Introduction

Orientia tsutsugamushi, the causative agent of scrub typhus, is an aerobic Gram negative obligate intracellular coccobacillus in the family Rickettsiaceae. It is transmitted to humans by the bite of the larval stage of the trombiculid mite ('Chigger') which typically feed on rodents. The bacteria propagate by vertical transovarial transmission within the mite line, which is the natural reservoir, and by horizontal transmission via mite bite to rodents and dead end hosts such as human. It is a major human pathogen in the Asia-Pacific region, causing up to 23% of febrile episodes in some rural areas. Over one billion are at risk and at least one million infections occur each year. Infection commonly presents as an acute febrile illness 7 to 10 days after bite. The severity of infection ranges from mild to multiorgan failure and death.

This MLST scheme was developed as a tool to understand the population genetic structure and ecology of *O. tsutsugamushi*, and to provide insights into the pathophysiology of scrub typhus. The shot-gun cloning and sequencing of *O. tsutsugamushi* strain UT76 (local strain in Thailand) was performed in collaboration with researchers from the Wellcome Sanger Institute. Using the scheme the role of homologous recombination in shaping the population structure of *O. tsutsugamushi* has been examined (**Sonthayanon P**, Peacock SJ, Chierakul W, Wuthiekanun v, Blacksell SD, Holden MTG, Bentley S D, Feil EJ, and Day NPJ. High rates of homologous recombination in the mite endosymbiont and opportunistic human pathogen *Orientia tsutsugamushi. PLoS Negl Trop Dis* 2010; 4(7):e752).

Using the database

Full details describing use of the database can be found at **http://bigsdb.readthedocs.org**.

The seven loci and the primers and conditions used for PCR

Orientia tsutsugamushi MLST scheme uses internal fragments of the following seven house-keeping genes:-

gpsA *glycerol-3-phosphate dehydrogenase*
mdh *malate dehydrogenase*
nrdF *ribonucleoside-diphosphate reductase beta subunit*
nuoF *NADH dehydrogenase chain F*
ppdK *pyruvate, phospate dikinase precursor*
sucB *dihydrolipoamide S-succinyltransferase*
sucD *succinyl-CoA synthase alpha chain*

PCR amplification and DNA sequencing: Two sets of primer pairs (outer primer set and inner primer set) used for nested-PCR of these genes are following:

Gene	Gene name	Outer primer	sequence (5'->3')	PCR Product size (bp)	Inner primer	sequence (5'->3')	PCR Product size (bp)	Sequence read size (bp)
gpsA	glycerol-3-phosphate dehydrogenase	gpsA_F gpsA_R	TCAGCCCATACTCAAGAAATCA GCAAATGCCACAATTTCCTT	572	gpsA_NF gpsA_NR	TCAGCTGCATACTAATAAAAA GATGCTTTACAGTTTTGACCA	510	390
mdh	malate dehydrogenase	mdh_F mdh_R	CCAAAGCAGTTGCTCAAGGT AGCTGCTGCTGGAGCATAAT	608	mdh_NF mdh_NR	AAAGCATGGGTATTGGTAAA TCCTCCATCTCTAGTTCTTTGT	512	348
nrdF	ribonucleoside-diphosphate reductase beta subunit	nrdF_F nrdF_R	TAAAGCATGGCACACTCAGC CTGTTCTGTCCAAACTTCAGGA	595	nrdF_NF nrdF_NR	AAATTCACTGGCTACCAGAA TGTTTCATCTCTAACTGACCA	500	384
nuoF	NADH dehydrogenase chain F	nuoF_F nuoF_R	ATCTGGTTCTATGGCAGTTGAC CATTTTGCGCCTCTTCTGAGTA	645	nuoF_NF nuoF_NR	AAAATCTGGCTTACGTGGT GAGTATTGTCGGAACTACAGC	520	360
ppdK	pyruvate, phospate dikinase precursor	ppdK_F ppdK_R	CAAAGGTGTAACACTTGCTCAGA TGGTGGTTCATCCATGATTTT	591	ppdK_NF ppdK_NR	TACCTATACCGCATGGTTTT ACTGCTTGAATAGCTTGGTG	528	396
sucB	dihydrolipoamide S-succinyltransferase	sucB_F sucB_R	CAGCAAAAGAAAGATGTTCAGC GGTTGCCAAAATGGTAGCAG	590	sucB_NF sucB_NR	ATTGGCACAACTAATCCAGA GCATAAAATCAATCCTGAGAA	537	411
sucD	succinyl-CA synthase alpha chain	sucD_F sucD_R	ATGTTCCTCCAGCTTTTGCT TCCAGCGCTTTTTAATGCTT	599	sucD_NF sucD_NR	TGAAGCTATTGATGCTGGTA AGCGCTTTTTAATGCTTCTA	562	411

First PCR amplification is carried out using the outer primer set and an annealing temperature of 55ºC for *mdh, nrdF, nuoF, ppdK, sucD,* and *sucB,* and 50ºC for *gpsA.* Five µl of the first PCR product is then used in a second PCR amplification using the inner primer set with the annealing temperature set at 50ºC for *sucD, nrdF, sucB, nuoF, ppdK* and 45ºC for *mdh* and *gpsA.*

Note the use of a nested PCR allows MLST to be carried out on DNA extracted directly from patient blood (without a culture step). One step PCR could be performed when DNA extracted from *in vitro* culture (bacterial isolation) using only inner primer set.

The single DNA fragments are purified and sequencing reactions are carried out, in each direction, using the inner PCR primers as sequencing primers.

Acknowledging the use of the MLST database in your publications.
Please acknowledge the use of this site in your publications as follows:
"We acknowledge the use of *Orientia tsutsugamushi* MLST database which is hosted at The Department of Zoology, University of Oxford, UK and is funded by The Wellcome Trust."

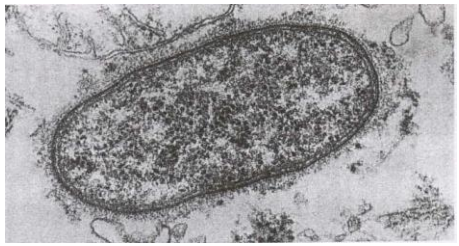

Figure 1. *Orientia tsutsugamushi* cell from electron microscope

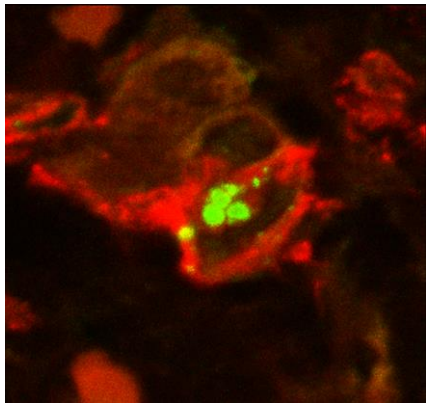

Figure 2. Multiple intracellular *O. tsutsugamushi* located within an HLADR-positive, antigen-presenting cell in the superficial dermis of an eschar from a patient with acute scrub typhus.(Paris, D.H., et al., *Orientia tsutsugamushi* in Human Scrub Typhus Eschars Shows Tropism for Dendritic Cells and Monocytes Rather than Endothelium. *PLoS Negl Trop Dis*, 2012. **6**(1): p. e1466)