choice experiment using general linear mixed models to test for the effects of sympatric versus allopatric hosts, sterile versus non-sterile seeds and their interaction as fixed factors on the lateral extension of the parasite root. The individual petri dish was included as a random factor, as was parasite population. The response variable (lateral extent of the parasite root system) was square-root transformed to meet the assumptions of normally distributed residuals and homoskedasticity. We also tested the effect of the side (right or left) on which the sympatric host was planted and the initial root length of the host seedlings as covariates, but both were removed as they were not significant.

Results

Early host and parasite size (four weeks) and survival

After four weeks of growth, the length of the longest leaf of a parasite was influenced by host origin ($F_{3,184.7}=5.64$, $p=0.0010$). In particular, parasites which grew with hosts from the same site (sympatric) were larger than those grown with other types of hosts (Tukey test in Fig. 3a). Cumulative leaf length of the hosts was reduced only in 'experienced' hosts, i.e. hosts from sites where the parasite occurs, whereas the size of hosts with no experience of the parasite was not affected (naïve host $\times$ parasite presence, Table 2a, Fig. 3b). Host leaf length was not related to parasite leaf length ($r^2=0.006$, $p=0.299$, Supporting information).

Of the established parasite seedlings, 29.4% died until harvest. In a generalized linear mixed model, there was some evidence for an influence of host type on parasite mortality ($\chi^2=6.54$, $df=3$, $p=0.089$): Mortality tended to be higher with naïve ($39 \pm 8\%$, mean $\pm$ SE) and sympatric hosts ($38 \pm 12\%$) than with far allopatric ($20 \pm 4\%$) or near allopatric hosts ($25 \pm 8\%$). Only 20 parasites flowered during the experiment. However, within each population only the largest individuals flowered, and plant biomass can be assumed to be a suitable fitness estimate. In the microscopic analysis of haustoria of a large parasite, some cells were destroyed due to freezing of the roots. Nevertheless, a continuous xylem bridge between host and parasite could be identified (Supporting information).

Parasite and host size at harvest (10 weeks)

The biomass of the surviving parasites depended on the type of host population ($F_{3,136.2}=3.35$, $p=0.021$). Parasites were largest when grown with hosts from the same site (sympatric) and smallest when grown with hosts from another site within the same region (Tukey test, Fig. 3c, Supporting information). These differences in the size of parasites were not the result of truncation of parasite size due to differential mortality, as the pattern remained qualitatively the same when the analysis was restricted to the largest parasite of each combination of host $\times$ parasite population (Supporting information), although this was not significant due to the strongly reduced

statistical power ($F_{3,50.77}=1.87$, $p=0.147$). Similarly, when the analysis was restricted to the four parasite populations of which plants survived in all factorial combinations, the effect of host origin on parasite biomass was similar as presented in Fig. 3c, but less statistically significant ($F_{3,76.6}=2.74$, $p=0.049$, Supporting information). The multiplicative fitness function (average biomass produced per parasite seedling planted) was influenced by host type ($F_{3,61.3}=3.19$, $p=0.030$) and showed a pattern very similar to that presented for biomass (Supporting information). Parasite fitness showed a quadratic relationship to the geographical distance between the sites of origin of host and parasite ($r^2=0.128$, $p=0.009$, Fig. 4).

Hosts were 16.3% smaller in the presence of a parasite, and this effect was similar for hosts from naïve or experienced populations (Table 2b, Fig. 3d). In an analysis restricted to experienced hosts, suppression tended to be stronger for sympatric than allopatric hosts ($F_{1,133}=3.41$, $p=0.067$), but did not differ between near and far allopatric hosts ($F_{1,133}=0.114$, $p=0.736$). Larger parasites had a stronger effect on host size than smaller parasites, but the variation explained was low ($r^2=0.032$, $p=0.033$, Supporting information).

Root allocation experiment

After four weeks, 17 of the 36 *Rhinanthus* seedlings had attached to the roots of their host. Attached parasites had larger leaves ($F_{1,34}=20.84$, $p < 0.001$, Fig. 5a) and higher biomass (16.1 ± 1.8 mm vs 10.2 ± 1.7 mm, $F_{1,34}=5.85$, $p=0.021$) than unattached parasites. Host shoot mass after four weeks of growth was not influenced by parasite presence or attachment (both $F_{1,69} < 2.49$, $p > 0.119$). However, there was a significant interaction between the effects of host naivety and parasite attachment on host shoot mass ($F_{1,68}=4.40$, $p=0.040$). The shoot mass of naïve hosts was suppressed by the attachment of parasites, while the shoot mass of experienced hosts was not affected (Fig. 5b). In contrast, the interaction of parasite presence and host naivety was not significant ($F_{1,68}=2.08$, $p=0.154$). Host root mass was reduced in the presence of a parasite ($F_{1,69}=8.10$, $p=0.006$), but did not differ between hosts with attached or not attached parasites ($F_{1,69}=0.027$, $p=0.869$, Fig. 5c). Host naivety did not influence root mass and was thus not included in the models.

In a general linear model, host root mass increased with host shoot mass (Table 3a). The presence of a parasite reduced root mass, but also changed the slope of the allometric relationship ($p=0.028$, Fig. 6a), as root mass was more strongly reduced in larger hosts. Average thickness of primary or lateral roots of the hosts was not influenced by parasite presence (both $F_{1,68} < 0.71$, $p > 0.40$), but was lower in hosts with attached parasites (primary root thickness: 0.460 ± 0.006 mm versus 0.424 ± 0.013 mm, $F_{1,68}=5.48$, $p=0.022$, lateral root thickness: 0.235 ± 0.003 mm versus 0.221 ± 0.006 mm, $F_{1,68}=4.25$, $p=0.043$). The total length of the lateral roots of a host increased with its root mass and this increase was stronger in the presence of a parasite (Table 3b, Fig. 6b).

Table 2. Results of general linear mixed models of parasite presence, experience of the host with the parasite ('naïve host') and their interaction on host performance (log-transformed cumulative leaf length after four weeks or host biomass after 10 weeks). Host population was included as a random factor in the analysis of early host size, see material and methods.

	df1	(a) 4 weeks			(b) 10 weeks		
		df2	F	p-value	df2	F	p-value
Naïve host	1	9.9	2.54	0.142	165	0.18	0.672
Parasite presence	1	206.2	0.19	0.662	165	4.27	0.040
Parasite $\times$ Naïve host	1	206.2	4.48	0.035	165	1.85	0.175

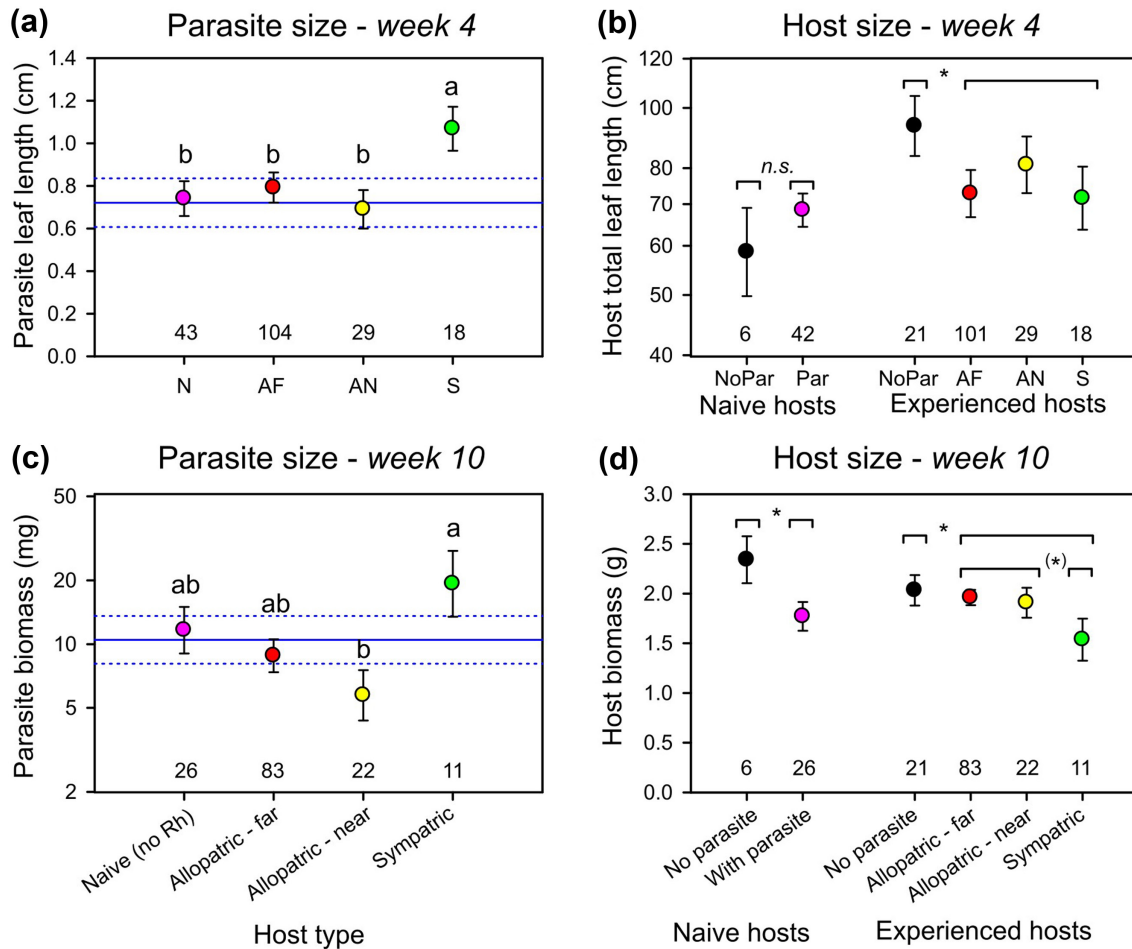


Figure 3. (a–b) Early size (4 weeks after planting) and (c–d) biomass at harvest (10 weeks after planting) of the parasite *R. alectorolophus* (a, c) and its host *P. lanceolata* (b, d). (a) Length of the longest leaf of the parasite when grown with hosts from sites where the parasite does not occur ('naïve', N), from sites with a parasite in a different region ('Allopatric – far', AF), from sites in the same region ('Allopatric – near', AN), and from the same sites as the parasite ('Sympatric', S). (b) Effect of parasite presence and origin of parasite population on cumulative leaf length of *P. lanceolata* from populations where the parasite occurs ('experienced hosts') or is absent ('naïve hosts'). (c) Biomass of parasites grown with different host types at the end of the experiment. (d) Biomass of different types of hosts grown with and without a parasite at the end of the experiment. Means $\pm$ 1 SE from models including parasite (a, c) or host population (b) as random factor. Note log-scale for host leaf length and parasite biomass. Numbers in the figures indicate the number of replicate plants per group. In (a) and (c), groups with different letters are significantly different ($p < 0.05$) based on a Tukey test. In (b) and (d), asterisks indicate a significant effect of the factor 'parasite presence' and its interaction with host naivety on host size in linear models, see Table 2 and of the contrast 'sympatric vs allopatric', *, $p < 0.05$; (*), $p < 0.10$. Blue horizontal lines in (a) and (c) indicate the mean $\pm$ 1 SE of the size of parasites grown without hosts for comparison.

In separate analyses of plants with a parasite, the responses of the hosts (slopes of the relationship between root mass and shoot mass and between root length and root mass) did not differ between hosts with attached or unattached parasites (both $F_{1,32} < 0.94$, $p > 0.341$).

Host choice experiment

Parasites did not generally grow in the direction of sympatric or allopatric hosts. There was an interaction between the two experimental runs (differing in host seed surface sterilization) and sympatric vs allopatric host origin (Table 4): Parasites grew more strongly towards sympatric host roots only in the

experiment in which seeds had not been surface sterilized (Fig. 7a–b).

Discussion

Rhinanthus grew largest and had the highest multiplicative fitness with hosts from the same site, suggesting local adaptation of the parasite to sympatric hosts. However, the relative quality of different types of allopatric populations as hosts changed over time. They were of similar quality after four weeks, whereas at harvest the near allopatric hosts (from a different site in the same region) were of particularly low quality

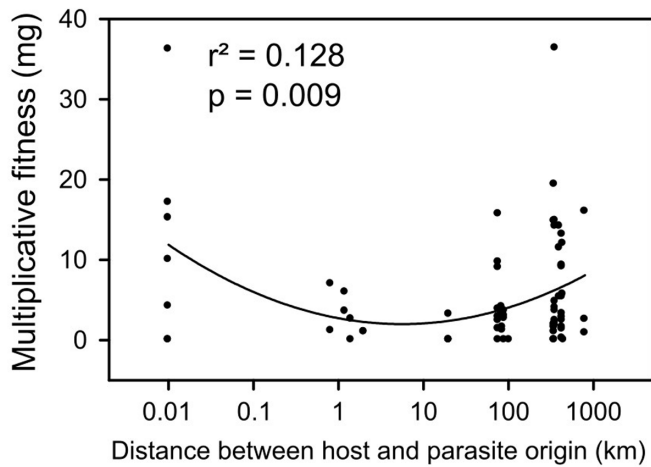


Figure 4. Relationship between multiplicative fitness of the parasite (biomass produced per seed sown) and the distance between the site of origin of host and parasite. Symbols show means for combinations of host and parasite population. Distance was set to 10 m for sympatric combinations. Distances > 50 km correspond to host plants from populations in other regions and from naïve host populations in Fig. 2. Note log-scale for distance.

for the parasites. The interaction between parasite and host can be understood by comparing patterns of parasite size and host suppression. Effects of sympatric and allopatric parasites on host size also changed over time, suggesting different mechanisms involved. We will first discuss separately the patterns of host and parasite performance observed after four and ten weeks, before we discuss the potential mechanisms involved in more detail.

Interactions between host and parasite after four weeks

Parasite seedlings growing together with sympatric hosts were larger than those growing with allopatric hosts already four weeks after planting. Leaf length of *Rhinanthus* species at this age is usually a very good predictor of parasite performance and final biomass (Matthies 2021), and the larger early size of parasites with sympatric hosts thus suggests local adaptation of parasites (Kaltz and Shykoff 1998). In our follow-up root allocation experiment 50% of the parasites had already attached to the host roots after four weeks of growth. Moreover, attached parasites were significantly larger than unattached parasites, suggesting that in our main experiment more parasites grown with sympatric than with allopatric hosts were attached after four weeks.

At the same time, the cumulative leaf length of hosts was reduced in the presence of a parasite, which is usually interpreted as host suppression by the parasite (Ameloot et al. 2005, Matthies 2021). However, there was no match between the effects of host type on parasite size and on host suppression: sympatric hosts were not suppressed more strongly than allopatric hosts, and hosts from naïve populations (i.e. from regions where the parasite does not occur) were not suppressed at all. Similarly, in the root allocation experiment, not only the successful attachment, but the mere presence of a parasite reduced host root mass after four weeks. This suggests that the reduced size of the potential hosts at this early stage was not a result of host suppression by the parasite. Instead, experienced hosts (sampled from sites where the parasite occurs) may have recognized the presence of *Rhinanthus* and started a

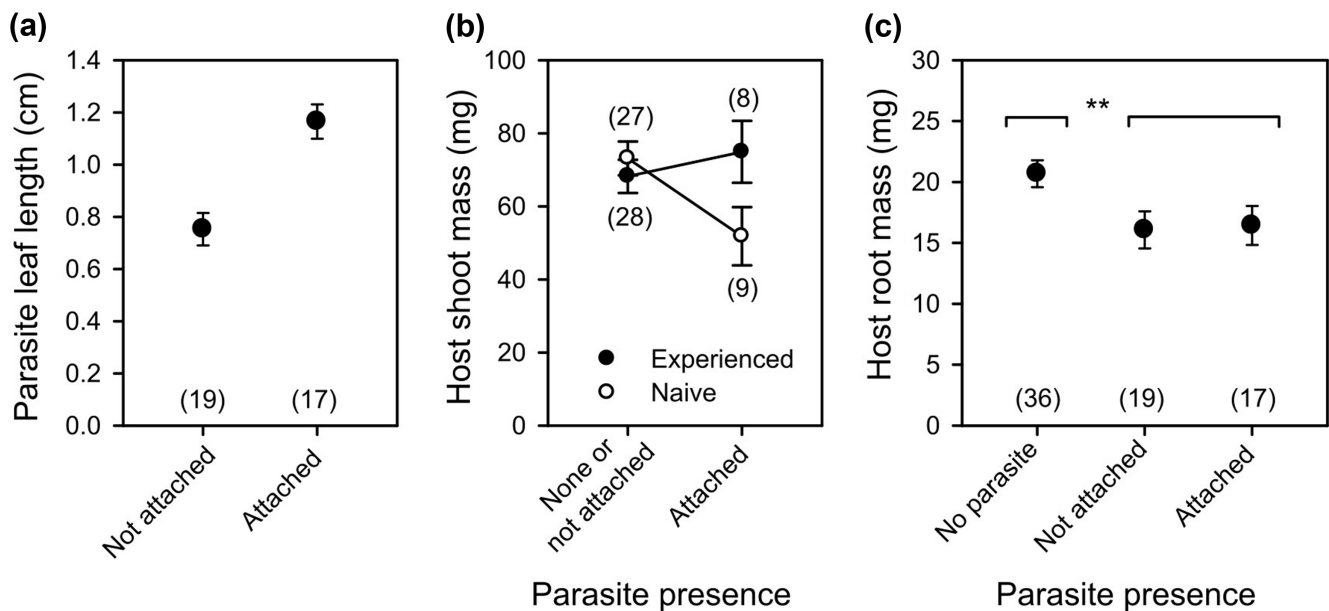


Figure 5. Effects of parasite attachment in the root allocation experiment on host traits after four weeks of growth together. (a) Effect of attachment to the host on the length of the longest parasite leaf. (b) Effect of attachment of a parasite on the shoot mass of *Plantago* seedlings from populations experienced with the parasite or not (naïve hosts). (c) Effect of parasite presence and parasite attachment on the root mass of seedlings of *Plantago*. Means $\pm$ 1 SE. Asterisks in (c) indicate significance ($p < 0.01$) of the factor 'parasite presence' in the ANOVA, see text for details. Numbers in brackets indicate the number of replicate plants.

Table 3. Results of general linear models of the effects of parasite presence on the allometric relationship between (a) log root and log shoot mass and (b) lateral root length and root mass of the host *Plantago*.

Source of variation	(a) Log root mass			Source of variation	(b) Lateral root length		
	df	F	p-value		df	F	p-value
Log shoot mass	1	247.36	< 0.001	Root mass	1	50.55	< 0.001
Parasite presence	1	4.14	0.046	Parasite presence	1	2.75	0.102
Shoot mass × parasite	1	5.04	0.028	Root mass × parasite	1	7.33	0.009
Residual	68			Residual	67		

response involving a reduction of their aboveground biomass. Such a response might be an induced defence mechanism that incurs costs on plant growth, as suggested by induced defences against herbivores (Redman et al. 2001, Ahonen et al. 2006, Moncalvillo and Matthies 2023). Alternatively, plants may have changed their patterns of biomass allocation to compensate for a potential loss of water and nutrients to the parasite. In the root allocation experiment, *Plantago* did not simply increase its root mass fraction as is typically observed in response to nutrient deficiency (Poorter et al. 2012). However, the production of less root mass with a similar total length of lateral roots in the root allocation experiment suggests that hosts respond to the presence of a parasite by producing more fine roots to increase nutrient uptake.

The results of the root allocation experiment highlight a difficulty in research on root-parasitic plants: the uncertainty about attachment to the host root. Parasite attachment (i.e. the formation of haustoria to a host root) had different effects on several host traits than the mere presence of a parasite, but had to be determined destructively by washing the entire root systems. Moreover, attachment does not imply that the connection to the host is functional (Rümer et al. 2007, Holá et al. 2017). Although successful attachment to a host may be deduced from an increase in parasite size and leaf greenness (Klaren and Janssen 1978), it is not possible without destructive root sampling to determine whether a parasite has unsuccessfully attempted to form connections to a host or not.

Interactions between host and parasite after ten weeks

After ten weeks of growth, patterns of local adaptation had changed. Parasites were still largest when grown with

sympatric hosts, but they were smallest when grown with hosts from another site in the same region and intermediate when grown with hosts from another region or with naïve hosts (Fig. 3c). A similar pattern was found for parasite multiplicative fitness, which was lowest when grown with hosts from intermediate geographic distances. Another study reported differences in the performance of parasites with near vs far sympatric hosts (Adiba et al. 2010), but in that case parasite performance changed monotonically with distance between host and parasite populations. To our knowledge, a bimodal pattern of parasite performance with relatedness to the hosts has not been reported before. It might suggest that not only the parasite adapts to its host, but the hosts also show some adaptation to the parasite (Fig. 1c, with the parasite adapting faster). The resulting pattern was a decreasing parasite performance with increasing familiarity to the hosts, except for parasites grown with hosts from the same site. The corresponding pattern of stronger suppression of sympatric than allopatric hosts was only marginally significant (Fig. 3d). We cannot be completely sure that hosts are locally adapted to the parasite, but we find this the most likely explanation of the bimodal pattern of parasite performance.

Plantago lanceolata has been found to be able to completely block haustoria of the closely related parasite *R. minor* (Rümer et al. 2007), although our results indicate that this defence is not absolute and can be overcome by some *Rhinanthus*. The relatively good performance of parasites with naïve hosts and the strong suppression of their biomass after 10 weeks may suggest that naïve hosts are less capable of such a defence. In contrast, hosts from the same region might be best adapted to recognize the attack by familiar parasite genotypes and block the formation of haustoria. Parasites even tended to be smaller with near-allopatric hosts than without

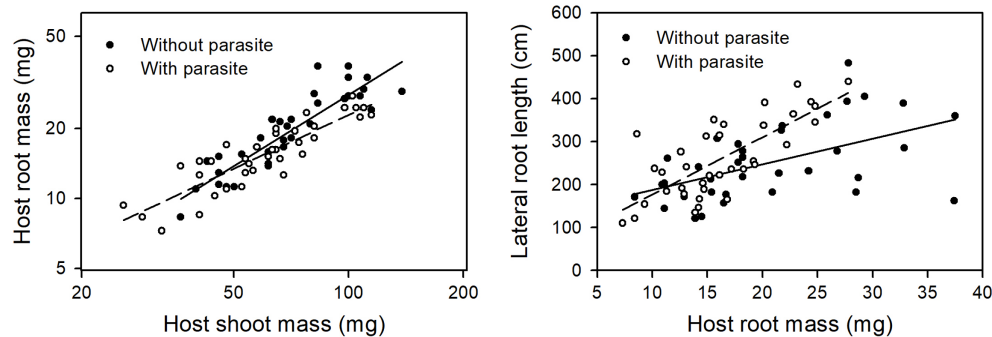


Figure 6. Effects of parasite presence on root traits of the host *Plantago* in the root allocation experiment. (a) Allometric relationship between root mass and shoot mass of hosts grown with (open symbols) or without the parasite *Rhinanthus* (filled symbols). Note log-scales of the axes. (b) Relationship between total lateral root length and root mass of the host. In both graphs slopes differ significantly, see Table 3.

Table 4. Results of a linear mixed model of the effects of two experimental runs (differing in whether host seeds were surface sterilized or not), sympatric versus allopatric origin of the host seedlings and their interactions on the lateral extension of the *Rhinanthus* root system grown in agar in petri dishes after seven days. Petri dish and parasite population were included as random factors.

Fixed factor	df _{nom}	df _{denom}	F	p-value
Experimental run	1	95.3	0.41	0.522
Sympatric host	1	98.0	0.20	0.654
Sympatric × Experiment	1	98.0	4.94	0.029

a host, suggesting suppression by *Plantago* via competition or allelopathy. However, sympatric parasites may have been able to overcome the host defence because they may have evolved faster, which we will discuss later in more detail. A strong interaction due to an evolutionary ‘arms race’ with high local adaptation of both host and parasites within populations is also supported by the tendency of higher mortality of parasites with sympatric than allopatric hosts – parasites were either adapted and grew relatively large, or the host was better adapted and the parasite died. However, it is important to note that the increased average size of parasites with sympatric hosts was not just an effect of truncation by differential mortality, but remained qualitatively the same when the analysis was restricted to the largest parasites of each combination of parasite × host population, or when a multiplicative fitness function combining mortality and biomass was analysed.

Possible mechanisms of host–parasite interaction

The mechanisms involved in the interaction between both species remain to be unravelled. It is likely that differences in the ability of induced defence between *Plantago* populations play a role, for example, when naïve hosts are less capable of a defence than host populations from the same region as

the parasites. A defence may include blocking haustoria by host cell fragmentation, as has been described for *P. lanceolata* (Rümer et al. 2007), or other morphological or chemical responses (Timko and Scholes 2013, Jhu and Sinha 2022). Although many parasites were not significantly larger than parasites grown without a host, in large hosts we found fully developed, functional haustoria, which – together with the large size of some parasites in pots with *Plantago* – illustrates the suitability of some *Plantago* genotypes as a host for some parasites. Variation among host genotypes in defence is known from some arable crops attacked by parasitic weeds (Goldwasser et al. 1999, Rich et al. 2004). Host specificity of parasites has often been linked to genetic differences among host and parasite lineages (Botanga and Timko 2006, Bellis et al. 2020), but environmental parental effects or epigenetic mechanisms have been suggested as an additional mechanism to explain host preferences of hemiparasites (Adler 2003) or the ability of parasitic weeds to quickly adapt to different crop species (Bruce and Gressel 2013). We used seed material collected in the field to include both genetic and environmental parental effects, although separating the two would be very interesting for a deeper mechanistic understanding of the interactions found.

Because of the very early positive effects of sympatric hosts on parasite size at a time when hosts were not yet consistently suppressed in their growth, we suspected that also differences in host recognition may be involved. Hemiparasites are known to recognize suitable host species by chemical cues (Yoder 2001, Mutuku et al. 2021), and host choice experiments in agar suggest that *Rhinanthus* can already at a very early stage grow more strongly in the direction of certain species than others (Sandner et al. 2022). Parasites might thus recognize *Plantago* from sympatric populations earlier as potential hosts and form haustorial connections, while parasites growing with other host populations continue to search for suitable hosts, thus

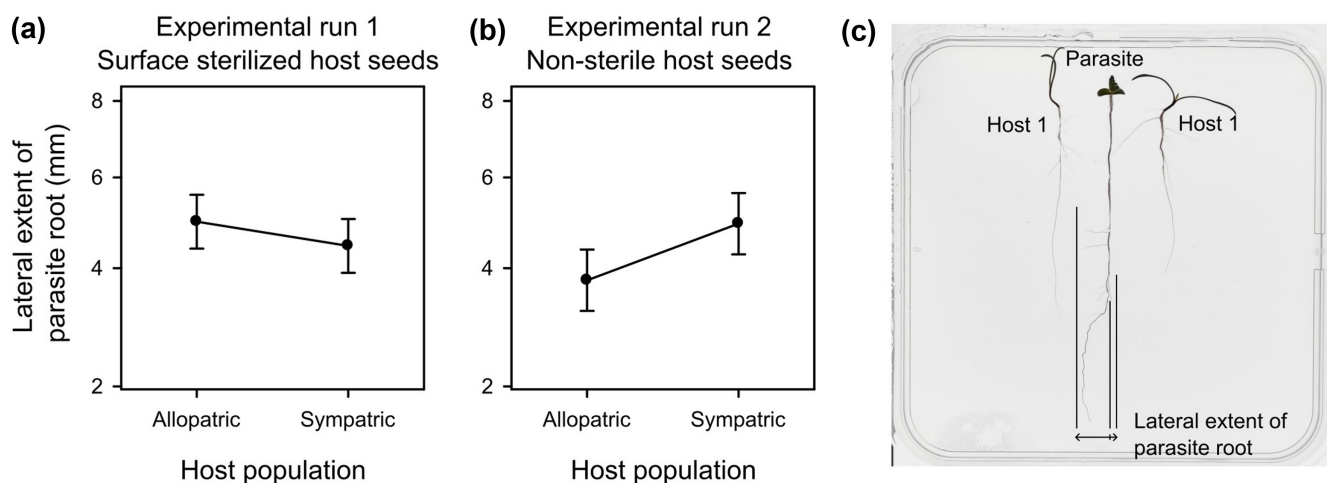


Figure 7. Host choice experiment. Lateral extent of the root system of *Rhinanthus* seedlings grown between two *Plantago* seedlings derived from (a) seeds that were surface sterilized ($n = 60$), and (b) seeds that were not sterilized ($n = 40$). Seeds originated from either allopatric or sympatric populations. Lines and error bars show mean and standard errors for the sympatric × sterilization (experimental run) effect (compare Table 4). (c) Image of a petri dish with a parasite seedling grown for seven days between two host seedlings, illustrating how the lateral extent of the parasite root system was measured.

allocating more resources to below-ground growth. However, this expectation was not confirmed in our host choice experiment. There was only slightly stronger root growth in the direction of sympatric hosts when host seeds were not surface sterilized (as in the main experiment), an effect that vanished when host seeds were surface sterilized, suggesting an effect of the microbiome. Microbiota can be transferred from seeds to the soil (Abdelfattah et al. 2023). Microbes of the seed surface are removed by surface-sterilization, leaving only endophytic communities (Azarbad et al. 2022). It is possible that parasites in our experiments recognized not sympatric hosts, but sympatric microbes carried with the host seeds. Moreover, host seedlings can produce root exudates as a specific reaction to the presence of certain microbes (e.g. strigolactones in response to mycorrhizal fungi), and parasitic plants can recognize these exudates to find a suitable host and form haustoria (Bouwmeester et al. 2007), a process that may be prevented by host seed sterilization. However, the early preference for sympatric *Plantago* seedlings was so weak that it is unlikely to explain the differences in parasite fitness observed in our experiment. Alternatively, hosts which were able to recognize the presence of parasites from root exudates may have been more likely to avoid contact with parasite roots or to start a defence reaction.

Stronger adaptation of parasites to local hosts than vice versa

At local scales (i.e. within our geographical regions), we found patterns of parasite (but not host) local adaptation. Why does the parasite win the arms race at the population scale? Firstly, models of coevolutionary dynamics predict that local adaptation of the parasites is more likely to be found when they have shorter generation times and higher gene flow than their hosts (Gandon et al. 1996, Kaltz and Shykoff 1998). In line with this hypothesis, *Rhinanthus* as an annual plant with a short-lived seed bank may evolve local adaptation more quickly than *Plantago*, which is potentially very long-lived, has an average lifespan of nearly three years (Roeder et al. 2017, Steiner et al. 2021), and a long-lived seed bank. However, the gene flow between populations is certainly higher for the common wind-pollinated host *Plantago* than for the more rare and bumble bee-pollinated parasite. This is supported by studies reporting 5–10 times higher genetic differentiation between populations of the related parasite *R. angustifolius* (Jonstrup et al. 2018) than between populations of *P. lanceolata* (Smith et al. 2020) at similar geographic distances (Supporting information). Higher gene flow (or ‘migration rate’) among host than parasite populations has been proposed to facilitate local adaptation of the host (Kaltz and Shykoff 1998, Mutikainen et al. 2000, Roth et al. 2012), because it would speed up the spread of newly acquired host-resistance alleles and thus give the host an advantage in the arms race. However, in the specific system, the higher gene flow among host populations may even slow down local adaptation, as parasites are absent in most *Plantago* populations and gene flow among populations may dilute adaptation of the host.

Secondly, the selection for adaptation to local genotypes of the interaction partner may be much stronger for the parasite, which has to reproduce within one season and whose fitness depends strongly on access to a suitable host, than for the perennial host. Our result is thus in line with other studies suggesting that local adaptation is more often found in parasites (including herbivores) than their hosts (Garrido et al. 2012). The mean suppression of host size (i.e. virulence) of 16% was similar to the mean suppression of 25 different host species by *Rhinanthus* in similar pot experiments (–19%, Matthies 2021). It should be noted that the situation in the current experiment of a parasite seedling encountering a *Plantago* seedling is different from the situation in natural populations, where *Rhinanthus* seeds germinate during winter and attach to their host roots in early spring, likely encountering large perennial host individuals. The effect of parasites on older *Plantago* may thus be different from that found in our study.

The fitness advantage for *Rhinanthus* of being able to use *Plantago* as a host is difficult to estimate. *Plantago* occurs in most parasite populations, but it always grows in a matrix including more suitable host species (i.e. most grass and legume species and several forbs, Matthies 2021). Most parasites unable to attack *Plantago* will thus have no problem to grow and reproduce, which might limit adaptation. A meta-analysis concluded that local adaptation is less likely in parasites with broader than those with narrower host ranges (Lajeunesse and Forbes 2002). However, other plant species are not only potential hosts for hemiparasites, but also competitors for light (Matthies 1995), and competition with non-hosts may thus lead to the competitive exclusion of good host species. There may thus be a strong selective advantage for hemiparasites not to specialize on one or few host species, but to use (and suppress in size) neighbours of all species. In natural grasslands, hemiparasites often attach to several host species simultaneously (Holá et al. 2017). In populations where *Plantago* is common, it may thus be an advantage for *Rhinanthus* to use this species as one of several hosts, and thus reduce competition for light by *Plantago*. *R. alectorolophus* is known to reduce the biomass of *Plantago* even in multi-species mixtures where better hosts are also present (Sandner and Matthies 2018). Moreover, hemiparasites receive different compounds from different host species, and a mixed diet has been found to increase the performance of hemiparasites compared to growth in monocultures (Marvier 1998, Sandner and Matthies 2018, but see Matthies 1996). It is thus possible that the ability to use *Plantago* as one of several hosts increases the performance of *Rhinanthus* in host mixtures.

Can generalist parasites adapt to individual host populations?

Our results do not support the predictions of a geographic mosaic of local adaptation (Lively et al. 1999) that due to fluctuations during coevolutionary dynamics, parasite local adaptation is found only in some years or populations, but not others. We could not test for differences in local adaptation among populations due to the low number of surviving

parasites, but our results suggest that similar patterns are found in all populations (Supporting information). This finding shows that predictions from host-parasite models based on specialized parasites may only to a limited degree be applied to hemiparasite–host systems. In classical models, increasing selection for defended host-genotypes leads to a decline of formerly virulent parasite genotypes until re-adaptation has taken place (Lively et al. 1999). In contrast, *Rhinanthus* genotypes unable to use *Plantago* may remain in the population using only other host species. This could allow the parasites to also adapt to the more resistant *Plantago* genotypes, either genetically via mutation and recombination, or via epigenetic mechanisms. Such adaptation may take place over several generations if the ability to use *Plantago* as one of several hosts increases parasite performance. Once connected to one or several good host species, *Rhinanthus* can produce new roots and haustoria to repeatedly probe different host- and non-host species for their suitability. The ability to use multiple hosts simultaneously is a special feature of parasitic plants and most similar to plant-herbivore interactions (Pennings and Callaway 2002), except that herbivores have to move between different host plants. While herbivorous insects will likely spend most of their time on suitable food plants, larger vertebrates are known to eat many different plant species, which may e.g. facilitate detoxification of secondary metabolites (Freeland and Janzen 1974, Marsh et al. 2006). Over the lifespan of the parasite, the constant probing of new potential host species can lead to the association of certain host cues with host quality, which might possibly even be transmitted to future generations. Adaptation of *Rhinanthus* to *Plantago* may thus also include trans-generational plasticity (Herman et al. 2012), e.g. if epigenetic mechanisms help the parasites to recognize *Plantago* as a host species and attach to it earlier, as has been found for host choice by *Bicyclus anynana* butterflies (Gowri et al. 2019). The role of genetic and epigenetic effects on host specificity in hemiparasitic plants should be addressed in future experiments (Bruce and Gressel 2013).

In conclusion, in contrast to earlier studies we found evidence for local adaptation of a hemiparasite to one of its host species. This may be due to the existence of defence mechanisms in *Plantago* that have to be overcome by *Rhinanthus*, while former studies used good and likely undefended host species. In contrast to parasite local adaptation in other parasite–host systems, this adaptation does not involve specialization of the generalist hemiparasite on this relatively poor host species. Similar processes may be found in parasite–hosts systems involving other hemiparasitic *Orobanchaceae*, but also holoparasitic plants like *Cuscuta* which may simultaneously attach to different host species (Koch et al. 2004). In such systems, simultaneous local adaptation of both partners may be possible, and coevolution with host species may thus take place even without specialization on individual species and increase the ability of the parasites to use many species in a mixed diet and control their abundance in the host community. Our results support the notion of Rowntree et al. (2011) that the interactions between hemiparasites and their hosts depend on the genetics of hemiparasite and hosts, which may have

implications for the conservation of rare hemiparasites as well as the effects of hemiparasites on plant community structure.

Acknowledgements – We thank Subject Editor Dr Simon Hart for comments on former versions of the manuscript. Open Access funding enabled and organized by Projekt DEAL.

Author contributions

Tobias M. Sandner: Conceptualization (lead); Formal analysis (lead); Investigation (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Milan J. Brand:** Investigation (equal); Writing – review and editing (supporting). **Manuel Hickler:** Investigation (equal); Writing – review and editing (supporting). **Lotte Korell:** Conceptualization (supporting); Investigation (equal); Writing – review and editing (equal). **Diethart Matthies:** Formal analysis (supporting); Investigation (supporting); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.wwpzgmssh> (Sandner et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

References

- Abdelfattah, A., Tack, A. J. M., Lobato, C., Wassermann, B. and Berg, G. 2023. From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. – *Trends Microbiol.* 31: 346–355.
- Adiba, S., Huet, M. and Kaltz, O. 2010. Experimental evolution of local parasite maladaptation. – *J. Evol. Biol.* 23: 1195–1205.
- Adler, L. S. 2003. Host species affects herbivory, pollination, and reproduction in experiments with parasitic *Castilleja*. – *Ecology* 84: 2083–2091.
- Ahonen, R., Puustinen, S. and Mutikainen, P. 2006. Host use of a hemiparasitic plant: no tradeoffs in performance on different hosts. – *J. Evol. Biol.* 19: 513–521.
- Ameloot, E., Verheyen, K. and Hermy, M. 2005. Meta-analysis of standing crop reduction by *Rhinanthus* spp. and its effect on vegetation structure. – *Folia Geobot.* 40: 289–310.
- Azarbad, H., Bainard, L. D., Agoussar, A., Tremblay, J. and Yergeau, E. 2022. The response of wheat and its microbiome to contemporary and historical water stress in a field experiment. – *ISME Commun.* 2: 1–69.
- Bates, D., Mächler, M., Bolker, B. and Walker, S. 2015. Fitting linear mixed-effects models using lme4. – *J. Stat. Softw.* 67: 1–48.
- Bellis, E. S., Kelly, E. A., Lorts, C. M., Gao, H., DeLeo, V. L., Rouhan, G., Budden, A., Bhaskara, G. B., Hu, Z., Muscarella, R., Timko, M. P., Nebie, B., Runo, S. M., Chilcoat, N. D., Juenger, T. E., Morris, G. P., dePamphilis, C. W. and Lasky, J. R. 2020. Genomics of sorghum local adaptation to a parasitic plant. – *Proc. Natl Acad. Sci. USA* 117: 4243–4251.

- Bettinger, A. 2013. Die floristische Kartierung Deutschlands. – In: NetPhyD & BfN. (ed.), Verbreitungsatlas der Farn- und Blütenpflanzen Deutschlands. Landwirtschaftsverlag, pp. 40–42.
- BfN 2022. Bundesamt für Naturschutz: distribution map from FloraWeb. – <https://floraweb.de/webkarten/karte.html?taxnr=4811>.
- Botanga, C. J. and Timko, M. P. 2006. Phenetic relationships among different races of *Striga gesnerioides* (Willd.) Vatke from West Africa. – *Genome* 49: 1351–1365.
- Bouwmeester, H. J., Roux, C., Lopez-Raez, J. A. and Bécard, G. 2007. Rhizosphere communication of plants, parasitic plants and AM fungi. – *Trends Plant Sci.* 12: 224–230.
- Brockhurst, M. A. and Koskella, B. 2013. Experimental coevolution of species interactions. – *Trends Ecol. Evol.* 28: 367–375.
- Brown, M. R., Moore, P. G. P. and Twyford, A. D. 2021. Performance of generalist hemiparasitic *Euphrasia* across a phylogenetically diverse host spectrum. – *New Phytol.* 232: 2165–2174.
- Bruce, T. J. A. and Gressel, J. 2013. Changing host specificities: by mutational changes or epigenetic reprogramming? – In: Joel, D. M., Gressel, J. and Musselman, L. J. (eds), *Parasitic Orobanchaceae*. Springer, pp. 231–241.
- Bullock, J. M., Moy, I. L., Coulson, S. J. and Clarke, R. T. 2003. Habitat-specific dispersal: environmental effects on the mechanisms and patterns of seed movement in a grassland herb *Rhinanthus minor*. – *Ecography* 26: 692–704.
- Cai, J., Zeng, Z., Connor, J. N., Huang, C. Y., Melino, V., Kumar, P. and Miklavcic, S. J. 2015. Rootgraph: a graphic optimization tool for automated image analysis of plant roots. – *J. Exp. Bot.* 66: 6551–6562. <https://doi.org/10.1093/jxb/erv359>
- Clifford, H. T. 1962. Insect pollination of *Plantago lanceolata* L. – *Nature* 193: 196.
- Ehrlén, J. and Lehtilä, K. 2002. How perennial are perennial plants? – *Oikos* 98: 308–322.
- Fox, J. and Weisberg, S. 2019. An R companion to applied regression, 3rd edn. – Sage
- Freeland, W. J. and Janzen, D. H. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. – *Am. Nat.* 108: 269–289.
- Gandon, S., Capowiez, Y., Dubois, Y. M. and Olivieri, I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. – *Proc. R. Soc. B* 263: 1003–1009.
- Garrido, E., Andraca-Gómez, G. and Fornoni, J. 2012. Local adaptation: simultaneously considering herbivores and their host plants. – *New Phytol.* 193: 445–453.
- Gibson, C. C. and Watkinson, A. R. 1989. The host range and selectivity of a parasitic plant: *Rhinanthus minor* L. – *Oecologia* 78: 401–406.
- Goldwasser, Y., Hershenhorn, J., Plakhine, D., Kleifeld, Y. and Rubin, B. 1999. Biochemical factors involved in vetch resistance to *Orobanche aegyptiaca*. – *Physiol. Mol. Plant Pathol.* 54: 87–96.
- Govier, R. N., Nelson, M. D. and Pate, J. S. 1967. Hemiparasitic nutrition in angiosperms. I. The transfer of organic compounds from host to *Odontites verna* (Bell.) Dum. (*Scrophulariaceae*). – *New Phytol.* 66: 285–297.
- Gowri, V., Dion, E., Viswanath, A., Piel, F. M. and Monteiro, A. 2019. Transgenerational inheritance of learned preferences for novel host plant odors in *Bicyclus anynana* butterflies. – *Evolution* 73: 2401–2414.
- Greischar, M. A. and Koskella, B. 2007. A synthesis of experimental work on parasite local adaptation. – *Ecol. Lett.* 10: 418–434.
- Gressel, J. and Joel, D. M. 2013. Weedy Orobanchaceae: the problem. – In: Joel, D. M., Gressel, J. and Musselman, L. J. (eds), *Parasitic Orobanchaceae*. Springer, pp. 309–312.
- Hartley, S. E., Green, P., Massey, F. P., Press, M. C. P., Stewart, J. A. and John, E. A. 2015. Hemiparasitic plant impacts animal and plant communities across four trophic levels. – *Ecology* 96: 2408–2416.
- Hautier, Y., Hector, A., Vojtech, E., Purves, D. and Turnbull, L. A. 2010. Modelling the growth of parasitic plants. – *J. Ecol.* 98: 857–866.
- Heide-Jørgensen, H. S. 2013. Introduction: the parasitic syndrome in higher plants. – In: Joel, D. M., Gressel, J. and Musselman, L. J. (eds), *Parasitic Orobanchaceae*. Springer, pp. 1–18.
- Herman, J. J., Sultan, S. E., Horgan-Kobelski, T. and Riggs, C. 2012. Adaptive transgenerational plasticity in an annual plant: grandparental and parental drought stress enhance performance of seedlings in dry soil. – *Integr. Comp. Biol.* 52: 77–88.
- Holá, E., Kocková, J. and Těšitel, J. 2017. DNA barcoding as a tool for identification of host association of root-hemiparasitic plants. – *Folia Geobot.* 52: 227–238.
- Jhu, M. Y. and Sinha, N. R. 2022. Parasitic plants: an overview of mechanisms by which plants perceive and respond to parasites. – *Annu. Rev. Plant Biol.* 73: 433–455.
- Jonstrup, A., Hedrén, M. and Andersson, S. 2016. Host environment and local genetic adaptation determine phenotype in parasitic *Rhinanthus angustifolius*. – *Bot. J. Linn. Soc.* 180: 89–103.
- Jonstrup, A., Andersson, S. and Hedrén, M. 2018. Genetic structure in parasitic *Rhinanthus angustifolius* is determined by geographical distance rather than habitat – implications for taxonomy and conservation. – *Nord. J. Bot.* 36: e01941.
- Kaltz, O. and Shykoff, J. A. 1998. Local adaptation in host–parasite systems. – *Heredity* 81: 361–370.
- Klaren, C. H. and Janssen, G. 1978. Physiological changes in the hemiparasite *Rhinanthus serotinus* before and after attachment. – *Physiol. Plant.* 42: 151–155.
- Koch, A. M., Binder, C. and Sanders, I. R. 2004. Does the generalist parasitic plant *Cuscuta campestris* selectively forage in heterogeneous plant communities? – *New Phytol.* 162: 147–155.
- Korell, L., Sandner, T. M., Matthies, D. and Ludewig, K. 2020. Effects of drought and N level on the interactions of the root hemiparasite *Rhinanthus alectorolophus* with a combination of three host species. – *Plant Biol.* 22: 84–92.
- Koskela, T., Salonen, V.-P. and Mutikainen, P. 2000. Local adaptation of a holoparasitic plant, *Cuscuta europaea*: variation among populations. – *J. Evol. Biol.* 13: 749–755.
- Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. 2017. lmerTest package: tests in linear mixed effects models. – *J. Stat. Softw.* 82: 1–26.
- Lajeunesse, M. J. and Forbes, M. R. 2002. Host range and local parasite adaptation. – *Proc. R. Soc. B* 269: 703–710.
- Lammi, A., Siikamäki, P. and Salonen, V. 1999. The role of local adaptation in the relationship between an endangered root hemiparasite *Euphrasia rostkoviana*, and its host, *Agrostis capillaris*. – *Ecography* 22: 145–152.
- Lenth, R. V. 2023. emmeans: estimated marginal means, aka least-squares means. – R package ver. 1.8.5, <https://CRAN.R-project.org/package=emmeans>.
- Lively, C. M. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. – *Am. Nat.* 153: S34–S47.
- Marsh, K. J., Wallis, I. R., McLean, S., Sorensen, J. S. and Foley, W. J. 2006. Conflicting demands on detoxification pathways

- influence how common brushtail possums choose their diets. – *Ecology* 87: 2103–2112.
- Marvier, M. 1998. A mixed diet improves performance and herbivore resistance of a parasitic plant. – *Ecology* 79: 1272–1280.
- Matthies, D. 1995. Parasitic and competitive interactions between the hemiparasites *Rhinanthus serotinus* and *Odontites rubra* and their host *Medicago sativa*. – *J. Ecol.* 83: 245.
- Matthies, D. 1996. Interactions between the root hemiparasite *Melampyrum arvense* and mixtures of host plants: heterotrophic benefit and parasite-mediated competition. – *Oikos* 75: 118–124.
- Matthies, D. 2017. Interactions between a root hemiparasite and 27 different hosts: growth, biomass allocation and plant architecture. – *Perspect. Plant Ecol. Evol. Syst.* 24: 118–137.
- Matthies, D. 2021. Closely related parasitic plants have similar host requirements and related effects on hosts. – *Ecol. Evol.* 11: 12011–12024.
- Moncalvillo, B. and Matthies, D. 2023. Performance of a parasitic plant and its effects on hosts depend on the interactions between parasite seed-family and host species. – *AoB Plants* 15: plac063.
- Mutikainen, P., Salonen, V., Puustinen, S. and Koskela, T. 2000. Local adaptation, resistance, and virulence in a hemiparasitic plant–host plant interaction. – *Evolution* 54: 433–440.
- Mutuku, J. M., Cui, S., Yoshida, S. and Shirasu, K. 2021. *Orobanchaceae* parasite–host interactions. – *New Phytol.* 230: 46–59.
- Oberdorfer, E. 2001. *Pflanzensoziologische Exkursionsflora für Deutschland und angrenzende Gebiete*, 8th edn. – Eugen Ulmer.
- Parker, C. 2012. Parasitic weeds: a world challenge. – *Weed Sci.* 60: 269–276.
- Pennings, S. C. and Callaway, R. M. 2002. Parasitic plants: parallels and contrasts with herbivores. – *Oecologia* 131: 479–489.
- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P. and Mommer, L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. – *New Phytol.* 193: 30–50.
- Pywell, R. F., Bullock, J. M., Walker, K. J., Coulson, S. J., Gregory, S. J. and Stevenson, M. J. 2004. Facilitating grassland diversification using the hemiparasitic plant *Rhinanthus minor*. – *J. Appl. Ecol.* 41: 880–887.
- Redman, A. M., Cipollini, D. F. and Schultz, J. C. 2001. Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. – *Oecologia* 126: 380–385.
- Rich, P. J., Grenier, C. and Ejeta, G. 2004. Striga resistance in the wild relatives of sorghum. – *Crop Sci.* 44: 2221–2229.
- Roeder, A., Schweingruber, F. H., Fischer, M. and Roscher, C. 2017. Growth ring analysis of multiple dicotyledonous herb species – a novel community-wide approach. – *Basic Appl. Ecol.* 21: 23–33.
- Roth, O., Keller, I., Landis, S. H., Salzburger, W. and Reusch, T. B. H. 2012. Hosts are ahead in a marine host–parasite coevolutionary arms race: innate immune system adaptation in pipefish *Syngnathus typhle* against *Vibrio* phylotypes. – *Evolution* 66: 2528–2539.
- Rowntree, J. K., Cameron, D. D. and Preziosi, R. F. 2011. Genetic variation changes the interactions between the parasitic plant–ecosystem engineer *Rhinanthus* and its hosts. – *Phil. Trans. R. Soc. B* 366: 1380–1388.
- Rümer, S., Cameron, D. D., Wacker, R., Hartung, W. and Jiang, F. 2007. An anatomical study of the haustoria of *Rhinanthus minor* attached to roots of different hosts. – *Flora Morphol. Distrib. Funct. Ecol. Plants* 202: 194–200.
- Sandner, T. M. and Matthies, D. 2017. Interactions of inbreeding and stress by poor host quality in a root hemiparasite. – *Ann. Bot.* 119: 143–150.
- Sandner, T. M. and Matthies, D. 2018. Multiple choice: hemiparasite performance in multi-species mixtures. – *Oikos* 127: 1291–1303.
- Sandner, T. M., Schoppa, L. and Matthies, D. 2022. Seedlings of a hemiparasite recognize legumes, but do not distinguish good from poor host species. – *Folia Geobot.* 57: 117–126.
- Sandner, T. M., Brand, M. J., Hickler, M., Korell, L. and Matthies, D. 2024. Data from: Local adaptation of a generalist hemiparasitic plant to one of its potential host plants. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.wwpzgmssh>.
- Seel, W. E. and Jeschke, W. D. 1999. Simultaneous collection of xylem sap from *Rhinanthus minor* and the hosts *Hordeum* and *Trifolium*: hydraulic properties, xylem sap composition and effects of attachment. – *New Phytolog.* 143: 281–298.
- Smith, A. L. et al. 2020. Global gene flow releases invasive plants from environmental constraints on genetic diversity. – *Proc. Natl Acad. Sci. USA* 117: 4218–4227.
- Steiner, U. K., Tuljapurkar, S. and Roach, D. A. 2021. Quantifying the effect of genetic, environmental and individual demographic stochastic variability for population dynamics in *Plantago lanceolata*. – *Sci. Rep.* 11: 23174.
- Těšitel, J. 2016. Functional biology of parasitic plants: a review. – *Plant Ecol. Evol.* 149: 5–20.
- Timko, M. P. and Scholes, J. D. 2013. Host reaction to attack by root parasitic plants. – In: Joel, D. M., Gressel, J. and Muselman, L. J. (eds.), *Parasitic Orobanchaceae*. Springer, pp. 115–141.
- Yoder, J. I. 2001. Host-plant recognition by parasitic *Scrophulariaceae*. – *Curr. Opin. Plant Biol.* 4: 359–365.
- Zhu, J., Buchmann, C. M. and Schurr, F. M. 2022. Improving measurements of the falling trajectory and terminal velocity of wind-dispersed seeds. – *Ecol. Evol.* 12: e9183.