

Fig. S1. Virulence measured in two different assays yields highly similar results. 32 evolved populations were tested for their virulence towards the nematode *C. elegans*. *Y* axis shows virulence tested in the environment the populations evolved in: populations that evolved on agar plates tested on agar plates, populations that evolved in liquid culture tested in liquid culture. *X* axis shows virulence when tested in the reciprocal environment: populations that evolved on agar plates tested in liquid culture, populations that evolved in liquid tested on agar plates. Virulence was quantified as percent nematodes killed at 24 h post infection, scaled to the ancestral wildtype, and averaged across three to six replicates per population. Individual dots represent average virulence of a population. Error bars denote the standard error of the mean. See Table S2 for details on statistical analysis.

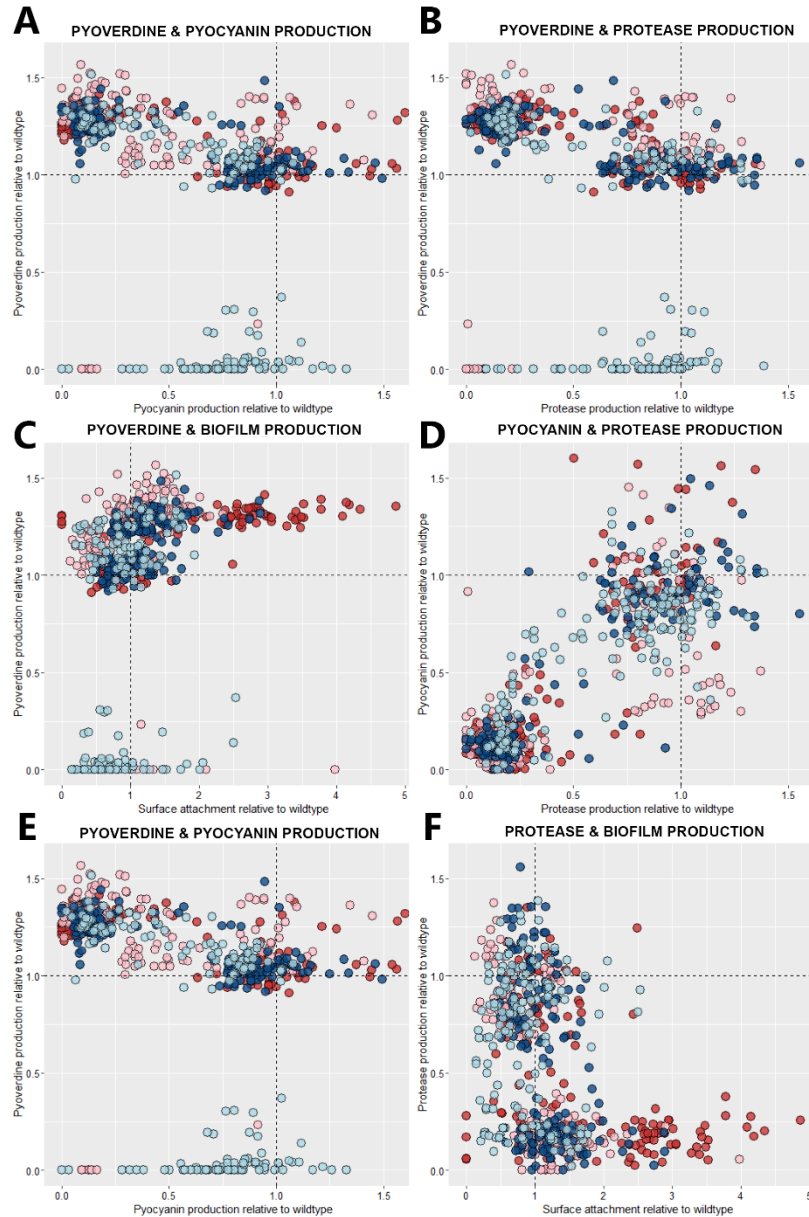


Fig. S2. Pairwise comparisons of the production of different secreted virulence factors. 640

evolved clones were tested for their expression of four secreted virulence factors. Plots each show pairwise comparisons of the production of two virulence factors for all clones. All values scaled to the ancestral wildtype. Colours represent the different environments the populations evolved in. Pyoverdine production against pyocyanin production (A), protease production (B) and biofilm production (C); pyocyanin against protease production (D) and biofilm production (E); protease production against biofilm production (F).

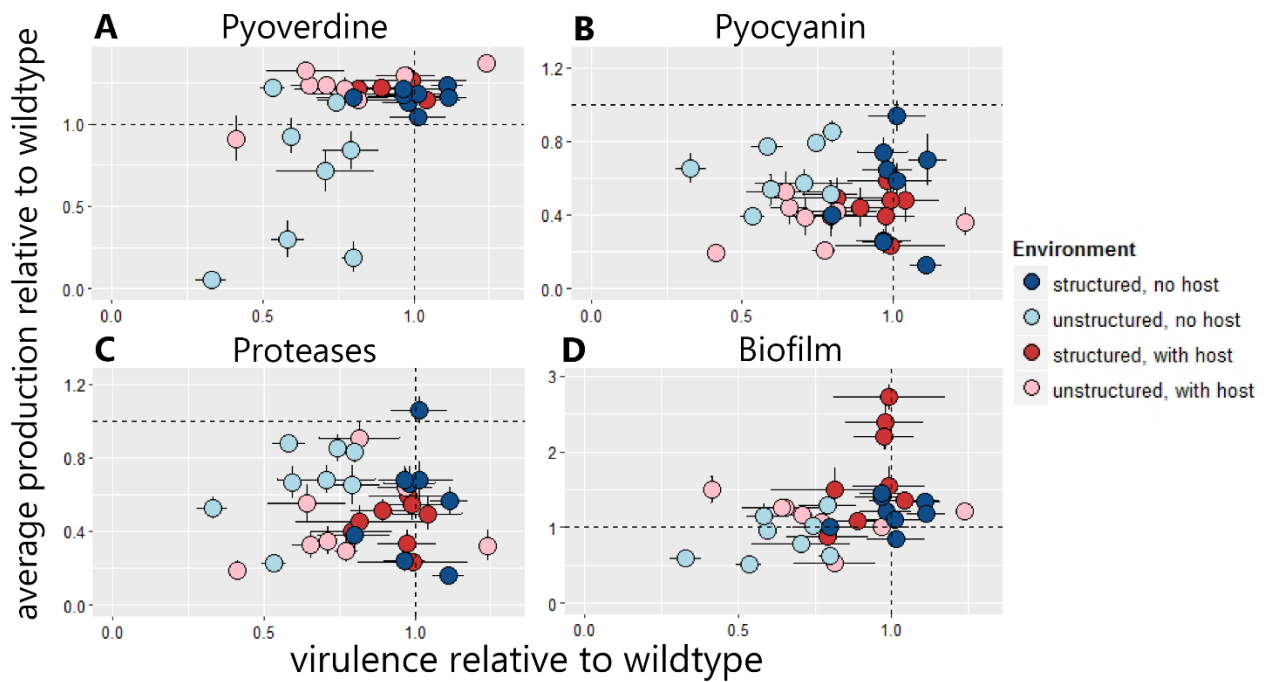


Fig. S3. No single virulence factor can fully explain evolved virulence levels. Virulence levels of 32 evolved populations were determined and plotted against average virulence factor production in 20 evolved clones per population. All values scaled to the ancestral wildtype. Error bars represent the standard error of the mean. (A) Virulence plotted against pyoverdine production. (B) Virulence plotted against pyocyanin production. (C) Virulence plotted against protease production. (D) Virulence plotted against production of surface-attached biofilms. Colours represent the different environments the populations evolved in.

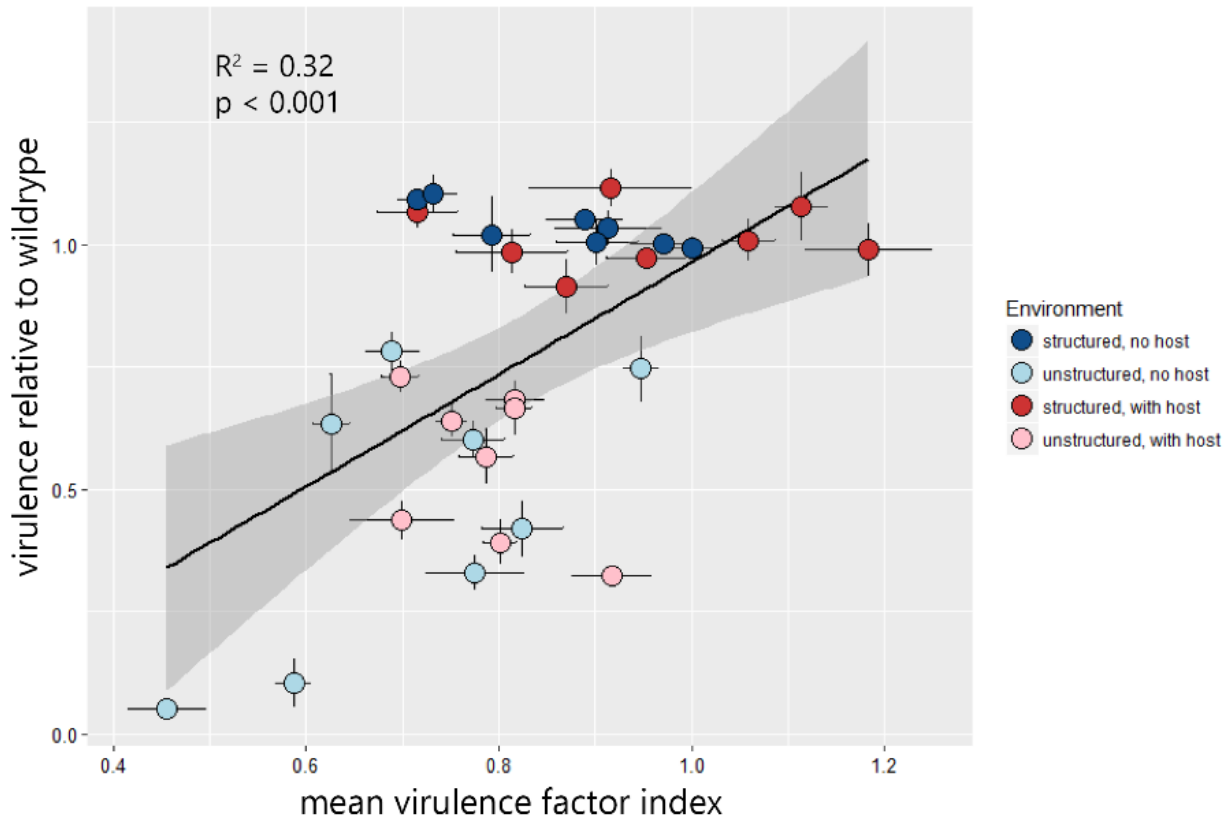


Fig. S4. Decrease in virulence explained by combining the expression profile of four virulence factors in evolved clones. For each of 640 evolved clones, the “virulence factor index” was calculated by summing over production levels of four secreted virulence factors and scaling to the ancestral wildtype. X axis shows average virulence indices, while y axis shows average virulence levels scaled to the wildtype. Individual dots represent average values across 20 clones for each evolved population, coloured by the environment they evolved in. Virulence was tested in the reciprocal environment: populations that evolved on agar plates tested in liquid culture, populations that evolved in liquid tested on agar plates. Virulence was quantified as percent nematodes killed at 24 h post infection, scaled to the ancestral wildtype, and averaged across three to six replicates per population. Error bars denote the standard error of the mean. See Table S2 for details on statistical analysis.

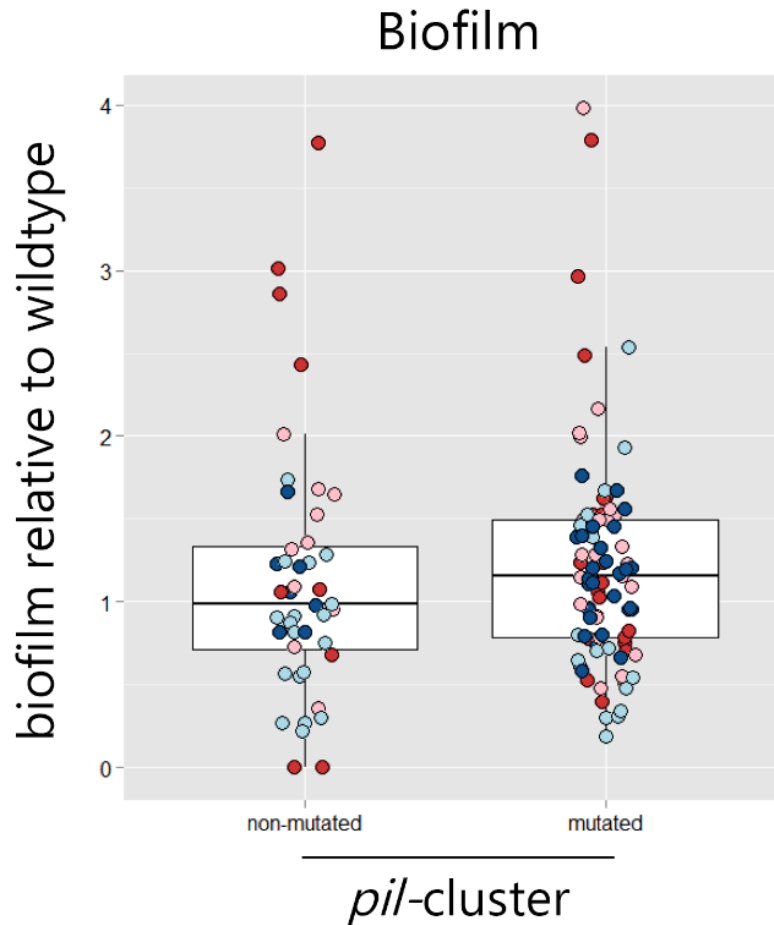


Fig. S5. Biofilm production not affected by mutations in the *pil* gene cluster. Whole genome sequencing was performed on 140 evolved clones, and SNPs and INDELs in genes related to biofilm production were tested for their effect on production levels of surface-attached biofilms. Clones with ≥ 1 detected SNP or INDEL in the respective gene are labelled “mutated”, clones with no SNPs or INDELs detected in this region are labelled “non-mutated”. Y axis values are scaled to biofilm production levels of the ancestral wildtype. We did not detect a difference in biofilm production levels in clones with mutations in the *pil* gene cluster (*pilM*, *pilQ*, *pilO*, *pilU*, *pilD*, *pilA*, *pilZ*, *pilY1*, *pilW*, *pilN*, *pilE*, *pilB*, *pilS*, *pilR*, *pilT*, *pilG*) when compared to clones without mutations in these genes ($p = 0.64$). See Table S2 for details on statistical analysis.