Lesley Sitter
lesley.sitter@lincolnuni.ac.nz

Bio-Protection Research Centre
Lincoln University
Lincoln, New Zealand

Lesley Sitter[1,2,3], Travis Glare[2,3] & Mark Hurst[1,2]
[1] Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand
[2] AgResearch Ltd, Lincoln, New Zealand
[3] Lincoln University, Lincoln, New Zealand

Bio-Protection
Bioprotection science for New Zealand

Evolutionary divergence of the insect disease-encoding *Serratia* plasmid pADAP

Introduction

The larval stage of the endemic New Zealand grass-grub (*Costelytra givenii*), causes significant damage to New Zealand's pastures. To date, two diseases of grass-grub larvae, instigated by strains of *Serratia entomophila* and *S. proteamaculns*, have been identified and are being used as commercial biocontrol agents. The two main virulence determinants of these *Serratia* strains, an Anti-feeding-prophage and an ABC-toxin-complex, are encoded on a 153-kb conjugative plasmid pADAP (amber disease associated plasmid).

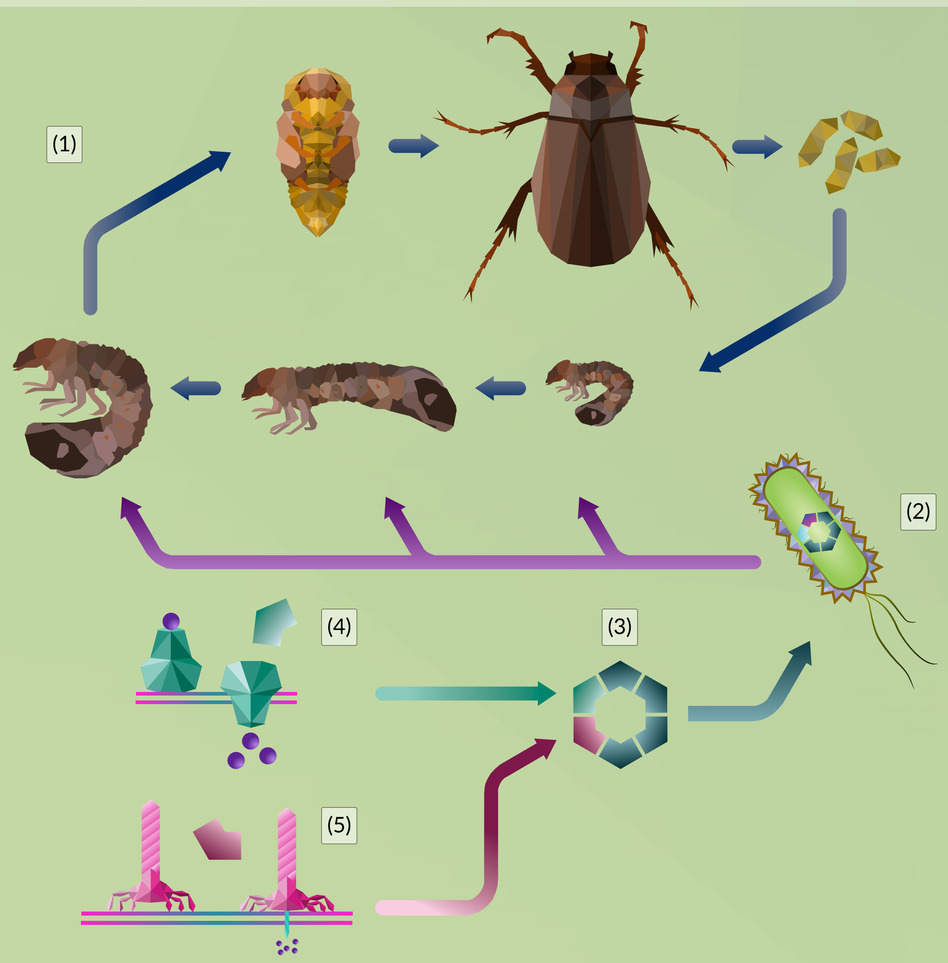


Figure 1. An overview of the life cycle of the *C. givenii* and the mechanics with which the *Serratia* genus can affect the larvae. (1) The grass grub is holometabolic, meaning it has four major stages in its life cycle; egg, larvae which goes through three instar stages (this stage is refered to as grub), pupa and adult beetle. (2) The *Serratia* species carrying pADAP-like plasmids are able to cause Amber disease in the grass grub during their larval stage. (3) The first characterized pADAP contained a backbone consisting of a replication and conjugation region, a Sef fimbriae cluster, a Sep-Tc PAI and an Afp PAI. (4) The SEP-Tc is an ABC-toxin complex that can form pores into target cells and release toxins and degrades gut enzymes and causes gut clearing. (5) The AFP is a tailocin-type secretion system, with host-specific tail fibers, able to inject a needle like structure into a cell to allow release of toxic compounds, causing an anti-feeding phenotype.

Research goals

A range of pADAP bearing *Serratia* isolates with divergent virulence have been identified. The pADAP plasmid (Type A) causes a chronic disease designated as Amber disease. AGR96X, a *S. proteamaculans* strain, containing a pADAP-like plasmid (Type B) with a divergent Afp, designated AfpX, causes rapid death after only several days. With the goal of defining evolutionary points of divergent between plasmid variants, and define potential co-evolution between plasmid and host, ~60 *Serratia* strains, that differed in disease phenology, microbiological characteristic and location of isolation were sequenced in order to characterize the genetic elements responsible, determine how acquisition and exchange of genetic elements on the plasmids have happened.

Figure 2. Strains that have the B genotype, as shown in figure 3, take only several days to induce death in grass-grub, whereas most other strains have a chronic or non-disease phenotype. In the figure from left to right you can see a grub that died after ~8 days from a hyper-path strain, a normal healthy grub, and a grub suffering from what is referred to as amber disease, a chronic disease that causes an amber discoloration in the gut and death after several months.

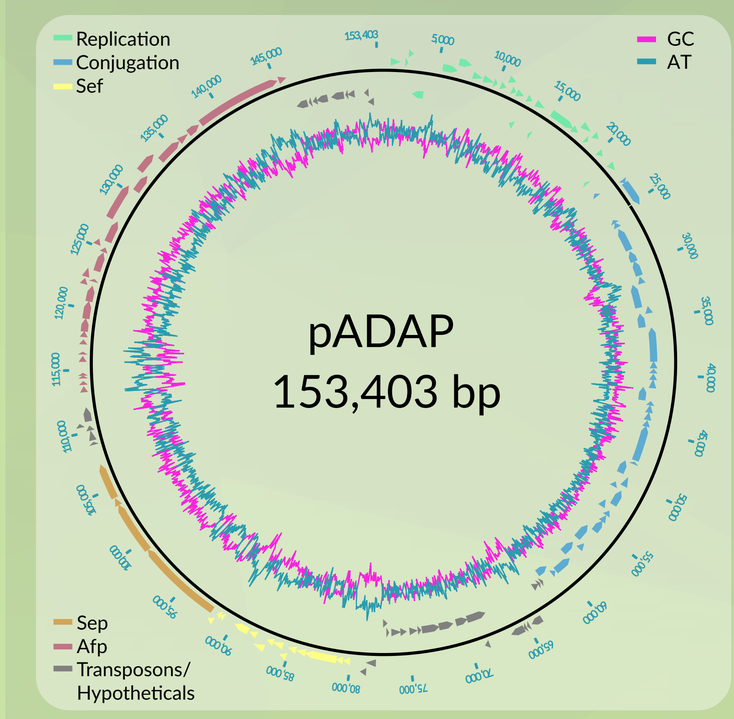


Figure 3. A schematic of the pADAP reference plasmid. This plasmid, originally isolated from a *S. entomophila* strain designated as A1MO2, contains a backbone consisting of a Replication and Conjugation region that spans from the *int2* gene till the *pilL* gene. It also contains a Sef fimbriae cluster, and a Sep-Tc cluster, which after comparative genomics appears to actually be one PAI, suggesting the fimbriae are needed for the Sep-Tc to perform its function correctly. The pADAP plasmid also contains a Afp PAI. Between the different regions several hypothetical genes and transposons can be found.

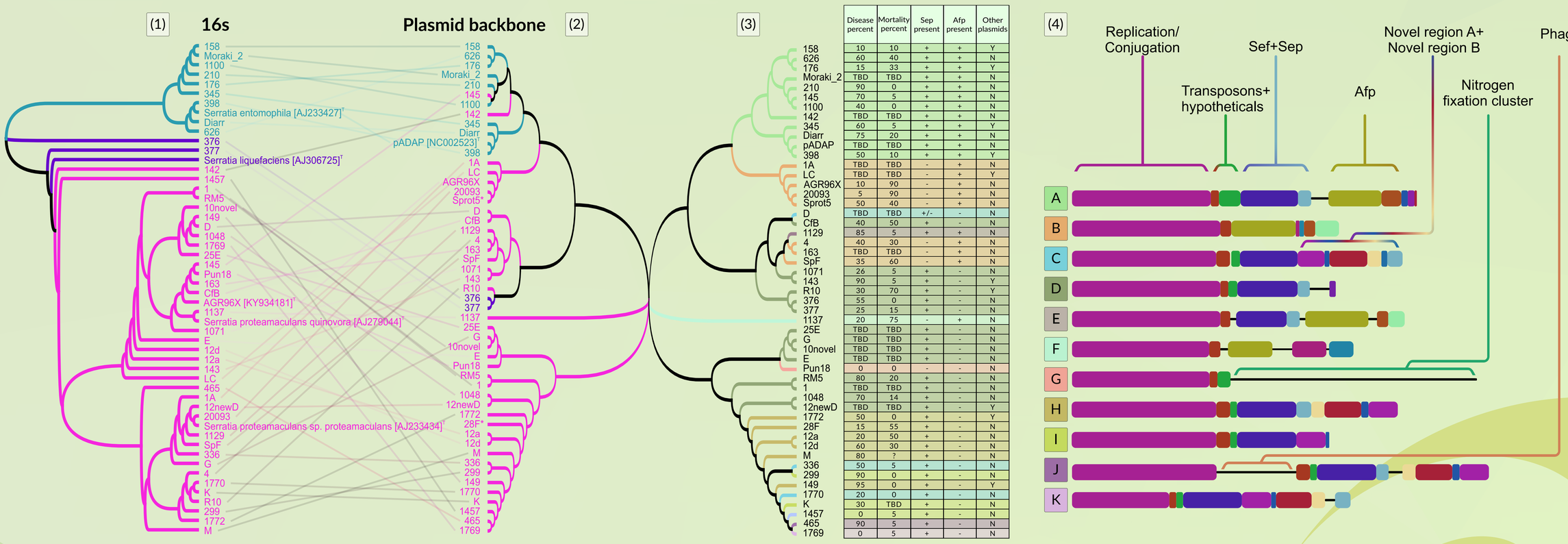
Discussion and future work

Phylogenetic analysis of the conserved plasmid "backbone", residing between a conserved integrase (*int2*) and the end of a conjugative pili gene cluster (*pilL*), shows clustering of all the *S. entomophila* plasmids.

In addition to virulence determinant variants, several novel gene clusters, ranging from putative accessory virulence determinants, toxin-antitoxin clusters, and secretion systems, were identified.

The envisioned goal is determining a timeline in which virulence factors were acquired or lost, and show *in vitro* that exchange of these elements can still take place.

Figure 4. An overview of the correlation between species of *Serratia*, plasmid backbone homology, phenotype and plasmid genotype. (1) A 16s based tree, representing the relationship of bacterial hosts where blue are *S. entomophilas*, purple are *S. liquefaciens* and pink are *S. proteamaculans*. (2) A tree build using the alignment of the conserved plasmid backbones (*int2* to *pilL*) where blue are *S. entomophilas*, purple are *S. liquefaciens* and pink are *S. proteamaculans*. (3) The backbone tree mirrored, colored with the 11 corresponding genotypes, slight genetic variations are present within each genotype, which are not shown. The associated table shows the disease and mortality percentage of the strain in bioassays, whether Afp and Sep are present on the plasmid and if the strain has additional plasmids. (4) A simplified overview of the genotypes and the regions of interest.



	Disease percent	Mortality percent	Sep present	Afp present	Other plasmids
158	10	10	+	+	Y
626	60	40	+	+	N
176	15	33	+	+	Y
Moraki_2	TBD	TBD	+	+	N
210	90	0	+	+	N
145	70	5	+	+	N
1100	40	0	+	+	N
142	TBD	TBD	+	+	N
345	60	5	+	+	Y
Diarr	75	20	+	+	N
pADAP	TBD	TBD	+	+	N
398	50	10	+	+	Y
1A	TBD	TBD	-	+	N
LC	TBD	TBD	-	+	Y
AGR96X	10	90	-	+	N
20093	5	90	-	+	N
Sprot5	50	40	-	+	N
D	TBD	TBD	+/-	-	N
CfB	40	50	+	-	N
1129	85	5	+	+	N
4	40	30	-	+	N
163	TBD	TBD	-	+	N
SpF	35	60	-	+	N
1071	26	5	+	-	N
143	90	5	+	-	Y
R10	30	70	+	-	Y
376	55	0	+	-	N
377	25	15	+	-	N
1137	20	75	-	+	N
25E	TBD	TBD	+	-	N
G	TBD	TBD	+	-	N
10novel	TBD	TBD	+	-	N
E	TBD	TBD	+	-	N
Pun18	0	0	-	-	N
RM5	80	20	+	-	N
1	TBD	TBD	+	-	N
1048	70	14	+	-	N
12newD	TBD	TBD	+	-	Y
1772	50	0	+	-	Y
28F	15	55	+	-	N
12a	20	50	+	-	N
12d	60	30	+	-	N
M	80	?	+	-	N
336	50	5	+	-	N
299	90	0	+	-	N
149	95	0	+	-	Y
1770	20	0	+	-	N
K	30	TBD	+	-	N
1457	0	5	+	-	N
465	90	5	+	-	N
1769	0	5	+	-	N

LINCOLN UNIVERSITY
TE WHARE WĀNAKA O AORAKI

agresearch
āta mātai, mātai whetū

www.bioprotection.org.nz