
Supplementary information

Host diversity slows bacteriophage adaptation by selecting generalists over specialists

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Supplementary Information

Supplementary Table 1. Host range analysis of the ancestor JB

<i>E. coli</i> strain	Phage titer (pfu/ml)
EPEC E2348/69	6.0×10^8
MG1655	-
REPEC E22	-
BL21	-

Supplementary Table 2. Sequences alignment using BLAST

Description	Max Score	Total score	Query cover	E value	Percent identity	Accession number
<i>Escherichia</i> phage N30, complete genome	34363	55967	95%	0.0	94.32	MH717098.1
<i>Escherichia</i> phage T7 genome assembly, chromosome: 1	14838	51492	89%	0.0	93.79	LR745710.1

Supplementary Table 3. GP17 mutation data table

Sr. No.	Treatment	Strain of isolation	Population by well position	Day of isolation	Generalist/ Specialist
1	3-host (EPEC, MG1655, REPEC)	REPEC	C8	Day 4	Generalist
2	3-host (EPEC, MG1655, REPEC)	REPEC	D9	Day 6	Generalist
3	3-host (EPEC, MG1655, REPEC)	REPEC	D8	Day 6	Specialist
4	3-host (EPEC, MG1655, REPEC)	REPEC	C8	Day 8	Generalist
5	3-host (EPEC, MG1655, REPEC)	REPEC	B8	Day 9	Generalist
6	3-host (EPEC, MG1655, REPEC)	REPEC	C8	Day 10	Specialist
7	3-host (EPEC, MG1655, REPEC)	REPEC	D8	Day 10	Specialist
8	3-host (EPEC, MG1655, REPEC)	REPEC	B8	Day 10	Specialist
9	3-host (EPEC, MG1655, REPEC)	REPEC	C9	Day 10	Specialist
10	3-host (EPEC, MG1655, REPEC)	REPEC	E8	Day 10	Specialist
11	3-host (EPEC, MG1655, REPEC)	MG1655	C8	Day 4	Specialist
12	3-host (EPEC, MG1655, REPEC)	MG1655	D9	Day 6	Generalist
13	3-host (EPEC, MG1655, REPEC)	MG1655	D8	Day 6	Generalist
14	3-host (EPEC, MG1655, REPEC)	MG1655	C8	Day 8	Generalist
15	3-host (EPEC, MG1655, REPEC)	MG1655	B8	Day 9	Generalist
16	3-host (EPEC, MG1655, REPEC)	MG1655	C8	Day 10	Generalist
17	3-host (EPEC, MG1655, REPEC)	MG1655	D8	Day 10	Specialist
18	3-host (EPEC, MG1655, REPEC)	MG1655	B8	Day 10	Specialist
19	3-host (EPEC, MG1655, REPEC)	MG1655	C9	Day 10	Generalist
20	3-host (EPEC, MG1655, REPEC)	MG1655	F8	Day 10	Generalist
21	3-host (EPEC, MG1655, REPEC)	EPEC	C8	Day 4	Generalist
22	3-host (EPEC, MG1655, REPEC)	EPEC	D8	Day 6	Specialist
23	3-host (EPEC, MG1655, REPEC)	EPEC	C8	Day 10	Specialist
24	3-host (EPEC, MG1655, REPEC)	EPEC	D8	Day 10	Specialist
25	3-host (EPEC, MG1655, REPEC)	EPEC	B8	Day 10	Specialist
26	3-host (EPEC, MG1655, REPEC)	EPEC	C9	Day 10	Specialist
27	3-host (EPEC, MG1655, BL21)	BL21	D10	Day 10	Generalist
28	3-host (EPEC, MG1655, BL21)	BL21	G10	Day 10	Generalist
29	3-host (EPEC, MG1655, BL21)	BL21	B11	Day 10	Generalist
30	3-host (EPEC, MG1655, BL21)	MG1655	D10	Day 10	Generalist
31	3-host (EPEC, MG1655, BL21)	MG1655	C11	Day 10	Generalist
32	3-host (EPEC, MG1655, BL21)	EPEC	D10	Day 10	Specialist
33	3-host (EPEC, MG1655, BL21)	MG1655	B10	Day 10	Specialist
34	3-host (EPEC, MG1655, BL21)	EPEC	H10	Day 10	Specialist
35	2-host (EPEC and MG1655)	MG1655	B2	Day 5	Generalist
36	2-host (EPEC and MG1655)	MG1655	B2	Day 10	Specialist
37	2-host (EPEC and MG1655)	MG1655	B4	Day 10	Specialist
38	2-host (EPEC and MG1655)	MG1655	C2	Day 10	Specialist

39	2-host (EPEC and MG1655)	MG1655	C6	Day 10	Specialist
40	2-host (EPEC and MG1655)	EPEC	A3	Day 4	Generalist
41	2-host (EPEC and MG1655)	EPEC	B2	Day 5	Specialist
42	2-host (EPEC and MG1655)	EPEC	C1	Day 10	Specialist
43	2-host (EPEC and MG1655)	EPEC	C6	Day 10	Specialist
44	2-host (EPEC and BL21)	BL21	C6	Day 10	Generalist
45	2-host (EPEC and BL21)	BL21	E6	Day 10	Specialist
46	2-host (EPEC and BL21)	BL21	H6	Day 10	Generalist
47	2-host (EPEC and BL21)	BL21	B7	Day 10	Generalist
48	2-host (EPEC and BL21)	BL21	E7	Day 10	Generalist
49	2-host (EPEC and BL21)	BL21	F7	Day 10	Generalist
50	2-host (EPEC and BL21)	EPEC	F6	Day 10	Specialist
51	2-host (EPEC and BL21)	EPEC	H6	Day 10	Specialist
52	2-host (EPEC and BL21)	EPEC	C7	Day 10	Specialist
53	2-host (EPEC and BL21)	EPEC	F7	Day 10	Specialist

Supplementary Table 4. Determination of expanded host range of evolved generalists from 3-host treatment EPEC/MG1655/REPEC. “Day” refers day of evolution experiment, “host of phage isolation” refers to the experimental procedure described in the legend of Fig. 4 whereby phage clones were isolated by plating whole-culture lysates onto plates containing a single host. “Generalist/specialist” refers to the designation experiments described in Fig. 4. Generalists are defined as clones that can infect more than one host. This table shows the results of spot assays of phage clone lysates spotted onto a bacterial lawn with a single host.

No.	Day	Host of phage isolation	Generalist/ Specialist	Host involved in the treatment			Host not involved in the treatment
				EPEC	MG1655	REPEC	BL21
1	4	EPEC	G	+	+	-	-
2	4	REPEC	G	+	-	+	-
3	4	MG1655	S	-	+	-	-
4	6	EPEC	G	+	+	-	-
5	6	MG1655	G	+	+	+	+
6	6	REPEC	G	+	-	+	+
7	6	EPEC	S	+	-	-	-
8	9	EPEC	G	+	+	-	-
9	9	MG1655	G	+	+	+	-
10	9	REPEC	G	+	+	+	+
11	10	EPEC	G	+	-	+	-
12	10	MG1655	G	+	+	+	+
13	10	REPEC	S	-	-	+	-
14	10	EPEC	S	+	-	-	-
15	10	MG1655	S	-	+	-	+
16	10	REPEC	S	-	-	+	-
17	10	EPEC	S	+	-	-	-
18	10	MG1655	S	-	+	-	-
19	10	REPEC	S	-	-	+	-

Supplementary Table 5. Determination of expanded host range of evolved generalists from 3-host treatment EPEC/MG1655/BL21 “Day” refers day of evolution experiment, “host of phage isolation” refers to the experimental procedure described in the legend of Fig. 4 whereby phage clones were isolated by plating whole-culture lysates onto plates containing a single host. “Generalist/specialist” refers to the designation experiments described in Fig. 4. Generalists are defined as clones that can infect more than one host. This table shows the results of spot assays of phage clone lysates spotted onto a bacterial lawn with a single host.

No.	Day	Host of phage isolation	Generalist/ Specialist	Host involved in the treatment			Host not involved in the treatment
				EPEC	MG1655	BL21	REPEC
1	10	BL21	G	+	+	+	+
2	10	MG1655	G	+	+	+	+
3	10	BL21	G	+	+	+	-
4	10	MG1655	G	+	+	-	-
5	10	BL21	G	+	+	+	-
6	10	EPEC	G	+	+	-	+
7	10	BL21	G	+	+	+	-
8	10	MG1655	S	-	+	-	-
9	10	EPEC	S	+	-	-	+
10	10	EPEC	S	+	-	-	-
11	10	EPEC	S	+	-	-	-

Supplementary Table 6 Parameter estimates for i_{sp}/i_{gen} .

We obtained broad parameter estimates for the infectivity of specialists (i_{sp}) and generalists (i_{gen}) from the three host treatments a (EPEC, MG1655, REPEC) and b (EPEC, MG1655, BL21). i_{sp} was estimated from the maximum measured infectivity (PFU) from a phage clone measured on its own strain of isolation. For example, i_{sp} for EPEC is the maximum PFU count of a clone isolated from EPEC, measured on EPEC. i_{gen} was estimated from the maximum measured infectivity of a phage clone isolated from different host. For example, i_{gen} for EPEC in the three host treatment “a”, is the highest infectivity measured on EPEC by a clone obtained from MG1655 or REPEC. We calculated the fold- advantage of the specialist over the generalist (i_{sp}/i_{gen}).

a.	i_{sp}	i_{gen}	i_{sp}/i_{gen}
EPEC	4×10^8	1.8×10^3	2.1×10^5
MG1655	1.3×10^{10}	7.6×10^2	1.7×10^7
REPEC	3.7×10^7	1.3×10^5	2.8×10^2
b.			
EPEC	5.3×10^8	5×10^8	10^0
MG1655	2.3×10^9	7.8×10^7	3×10^1
BL21	1.6×10^8	3.1×10^7	5×10^0

Supplementary Table 7. List of primers

Target	Primer Name	Sequence
Gp17	Tail Fibre-F	CTCTACCAAGGAGTTAGTACC
Gp17	Tail Fibre-R	CAATCGGTCCCACAAGATGC