

Local adaptation of *Legionella pneumophila* within a hot distribution water system increase resistance to copper

Supplemental Material

Table S1: Mean culture count results (n = 3) for the controls without copper over the duration of the assay

Table S2: Comparison of 45 isolate pairs by a one-way ANOVA analysis

Table S3: Percent viability measured in the control water for all tested strains

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Table S6: Strains included in the phylogenetic analysis

Figure S1: High-level phylogenetic tree comparing 96 completed *L. pneumophila* genomes, including, Philadelphia-1, Lens, Corby, Lorraine and Paris

Table S1: Mean culture count results (n = 3) for the controls without copper over the duration of the assay

Isolate ID	Sampling time (days)						
	0	1	2	3	7	14	28
JR32	1.5E+08	1.2E+08	1.3E+08	1.6E+08	1.5E+08	1.1E+08	2.5E+07
ID67501-2	7.0E+08	3.7E+08	7.0E+08	8.7E+08	5.7E+08	4.7E+08	9.3E+07
ID76826-3	7.0E+08	3.7E+08	7.0E+08	4.7E+08	3.7E+08	1.8E+08	1.7E+08
ID87726-5	7.0E+08	3.7E+08	7.0E+08	7.7E+08	1.1E+09	3.7E+08	1.5E+08
ID144141	1.9E+08	1.6E+08	1.3E+08	1.1E+08	1.2E+08	1.5E+08	8.0E+07
ID135110	1.4E+07	1.2E+07	1.1E+07	9.7E+06	7.3E+06	5.7E+06	2.4E+06
ID144150	1.5E+08	1.2E+08	1.3E+08	1.6E+08	1.5E+08	1.7E+08	8.3E+07
ID120145	1.3E+08	1.6E+08	1.7E+08	1.1E+08	1.2E+08	1.2E+08	1.7E+07
ID120112	1.9E+08	1.6E+08	1.3E+08	1.1E+08	1.2E+08	2.2E+08	1.6E+08
ID120292	1.9E+08	1.6E+08	1.3E+08	1.1E+08	1.2E+08	2.3E+08	1.9E+08

Table S2: Comparison of 45 isolate pairs by a Kruskal Wallis analysis

Uncorrected Dunn's test	Mean rank diff.	Significant?	Summary	Individual P Value	
JR32 vs. 1185.W.F	9.333	No	ns	0.1938	A-B
JR32 vs. 1427.B.F	5.667	No	ns	0.4301	A-C
JR32 vs. 378.W.F	18.33	Yes	*	0.0107	A-D
JR32 vs. 1427.W.HE	21.33	Yes	**	0.003	A-E
JR32 vs. 1427.C	15.33	Yes	*	0.0328	A-F
JR32 vs. 1427.B.HE	-5.667	No	ns	0.4301	A-G
JR32 vs. 284.W	12.33	No	ns	0.0859	A-H
JR32 vs. 62.C	3.667	No	ns	0.6097	A-I
JR32 vs. 62.W	-2	No	ns	0.7807	A-J
1185.W.F vs. 1427.B.F	-3.667	No	ns	0.6097	B-C
1185.W.F vs. 378.W.F	9	No	ns	0.2102	B-D
1185.W.F vs. 1427.W.HE	12	No	ns	0.0948	B-E
1185.W.F vs. 1427.C	6	No	ns	0.4035	B-F
1185.W.F vs. 1427.B.HE	-15	Yes	*	0.0368	B-G
1185.W.F vs. 284.W	3	No	ns	0.6762	B-H
1185.W.F vs. 62.C	-5.667	No	ns	0.4301	B-I
1185.W.F vs. 62.W	-11.33	No	ns	0.1146	B-J
1427.B.F vs. 378.W.F	12.67	No	ns	0.0778	C-D
1427.B.F vs. 1427.W.HE	15.67	Yes	*	0.0292	C-E
1427.B.F vs. 1427.C	9.667	No	ns	0.1783	C-F
1427.B.F vs. 1427.B.HE	-11.33	No	ns	0.1146	C-G
1427.B.F vs. 284.W	6.667	No	ns	0.3533	C-H
1427.B.F vs. 62.C	-2	No	ns	0.7807	C-I
1427.B.F vs. 62.W	-7.667	No	ns	0.2858	C-J
378.W.F vs. 1427.W.HE	3	No	ns	0.6762	D-E
378.W.F vs. 1427.C	-3	No	ns	0.6762	D-F
378.W.F vs. 1427.B.HE	-24	Yes	***	0.0008	D-G
378.W.F vs. 284.W	-6	No	ns	0.4035	D-H
378.W.F vs. 62.C	-14.67	Yes	*	0.0411	D-I
378.W.F vs. 62.W	-20.33	Yes	**	0.0046	D-J
1427.W.HE vs. 1427.C	-6	No	ns	0.4035	E-F
1427.W.HE vs. 1427.B.HE	-27	Yes	***	0.0002	E-G
1427.W.HE vs. 284.W	-9	No	ns	0.2102	E-H
1427.W.HE vs. 62.C	-17.67	Yes	*	0.0139	E-I
1427.W.HE vs. 62.W	-23.33	Yes	**	0.0012	E-J
1427.C vs. 1427.B.HE	-21	Yes	**	0.0035	F-G
1427.C vs. 284.W	-3	No	ns	0.6762	F-H
1427.C vs. 62.C	-11.67	No	ns	0.1043	F-I
1427.C vs. 62.W	-17.33	Yes	*	0.0158	F-J
1427.B.HE vs. 284.W	18	Yes	*	0.0122	G-H
1427.B.HE vs. 62.C	9.333	No	ns	0.1938	G-I
1427.B.HE vs. 62.W	3.667	No	ns	0.6097	G-J
284.W vs. 62.C	-8.667	No	ns	0.2276	H-I
284.W vs. 62.W	-14.33	Yes	*	0.046	H-J
62.C vs. 62.W	-5.667	No	ns	0.4301	I-J

Table S3: Percent viability measured in the control water for all tested strains

Time	JR32	1185.W.F	378.W.F	1427.C	1427.B.F	1427.W.HE	1427.B.HE	284.W	62.C	62.W
1h	90%	99%	92%	49%	99%	96%	99%	97%	80%	81%
24h	83%	100%	77%	45%	82%	97%	100%	96%	93%	89%
48h	81%	n.m.	67%	100%	91%	98%	100%	96%	87%	96%
72h	87%	77%	88%	71%	100%	97%	100%	97%	76%	87%
168h	86%	86%	80%	92%	99%	88%	n.m.	95%	84%	86%
336h	86%	76%	75%	97%	98%	n.m.	99%	96%	84%	79%
672h	79%	78%	89%	97%	97%	n.m.	96%	98%	70%	96%

Table S4: Single nucleotides polymorphism identified

Position in NC_021350	Isolate Strain					Annotation	Gene	Description
	NC_021350	1427.B.F	1427.B.HE	1427.C	1427.W.HE			
22618	A	A	A	G	A	K41R (AAA→AGA)	LP6_RS00090 →	TolC family protein
138963	G	A	G	G	G	M172I (ATG→ATA)	LP6_RS00620 →	ABC transporter permease subunit
238758	A	A	G	G	G	Y42C (TAT→TGT)	LP6_RS01070 →	protein kinase
305099	G	G	A	G	G	A175T (GCC→ACC)	LP6_RS01320 →	hypothetical protein
400586	T	T	A	T	A	K728* (AAA→TAA)	LP6_RS01805 ←	Ipg0365 family Dot/Icm T4SS effector
676192	G	A	A	A	G	G514S (GGT→AGT)	wipB →	Dot/Icm T4SS effector WipB
706918	T	T	T	C	T	D30G (GAT→GGT)	rpe ←	ribulose phosphate 3 epimerase
863301	T	T	C	C	C	intergenic (+31/ 83)	LP6_RS03955 → / → LP6_RS03960	hypothetical protein/IS4 family transposase
941080	A	A	A	T	A	E234D (GAA→GAT)	LP6_RS04325 →	cytochrome c biogenesis protein CcsA
1024041	A	A	G	A	G	H429H (CAT→CAC)	lidA ←	Dot/Icm T4SS effector LidA
1051477	T	A	T	T	T	F577L (TTT→TTA)	ligA →	NAD dependent DNA ligase LigA
1114928	C	C	T	T	T	intergenic (+168/ 104)	prs → / → LP6_RS05135	ribose phosphate pyrophosphokinase/copper translocating P type ATPase
1124675	G	G	A	A	A	L133L (TTG→TTA)	LP6_RS05165 →	hypothetical protein
1203009	G	G	A	A	A	A154T (GCT→ACT)	LP6_RS05520 →	efflux RND transporter periplasmic adaptor subunit
1342177	C	T	T	T	C	E203E (GAG→GAA)	LP6_RS06130 ←	LysE family transporter
1434172	G	G	A	G	G	E563E (GAG→GAA)	LP6_RS06610 →	Tfp pilus assembly protein FimV (twitching motility)
1443541	G	A	A	A	G	intergenic (+64/ 102)	LP6_RS06650 → / → LP6_RS06655	cysteine tRNA ligase/DUF3413 domain containing protein
1468941	T	T	A	A	A	Q144L (CAA→CTA)	lbtU ←	siderophore legiobactin import outer membrane protein LbtU
1503327	A	G	A	A	A	intergenic (69/+84)	LP6_RS06885 ← / ← sidG	SGNH/GDSL hydrolase family protein/Dot/Icm T4SS effector SidG
1688131	T	T	T	C	T	F835F (TTT→TTC)	LP6_RS07705 →	EAL domain containing protein
1902159	C	T	T	T	C	A513V (GCT→GTT)	LP6_RS08630 →	kinectin 1
1903355	G	G	A	A	A	D306N (GAT→AAT)	ppeB →	Dot/Icm T4SS effector PpeB
2011194	A	A	A	T	T	intergenic (+178/+7)	rvfA → / ← mavB	Dot/Icm T4SS effector RvfA/Dot/Icm T4SS effector MavB
2011196	T	T	T	G	G	intergenic (+180/+5)	rvfA → / ← mavB	Dot/Icm T4SS effector RvfA/Dot/Icm T4SS effector MavB
2486659	T	T	C	C	C	S263S (TCA→TCG)	LP6_RS11310 ←	rod shape determining protein
2571690	G	G	A	A	A	I196I (ATC→ATI)	LP6_RS11625 ←	hypothetical protein
2764264	A	A	C	A	A	intergenic (+42/+68)	LP6_RS12515 → / ← LP6_RS12520	hypothetical protein/M23 family metalloproteinase
3050179	A	A	G	A	G	F47S (TIT→TCT)	LP6_RS13810 ←	phenylalanine 4 monooxygenase
3421575	G	A	G	G	G	A841V (GCT→GTT)	LP6_RS15515 ←	Ipg2975 family Dot/Icm T4SS effector

Table S5: Concentration of the ions added to Milli-Q water buffered to pH 7.6. Ion concentration was obtained from the Montreal City water quality report (Ville de Montréal, 2015)¹

	Final Concentration (mg/L)	Ion source	Salt solution concentration (mg/L)	Volume of salt solution added for final volume of 1L
Aluminum	0.07	$KAl(SO_4)_2 \bullet 12H_2O$	1226	1
Calcium	31.95	$CaCl_2 \bullet 2H_2O$	117189	1
Chloride	24.70	(KCl + $CaCl_2 \bullet 2H_2O$)	-	-
Fluoride	0.13	NaF	287	1
Iron	0.02	$FeCl_3 \bullet 6H_2O$	960	0,1
Magnesium	8.21	$MgSO_4$	40648	1
Nitrate (NO ₃)	0.33	$Ca(NO_3)_2 \bullet 4H_2O$	628	1
Potassium	1.58	KCl	3012	1
Sulfate	22.15	$KAl(SO_4)_2 \bullet 12H_2O$ + $MgSO_4$	-	-

¹Ville de Montréal, 2015. Qualité de l'eau potable produite par les usines Atwater et Charles-J.-Des-Baillets et distribuée en réseau, p. 6.

Table S6: Strains included in the phylogenetic analysis

Accession	Name	Included in Fig. 4	Accession	Name	Included in Fig. 4
NC_002942	Philadelphia_1	Yes	NZ_CP015949	E3_N	No
NC_016811	ATCC_43290	Yes	NZ_CP015950	E4_N	No
NC_020521	LPE509	Yes	NZ_CP015951	E5_N	No
NC_021350	Thunder_Bay	Yes	NZ_CP015953	E6_N	No
NZ_CP015927	Philadelphia_1_ATC	Yes	NZ_CP015954	E7_O	No
NZ_CP015928	Philadelphia_1_CDC	Yes	NZ_CP015955	E8_O	No
NZ_CP015929	Philadelphia_2	Yes	NZ_CP015956	E9_O	No
NZ_CP015930	Philadelphia_3	Yes	NZ_CP016029	Pontiac	No
NZ_CP015931	Philadelphia_4	Yes	NZ_CP016030	OLDA	No
NZ_CP021267	Burlington_1	Yes	NZ_CP016868	FF1102	No
NZ_CP045974	SBT211	Yes	NZ_CP016870	FF1103	No
NZ_LR133933	NCTC12180	Yes	NZ_CP016872	FF1104	No
NZ_LR134332	NCTC11193	Yes	NZ_CP016873	FF1105	No
NZ_LT598657	Lpm7613	Yes	NZ_CP016874	FFI329	No
NZ_LT632616	ST37	Yes	NZ_CP016876	FFI337	No
	1427.B.F	Yes	NZ_CP017457	Detroit-1	No
	1427.W.HE	Yes	NZ_CP017458	Dallas_1E	No
	1427.B.HE	Yes	NZ_CP017601	D6026	No
	1427.C	Yes	NZ_CP017602	D5945	No
NC_006368	Paris	No	NZ_CP021256	D-4954	No
NC_006369	Lens	No	NZ_CP021257	Lansing_3	No
NC_009494	Corby	No	NZ_CP021258	D-5744	No
NC_014125	2300/99_Alcoy	No	NZ_CP021259	D-7708	No
NC_018139	Lorraine	No	NZ_CP021260	NY24	No
NC_018140	HL06041035	No	NZ_CP021261	NY23	No
NZ_CP011105	L10-023	No	NZ_CP021262	U8W	No
NZ_CP012019	Toronto-2005	No	NZ_CP021263	D-3137	No
NZ_CP014255	F-4185	No	NZ_CP021264	D-5387	No
NZ_CP014256	D-7158	No	NZ_CP021265	Los_Angeles_1	No
NZ_CP014257	D-7119	No	NZ_CP021266	Knoxville_1	No
NZ_CP014759	F4468	No	NZ_CP021268	Birmingham_1	No
NZ_CP014760	F4469	No	NZ_CP021269	Bellingham_1	No
NZ_CP015342	D-7632	No	NZ_CP021270	D-7787	No
NZ_CP015343	D-7631	No	NZ_CP021272	D-5265	No
NZ_CP015344	D-7630	No	NZ_CP021274	D-4040	No
NZ_CP015925	E10_P	No	NZ_CP021277	D-4058	No
NZ_CP015926	E11_U	No	NZ_CP021279	F-4198	No
NZ_CP015932	C1_S	No	NZ_CP021281	Flint_2	No
NZ_CP015933	C2_S	No	NZ_CP021283	Allentown_1	No
NZ_CP015934	C3_O	No	NZ_CP021286	Albuquerque_1	No
NZ_CP015935	C4_S	No	NZ_CP040987	FDAARGOS_779	No
NZ_CP015936	C5_P	No	NZ_LR134176	NCTC12179	No
NZ_CP015937	C6_S	No	NZ_LR134380	NCTC12273	No
NZ_CP015938	C7_O	No	NZ_LS483410	NCTC11404	No
NZ_CP015939	C8_S	No	NZ_LS483412	NCTC12272	No
NZ_CP015941	C9_S	No	NZ_LT632614	ST62	No
NZ_CP015944	C10_S	No	NZ_LT632615	ST23	No
NZ_CP015945	C11_O	No	NZ_LT632617	ST42	No
NZ_CP015946	E1_P	No	NZ_LT906452	NCTC11985	No
NZ_CP015947	E2_N	No	NZ_LT906476	NCTC11286	No

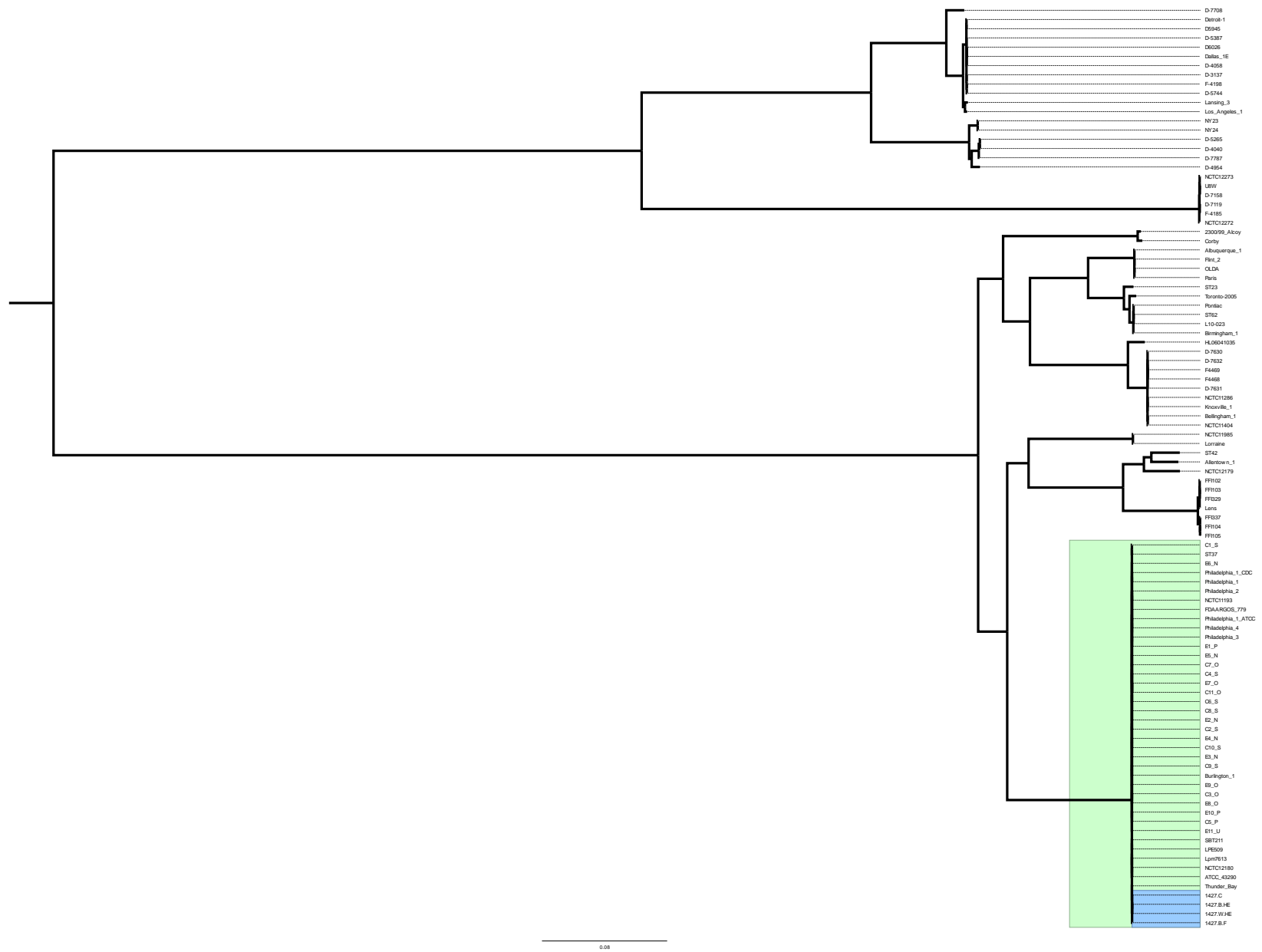


Figure S1: High-level phylogenetic tree comparing 96 completed *L. pneumophila* genomes, including, Philadelphia-1, Lens, Corby, Lorraine and Paris