Extended Data and Supplementary Information

The emergence of virulence via the collective action of bacterial effectors

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Extended Data:

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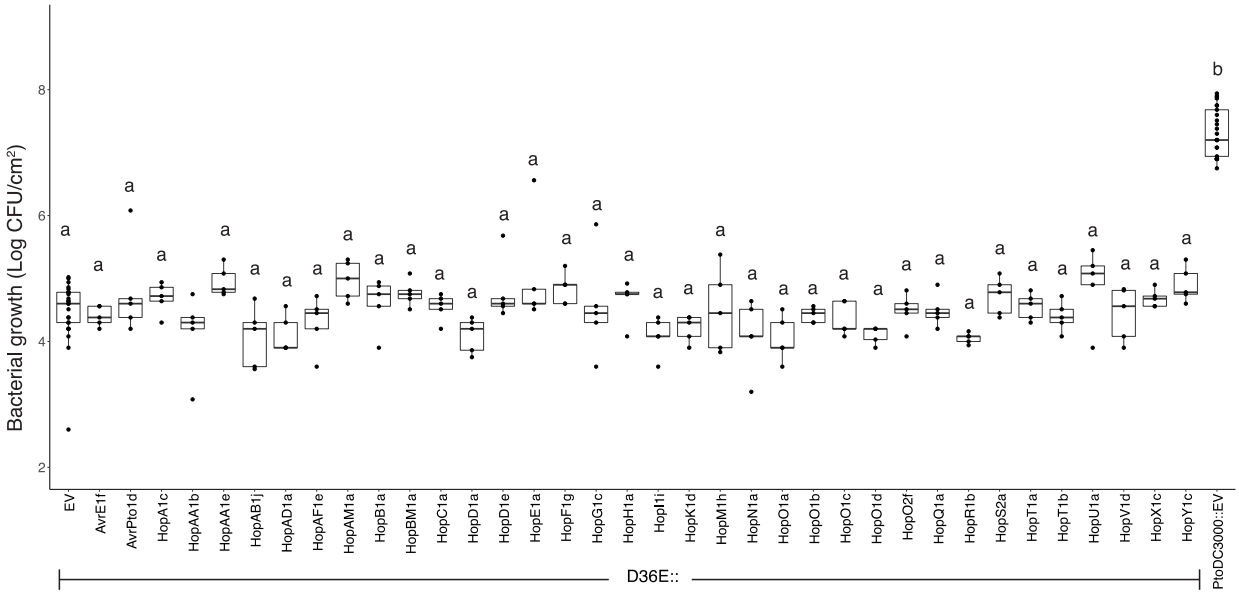
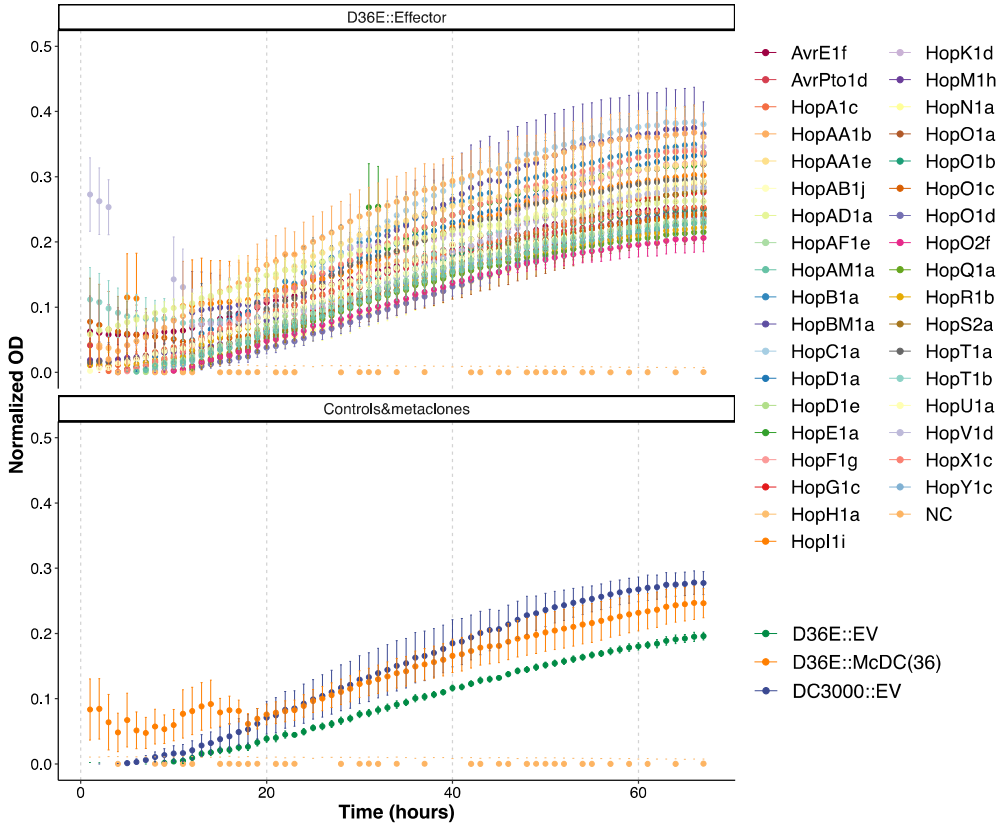
Supplementary Information:

Supplementary Table 1. All construct sequences used in this study and associated metadata (Excel format)

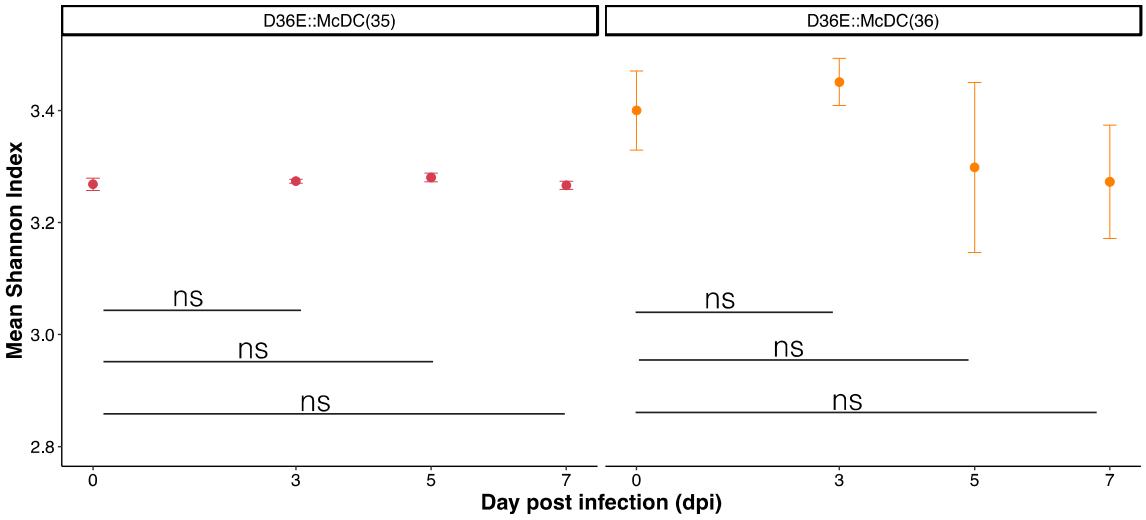
Supplementary File 2. All construct sequences used in this study and associated metadata (Excel format)



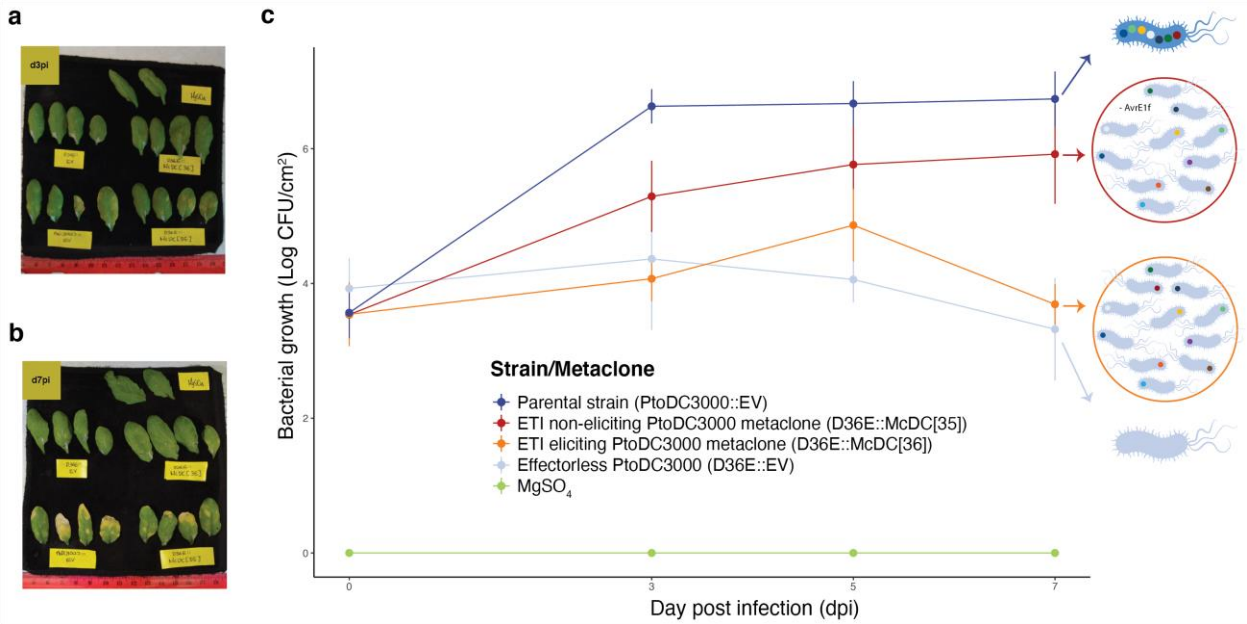
Extended Data Figure 1. *In-planta* fitness of individual effector clones. Bacterial growth was quantified on day 6 post spray infection for all effector clones that comprise the well-expressed effector arsenal of the pathogen PtoDC3000. The effector-less mutant strain derivative of PtoDC3000 and the wildtype pathogen PtoDC3000 each harboring an empty vector were used as a negative and positive controls, respectively. No significant deviations from the mean of the effector-less mutant were detected. All comparisons were performed via ANOVA and post-hoc correction Tukey-Kramer HSD test with 95% confidence interval.



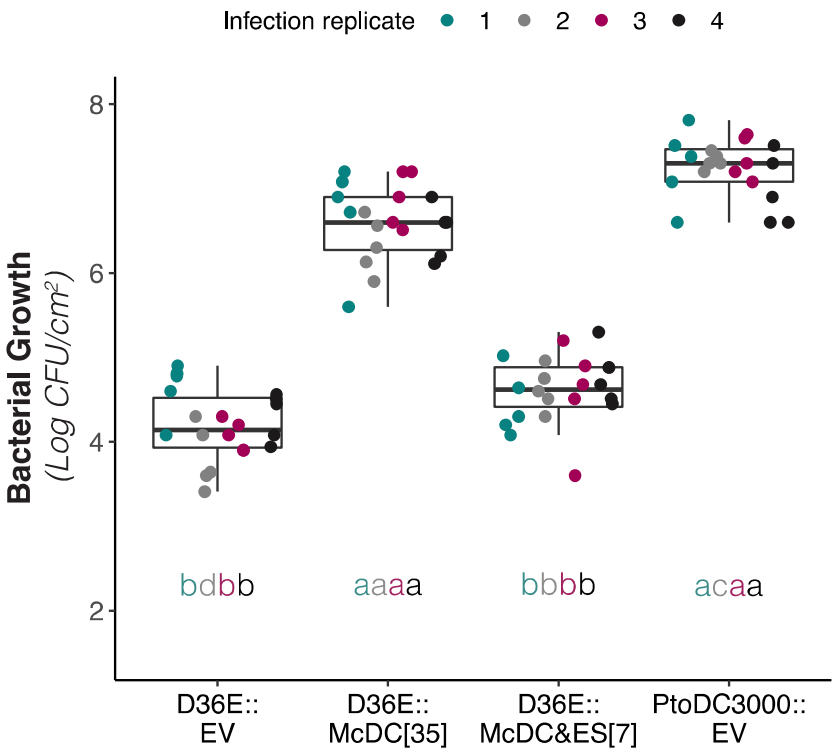
Extended Data Figure 2. *In-vitro* growth curves of individual effector clones composing the PtoDC3000 metaclone D36E::McDC[36], and control strains harboring empty vectors. Data points represent the average optical density (OD) measured at 600nm across 3 technical replicates grown in rich media (KB) with kanamycin. Outliers were excluded (SE > 0.07) and were likely driven by variation in measurements due to condensation, which was noticeable during lag phase predominantly over the first 10 hours. This, however, did not impact the overall trajectory of the growth curves. Harboring any of the individual effector plasmids did not substantially burden or benefit of D36E *in vitro*.



Extended Data Figure 3. Mean Shannon's diversity index for metaclones does not deviate significantly throughout disease progression. Diversity calculations were based on the relative abundance of each D36E::effector barcode composing the metaclones for the pathogen PtoDC3000. Barcode sequence diversity was reported as the mean Shannon index across four biological replicates and error bars represent their standard error. No significant differences were found between any stage of infection and the mean barcode diversity of the inoculum (Bonferroni-corrected Student's t-tests ($p>0.05$)). Error bars represent standard error across 4 biological replicates. Higher variance across replicates is observed in the ETI-eliciting metaclone D36E::McDC[36], particularly on the latter stages of the infection process, which can be attributed to smaller population sizes and increased drift in the metaclone composition.



Extended Data Figure 4. Mutualism-dependent virulence emergence after syringe pressure infiltration assays in *A. thaliana*. a, Host symptoms from representative leaves before tissue harvesting at 3- and **b**, 7- days post infection. **c**, Endophytic bacterial growth per cm^2 of harvested tissue, estimated at 0-, 3-, 5- and 7-days post infection. Diagrams on the right represent the corresponding population and genetic makeup of each infection.



Extended Data Figure 5. Metaclone representing the effector allele overlap between PtoDC3000 and PmaES4326, McDC&ES[7], fails to match fitness of PtoDC3000 and the non-ETI-eliciting metaclone D36E::McDC[35]. D36E::McDC&ES[7] shows a fitness significantly closer to that of the polymutant D36E::EV and does not restore the growth levels typical of their wildtype parental strain PtoDC3000 or its non-ETI eliciting metaclone D36E::McDC[35]. ANOVA and post-hoc Tukey-Kramer HSD with confidence interval of 95% was performed separately for 4 independent experiments.

Extended Data Table 1. Effector alleles used in the construction of metaclones.

Effector Allele [a]	McDC[ ]	McES[ ]	Expression [b]
AvrE1f	*	0	1
AvrE1i	0	*	1
AvrPto1d	1	0	1
HopA1c	1	0	1
HopAA1b	1	1	1
HopAA1e	1	0	1
HopAA1k	0	1	1
HopAB1j	1	0	1
HopAB1p	0	1	1
HopAB1w	0	1	1
HopAD1a	1	1	1
HopAF1e	1	0	1
HopAF1h	0	1	1
HopAL1b	0	1	0
HopAM1a	1	0	1
HopAQ1a	0	1	0
HopAS1e	0	1	0
HopAZ1d	0	1	1
HopB1a	1	0	1
HopB2k	0	1	0
HopBD1a	0	1	1
HopBD1g	0	1	1
HopBG1a	0	1	1
HopBM1a	1	0	0
HopBM1b	0	1	1
HopBO1d	0	1	1
HopC1a	1	0	1
HopD1a	1	1	1
HopD1e	1	1	1
HopD2b	0	1	1
HopE1a	1	0	1
HopF1g	1	0	1
HopG1c	1	0	1
HopH1a	1	0	1
HopI1g	0	1	1
HopI1i	1	0	1

HopK1d	1	0	1
HopM1h	1	0	1
HopM1v	0	1	1
HopN1a	1	0	1
HopO1a	1	0	1
HopO1b	1	*	1
HopO1c	1	0	1
HopO1d	1	0	1
HopO2f	1	0	0
HopQ1a	1	1	1
HopR1b	1	0	1
HopR1g	0	1	1
HopS2a	1	0	0
HopT1a	1	1	1
HopT1b	1	0	1
HopU1a	1	0	1
HopV1d	1	1	1
HopW1g	0	1	1
HopX1c	1	0	1
HopX1g	0	1	1
HopY1c	1	0	1
HopZ1b	0	1	1
Total [c]	**35**	**28**	**51**

[a] Unique effectors carried by the PtoDC3000 metaclones McDC[] and PmaES4326 metaclones McES[]. 0 = allele absent; 1 = allele present; * = ETI-eliciting allele that were removed from the non-ETI-eliciting metaclones; light grey shading = same effector family but different allele in the two metaclones; dark grey shading = same allele in the two metaclones.

[b] Expression of the cloned allele constructs (*16*): 0 = not expressed; 1 = expressed.

[c] Total count indicate total unique alleles for each metaclone, excluding ETI-elicitors.

Extended Data Table 2. Effector-specific barcode primer pairs design to contain Nextera overhang adapters.

(5´ > 3´)	**Overhang adapter**	**Locus specific sequence**	**Final Tm °C**
Forward	TCGTCGGCAGCGTCAGA TGTGTATAAGAGACAG	GGCGCATACCCTTACGA TGTTCCTGATT	61
Reverse	GTCTCGTGGGCTCGGAGA TGTGTATAAGAGACAG	GCGCGAGCAGGGGAATT GATCC	57