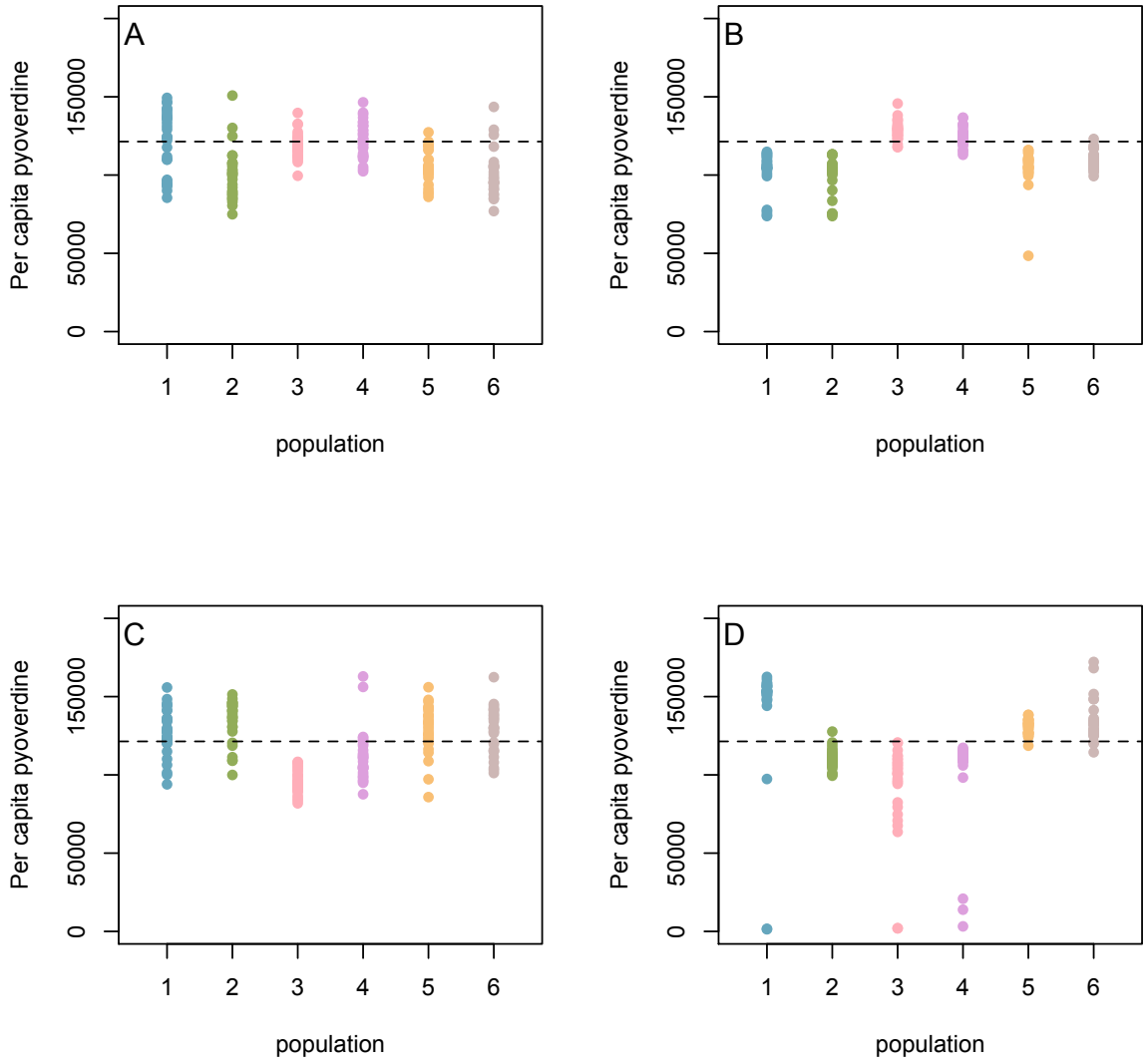

Figure S1

Endpoint per capita pyoverdine production for 30 isolated colonies (indicated here as individual dots) per evolving population. Horizontal line indicates ancestral pyoverdine production. Populations were evolved under four treatments: A) iron-rich, B) iron-limited C) iron-rich & phage, D) iron-limited & phage. Within-population variance in pyoverdine production increased only under iron-limitation and in the presence of phage (LMER; phage x iron interaction, $X^2_{1,9}$=12.22, p=0.0004), irrespective of time (LMER non-significant phage x iron x transfer interaction $X^2_{1,11}$=0.4591, p=0.4981). Colours represent different evolving populations (1-6) that can be cross-referenced with other figures in this manuscript

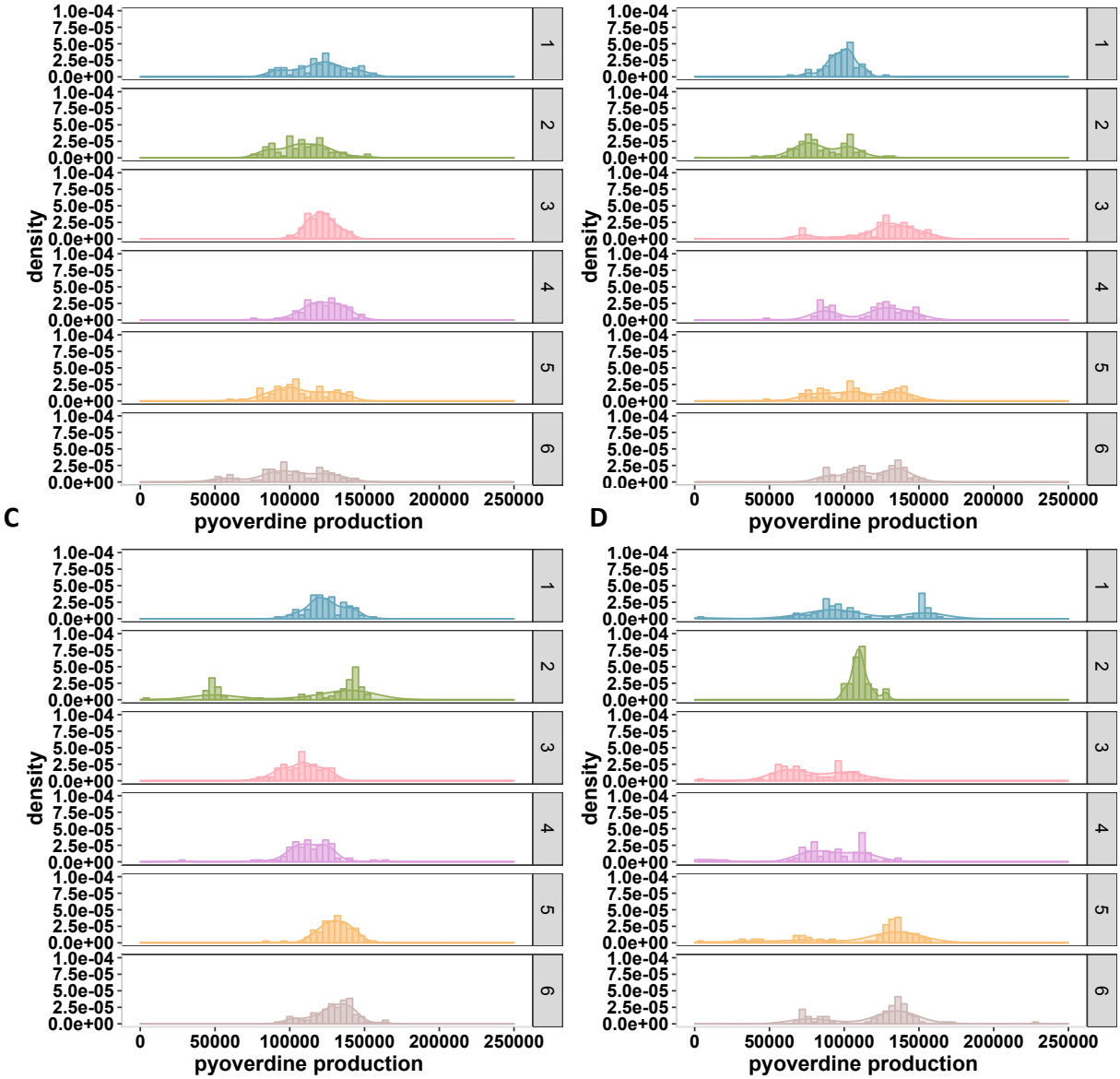

Figure S2

Density histogram illustrating variation in pyoverdine production within each evolving population (labeled 1-6 on right side panel). Populations were evolved under four treatments: A) iron-rich, B) iron-limited C) iron-rich & phage, D) iron-limited & phage. Data are per capita pyoverdine production for 30-90 isolated colonies per treatment (pooling all timepoints for each treatment). Within-population variance in pyoverdine production increased only under iron-limitation and in the presence of phage (LMER; phage x iron interaction, $X^2_{1,9}$=12.22, p=0.0004), irrespective of time (LMER non-significant phage x iron x transfer interaction $X^2_{1,11}$=0.4591, p=0.4981). Colours represent different evolving populations (1-6) that can be cross-referenced with other figures in this manuscript.

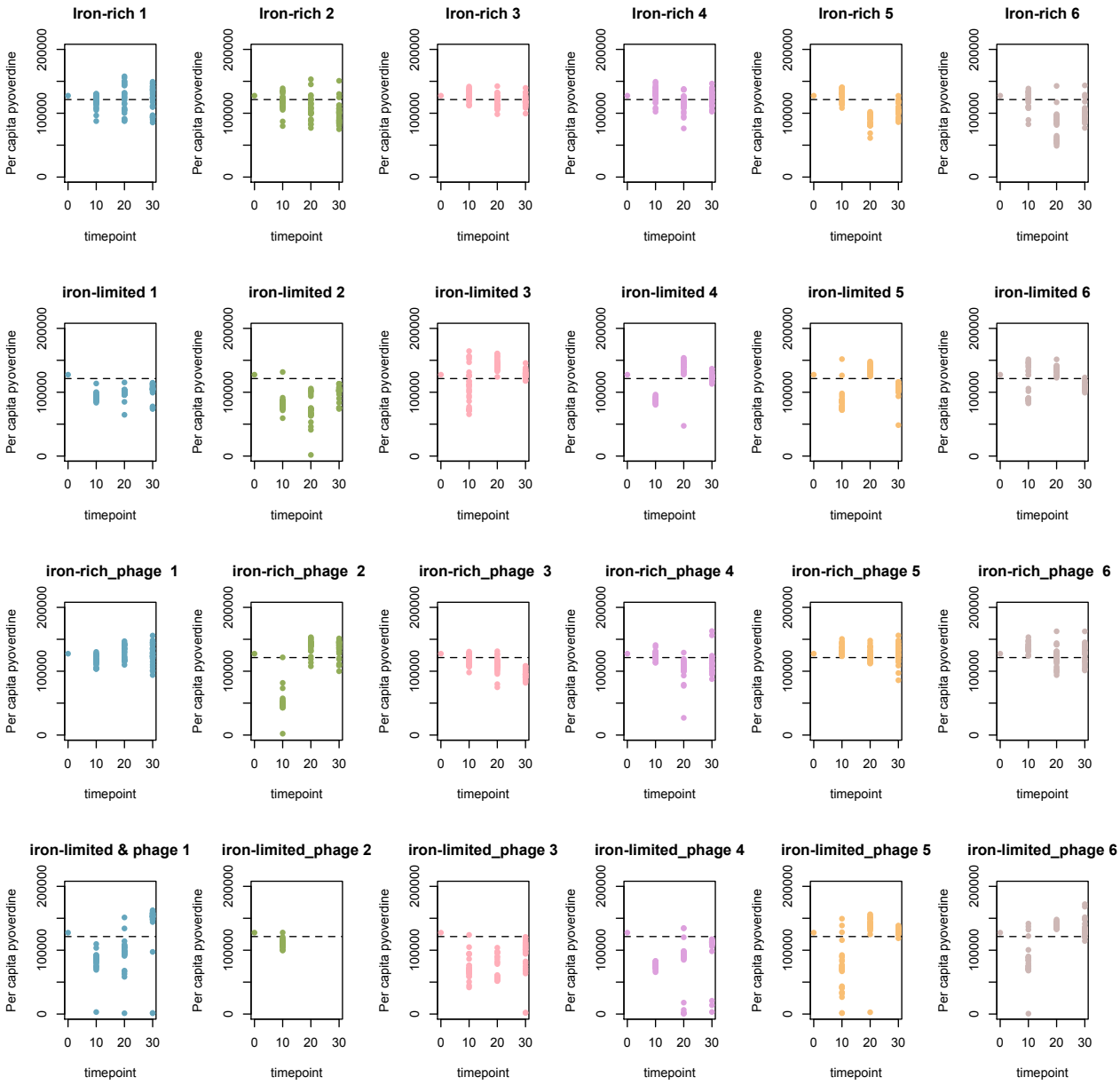

Figure S3

Per capita pyoverdine production for every isolated colony through time per evolving population. Horizontal line indicates ancestral pyoverdine production. Iron limitation and phage presence both independently reduced *per capita* pyoverdine, but this effect was less strong over time (LMER; iron x transfer interaction, $X^2_{1,9}$= 8.1656, p=0.004, phage x transfer interaction, $X^2_{1,9}$= 4.2632, p=0.03). Within-population variance in pyoverdine production increased only under iron-limitation and in the presence of phage (LMER; phage x iron interaction, $X^2_{1,9}$=12.22, p=0.0004), irrespective of time (LMER non-significant phage x iron x transfer interaction $X^2_{1,11}$=0.4591, p=0.4981). Colours represent different evolving populations (1-6) that can be cross-referenced with other figures in this manuscript

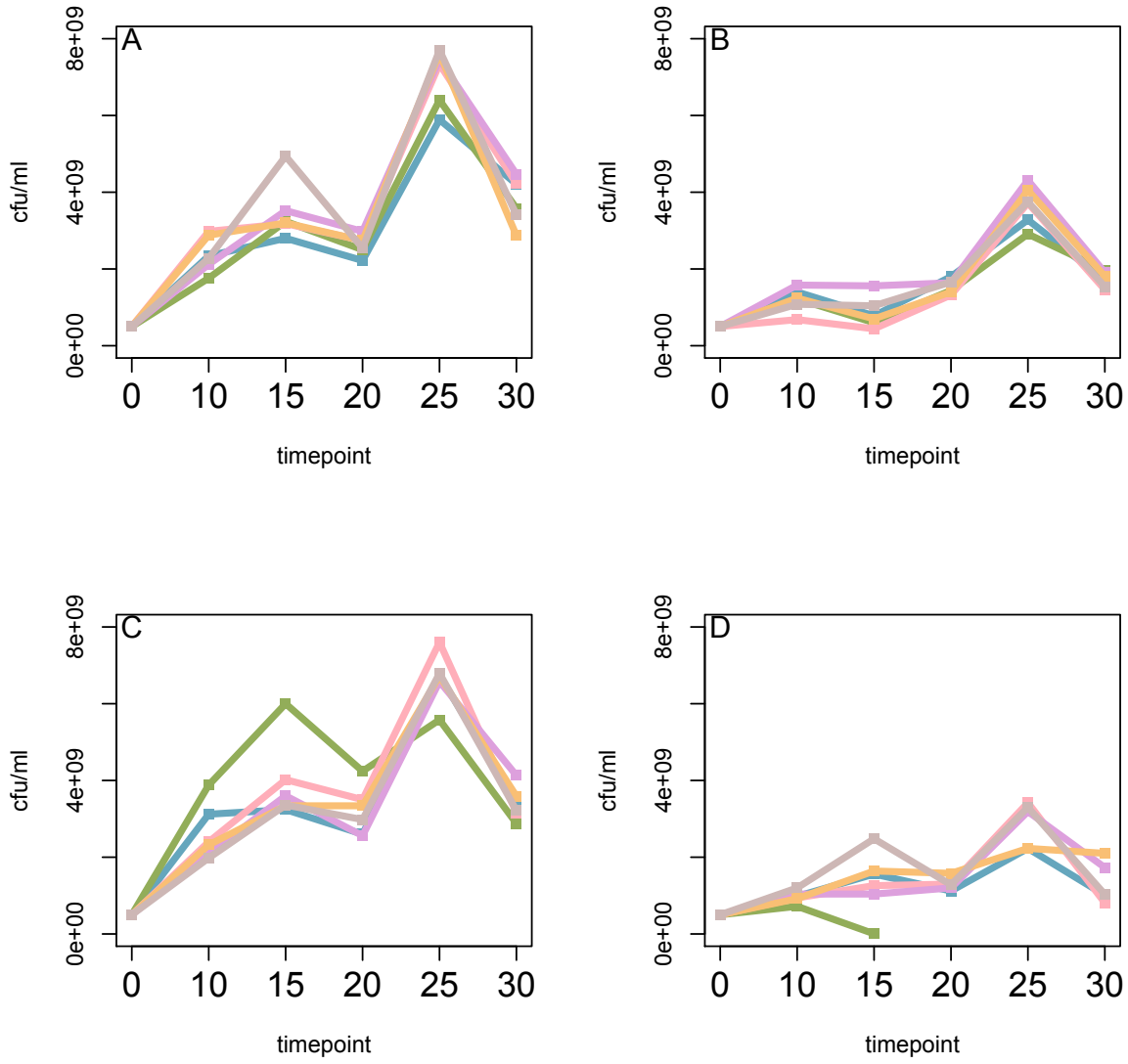

Figure S4

Density (colony forming units; CFU/ml) of each evolving population through time. Each line depicts a single evolving population from one of four treatments: A) iron-rich, B) iron-limited C) iron-rich & phage, D) iron-limited & phage. Colours represent different evolving populations (1-6) that can be cross-referenced with other figures in this manuscript. Note that one population (green) in the iron-limited and phage treatment (D) dropped to very low density at timepoint 15 and was extinct by timepoint 20. We find that while phages have no effect on population density (LMER; $X^2_{1,8}$= 0.0727, p=0.79), the presence of iron resulted in significant increases in population density, that became more pronounced with time (LMER; significant time x iron interaction $X^2_{1,8}$= 14.1 p<0.001).

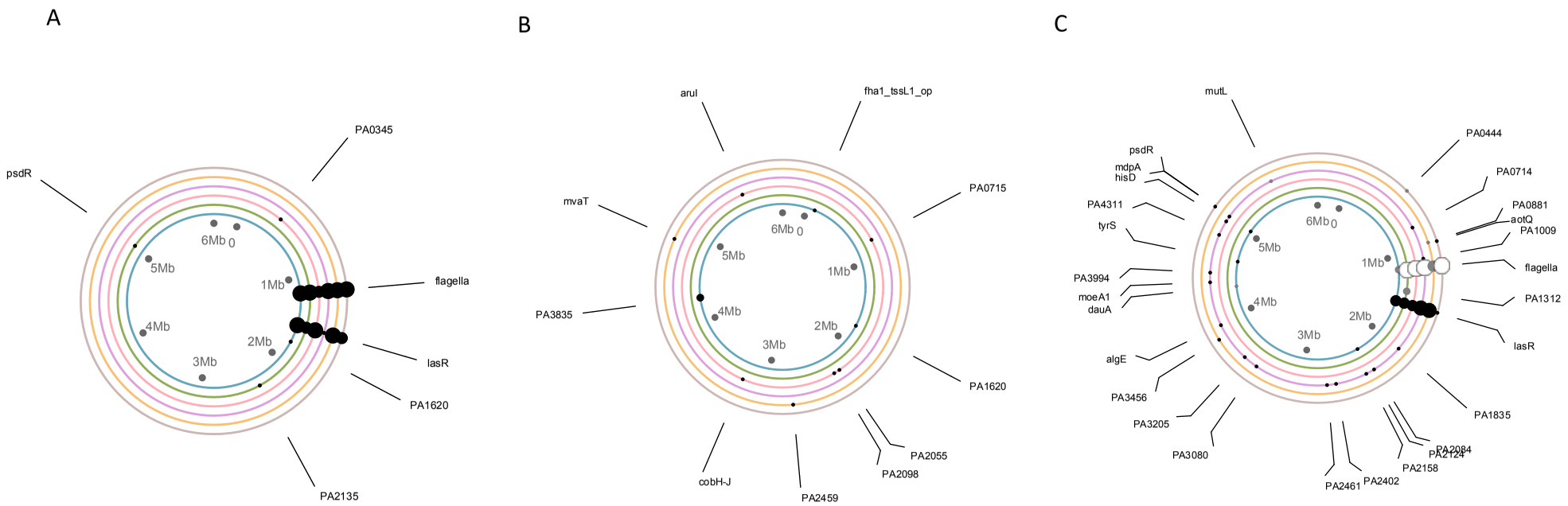

Figure S5:

Genetic loci under positive selection in populations evolving under iron-rich (A), iron-limited (B) and iron-rich with phage (C) conditions. Each concentric circle represents a replicate population. Concentric circles correspond to populations 1-6, from the innermost to outermost circle, respectively. Positions around each concentric circle correspond to positions around the PAO1 published and annotated chromosome. Small black dots around these circles indicate the occurrence of an indel or SNP, grey dots represent phage integration events in those regions, and white dots indicate both. Four colonies were selected in total per population: Dot size corresponds to the number of colonies in which a given mutation was observed. When two genes are mentioned, the mutation is intergenic. A complete list of mutations are provided in Table S3.

Table S1:

Occurrences (n=17) of pyoverdine non-producers over the course of the selection experiment and associated pyoverdine production as a percentage of ancestral levels.

Treatment	Population	Timepoint	% Anc pvd
Iron-limited +phage	1	1	0,025516704
Iron-rich +phage	2	1	0,017648184
Iron-limited +phage	5	1	0,012863322
Iron-limited +phage	5	1	0,012911543
Iron-limited +phage	6	1	0,005262883
Iron-limited +phage	1	2	0,010712212
Iron-limited	2	2	0,014854685
Iron-limited +phage	4	2	0,01515838
Iron-limited +phage	4	2	0,013881452
Iron-limited +phage	4	2	0,005667916
Iron-limited +phage	4	2	0,005404292
Iron-limited +phage	5	2	0,022721094
Iron-limited +phage	1	3	0,013659019
Iron-limited +phage	1	3	0,011286403
Iron-limited +phage	3	3	0,016460024
Iron-limited +phage	3	3	0,016616551
Iron-limited +phage	4	3	0,026403592

Table S2: Comprehensive list of mutations for high and low producers from iron limited populations evolving with phage. Mutations are classified as being caused by phage insertion (INS) or genomic mutations (SEQ). Pyoverdine production is listed for each clone (relative to ancestor).

Classification	Population	Disrupted loci	Type of mutation	Rel. pyoverdine production	Clone ID
High	1	phzG2-PA1906	INS	1.379401921	76
High	1	phzG2-PA1906	INS	1.393084233	73
High	1	mvaT	SEQ	1.379401921	76
High	1	cobH	SEQ	1.393084233	73
High	1	mvaT	SEQ	1.393084233	73
High	1	PA3340 / PlpD	SEQ	1.393084233	73
High	2	flgE	INS	1.033998351	78
High	2	flgJ	INS	1.093753793	77
High	3	PA4772	INS	1.030314031	84
High	3	PA4772	INS	1.033920354	82
High	4	PA0460	INS	0.994430857	88
High	4	flgF	INS	1.004802133	86
High	4	pvdS_binding_site	SEQ	1.004802133	86
High	5	PA2319-gntR	INS	1.18852379	90
High	5	PA2319-gntR	INS	1.279271313	89
High	5	TssL1	SEQ	1.279271313	89
High	6	pvcD	INS	1.174698274	93
High	6	phzG1-phzS	INS	1.213808147	96
High	6	PA2228	SEQ	1.174698274	93
High	6	cobH	SEQ	1.213808147	96
High	6	PA2228	SEQ	1.213808147	96
Low	1	gacS	INS	0.014209585	74
Low	1	gacS	INS	0.011742176	75

Table S2: Comprehensive list of mutations for high and low producers from iron limited populations evolving with phage. Mutations are classified as being caused by phage insertion (INS) or genomic mutations (SEQ). Pyoverdine production is listed for each clone (relative to ancestor).

Low	1	fliF	SEQ	0.014209585	74
Low	1	ftsY	SEQ	0.011742176	75
Low	1	pvdI	SEQ	0.011742176	75
Low	1	pqsR	SEQ	0.014209585	74
Low	1	pvdA	SEQ	0.014209585	74
Low	2	flgE	INS	0.859407448	80
Low	2	flgG	INS	0.852192054	79
Low	2	tonB2	SEQ	0.859407448	80
Low	3	fliI	INS	0.017125525	81
Low	3	fliI	INS	0.017286519	83
Low	3	argA	SEQ	1.033920354	82
Low	3	PA1238	SEQ	0.017125525	81
Low	3	PA2553	SEQ	0.017125525	81
Low	3	pvdS_binding_site	SEQ	0.017125525	81
Low	3	pvdS_binding_site	SEQ	0.017286519	83
Low	4	flgF	INS	0.027462498	85
Low	4	flgF	INS	0.118892482	87
Low	4	pvdS_binding_site	SEQ	0.027462498	85
Low	4	pvdS_binding_site	SEQ	0.118892482	87
Low	5	pvdD	INS	0.013378002	91
Low	5	pvdD	INS	0.013428153	92
Low	5	pqsR	SEQ	0.013378002	91
Low	5	pqsR	SEQ	0.013428153	92
Low	6	pqsA	INS	0.005473458	95
Low	6	pqsA	INS	0.58297409	94

Table S3: Comprehensive list of mutations for control treatments.

Treatment	Population	Disrupted loci	Type of mutation	Number of clones with given mutation
Iron-rich	1	flagella	SEQ	4
Iron-rich	1	lasR	SEQ	4
Iron-rich	1	PA1620	SEQ	1
Iron-rich	2	flagella	SEQ	4
Iron-rich	2	lasR	SEQ	3
Iron-rich	2	PA2135	SEQ	1
Iron-rich	2	psdR	SEQ	1
Iron-rich	3	flagella	SEQ	3
Iron-rich	3	lasR	SEQ	4
Iron-rich	3	PA0345	SEQ	1
Iron-rich	4	flagella	SEQ	4
Iron-rich	4	lasR	SEQ	1
Iron-rich	5	flagella	SEQ	4
Iron-rich	5	lasR	SEQ	4
Iron-rich	6	flagella	SEQ	4
Iron-rich	6	lasR	SEQ	3
Iron-limited	1	fha1_tssL1_op	SEQ	1
Iron-limited	1	PA1620	SEQ	1
Iron-limited	1	PA3835	SEQ	2
Iron-limited	3	PA0715	SEQ	1
Iron-limited	3	PA2055	SEQ	1
Iron-limited	3	PA2098	SEQ	1
Iron-limited	3	aruI	SEQ	1
Iron-limited	3	cobH-J	SEQ	1
Iron-limited	5	PA2459	SEQ	1
Iron-limited	5	mvaT	SEQ	1
Iron-rich & Phage	1	dauA	PHAGE	1
Iron-rich & Phage	1	flagella	PHAGE	2
Iron-rich & Phage	1	lasR	SEQ	3
Iron-rich & Phage	1	mdpA	SEQ	1
Iron-rich & Phage	1	PA2124	SEQ	1
Iron-rich & Phage	1	tyrS	SEQ	1
Iron-rich & Phage	2	flagella	PHAGE	1
Iron-rich & Phage	2	flagella	SEQ	3
Iron-rich & Phage	2	lasR	SEQ	3
Iron-rich & Phage	2	PA1312	PHAGE	2
Iron-rich & Phage	3	flagella	PHAGE	2
Iron-rich & Phage	3	flagella	SEQ	2
Iron-rich & Phage	3	lasR	SEQ	3

Table S3: Comprehensive list of mutations for control treatments.

Iron-rich & Phage	4	algE	SEQ	1
Iron-rich & Phage	4	flagella	PHAGE	2
Iron-rich & Phage	4	flagella	SEQ	2
Iron-rich & Phage	4	hisD	SEQ	1
Iron-rich & Phage	4	lasR	SEQ	4
Iron-rich & Phage	4	moeA1	SEQ	1
Iron-rich & Phage	4	mutL	PHAGE	1
Iron-rich & Phage	4	PA0714	SEQ	1
Iron-rich & Phage	4	PA1009	SEQ	1
Iron-rich & Phage	4	PA1835	SEQ	1
Iron-rich & Phage	4	PA2084	SEQ	1
Iron-rich & Phage	4	PA2158	SEQ	1
Iron-rich & Phage	4	PA2402	SEQ	1
Iron-rich & Phage	4	PA2461	SEQ	1
Iron-rich & Phage	4	PA3080	SEQ	1
Iron-rich & Phage	4	PA3205	SEQ	1
Iron-rich & Phage	4	PA3994	SEQ	1
Iron-rich & Phage	4	PA4311	SEQ	1
Iron-rich & Phage	4	psdR	SEQ	1
Iron-rich & Phage	5	flagella	PHAGE	3
Iron-rich & Phage	5	lasR	Seq	4
Iron-rich & Phage	5	PA0881	PHAGE	1
Iron-rich & Phage	5	PA3456	SEQ	1
Iron-rich & Phage	6	aotQ	SEQ	1
Iron-rich & Phage	6	flagella	SEQ	1
Iron-rich & Phage	6	lasR	SEQ	1
Iron-rich & Phage	6	PA0444	PHAGE	1
Iron-rich & Phage	6	psdR	SEQ	1

Table S4: Linear mixed models used in this study *(a)* with associated effect sizes and p-values *(b)*. Note that when an interaction was significant, it did not make sense to further simplify the model. Hence, we only see explanatory variables appearing individually when it was no longer a component of a significant higher-order interaction. We used anova comparisons at each step of simplification.

(a)

Explanatory variables	**Fixed effects (including 2- and 3- way interactions)**	**Random effects**	**Error structure**
Model1: Per capita pyoverdine production	Iron x Phage x Time	Time | Population	Gaussian
Model 2: Population variance in pyoverdine production (log transformed)			
Model 3: Population density (CFU/ml)			
Model 4: Number of overproducers			Poisson

(b)

Model 1

Response variable: *Per capita* pyoverdine production

	DF	Chi-squared	P-value
Iron X Phage X Time	1,11	3.00E-04	9.86E-01
Iron X Phage	1,10	1.33E+00	2.49E-01
Phage X Time	1,9	4.26E+00	3.90E-02
Iron X Time	1,9	8.17E+00	4.27E-03

Model 2			
Response variable: Population variance in pyoverdine production (log transformed)			
	DF	Chi-squared	P-value
Iron X Phage X Time	1,11	4.59E-01	4.98E-01
Iron X Phage	1,9	1.23E+01	4.40E-04
Phage X Time	1,9	1.89E+00	1.70E-01
Iron X Time	1,10	7.73E-01	3.79E-01
Time	1,8	6.18E-01	4.32E-01

Model 3			
Response variable: Population density (CFU/ml)			
	DF	Chi-squared	P-value
Iron X Phage X Time	1,11	4.20E-03	9.49E-01
Iron X Phage	1,10	5.16E-01	4.73E-01
Phage X Time	1,9	9.53E-01	3.29E-01
Iron X Time	1,8	1.41E+01	1.70E-04
Phage	1,8	7.27E-02	7.88E-01

Model 4			
Response variable: Number of overproducers			
	DF	Chi-squared	P-value
Iron X Phage X Time	1,10	1.72E+00	1.89E-01
Iron X Phage	1,8	9.17E-01	3.40E-01
Phage X Time	1,8	5.36E+00	2.00E-02
Iron X Time	1,9	4.79E-02	8.27E-01
Iron	1,7	6.31E-01	4.27E-01