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Within-host adaptation alters priority effects within the tomato phyllosphere microbiome

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To predict the composition and function of ecological communities over time, it is essential to understand how in situ evolution alters priority effects between resident and invading species. Phyllosphere microbial communities are a useful model system to explore priority effects because the system is clearly spatially delineated and can be manipulated experimentally. We conducted an experimental evolution study with tomato plants and the early-colonizing bacterium species *Pantoea dispersa*, exploring priority effects when *P. dispersa* was introduced before, simultaneously with or after competitor species. *P. dispersa* rapidly evolved to invade a new niche within the plant tissue and altered its ecological interactions with other members of the plant microbiome and its effect on the host. Prevailing models have assumed that adaptation primarily improves the efficiency of resident species within their existing niches, yet in our study system, the resident species expanded its niche instead. This finding suggests potential limitations to the application of existing ecological theory to microbial communities.

Priority effects, an ecological phenomenon whereby community assembly outcomes depend on the order of species arrival, can play a critical role in the assembly, stability and function of ecological communities[1]. Understanding how resident communities resist and/or facilitate invasions by arriving species can be useful for guiding ecosystem restoration and promoting resistance to invasive species[2,3]. Systems in which community assembly spans multiple generations open the potential for evolution to play a role as well. This is probably true for microbial communities, suggesting that models recognizing only ecological processes may not sufficiently capture microbiome assembly dynamics[4]. Currently, we have little understanding of how microbiomes evolve within hosts or how within-host selection alters interactions between resident species and invading species.

Prevailing eco-evolutionary models assume that species that colonize hosts early in succession evolve to occupy their niches more efficiently, providing an additional competitive advantage against invaders[5]. Early-arriving species would thus resist invasion through both ecological and evolutionary processes, implying that coexistence of competing species should be low and that communities should be highly resistant to change after initial establishment. Known as the community monopolization hypothesis, this model has received support from several studies of microbial populations evolving in laboratory culture[6–8]. Yet observations of microbiomes in nature often reveal continuous replacement among strains and species[9,10], questioning the extent to which adaptation can increase colonization resistance within hosts.

The phyllosphere, or above-ground plant tissues, provides many advantages for studying microbiome assembly. This plant compartment is clearly delineated from the surrounding environment, supports a diversity of microbial species and plays an important role both in individual plant fitness and global nutrient cycling[11]. To interrogate priority effects in this system, we examined the adaptive potential of the early-colonizing bacterium *Pantoea dispersa* on the leaves of tomato plants (*Solanum lycopersicum*). This species is found on seeds and in the phyllosphere of juvenile and adult plants. In a previous study, *P. dispersa* was consistently detected in the microbiomes of 2-week-old tomato seedlings[12], suggesting that it experiences ample opportunity in nature to colonize new plants. However, the relationship between

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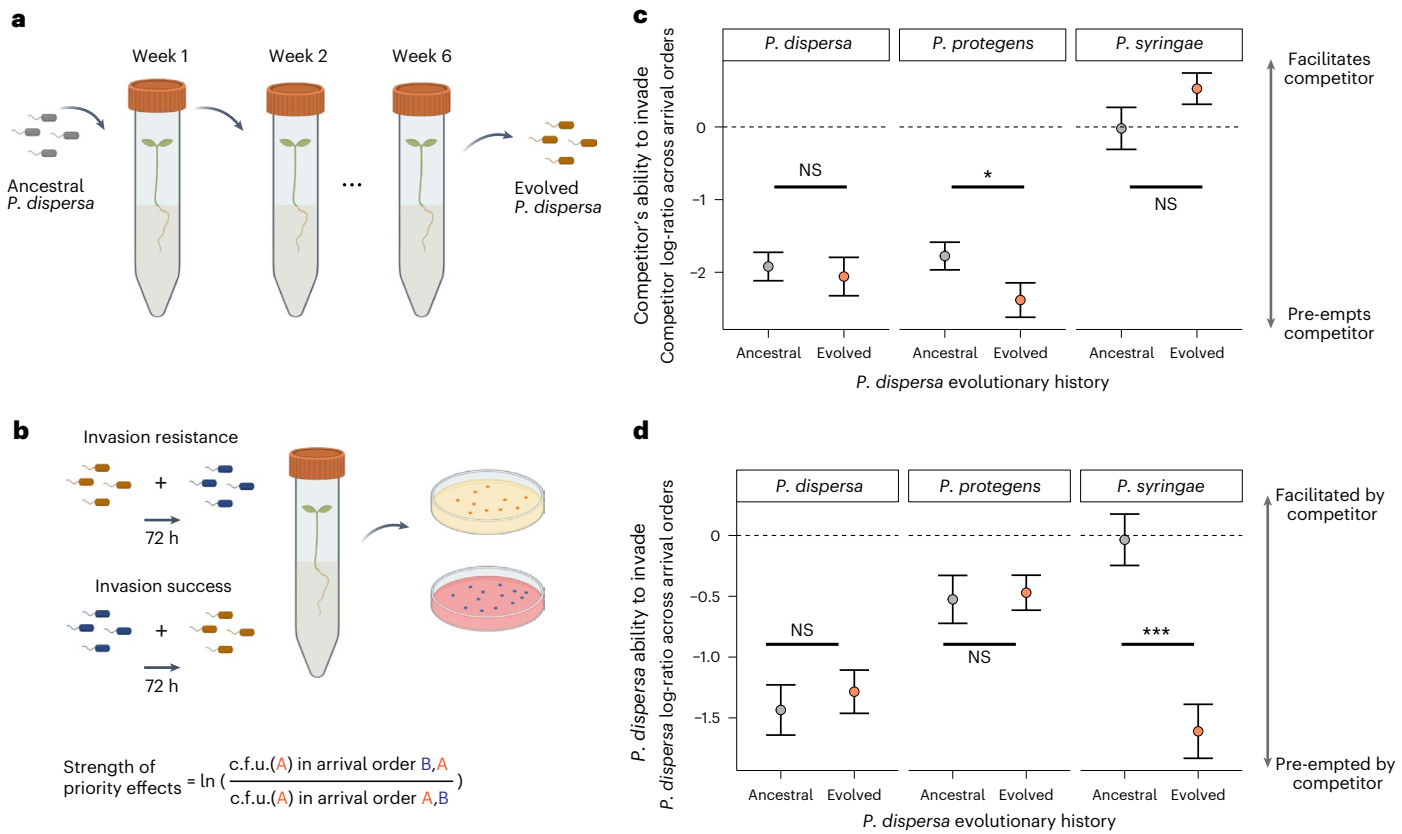

Fig. 1 | Within-host evolution of the early colonizer *P. dispersa* alters priority effects among bacterial strains. a, Schematic of experimental evolution protocol. Six replicate populations of *P. dispersa* were each inoculated onto tomato seedlings via flooding. Seedlings were incubated for 1 week, then harvested to isolate bacteria for the next generation of inoculum. **b**, Schematic of arrival order experiments. Tomato seedlings were inoculated with either evolved or ancestral *P. dispersa* and a competitor (*P. dispersa*, *P. protegens* or *P. syringae*). Seedlings were harvested 72 h after the addition of the second strain and plated on selective media to quantify population sizes. **c**, Competitor's ability to invade in arrival order experiments. Evolved populations of *P. dispersa* were more resistant to invasion by *P. protegens* (two-sided *t*-test, $t = 1.99$, degrees of freedom (d.f.) = 63.05, $P = 0.05$). **d**, *P. dispersa* ability to invade in arrival order experiments. Evolved populations of *P. dispersa* were less successful at invading *P. syringae* (two-sided *t*-test, $t = 5.14$, d.f. = 69.83, $P = 2.38 \times 10^{-6}$). Grid panels in **c** and **d** indicate competitor identity and asterisks indicate level of significance: $0.05 < P \leq 1$ (not significant, NS); $0.01 < P \leq 0.05$ (*); $0.001 < P \leq 0.01$ (**); $0 < P \leq 0.001$ (***). Error bars, means ± s.e. (standard error, $n = 6$ replicates per treatment).

P. dispersa and its host organisms may be context- or strain-dependent. It can promote plant growth and protect against pathogens[12,13], yet has also been reported as an opportunistic pathogen of both plants and animals[14,15]. Using experimental evolution and arrival order manipulations, we asked how selection for increased host association in *P. dispersa* altered priority effects and plant–microbe interactions.

Results and discussion

Priority effects shape microbial colonization outcomes in the tomato phyllosphere

We first asked whether priority effects might play an important role in phyllosphere microbiome assembly by introducing *P. dispersa* to tomato seedlings before, simultaneously with or after a competitor. To capture a range of expected overlap in niches and potential outcomes on host plant phenotype, we selected the following competitors: (1) a closely related strain of the same species *P. dispersa*, (2) the plant-protective bacterium *Pseudomonas protegens* and (3) the plant pathogen *Pseudomonas syringae*, a causative agent of bacterial speck and promoter of frost injury in plants[16,17]. In the first two cases, the eventual community composition strongly depended on arrival order, indicative of priority effects. In contrast, *P. dispersa* and *P. syringae* reached similar proportions regardless of their arrival order, possibly reflecting differences in their life cycles and spatial localization in the plant tissue (Extended Data Fig. 1). *P. syringae* inhabits mainly the above-ground tissues of plants. The pathogenic strain used in this study initially colonizes leaf surfaces as a weak epiphyte. Upon reaching a threshold population size, it enters the leaf apoplast, where it has specialized to multiply and cause disease[18]. In contrast, *P. dispersa* colonizes a wider variety of plant tissues (for example, roots, leaves, stems and seeds) and has been detected both on plant surfaces[12,19] and within the root and stem tissue[13].

As arrival order is inevitably correlated with the amount of time between inoculation and harvest in such experiments, it is possible that early arrival appears advantageous only because of population growth, independent of any interactions with other strains. We excluded this possibility by measuring the growth of each strain on plants in the absence of competition, which indicated that the duration of the experiment was sufficient for all strains to reach carrying capacity (Extended Data Fig. 2). We observed no differences in plant growth across any of these inoculations (Extended Data Fig. 3).

Experimental evolution alters the direction and magnitude of priority effects

To select for increased host association, we repeatedly inoculated tomato seedlings grown in sterile microcosms with replicate populations of *P. dispersa* and allowed the bacteria to associate with the plant

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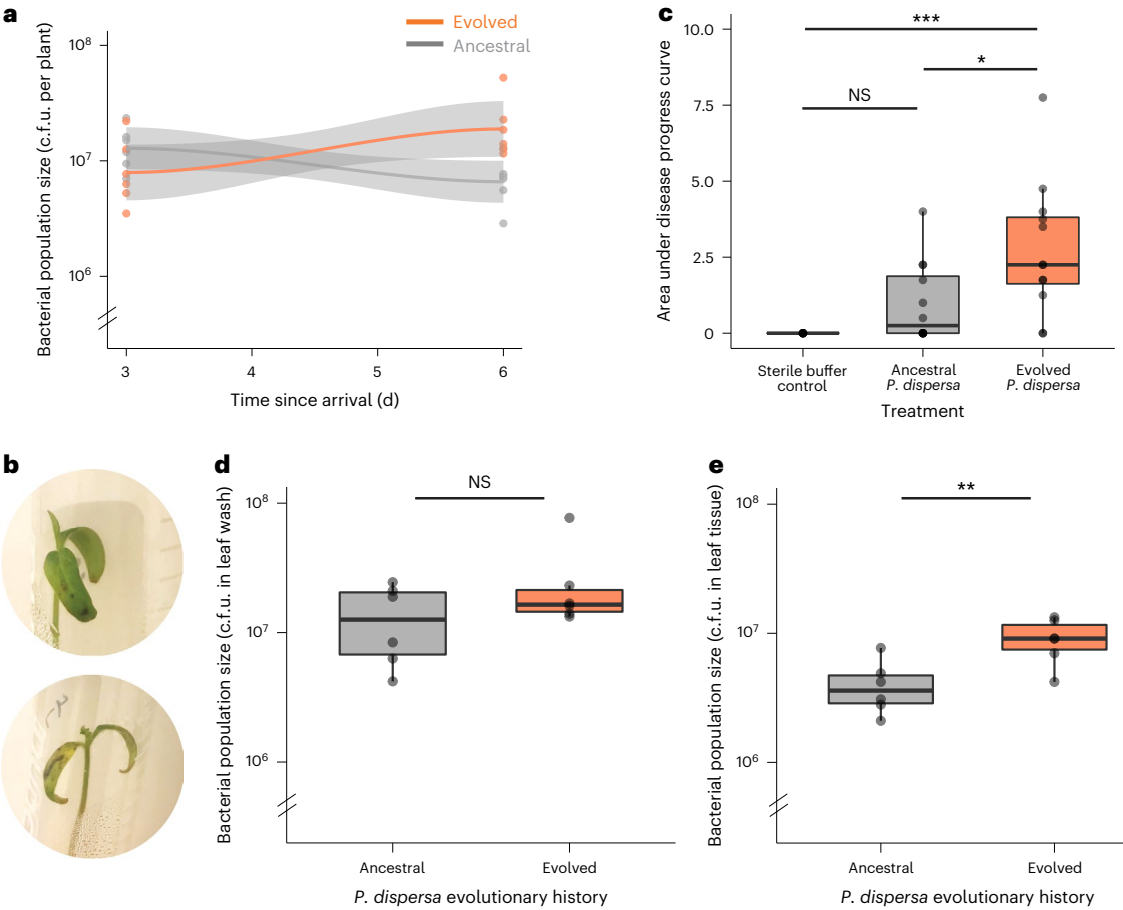

Fig. 2 | Changes in *P. dispersa* colonization dynamics over experimental evolution. a, Life history in the absence of competition. Population sizes of *P. dispersa* after inoculation via flooding. Interaction term between time since arrival and evolutionary history: analysis of variance (ANOVA; two-sided), $F = 12.07$, degrees of freedom (d.f.) = 1, $P = 0.0024$. Error bands represent 95% confidence intervals. **b**, Images of seedlings inoculated with evolved *P. dispersa* populations. **c**, Quantitative assay of symptom severity. A cumulative measure of symptom severity was calculated as the area under the curve of daily symptom scores taken blindly with respect to treatment for 5 d after inoculation ($n = 30$ seedlings). Symptom scores were higher in plants inoculated with evolved *P. dispersa* than in ancestral *P. dispersa* (ANOVA with Tukey's honestly significant difference (HSD) test correction, mean difference = 1.77, adjusted $P = 0.015$) or sterile buffer (ANOVA with Tukey's HSD test correction, mean difference = 2.75, adjusted $P = 0.00018$). **d**, Bacterial population sizes on leaf surface. Seedlings were submerged in sterile buffer, sonicated and vortexed thoroughly. Population sizes in leaf wash were determined via dilution plating. There was no difference in population sizes between evolved and ancestral populations ($n = 12$ seedlings, two-sided *t*-test, $t = 1.194$, $P = 0.276$). **e**, Bacterial population sizes in leaf tissue. Following the sonication described in **d**, the remaining bacteria associated with seedlings were isolated by homogenizing the seedling in sterile buffer. Evolved populations reached higher population sizes in the leaf tissue than did ancestral populations ($n = 12$ seedlings, two-sided *t*-test, $t = 3.23$, d.f. = 9.93, $P = 0.0091$). Box plots represent the first quartile, median and third quartile. Whiskers represent 1.5× the interquartile range (IQR) (or minima/maxima if no points exceeded 1.5× IQR). Asterisks indicate level of significance: $0.05 < P \leq 1$ (NS); $0.01 < P \leq 0.05$ (*); $0.001 < P \leq 0.01$ (**); $0 < P \leq 0.001$ (***). Note truncated *y* axes and logarithmic scale in **a**, **d** and **e**. Each experiment included one or more seedlings inoculated with sterile buffer and harvested alongside bacteria-treated plants. No bacterial colonies were recovered from these controls.

for 1 week. Bacteria that successfully colonized the plant were harvested and used to inoculate the next generation of seedlings (Fig. 1a). Following the experimental evolution, we asked whether within-host selection had altered the ability of *P. dispersa* to resist invasion and/or invade established populations of competitors (Fig. 1b).

After 6 weeks of experimental evolution within tomato seedlings, populations of *P. dispersa* were significantly more resistant to invasion by *P. protegens* than were their ancestral counterparts, consistent with the suggestion that within-host adaptation can strengthen priority effects by early colonizers (Fig. 1c, Extended Data Fig. 5a). In contrast, evolved populations of *P. dispersa* did not improve their ability to invade established populations of competitors (Fig. 1d, Extended Data Fig. 5b). Where evolution of invasion success has been reported in the literature, populations were passaged within cultures that had been preconditioned by competitors[7], suggesting that adaptation to the host environment alone may not be sufficient to overcome competition-mediated priority effects. Finally, evolved populations of *P. dispersa* were significantly less effective than their progenitors at colonizing plants after the establishment of *P. syringae* (Fig. 1d, Extended Data Fig. 5b). This final observation was unexpected given that *P. syringae*, an endophytic plant pathogen, had a substantially different life cycle than the other strains in the study and had not previously shown signs of direct competition with *P. dispersa*.

Phenotypic and genetic parallelism underlie adaptation to the phyllosphere

To understand this previously unobserved priority effect between *P. syringae* and *P. dispersa*, we first examined the colonization dynamics of ancestral and evolved *P. dispersa* in the absence of competition. The evolved populations appeared to have undergone a life history

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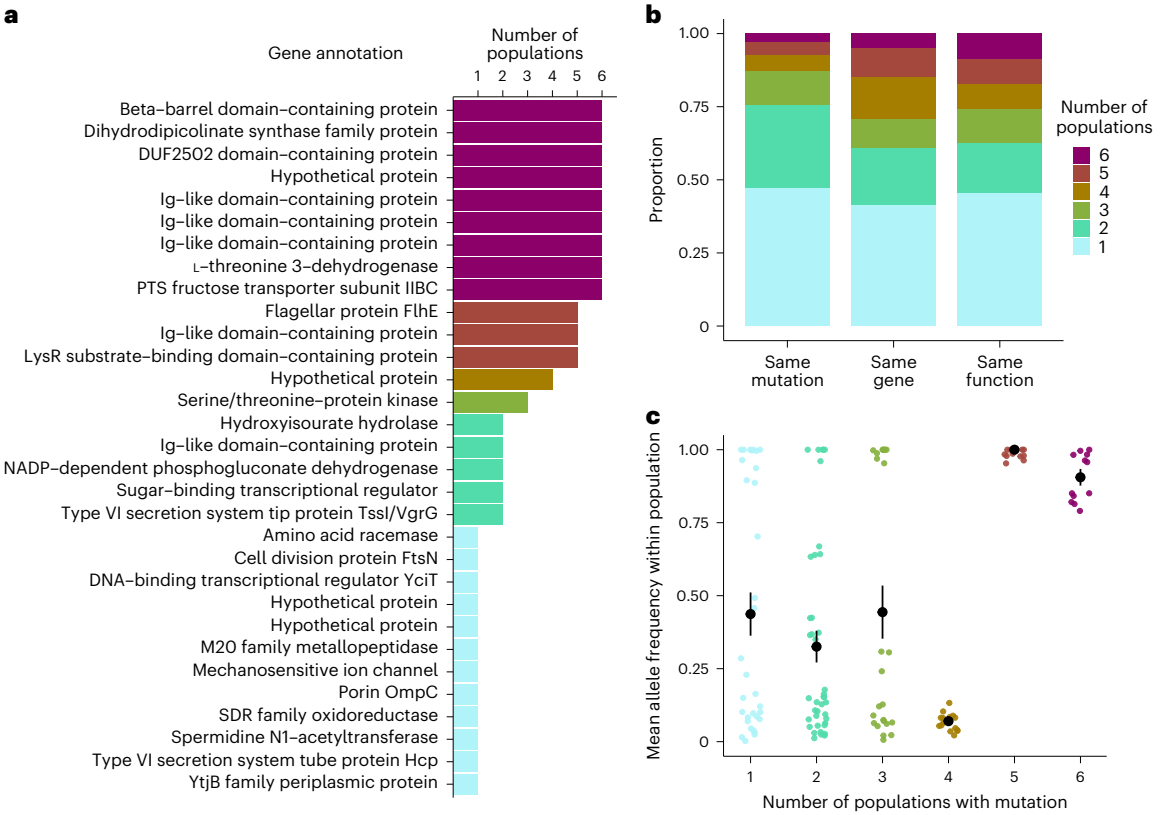

Fig. 3 | Genetic changes in *P. dispersa* during experimental evolution. **a**, Functional annotations of genes fixed in one or more populations. Gene product annotations are displayed according to the *P. dispersa* genome assembly (NCBI 22561). **b**, Evolutionary parallelism increases across broader levels of categorization. The leftmost bar indicates the proportion of mutations observed in the experiment that were unique or shared among two, three, four, five or six populations at any allele frequency. Middle bar indicates the extent of overlap in genes that accumulated mutations, whether they were at the same or different positions. The rightmost bar indicates the extent of overlap in gene annotations that accumulated mutations, whether they were in the same or different genes. **c**, Frequencies of mutations within and across populations. Mutations arising in parallel tended to reach higher allele frequencies within populations (generalized linear model with binomial distribution, $z = 4.02$, degrees of freedom (d.f.) = 212, $P = 1.04 \times 10^{-13}$). Error bars, means ± s.e. (standard error, $n = 218$ mutations).

shift, reaching higher population sizes than their ancestor but only after a period of slow growth (Fig. 2a). Additionally, many plants inoculated with either ancestral or evolved *P. dispersa* showed phenotypes reminiscent of disease, such as specks and chlorosis on cotyledons, that were not present in plants inoculated only with sterile buffer (Fig. 2b). To further examine this phenomenon, we adopted a quantitative assay of leaf symptoms previously used to study plant pathogens[10]. We inoculated plants with either ancestral or evolved populations of *P. dispersa* and recorded symptom scores on a daily basis for 5 days (d), blindly with respect to treatment. This experiment revealed that evolved *P. dispersa* was associated with more pronounced symptom development on leaves (Fig. 2c). We next asked whether it would be possible to leverage priority effects to mitigate the plant symptoms associated with colonization of the evolved *P. dispersa*. When a competing strain of *P. dispersa* was introduced 3 d before the evolved strain, symptom progression was reduced, indicating that priority effects can impact not only community composition but also functional properties (Extended Data Fig. 4).

On the basis of the above observations, we suggested that *P. dispersa* had evolved to exploit a new niche on the plant. As the colonization success of evolved *P. dispersa* depended on whether *P. syringae* had previously established, it seemed plausible that *P. dispersa* had shifted towards a more endophytic lifestyle. We inoculated plants with either ancestral or evolved *P. dispersa* as before, then sampled populations in a spatially resolved manner as follows. First, plants were submerged in sterile buffer and placed in an ultrasonic bath to dislodge bacterial cells from the leaf surface. Then, the sonication buffer was removed and plants were homogenized to isolate the remaining bacterial cells associated with the plant. Ancestral and evolved populations of *P. dispersa* reached similar population sizes in the leaf wash, but the evolved populations were more abundant within the leaf homogenate (Fig. 2d,e).

A total of 6 weeks of experimental evolution within tomato seedlings considerably altered the temporal and spatial colonization dynamics of *P. dispersa* populations. Although these changes were small, they were sufficient to affect priority effects among bacterial strains as well as the effect of *P. dispersa* on its host. Such observed changes could be driven either by genetic adaptation or by non-genetic, plastic shifts in the phenotype of *P. dispersa*. To investigate whether our experimentally evolved populations accumulated genetic changes, we deep-sequenced the ancestor of the evolution experiment and the six final populations. We detected 72 mutations across all populations located within 31 genes (Fig. 3a). In addition to the fixed mutations, 110 other alleles arose to intermediate frequencies but did not fix in any population (Supplementary Table 1). In many cases, mutations within the same gene or even at the same position arose repeatedly across populations, suggestive of evolutionary parallelism (Fig. 3b). Mutations arising in parallel across populations tended to reach higher allele frequencies, further suggesting that they were under selection (Fig. 3c).

The most common annotation for genes altered in experimental evolution was proteins containing an immunoglobulin (Ig)-like domain.

This structure is widespread throughout living organisms and appears in bacterial proteins with a wide range of functions, including adhesion, nutrient uptake and pathogenic invasion into host cells[20]. Other genes were commonly implicated in sugar and amino acid metabolism (for example, L-threonine 3-dehydrogenase, PTS fructose transporter) and motility (for example, flagellar protein FlhE), both important aspects of plant–microorganism interactions[11,21].

Conclusions

The phenotypic and genetic changes observed in this experiment suggest the evolution of a more intimate association with the plant tissue, possibly mediated by exploitation of plant stomata or improved persistence within other protected sites on the leaf. As in any experimental evolution study, it is important to consider why, if the observed traits are beneficial and evolve readily, the organism has not already acquired them in nature. *P. dispersa* appears to be a widely distributed species that has been isolated from plants, animals and non-host habitats[14,15,22]. It is plausible that improved colonization of tomato seedlings comes with a trade-off at other life stages, on other host species or in other environments. Another consideration is that the microcosms in this study represent certain conditions (for example, nutrient limitation and high humidity) that may promote stomatal opening or otherwise make plants particularly vulnerable to exploitation. Indeed, high humidity has previously been shown to promote overgrowth and disease symptoms by endogenous (non-pathogenic) microbiota[23].

Eco-evolutionary models often describe community assembly as a race between local adaptation by resident species and invasion by new species[5,24]. They suggest that communities of small, passive dispersers with fast evolutionary rates (such as bacteria) are prone to strong monopolization by early arrivers[5]. These predictions are difficult to reconcile, however, with observed fluctuations and replacements in the composition of natural microbial communities. Here, we show that an early colonizer evolved to exploit a new niche within a short time. This niche shift coincided with changes to both the nature and intensity of ecological interactions with other members of the plant microbiome. Although this study did not explicitly link genetic changes to niche occupancy, the observed parallel response to selection across experimental populations suggests that the two are related. Our findings imply that the plant environment is sufficiently complex and heterogeneous in chemical and physical structure to present possibilities for microbial populations to adapt beyond simply improving their efficiency within their existing niche space. To understand the predictability of community assembly, it will be critical to further develop theory and data that can fully describe the adaptive landscapes for colonizing populations[25,26].

Methods

Bacterial strains and selective markers

This study included the following bacterial strains: *P. dispersa* strain ZM1 (originally isolated from tomato plants as reported previously[12]), *P. protegens* strain ZDW1 (isolated in this study) and *P. syringae* pv. tomato DC3000 (provided by G. Preston, University of Oxford). To distinguish competing populations of *P. dispersa* strain ZM1, antibiotic-resistant strains were selected as follows. A total of 500 µl of overnight culture was inoculated into sterile King's B Medium with either 4 µg ml^{-1} of rifampicin or 2 µg ml^{-1} of chloramphenicol. The inoculated culture was incubated overnight at 28 °C and the process was repeated with an increasing antibiotic concentration until resistance was achieved at the final concentrations of 20 µg ml^{-1} of rifampicin or 10 µg ml^{-1} of chloramphenicol. Strains were then selected for equal competitive ability as their antibiotic-sensitive counterparts in vitro by repeatedly coculturing resistant and sensitive bacteria and measuring the ratios they reached in 24 hours (h) until there were no significant costs of the resistance marker. A single colony of each strain was selected to grow the ancestral stock, which was subsequently used to initiate six experimental evolution populations. This selection procedure resulted in several other genetic differences aside from the antibiotic resistance mutations, which could potentially affect leaf colonization and competition. Since the aim of this process was to generate a closely related and phenotypically distinguishable competitor for focal *P. dispersa*, isogenic populations were not required. Genetic differences between the two strains are listed in Supplementary Table 2.

Arrival order experiments

Tomato seeds (*S. lycopersicum* cv. 'Moneymaker') were surface-sterilized in 2.7% bleach (sodium hypochlorite) solution for 20 min, then washed three times with 10 mM $MgCl_2$ to remove residual bleach. Each seed was placed in a loosely capped, sterile 15 ml tube with 7 ml of 1% water agar. Tubes were covered in foil and maintained in a 21 °C chamber until shoot emergence, then moved to a 28 °C growth chamber with a 15 h day:9 h night cycle.

Seedlings were flooded 9–12 d after planting, depending on the experiment. To prepare inocula, overnight cultures were pelleted at 3,500*g* and washed with 10 mM $MgCl_2$ to remove residual media. Each culture was diluted to an optical density (OD_{600}) of 0.0015, ~10^7 colony-forming units (c.f.u.) per ml. The surfactant Silwet L-77 was added at a concentration of 0.015% to facilitate leaf colonization. Tubes were immediately placed on an orbital shaker for 4 min, then the inocula were poured off and the seedlings were allowed to dry in a biosafety cabinet. Each flooding trial included one or more seedlings treated with only 10 mM $MgCl_2$ and Silwet L-77 to ensure that reagents were sterile.

At 3 d after the addition of the second species, seedlings were individually weighed and then harvested. Two sterile ceramic beads and 7 ml of 10 mM $MgCl_2$ were added to each tube and tubes were agitated in a FastPrep-24 5 G system (MP Biomedicals catalogue no. 116005500) at 4 m s^{-1} for 60 s to homogenize plant tissue. Leaf homogenate was independently diluted twice per sample and each replicate dilution series was plated on both rifampicin- and chloramphenicol-supplemented King's B agar plates to distinguish strains in mixed inoculations. Bacterial population sizes were quantified by counting c.f.u. after 2 d of incubation at 28 °C.

Experimental evolution in planta

An overnight culture of *P. dispersa* was pelleted at 3,500*g* and washed with 10 mM $MgCl_2$ to remove residual media. Inocula were prepared by resuspending pellets in 10 mM $MgCl_2$ to a bacterial OD_{600} of 0.0025 and adding 0.015% of the surfactant Silwet L-77 to facilitate leaf colonization. Seedlings were flooded as described above and maintained in the growth chamber for 7 d. At the end of each week, seedlings were collected with sterile forceps into sterile 15 ml Eppendorf tubes and homogenized in sterile 10 mM $MgCl_2$ as above. Leaf homogenate was diluted and plated on rifampicin-supplemented King's B agar plates. For each experimental evolution population, 100 colonies were individually picked from plates, mixed and grown overnight to generate inocula for the following generation of seedlings.

Plant symptom quantification

In all cases where symptoms were measured, seedlings were grown in 15 ml tubes and flooded with bacteria at OD_{600} = 0.0015, as described above. Instead of harvesting 3 d after inoculation to measure bacterial abundances, these seedlings were maintained in the growth chamber to track symptom development on a daily basis. In accordance with previous plant pathology studies, symptoms were scored blindly with respect to treatment using scores that describe different levels of symptom severity[27]. Scores in this study were as follows: no symptoms (level 0), mild speckling (level 1), extensive speckling and/or chlorosis (level 2) and leaf necrosis and/or detachment (level 3). Individual leaflets on the same seedling were scored separately, then combined into an average symptom severity per plant for all analyses and figures to avoid pseudoreplication.

Genome sequencing

The six evolved populations of *P. dispersa*, their ancestral stock and the competitor strain of *P. dispersa* were grown overnight in liquid culture to prepare for DNA extraction. Bacterial DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN catalogue no. 69504). Sample libraries were prepared using the Illumina DNA Prep kit and IDT 10 base pairs (bp) UDI indices and sequenced at an estimated coverage of 133× on an Illumina NextSeq 2000 at SeqCenter. Demultiplexing, quality control and adaptor trimming were performed with bcl-convert (v.3.9.3). Paired-end reads were filtered and trimmed using Trimmomatic (v.0.39). Reads shorter than 25 bp or with an average quality score <20 in a 4 bp sliding window were discarded. Reads were mapped to the *P. dispersa* genome (entry 22561 in the NCBI genome database) and variants were called using breseq (v.0.35.4), a pipeline for identifying fixed and polymorphic genetic variation within microbial populations[28,29]. To avoid false positive calls from repetitive elements, mutations were filtered to exclude highly polymorphic regions (five or more mutations in a 50 bp sliding window within a population). All analyses focused on non-synonymous mutations within coding regions of the genome.

Statistical analyses

Bacterial population sizes at harvest were determined on the basis of colony counts on selective media. Colony counts of single-species controls were checked for cross-contamination or additional resistance evolution, neither of which was observed in the study. Colony counts were log-transformed before statistical analysis to meet assumptions of normality.

We calculated P_{ij}, a previously developed metric for quantifying the strength of priority effects[30], as follows. Here, $D(i)_{ji}$ represents the population size reached by species i when it was introduced after species j and $D(i)_{ij}$ represents its population size when i was introduced before j. This coefficient takes on negative values if the population size of species i is smaller when arriving after j than when arriving before; that is, if competition outcomes between i and j are arrival order-dependent.

$$P_{ij} = \ln\left[\frac{D(i)_{ji}}{D(i)_{ij}}\right]$$

We also calculated an alternative metric P'_{ij}, as previously described[30], where $D(i)_{0i}$ and $D(i)_{i0}$ represent the single-species controls of species i that were inoculated and sampled at the same time as the corresponding cocolonization treatments:

$$P'_{ij} = \ln\left[\frac{D(i)_{ji}}{D(i)_{0i}}\right] - \ln\left[\frac{D(i)_{ij}}{D(i)_{i0}}\right]$$

P'_{ij} is similar to P_{ij} but accounts for the growth of species i alone. We found that the two metrics were highly correlated and gave qualitatively similar results (Extended Data Fig. 5).

Plant symptom progression was analysed by calculating the area under the disease progression curve, a cumulative measure of symptom severity over time[31]. Differences in cumulative symptom progression over time were assessed using Welch's t-test that compared the area under the disease progression curve across bacterial treatments.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The 16S ribosomal RNA sequences of all bacterial strains in this study and whole-genome sequences of the ancestral and evolved *P. dispersa* populations have been deposited to the NCBI Sequence Read Archive (BioProject ID PRJNA865524) and the figshare repository https://doi.org/10.6084/m9.figshare.20696560. All other datasets generated in this study are available in the GitHub public repository https://github.com/reenadebray/ecoevopriority.

Code availability

The code used to analyse the data in this study is available in the GitHub public repository https://github.com/reenadebray/ecoevopriority.

References

1. Fukami, T. Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annu. Rev. Ecol. Evol. Syst.* **46**, 1–23 (2015).
2. Weidlich, E. W. A. et al. Priority effects and ecological restoration.*Restor. Ecol.* **29**, e13317 (2021).
3. Young, T. P., Stuble, K. L., Balachowski, J. A. & Werner, C. M. Using priority effects to manipulate competitive relationships in restoration: using priority effects in restoration. *Restor. Ecol.* **25**, S114–S123 (2017).
4. Koskella, B., Hall, L. J. & Metcalf, C. J. E. The microbiome beyond the horizon of ecological and evolutionary theory. *Nat. Ecol. Evol.* **1**, 1606–1615 (2017).
5. Urban, M. C. & De Meester, L. Community monopolization: local adaptation enhances priority effects in an evolving metacommunity. *Proc. R. Soc. B* **276**, 4129–4138 (2009).
6. Nadeau, C. P., Farkas, T. E., Makkay, A. M., Papke, R. T. & Urban, M. C. Adaptation reduces competitive dominance and alters community assembly. *Proc. R. Soc. B* **288**, 20203133 (2021).
7. Chappell, C. R. et al. Wide-ranging consequences of priority effects governed by an overarching factor. *eLife* **11**, e79647 (2022).
8. Piccardi, P., Alberti, G., Alexander, J. M. & Mitri, S. Microbial invasion of a toxic medium is facilitated by a resident community but inhibited as the community co-evolves. *ISME J.* **16**, 2644–2652 (2022).
9. Garud, N. R., Good, B. H., Hallatschek, O. & Pollard, K. S. Evolutionary dynamics of bacteria in the gut microbiome within and across hosts. *PLoS Biol.* **17**, e3000102 (2019).
10. Meyer, K. M. et al. Plant neighborhood shapes diversity and reduces interspecific variation of the phyllosphere microbiome. *ISME J.* **16**, 1376–1387 (2022).
11. Vorholt, J. A. Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* **10**, 828–840 (2012).
12. Morella, N. M., Zhang, X. & Koskella, B. Tomato seed-associated bacteria confer protection of seedlings against foliar disease caused by *Pseudomonas syringae*. *Phytobiomes J.* **3**, 177–190 (2019).
13. Jiang, L. et al. Potential of *Pantoea dispersa* as an effective biocontrol agent for black rot in sweet potato. *Sci. Rep.* **9**, 16354 (2019).
14. Toh, W. K., Loh, P. C. & Wong, H. L. First report of leaf blight of rice caused by Pantoea ananatis and Pantoea dispersa in Malaysia. *Plant Dis.* **103**, 1764 (2019).
15. Asai, N. et al. *Pantoea dispersa* bacteremia in an immunocompetent patient: a case report and review of the literature. *J. Med. Case Rep.* **13**, 33 (2019).
16. Yunis, H. Two sources of resistance to bacterial speck of tomato caused by *Pseudomonas* tomato. *Plant Dis.* **64**, 851 (1980).
17. Lindow, S. E., Arny, D. C. & Upper, C. D. Bacterial ice nucleation: a factor in frost injury to plants. *Plant Physiol.* **70**, 1084–1089 (1982).
18. Xin, X.-F., Kvitko, B. & He, S. Y. *Pseudomonas syringae*: what it takes to be a pathogen. *Nat. Rev. Microbiol.* **16**, 316–328 (2018).
19. Völksch, B. & Sammer, U. in *Pseudomonas syringae Pathovars and Related Pathogens—Identification, Epidemiology and Genomics* (eds Fatmi, M. et al.) 111–116 (Springer, 2008).

20. Bodelón, G., Palomino, C. & Fernández, L. Á. Immunoglobulin domains in *Escherichia coli* and other enterobacteria: from pathogenesis to applications in antibody technologies. *FEMS Microbiol. Rev.* **37**, 204–250 (2013).
21. Haiko, J. & Westerlund-Wikström, B. The role of the bacterial flagellum in adhesion and virulence. *Biology* **2**, 1242–1267 (2013).
22. Selvakumar, G. et al. Characterization of a cold-tolerant plant growth-promoting bacterium *Pantoea dispersa* 1A isolated from a sub-alpine soil in the North Western Indian Himalayas. *World J. Microbiol. Biotechnol.* **24**, 955–960 (2008).
23. Xin, X.-F. et al. Bacteria establish an aqueous living space in plants crucial for virulence. *Nature* **539**, 524–529 (2016).
24. Vanoverbeke, J., Urban, M. C. & Meester, L. Community assembly is a race between immigration and adaptation: eco-evolutionary interactions across spatial scales. *Ecography* **39**, 858–870 (2016).
25. Lieberman, T. D. Detecting bacterial adaptation within individual microbiomes. *Philos. Trans. R. Soc. B* **377**, 20210243 (2022).
26. Wong, D. P. G. H. & Good, B. H. Quantifying the adaptive landscape of commensal gut bacteria using high-resolution lineage tracking. Preprint at *bioRxiv* https://doi.org/10.1101/2022.05.13.491573 (2023).
27. Rajendran, D. K. et al. Visual analysis for detection and quantification of *Pseudomonas cichorii* disease severity in tomato plants. *Plant Pathol. J.* **32**, 300–310 (2016).
28. Deatherage, D. E. & Barrick, J. E. in *Engineering and Analyzing Multicellular Systems: Methods and Protocols* (eds Sun, L. & Shou, W.) 165–188 (Springer, 2014).
29. Deatherage, D. E., Traverse, C. C., Wolf, L. N. & Barrick, J. E. Detecting rare structural variation in evolving microbial populations from new sequence junctions using breseq. *Front. Genet.* **5**, 468 (2015).
30. Vannette, R. L. & Fukami, T. Historical contingency in species interactions: towards niche-based predictions. *Ecol. Lett.* **17**, 115–124 (2014).
31. Jeger, M. J. & Viljanen-Rollinson, S. L. H. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. *Theor. Appl. Genet.* **102**, 32–40 (2001).

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Author contributions

R.D. and B.K. conceived the study. R.D., A.C., X.Z. and E.A.D.-W. collected the data. R.D. performed the statistical analyses and drafted the manuscript. All authors contributed to the manuscript revision.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-023-02040-w.

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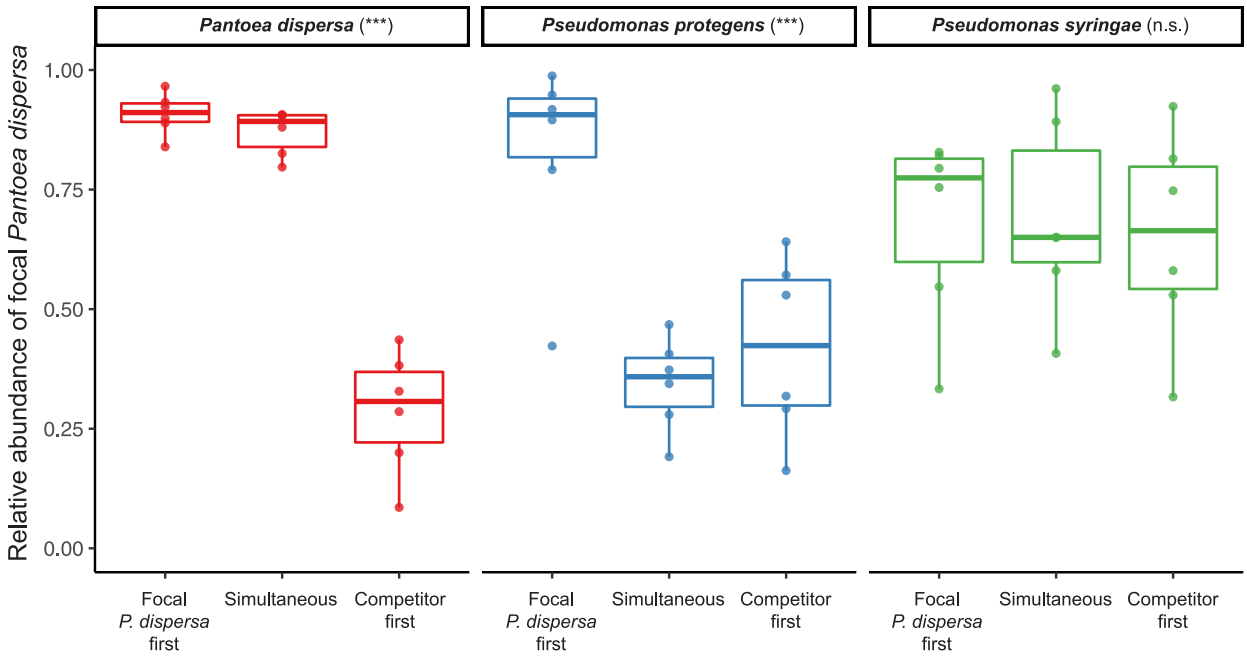

Extended Data Fig. 1 | Priority effects among bacteria in the tomato phyllosphere. Relative abundance of *P. dispersa* on tomato seedlings when co-inoculated with either *P. dispersa*, *P. protegens*, or *P. syringae* across varying arrival orders. Seedlings were flooded either with both strains simultaneously or 72 h apart. Seedlings were harvested another 72 h after the addition of the final strain, and homogenized plant tissue was plated on selective media to count colonies. Arrival order significantly impacted strain proportions when both strains were *P. dispersa* (ANOVA (two-sided), $F = 107.43$, d.f. = 34, $P = 1.29 \times 10^{-9}$), or when *P. dispersa* was co-inoculated with *P. protegens* (ANOVA (two-sided), $F = 13.48$, d.f. = 34, $P = 0.0004$), but not when co-inoculated with *P. syringae*. Box plots represent the first quartile, median, and third quartile ($n = 6$ replicates per treatment). Whiskers represent 1.5 times the IQR (or minima/maxima if no points exceeded 1.5× IQR).

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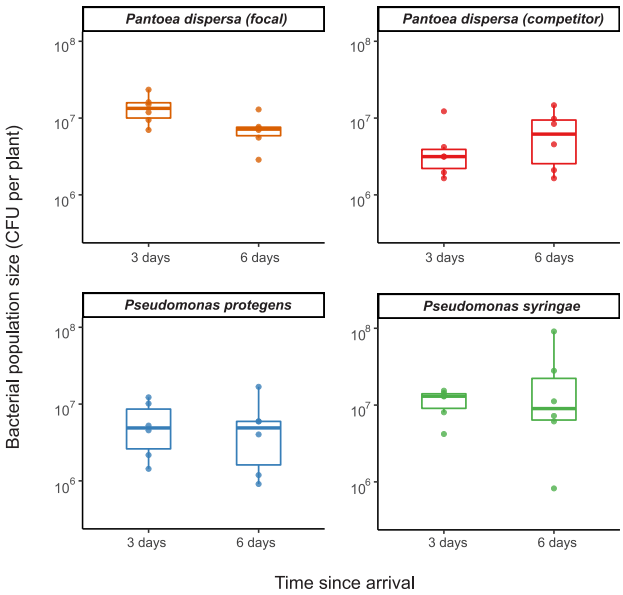

Extended Data Fig. 2 | Experimental duration is sufficient to reach carrying capacity on tomato seedlings. Population sizes of *P. dispersa*, *P. protegens*, and *P. syringae* when inoculated individually onto plants. Seedlings were either harvested directly after 72 h (as a control for the simultaneous co-inoculation treatments) or flooded with sterile buffer after 72 h and harvested after 144 h (as a control for the staggered co-inoculation treatments). At harvest, homogenized plant tissue was plated on selective media to count colonies. None of the strains grew significantly between 3 and 6 d after arrival, although the focal strain of *P. dispersa* decreased slightly in population size over this time (ANOVA (two-sided), $F = 6.28$, d.f. = 10, $P = 0.031$). Box plots represent the first quartile, median, and third quartile ($n = 6$ replicates per treatment). Whiskers represent 1.5 times the IQR (or minima/maxima if no points exceeded 1.5× IQR).

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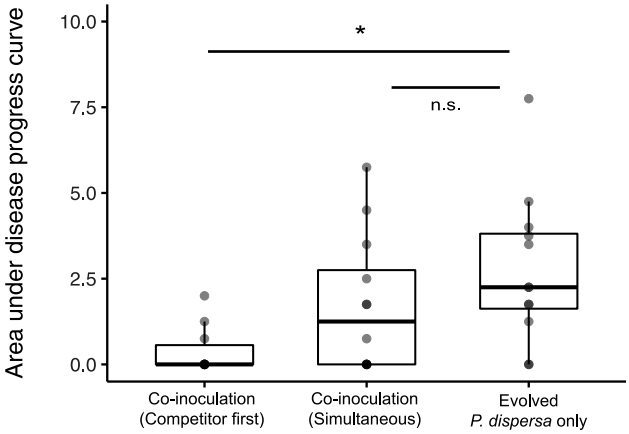

Extended Data Fig. 3 | No differences in seedling biomass across treatments. Seedlings were weighed at harvest (72 h after second inoculation). No significant differences were observed with respect to species identity or evolutionary history. Box plots represent the first quartile, median, and third quartile ($n = 12$ replicates per treatment). Whiskers represent 1.5 times the IQR (or minima/maxima if no points exceeded 1.5× IQR).

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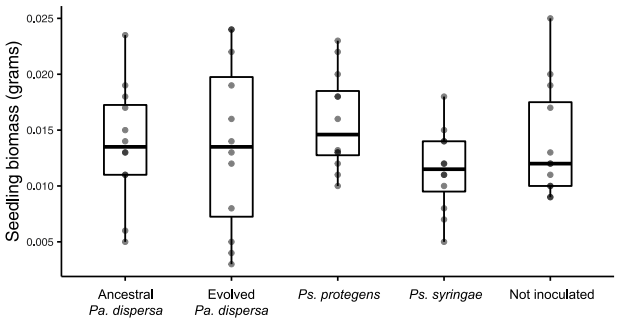

Extended Data Fig. 4 | Consequences of priority effects for plant symptom severity. Seedlings were inoculated with evolved populations of *P. dispersa*, which were previously associated with lesions and discoloration of the leaf tissue, and a competing strain of *P. dispersa* that does not cause such symptoms. A cumulative measure of symptom severity was calculated as the area under the curve of daily symptom scores taken blindly with respect to treatment for 5 d after inoculation. Each evolved population of *P. dispersa* was replicated twice in each treatment category ($n = 12$ seedlings per treatment), but symptom scores were subsequently averaged for each biological replicate to avoid pseudoreplication ($n = 6$ per treatment in statistical analysis). Co-inoculation with competing *P. dispersa* suppressed symptom development, but only if the competing *P. dispersa* arrived first (ANOVA with Tukey's HSD correction, mean difference = 2.35, adjusted $P = 0.012$). Asterisks indicate level of significance: $0.05 < P \leq 1$ (NS); $0.01 < P \leq 0.05$ (*); $0.001 < P \leq 0.01$ (**); $0 < P \leq 0.001$ (***). Box plots represent the first quartile, median, and third quartile. Whiskers represent 1.5 times the IQR (or minima/maxima if no points exceeded 1.5× IQR., median, and third quartile. Whiskers represent 1.5 times the IQR (or minima/maxima if no points exceeded 1.5× IQR.

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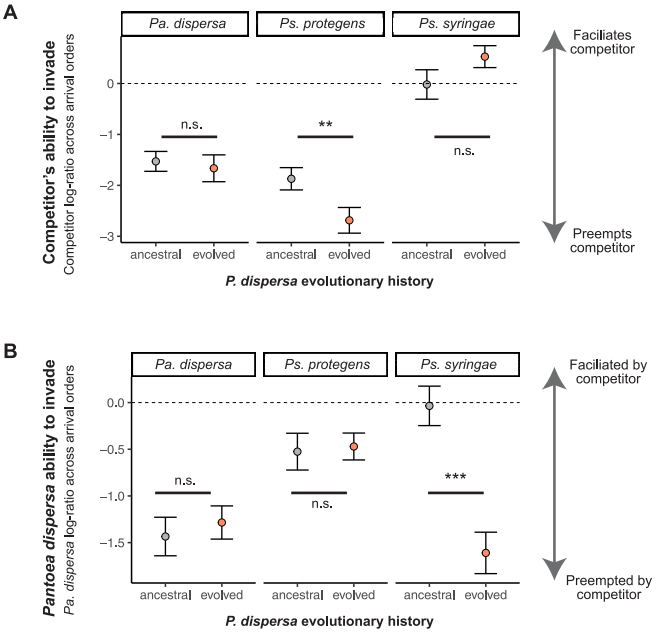

Extended Data Fig. 5 | An alternative measure of priority effects recapitulates effects of experimental evolution. (**a**) Competitor's ability to invade in arrival order experiments. Evolved populations of *P. dispersa* were more resistant to invasion by *P. protegens* (two-sided *t*-test, $t = 2.93$, d.f. = 40.58, $P = 0.0056$). (**b**) ***P. dispersa*** ability to invade in arrival order experiments. Evolved populations of *P. dispersa* were less successful at invading *P. syringae* (two-sided *t*-test, $t = 10.18$, d.f. = 35, $P = 2.65 \times 10^{-12}$). Grid panels indicate competitor identity and asterisks indicate level of significance: $0.05 < P \leq 1$ (NS); $0.01 < P \leq 0.05$ (*); $0.001 < P \leq 0.01$ (**); $0 < P \leq 0.001$ (***). Error bars represent means ± standard error ($n = 6$ replicates per treatment).

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No custom algorithms or software were used to collect the data in this study.
Data analysis	The following software tools were used to analyze the genomic data: bcl-convert (v3.9.3), Trimmomatic (v0.39), breseq (v0.35.4). R version 4.2.1 was used to run statistical tests and construct plots of the data in this study. Custom code is available on GitHub (https://github.com/reenadebray/ecoevopriority)

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The 16S rRNA sequences of all bacterial strains in this study and whole-genome sequences of the ancestral and evolved Pantoea dispersa populations have been

deposited to the NCBI Sequence Read Archive (BioProject ID PRJNA865524) and the figshare repository https://doi.org/10.6084/m9.figshare.20696560. All other datasets generated in this study are available in the Github public repository https://github.com/reenadebray/ecoevopriority.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Six populations of the early-colonizing bacterium Pantoea dispersa strain ZM1 were experimentally adapted to tomato seedlings via a passaging protocol. The strength of priority effects between Pantoea dispersa ZM1 and three other bacterial strains within the tomato phyllosphere microbiome was evaluated using staggered arrival experiments with either the experimentally evolved populations or their progenitor.
Research sample	The study system was comprised of MoneyMaker tomato plants and three species of bacteria: Pantoea dispersa strain ZM1, Pseudomonas protegens strain ZDW1, and Pseudomonas syringae pv. tomato DC3000. The plant phyllosphere was selected as a study system for community assembly because it is clearly delineated from the surrounding environment, highly culturable, and plays an important role in plant fitness. The three species of bacteria were chosen to capture a range of expected overlap in niches and potential effects on plant phenotype: One was the same species as the focal bacterial species, another was a plant-protective bacterium, and the third was a plant pathogen.
Sampling strategy	Seedlings were grown in sterile microcosms within a climate- and light-controlled growth chamber. The majority of experiments included six replicates per treatment group, based on pilot work indicating that the sample size was sufficient to capture priority effects in this system. For symptom scores, each evolved population was replicated twice in the experiment, and then symptom scores were averaged for each biological replicate prior to analysis to avoid pseudoreplication.
Data collection	Population sizes were quantified via serial dilution and colony plating on selective media. Colony counts were recorded two days after plating. Symptom scores were assessed based on a quantitative assay previously used to study leaf pathogens. All samples were assigned randomized identification numbers throughout the experiment so that data were collected blindly with respect to treatment.
Timing and spatial scale	Experimental evolution was conducted from December 2019 to June 2020. Arrival order experiments were conducted from May 2021 to March 2022.
Data exclusions	No data were excluded from data analysis.
Reproducibility	Experiments were not repeated.
Randomization	Each seedling was assigned a random identification number. Microcosms were arranged according to the random identification number to ensure that any spatial variation (e.g. in lighting or microclimate) would be randomly distributed across treatments. Seedlings were harvested according to their identification number to ensure any effects of processing order would be randomly distributed across treatments.
Blinding	Each seedling was assigned a random identification number. All data were collected blindly to minimize observer bias, and identification numbers were only connected to treatments after the study was completed.

Did the study involve field work? ☐ Yes ☒ No

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Methods

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