Figures and figure supplements

Harbouring public good mutants within a pathogen population can increase both fitness and virulence

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Figure 1. Invertase production in M. oryzae is a cooperative trait. (a) \( \Delta \text{inv}1 \) has growth defects on sucrose minimal media, with functional complementation restoring invertase synthesis and growth morphology of \( \Delta \text{inv}1:INV1 \), confirming the function of \( INV1 \). (b) Invertase deficiency resulted in a fitness reduction on sucrose (mean ± s.e.m.) with respect to conidia (\( p < 0.0001, n = 12, \) two-sided 2-sample t-test for unequal variance) and biomass (\( p < 0.0001, n = 9, \) two-sided 2-sample t-test for unequal variance). (c) This was confirmed to be caused by invertase production deficiency tested by enzymatic assay of culture filtrate under different induction treatments (units are \( \mu \) moles of glucose / fructose liberated from sucrose per minute) mean ± s.e.m., \( n = 3 \). \( INV1 \) expression in Guy11 is sucrose induced and glucose repressed, with constitutive expression remaining in non-yielding environments (glucose). (d) \( INV1 \) production is an exploitable secreted product as \( \Delta \text{inv}1 \) could generate significantly more biomass in the supernatant of Guy11 than in the supernatant of \( \Delta \text{inv}1 \) (\( p < 0.0001, n = 9, \) two-sample t-test for equal variance). (e) The non-producer, \( \Delta \text{inv}1 \), gains a fitness advantage over invertase producers in a low-structured environment (\( p < 0.003 \) at each initial frequency, one-sample t-test, \( n = 9 \), mean ± s.e.m.). A small amount of x-axis noise was added to help visualize data points.

DOI: 10.7554/eLife.18678.002
Transform into *Magnaporthe oryzae*

Gene replacement by homologous recombination:

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**Figure 1—figure supplement 1.** Targeted gene deletion of *INV1* in *Magnaporthe oryzae*. (a) Schematic of fusion PCR based split-marker targeted gene deletion of invertase *INV1*. Gene replacement was achieved by replacing putative invertase genes with a 2.8 kb sulfonylurea resistance allele (*ILV1*). In a first round of PCR, a 1 kb genomic fragment upstream (LF) and downstream (RF) of the ORF were amplified. Separately, 1.6 kb overlapping fragments of the 5’ and 3’ end of the sulfonylurea resistance gene cassette (*ILV1*) were amplified. Amplicons produced were fused in a second round of PCR to produce two larger fragments of 2.6 kb. The constructs were used to transform *M. oryzae* (Strain ToxA:sGFP background Guy11) and gene replacement achieved by homologous recombination. (b) Gene replacement was confirmed using digoxigenin (DIG)-labelled Southern blot analysis by fragment size differentiation following restriction endonuclease digestion of genomic DNA with *XhoI*. Blots were probed with the left flank region (LF, 1 kb upstream of the ORF) using primers INV1_50.1 and INV1_M13F (Supplementary file 1). Analysis showed a 1.4 kb size difference with wild-type genotype band at 4.2 kb with mutants at 5.6 kb. Transformants 11 and 15 shown are positive knock-out strains.

DOI: 10.7554/eLife.18678.003
Figure 1—figure supplement 2. Functional complementation of *Saccharomyces cerevisiae* invertase deletion strain DBY1701 with *M. oryzae* INV1. To verify its function, the ORF of *M. oryzae* INV1 was cloned into the yeast expression vector NEV-E with the PMA1 promoter being replaced with the constitutive GPD promoter. This vector was expressed in the invertase deletion strain DBY1701. This partially restored the growth rate of the deletion strain in sucrose limited media (dark red). The empty vector was transformed into DBY1701 as a negative control (blue). The ancestral SUC2 invertase bearing strain (DBY1034) with the empty vector was used as the positive control (pink) (n = 3, mean ± 95% CI). BLASTp of *M. oryzae* INV1: *S. cerevisiae* S288c invertase SUC2: Identities = 31%, E-value = 3e−10.

DOI: 10.7554/eLife.18678.004
The relative fitness of producers (Guy11) in a spatially structured population. In sufficiently structured environments (see Materials and methods) the fitness of producers shows the negative frequency-dependence (Linear model: Adj. $R^2 = 0.4756$, $\beta = -0.5320$, $p=1.98e-06$, $F_{(1,34)} = 32.74$), whereby producers gain a selective advantage when rare in a population consisting predominantly of non-producers. Moreover, the data suggest that producers and non-producers can coexist at some intermediate frequency in the sufficiently structured environment. However, this measure assumes constant fitness differences between competitors and so is not necessarily a predictor of long-term equilibrium frequencies (Ribeck and Lenski, 2015). Open circles show data points, closed circles show mean ± s.e.m., $n = 9$. Line is linear model ± s.e.m.

DOI: 10.7554/eLife.18678.005
Figure 2. Virulence and pathogen fitness measurements of Δinv1, Guy11 and a mixed inoculum. (a) In planta fitness of *M. oryzae* during infection was evaluated by leaf spot inoculation (mean ± s.e.m., n = 42). Fitness was quantified by the number of conidia recovered per lesion at the end of the disease cycle. Infections with Guy11 produced significantly more conidia than pure non-producer (Δinv1) infections (**p<0.0001, W = 66, two-sided Mann-Whitney U test, n = 42). In addition, applying existing social theory we hypothesize that the number of conidia recovered per mixed Guy11 and Δinv1 disease lesions is not higher than the number of conidia recovered from Guy11-only disease lesions. However, we can reject this hypothesis using properties of Boolean algebra: analysis of raw data (*p<0.0365, W = 1174, two-sided Mann-Whitney U-test with Bonferroni correction, n = 42, see Appendix 1A for detailed analysis) and log-transformed data (Figure 2—figure supplement 4). (b) Disease virulence of *M. oryzae* during infection was also evaluated by spot inoculation (mean±s.e.m., n = 20). It showed reduced virulence, as measured by lesion area, of Δinv1 compared to Guy11 (*p<0.00003, two-sided 2-sample t-test for unequal variances, mean±s.e.m., n = 20). Guided by the data in panel (a), we also confirm that fitness positively correlates with virulence and that mixed populations of Guy11 and Δinv1 also have higher virulence than pure Guy11 infections (**p<0.0032, two-sided 2-sample t-test for unequal variances, n = 20). Example lesions (7d) from leaf spot infections from pure and mixed populations, scale bar = 3 mm, with ImageJ analysis of images from which lesion areas were measured. Images of all replicates can be seen in Figure 2—figure supplement 1. (c) Live cell imaging of mixed strain infection (48 hr.p.i.) of rice sheath epidermal cells indicating close proximity of co-infecting strains; this suggests interactions and invertase exploitation is possible, scale bar = 50 μm. (d) Epifluorescence micrograph of sporulating lesion from mixed infections (9d) with DIC, RFP (wildtype) and GFP (Δinv1) conidia, indicating the presence of both strains within conidia populations at the end of the infection cycle, scale bar = 200 μm.

DOI: 10.7554/eLife.18678.006
Figure 2—figure supplement 1. Extended summary of Figure 2. Disease virulence of Magnaporthe oryzae from attached leaf spot inoculations of Guy11, Δinv1, and a mixture of Guy11 (80%) + Δinv1 (20%). (a) Images of disease lesions captured with Epson Expression 1680 Pro scanner after 7d, pale blue lines indicate boundaries of lesion area quantified, Scale bar = 7 mm. (b) Disease lesion area quantified with image analysis software (ImageJ). Δinv1 imposed reduced virulence as measured by lesion area compared to Guy11 (*p<0.00003, two sided 2-sample t-test for unequal variance, n = 20). Mixed strain infection containing Guy11 at 80% was more virulent than pure Guy11 (**p<0.0032, two sided 2-sample t-test for unequal variance, n = 20). (c) This virulence difference was as a result of different pathogen population fitness as measured by the number of conidia generated at the end of the experiment. Mixed strain infection containing Guy11 at 80% generated significantly more conidia than pure Guy11 (**p<0.0032, two sided 2-sample t-test for unequal variance, n = 20).
disease cycle where Guy11 was fitter than Δinv1 (*p<0.0001) with mixed infections with 80% Guy11 being fitter than pure Guy11 populations (**p<0.0365, Mann-Whitney U-test with Bonferroni correction, n = 42), all treatments shown in Figure 2a.

DOI: 10.7554/eLife.18678.007
Figure 2—figure supplement 2. Strains were distinguishable by the presence of fluorescent protein tag. (a) Wild-type invertase producer strain Guy11 was tagged with ToxA:3xmCherry and the non-invertase producing strain Δinv1 was tagged with ToxA:sGFP. Bright field (DIC) image (top left) with RFP (top right) GFP (bottom left) and overlaid (bottom right). Scale bar = 30 μm. (b) These tags were selectively neutral in wild-type strain Guy11 (Two sample t-test) based on biomass (grey bars, \( p=0.3319 \), df = 10) and conidia (white bars, \( p=0.5845 \), df = 18) production on MM Sucrose (mean ± s.e.m.). DOI: 10.7554/eLife.18678.008
Figure 2—figure supplement 3. The disease cycle of Magnaporthe oryzae. Infection of rice starts and finishes with the release of conidia, the infecting agent analogous to many microbial diseases. Conidia initiate disease by germinating to form a specialised structure, the appressorium, which generates the mechanical force to enable penetration of the host. From the appressorium, primary invasive hyphae grow into the host cells. Hyphae proliferate through host cells enabling the pathogen to colonise the tissue and exploit nutrients. Disease lesions form on the plant from which the fungus erupts and conidia are generated for transmission. Images shown are (clockwise) a three celled conidium, a germinated conidia forming an appressorium in vitro on an inducing hydrophobic surface, live-cell image of a successfully penetrated rice plant (CO39 cultivar) leaf sheath 26 hr.p.i (DIC), a proliferating cytoplasmic GFP expressing strain of M. oryzae, ramifying through rice plant cells, and disease lesion on rice leaf 7 d.p.i.

DOI: 10.7554/eLife.18678.009
Log-transformation of the data in Figure 2a showing pathogen fitness measurement of \( \Delta \text{inv}1 \), Guy11 and a mixed inoculum. The in planta infection data from Figure 2a was log-transformed to better meet the assumption of normality. All 210 data points shown (open grey circles - 42 replicates x five inoculum conditions = 210) were log transformed and small artificial noise was added to the x-axis for visualisation. Closed black circles denote mean ± s.e.m. Applying the existing social theory we hypothesize that the number of conidia recovered per mixed Guy11 and \( \Delta \text{inv}1 \) disease lesions is not higher than the number of conidia recovered from Guy11-only disease lesions. However, we can reject this hypothesis using properties of Boolean algebra (*p<0.017, two sample 2-sided t-test for unequal variance with Bonferroni correction, n = 42, see Appendix 1A for details of the analysis). DOI: 10.7554/eLife.18678.010
Figure 2—figure supplement 5. Micrograph of sporulating lesion from mixed strain leaf spot infection. (a) DIC image of sporulating lesion nine days post inoculation. (b) Magnification of region from (a). (c) Region from (b) with overlaid GFP (showing Δinv1) and RFP (wt) epifluorescence (scale bar 200 μm).

DOI: 10.7554/eLife.18678.011
Figure 3. Multi-trait interactions during sucrose metabolism by *M. oryzae*. In addition to public good invertase production (*Figure 1*), we found evidence of a rate-efficiency trade-off where resources are used less efficiently when abundant, applicable to growth on glucose (●) (\( \mu = 0.8, p < 0.0001, \) Spearman rank correlation, significance level \( \alpha = 0.0005 \)) and sucrose (○) (\( \mu = 0.5, p < 0.05, \) Spearman rank correlation, significance level \( \alpha = 0.0025 \)). Efficiency units are conidia generated per molecule of saccharide, growth rate is calculated from the Malthusian growth parameter (mean ± s.e.m., \( n = 5 \)) which were controlled by varying uptake rates by culturing on varying resource concentrations (1, 0.5, 0.125 and 0.03125 % w/v). Lines (solid = glucose, dashed = sucrose) represent a fit to data of a trade-off geometry directly inferred from the biophysical mechanisms that cause trade-offs *Meyer et al., 2015* (Materials and methods). Typical parameter estimates can be seen in *Figure 3—source data 1*.

**Source data 1.** Typical parameter estimates obtained by fitting the geometric form of the rate-efficiency trade-off *Meyer et al., 2015* to data in *Figure 3* of the main text.

DOI: 10.7554/eLife.18678.013
Figure 4. Population fitness of INV1 producing Guy11 and the Δinv1 mutant in axenic and mixed-strain populations of intermediate frequencies. (a, b, d) Populations were established by inoculation with $1 \times 10^5$ conidia with varying initial frequencies of invertase producers and non-producers, with population fitness being assessed by the number of conidia recovered per plate. (a), on 1% sucrose agar media (mean ± s.e.m., n = 9). Single genotype populations of Guy11 produced more conidia than the Δinv1 mutant (p<0.0002, two-sided 2-sample t-test, n = 9). In addition, as when analyzing the in vivo data in Figure 2a, we hypothesize that the number of conidia recovered per mixed Guy11 and Δinv1 populations is not higher than the number of conidia recovered from Guy11-only populations. However, we can reject this hypothesis using properties of Boolean algebra (*p<0.025, **p<0.004, two-sided 2-sample t-test with Bonferroni correction, n = 9, see Appendix 1A for detailed analysis). (b) Population fitness on 0.01% sucrose agar media to remove the influence of a rate-efficiency trade-off. In this case there was no significant difference amongst fitnesses of mixed populations of producers and non-producers and single genotype populations of producers (p>0.75, F(4, 40) =0.48, one-way ANOVA, n = 9, detecting effect size of 0.79 with the probability of Type II error of 0.01). (c) Population fitness in 1% sucrose liquid media to minimise population spatial structure. There was no significant difference amongst fitnesses of mixed populations of producers and non-producers and pure producer populations (p>0.85, F(4, 40) = 0.33, one-way ANOVA, n = 9, detecting effect size of 0.62 with the probability of Type II error of 0.01). Cultures were prepared using a mycelial homogenate and fitness measured as biomass production (dry weight). (d) Population fitness on 1% glucose agar media to remove the need for invertase mediated metabolism and hence spatial heterogeneity in hexoses. There was no significant difference amongst fitnesses of mixed populations of producers and non-producers and pure producer populations (p>0.9, F(4, 40) = 0.24, one-way ANOVA, n = 9, detecting effect size of 0.55 with the probability of Type II error of 0.01).

DOI: 10.7554/eLife.18678.014
Figure 4—figure supplement 1. The interactions between two social traits: public goods production and self-restraint; theoretical results. (a) Initial distribution of producers and non-producers, for producer frequency 0.4. Normalised final population size after exhaustion of resources as a function of initial producer frequency (b) in the spatially structured environment and in presence of rate-efficiency trade-off, (c) in the spatially structured environment and in the absence of rate-efficiency trade-off, (d) in homogeneous environment and in the presence of rate-efficiency trade-off.

DOI: 10.7554/eLife.18678.015