

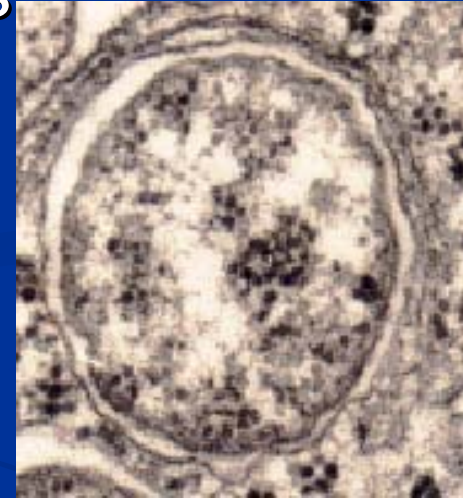
Genomics & Biology of *Wolbachia* wPip in *Culex*

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Wolbachia pipientis in *Culex*

- Intracellular maternally inherited bacterium first described in *Culex pipiens*
- *C. quinquefasciatus* is sibling species found in tropics, can interbreed with *pipiens*
- transmits Bancroftian filariasis
- **cytoplasmic incompatibility**
- high complexity of crossing types

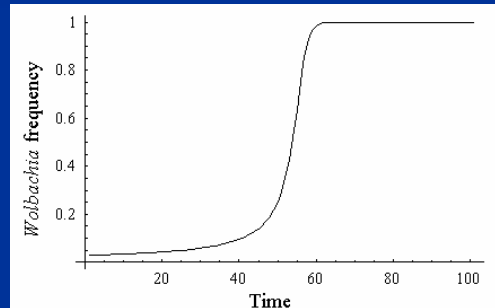


Wolbachia infection in **red**

female x male



female x **male**



female x **male**



Incompatible

female x male



Bidirectional cytoplasmic incompatibility (CI)
between *Wolbachia*-infected populations 1 & 2

female1 x **male1**



female2 x **male2**



female1 x **male2**



Incompatible

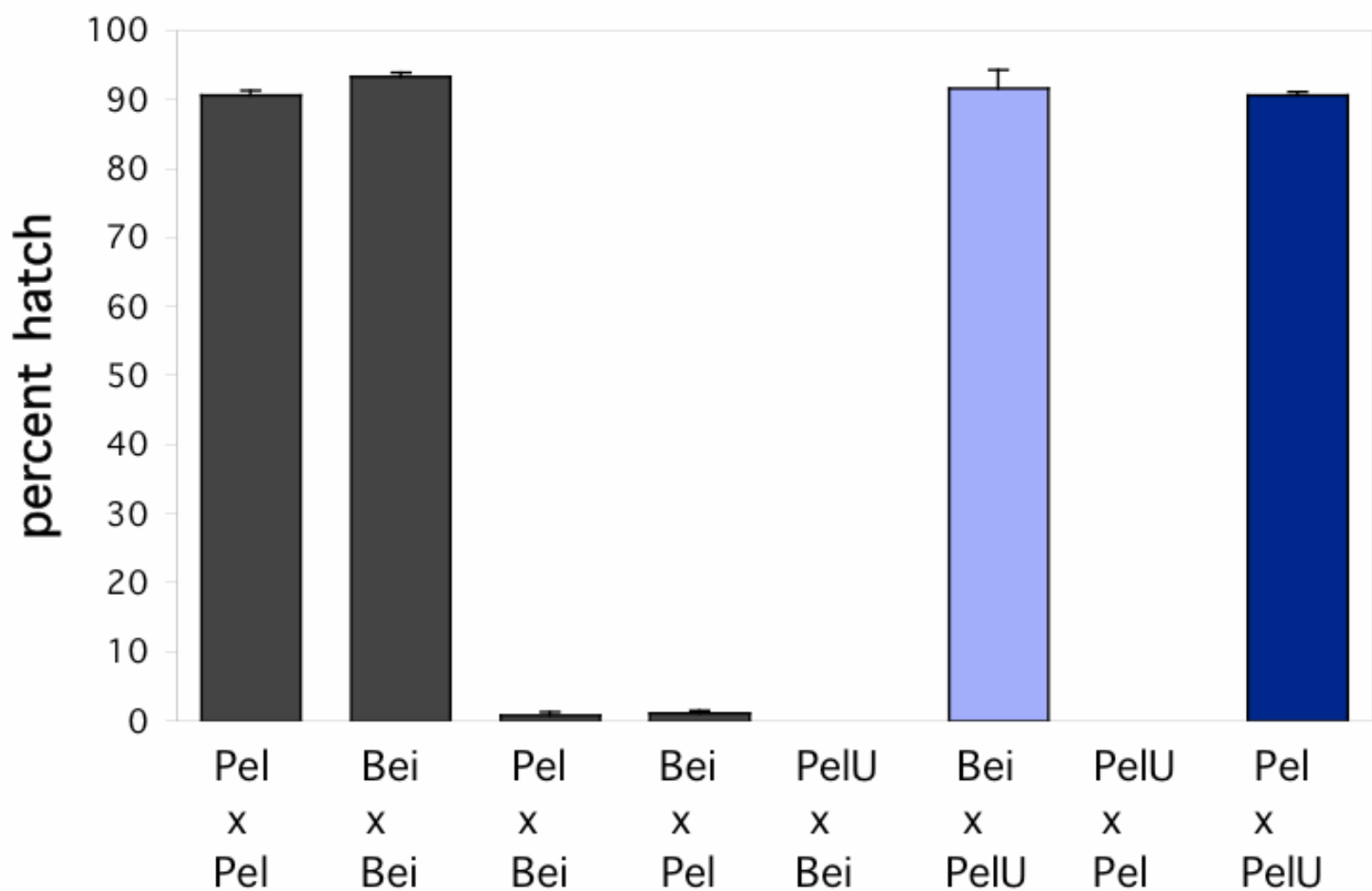
female2 x **male1**



Incompatible

Strain (Origin)	Species / form	<i>wsp</i> differences	<i>ND4</i> differences
Bei (Beijing, China)	<i>pallens</i> / <i>quinqs.</i>	reference sequence	reference sequence
Col (Colombia)	<i>quinquefasciatus</i>	0	0
Gzh (Guangzhou , China)	<i>quinquefasciatus</i>	0	0
Mol (Beijing, China)	<i>molestus</i>	0	0
Mosh (Moshi, Tanzania)	<i>quinquefasciatus</i>	0	0
Muh (Muheza, Tanzania)	<i>quinquefasciatus</i>	0	0
Pel (Sri Lanka)	<i>quinquefasciatus</i>	0	0
Thai (Bangkok, Thailand)	<i>quinquefasciatus</i>	0	0

At least 4 distinct crossing types; uni- / bi-directional, partial / complete CI



how are complex crossing types produced in the *Culex pipiens* group?

- *Wolbachia* variability - genome & comparative sequencing, Pel strain chosen
- Host effects - backcrossing

Genome sequencing of *wPip* (clade B) - funded by Wellcome Beowulf

Wolbachia DNA purification: Methods attempted:

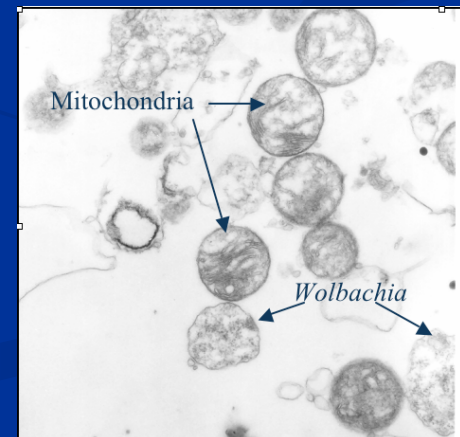
Pulsed-field electrophoresis - major yield loss

Renografin gradient purification - Insufficient separation of *Wolbachia* from host material

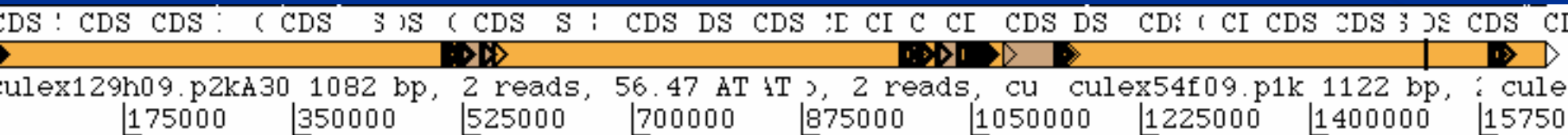
- Centrifugation/ Unit gravity filtration through a 5 μM pore size filter
 - Use of preblastoderm embryos

gene	Amplification
<i>wsp</i> , <i>ftsZ</i> (<i>Wolbachia</i>)	+
S7 (<i>Culex</i> gDNA)	-
ND4 (<i>Culex</i> mDNA)	(-)

50 extractions (2,500 embryo rafts) required



shotgun genome sequencing completed
at Sanger Institute (around 1.6Mb genome)
www.sanger.ac.uk/projects/W_pipientis



only ~80% sequence identity with *wMel*

Currently in assembly

large insert library was needed for finishing -
repeats larger than mean insert size

175000

350000

525000

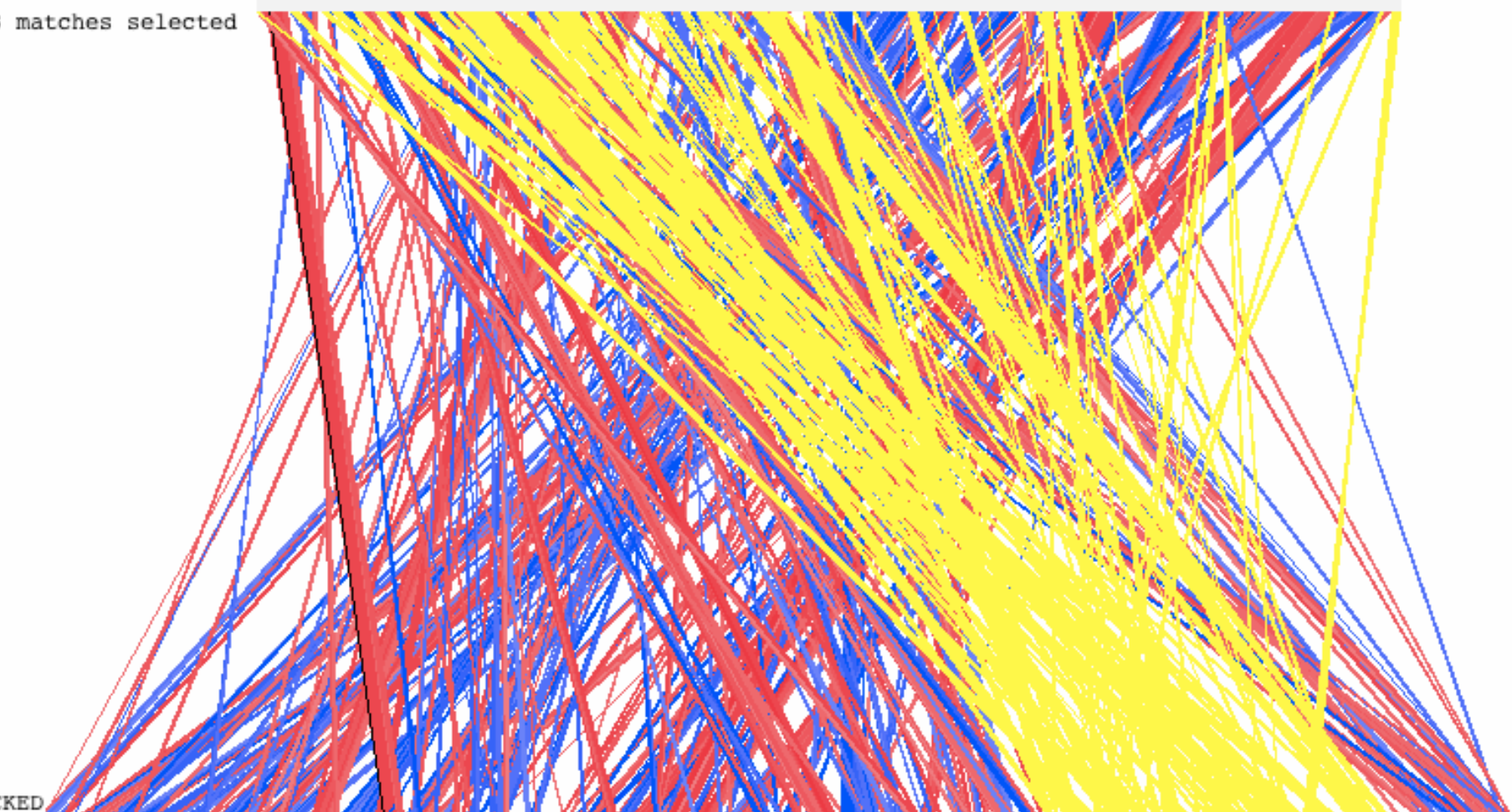
700000

875000

1050000

1225000

matches selected



KED

zulex90d07.q1k 459418 bp, 6018 su zulex101q02.plk 417936 bp, zulex101q02.plk 417936 bp, zulex101q02.plk 417936 bp

175000

350000

525000

700000

875000

1050000

1225000

1400000

1575000

Ankyrin repeat-encoding genes in *Wolbachia*

Ankyrins - tandemly repeated modules of about
30-33 amino acids - mainly eukaryotes

functionally diverse proteins

- Protein-protein interactions
- Transcription factors
- Interactions with cytoskeleton
- **Cell cycle regulators**

No. nucleotides compared	Pel / Bei % divergence: DNA (predicted amino acids)
439	0
463	0
599	0
612	0
339	0
397	0
1183	0
723	0
255	0
274	0
433	0
391	0
798	9.6 (8.4)
557	0
453	3.1 (3.4)
487	1.2 (0)
219	0
648	0

**Sequence comparison of
18 ankyrin repeat-
encoding genes between
bidirectionally
incompatible
Pel and Bei colonies**

pk1 aa alignment - Pel vs Bei

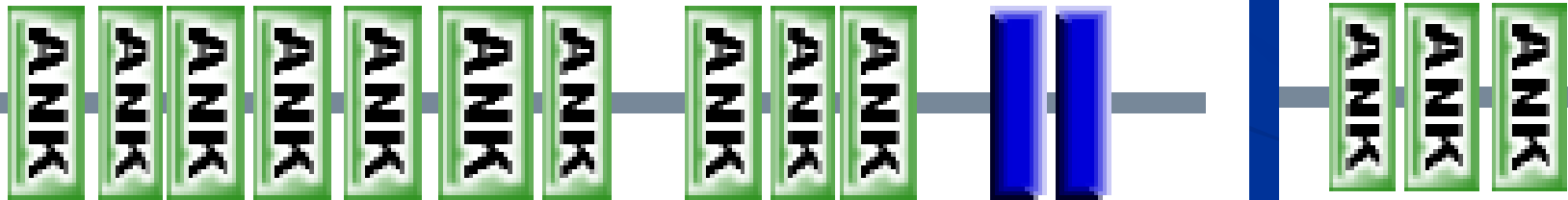
S A HLSLELIKCLINQP**G L**DVNV RGLNGKTP LHC AIEFDELS MVDLL LTK
A D NLSLELIKCLINQPG**V**DVNV RGLNGKTP LHC AIEFDELS MVDLL LTK

 NINPFV EDNDG KTS LDYAKE**G K**KA EIL**K**ALINNKY GSEQ D**S**LLHLAAM**V**
 NINPFV EDNEG KTS LDYAKE**G E**KA EIL**Q**ALINNKY GSEQ D**N**LLHLAAM**I**

 EVNAVRYLI**N R**GIDVNSRNA**I F**H TPLHLAAGIGH**V E**VVKILVREGNAEI
 EVNAVRYLI**G K**GVDVNV RNA**L H**H TPLHLAAGIGH**E N**VVKILVKEGN AEI

 VFDARNQTPMHYAVNNKKLEIVKLLL**E**LGADVNSAR**V**GQNSMKLSPI**H**I
 VFDARNQTPMHYAVNNKKLEIVKLLL**K**LGADVNSAR**I**GQNSMKLSP**V**H I

 VSNTNYDERDLCLDILKCL**I R**EPNAQVNLQDYENKTP LHYAERLKTIEV
 VSNTNYDERDLCLDILKCL**I K**EPNAQVNLQDYENKTP LHYAERLKTIEV



pk1

.p□□

Pk 'A'

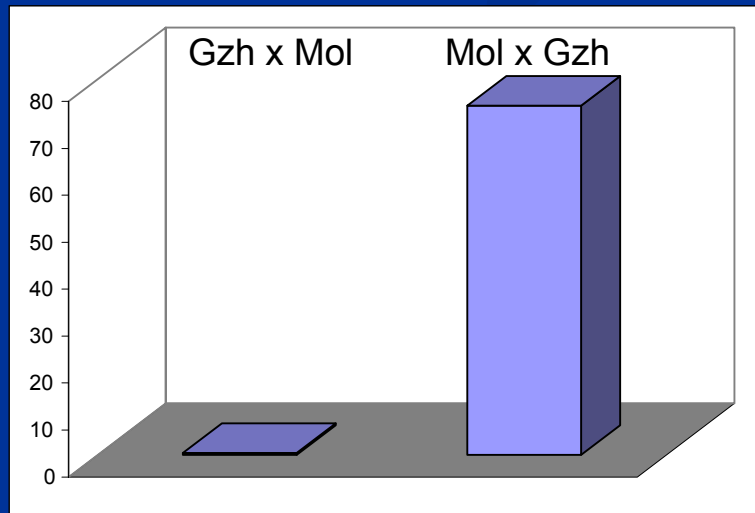
Pel, Sumo (Sri Lanka)

Mol (China)

Pk 'B'

Bei (China), Col (Colombia)

Thai, Muh (Tanzania), Gzh (China)



pk1. pk2 are **prophage-associated**

WO-B phage in *wMel* 66Kb, mosaic lambdoid / P2

are phage virions produced from prophage?

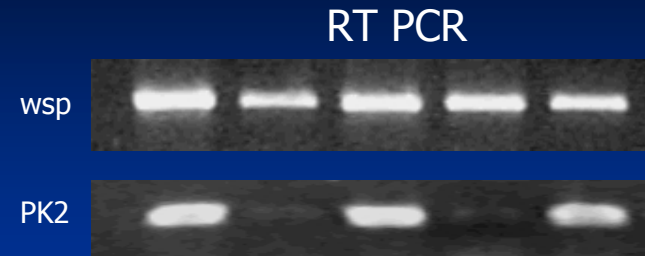
- Purification of bacteriophage WO :
 - Preblastoderm embryos or larvae
 - filtration through 0.22 μ M filter

gene	PCR Amplification
WO capsid, Pk1, PK2 (phage)	+
<i>wsp</i> & <i>ftsZ</i> (<i>Wolbachia</i>)	-
S7 (<i>Culex</i> gDNA)	-
ND4 (<i>Culex</i> mDNA)	-

Can paternal transmission of phage WO occur?

- F₁ generation (1st instar larvae + adult) from crossing experiments assayed for pk1 gene variant
 - **No paternal *pk* variant amplified** (maternal *pk1* variant only amplified in all cases)
- Mating of *Wolbachia*-uninfected virgin Pel U x Pel
 - PCR analysis of pooled spermathecae (3 days post mating), no amplification of phage WO
- Paternal transmission of phage not observed
- **PK1 gene variant population cage experiment**
PCR analysis of for individuals with both variants

Expression of ANK genes *pk1* & *pk2*

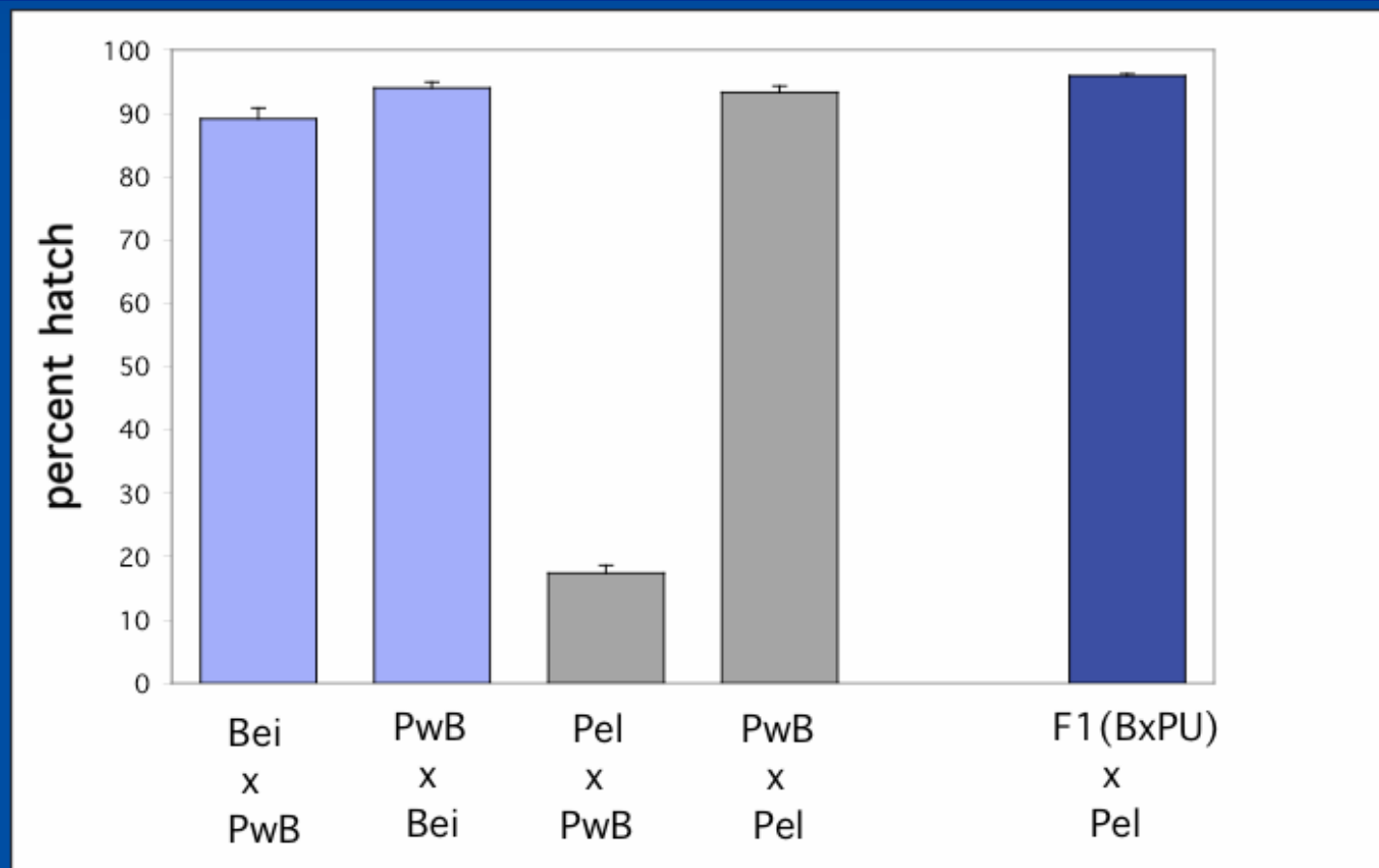


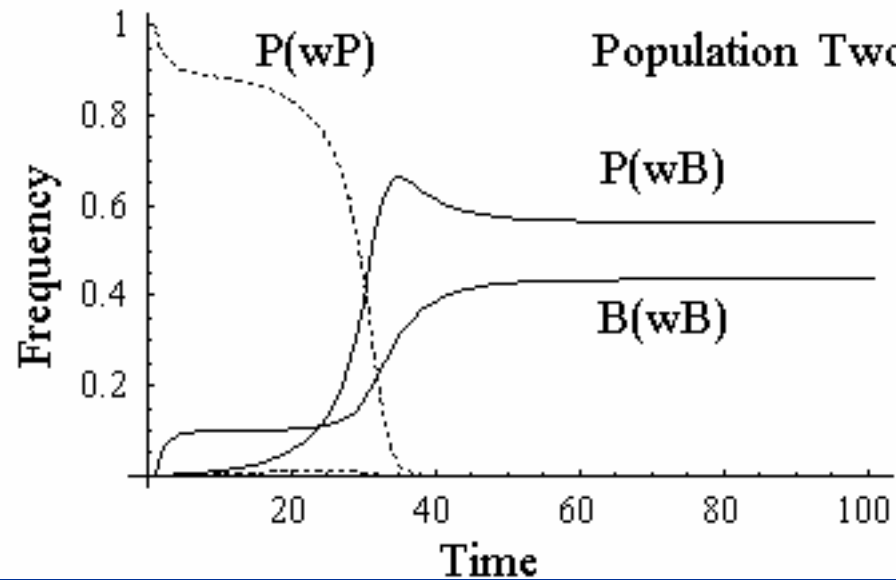
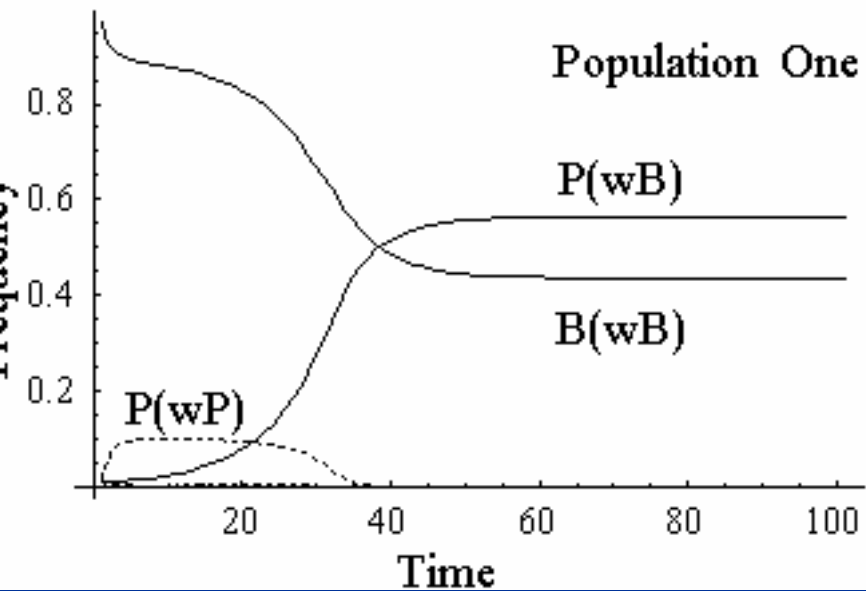
	WO capsid	<i>pk1</i>	<i>pk2</i>
Pka females	+	+	+
Pka males	+	+	-
Pkb females	+	+	+
Pkb males	+	+	+
PeIU	-	-	-

Host Effects on CI in *Culex*

Bei females crossed with PelU males;
backcrossing for 6 generations

More than 99% Pel genome, with Bei *Wolbachia*: PwB





host effect: cryptic unidirectional CI
 Evolutionary implications

Oxford

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Arun Raju

Dr Song Shewu

Dr Hoang Kim Phuc

*Liverpool School of
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Amy Lynd

Dr Ben Makepeace

Dr Julie Earl

Andy Steven

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