Effects of bottlenecks on immunity genes in NZ's threatened birds

PI: Ian Jamieson

AI: Catherine Grueber (postdoc)

PhD student: Gabby Knafler

Collaborators:

- Bruce Robertson (Otago)
- Zoe Grange (PhD), Brett Gartrell, Nigel French, Nicky Nelson, DOC
- Bethany Jackson (PhD), Richard Jacob-Hoff (Auckland Zoo), DOC

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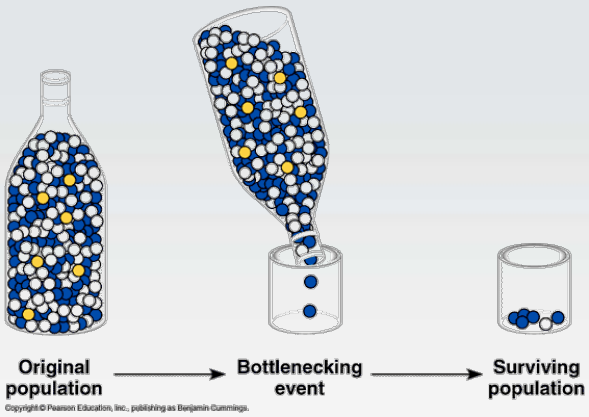
Effects of bottlenecks on immunity genes in NZ's threatened birds

Background to Strategic Initiative



- Bottleneck ➔ Loss of genetic diversity
  ➔ Loss of evolutionary potential / ability to adapt
  (e.g. impaired immune response)
- Neutral markers (e.g. microsatellites) used as surrogate for coding regions
- Longer-term consequences of loss of immune diversity under-appreciated
- Disease issues increase when threatened species are translocated outside their historic range
  - Takahe, Saddleback, Kakapo

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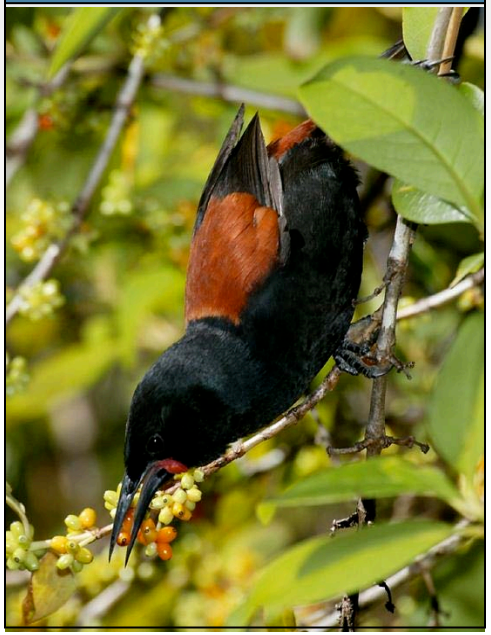
Bottlenecks and immunity genes

Translocated populations far from source populations:

Takahe
Lowest diversity

S.I. Saddleback
Low-Moderate diversity



Kakapo
Variable diversity

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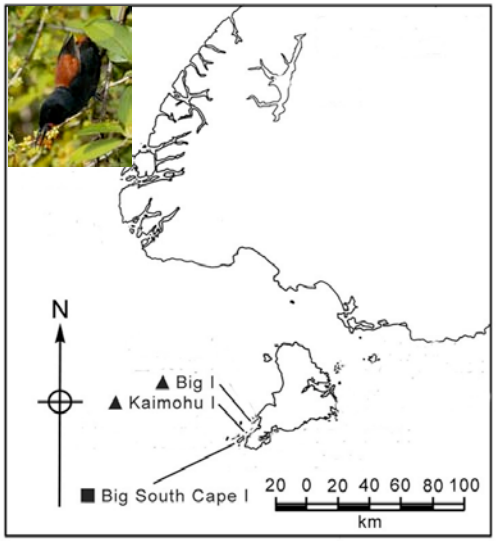
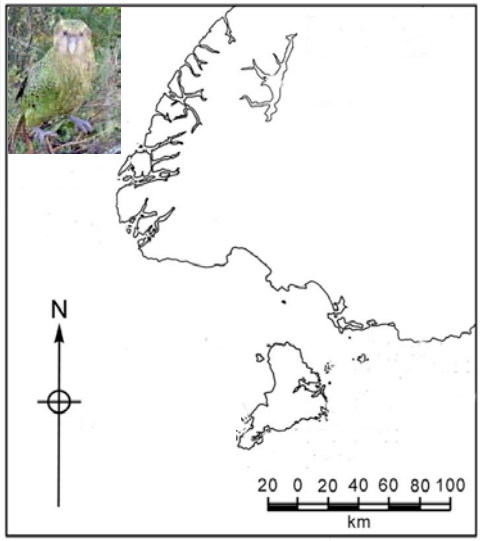
Bottlenecks and immunity genes

Translocated populations far from source populations:

Takahe Lowest diversity	S.I. Saddleback Low-Moderate diversity	Kakapo Variable diversity





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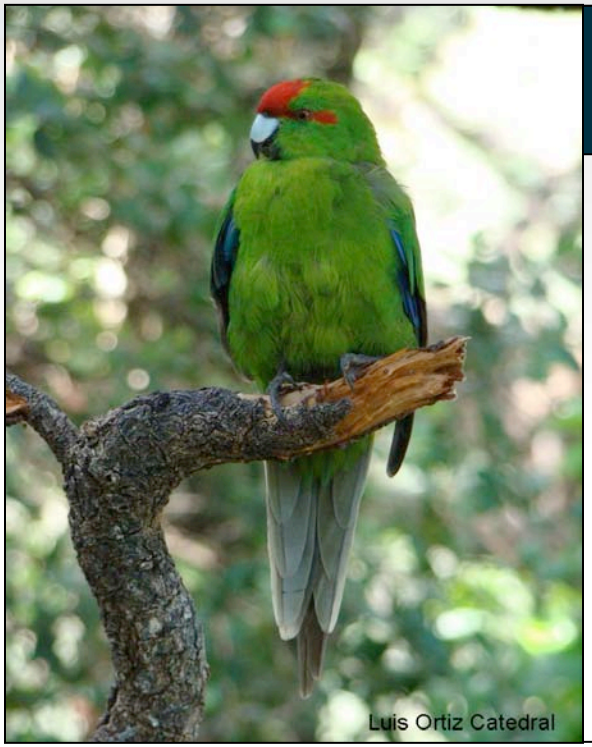
Bottlenecks and immunity genes

Variation in immunity genes and incidence of BFDV in kakariki



Bethany Jackson & Richard Jacob-Hoff
Gabby Knafler, Catherine Grueber & Bruce Robertson

- Quantify immunogenetic variation within and between LBI/Hauturu (source) and Tiritiri Matangi (bottleneck).
- Relate genetic data to disease and immunity data at individual level

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Effects of bottlenecks on immunity genes in NZ's threatened birds

Goal of Strategic Initiative

- Highlight relationship between disease susceptibility and loss of diversity in immunity genes in four high profile NZ species:
  - Takahe, Saddleback, Kakapo, Kakariki (BFDV)
- Immunity genes:
  - Major Histocompatibility Complex (MHC);
  - Toll-like Receptors (TLRs)

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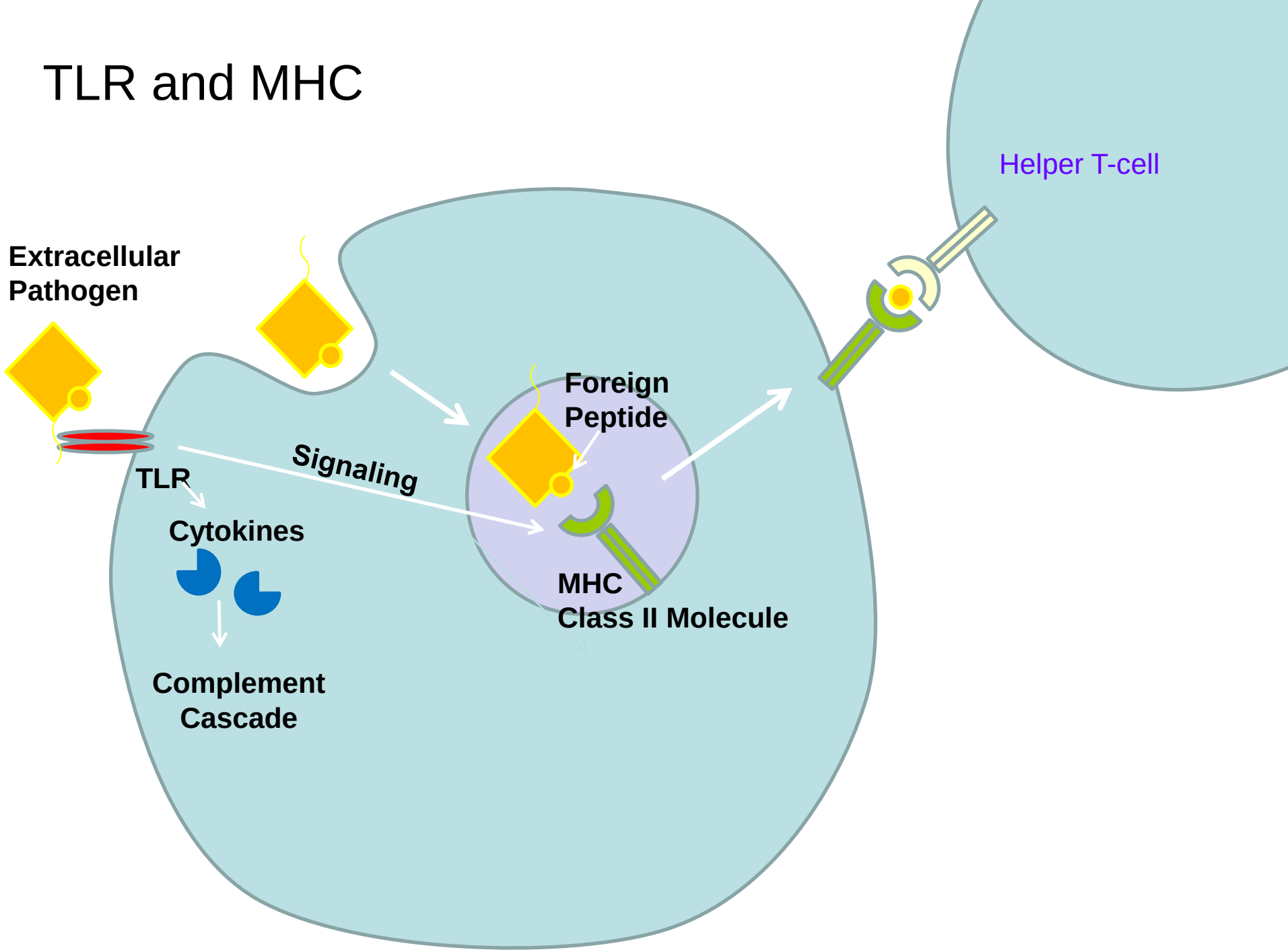
TLR and MHC

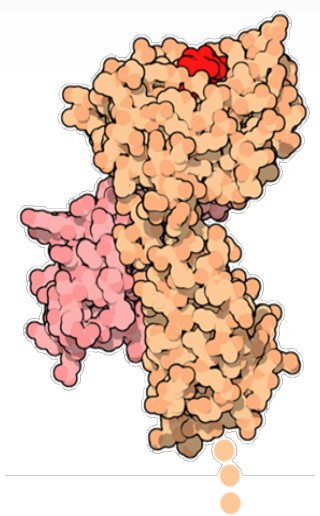

Helper T-cell
Extracellular
Pathogen
Foreign
Peptide
Signaling
TLR
Cytokines
MHC
Class II Molecule
Complement
Cascade

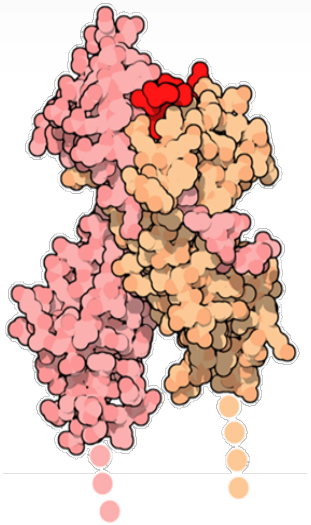
Immunity genes

Major histocompatibility complex (MHC)

- Encode cell-surface proteins responsible for presentation of foreign peptides
- Evidence that balancing selection plays a role in evolution
- Well-studied in molecular ecology of wild populations
- Technically challenging to work with in birds, especially passerines

Class I





Class II

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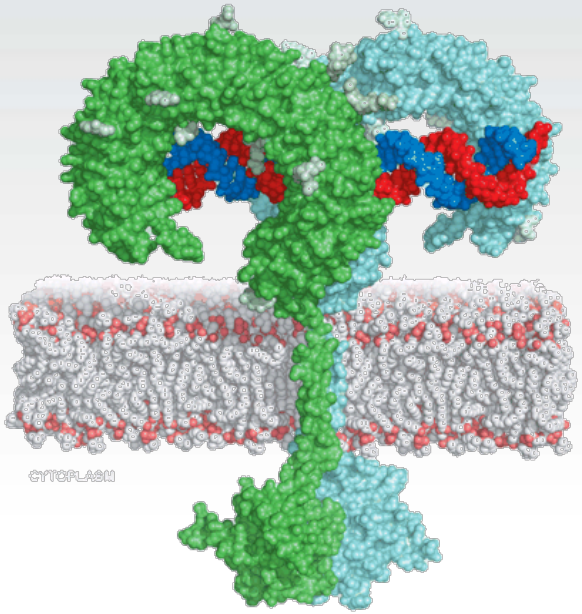
Immunity genes

Toll-like receptors (TLR)



- Ancient family of innate immunity receptors that bind pathogen-associated molecular patterns
- 10 TLRs in birds: diversity of pathogens
- Some evidence of a role of balancing selection
- Sequence variation → variation in immune function
- A way to diversify the genetic diversity toolkit

- Fewer technical challenges:
  - More conserved than MHC
  - Can be typed using Sanger sequencing or SNP-typing platforms
  - Standard statistics and diversity metrics can be calculated

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Diversity of 10 avian TLRs

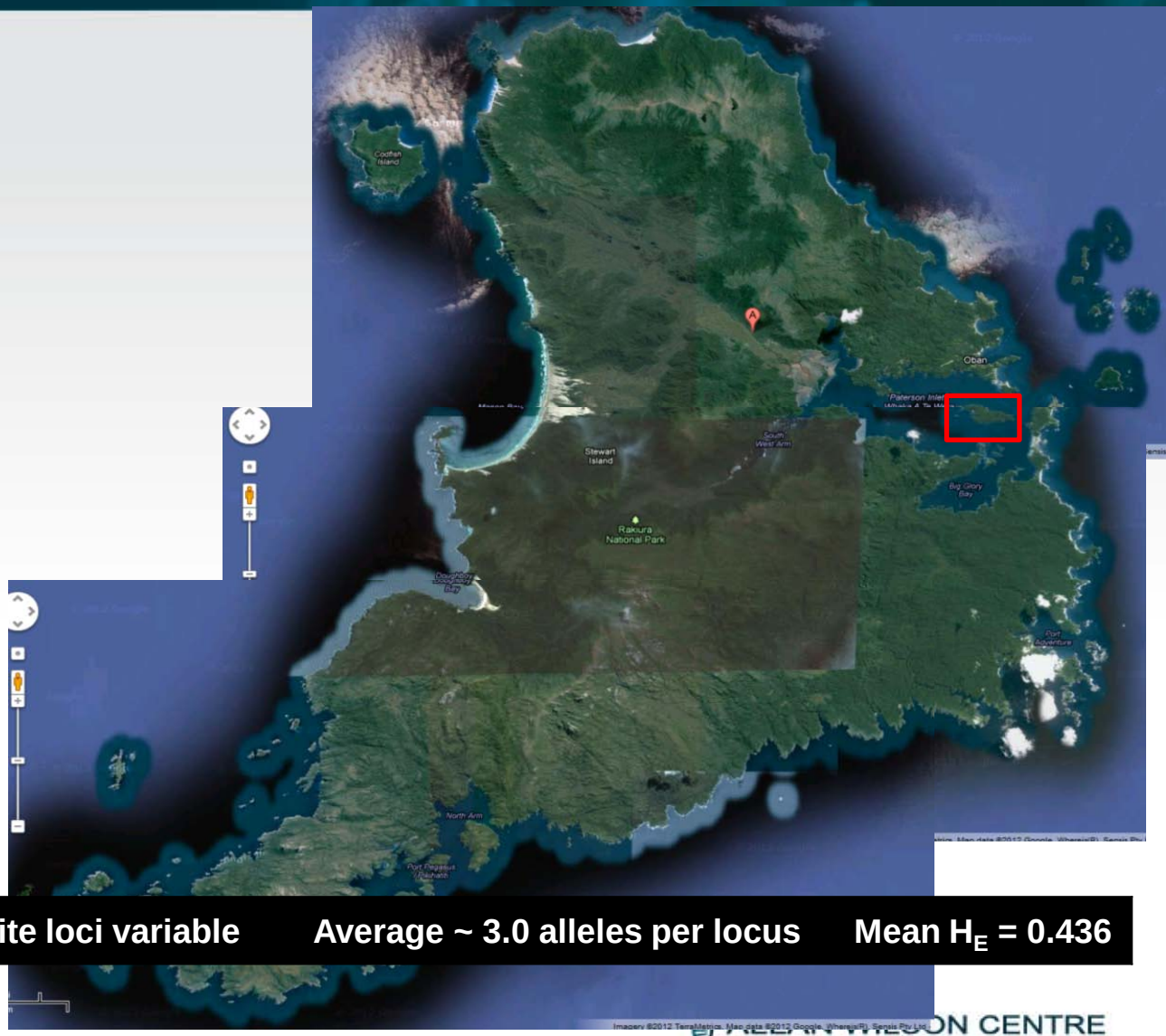


Locus	Presumed pathogen targets	House Finch Most N = 8	Lesser Kestrel Most N = 8	Stewart Is robin N = 12 founders
TLR1LA	Lipoprotein e.g. Mycoplasma	62 (N = 51)	11	2
TLR1LB		20 (N = 13)	15	2
TLR2A	Peptidoglycan of Gram+ bacteria Eukaryote fungi & other parasites?	8	5	1
TLR2B		11	3	2
TLR3	Viruses with dsRNA	9	2	1
TLR4	Lipopolysaccharide of Gram- bacteria, plus other things	14	7	5
TLR5	Flagellate bacteria	3	16	3
TLR7	Viruses with ssRNA e.g. avian influenza	15	4	> 2?
TLR15	Uncertain, Gram+ and Gram- bacteria?	16	14	2
TLR21	CpG motifs of bacterial and viral DNA (“functional homolog” of mammal TLR9)	2 (N = 4)	3	3
Mean		16.0	8.0	2.6 (poly)

Alcaide & Edwards *Mol. Biol. Evol.* (2011)

Grueber, Wallis, King & Jamieson *PLoS One* (2012)

TLRs and fitness: an example



Microsats: 53% of microsatellite loci variable Average ~ 3.0 alleles per locus Mean H_E = 0.436

Grueber, Wallis & Jamieson *Molecular Ecology* (in press)

TLRs and fitness: an example

Effects of TLR genotypes on survival

- Juvenile first-year survival is the robins' most vulnerable period
- Affected by inbreeding and microsatellite heterozygosity

- In general, TLR heterozygosity doesn't appear to be associated with survival
- Nor particular alleles – but what about TLR genotypes?

1. Base model is the effect of inbreeding coefficient (from pedigrees), and year of hatch, on juvenile survival.
2. Then, for the three TLRs with >2 alleles, make new models with each of the genotypes
3. Rank the models by AIC_C to compare them.

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Grueber, Wallis & Jamieson *Molecular Ecology* (in press)

TLRs and fitness: an example

TLR4 (5 alleles)

Model	β_{ij} (± SE_β)	AIC_C	ΔAIC_C	w_i	N_{ij}	$Survival_{ij}$
Base + BE	**1.007 (0.448)**	**667.6**	**-**	**0.511**	**60**	**90%**
Base + AA	0.463 (0.303)	671.4	3.82	0.076	95	84%
Base	-	672.4	4.79	0.047	645	78%
Base + BD	-0.443 (0.394)	672.7	5.14	0.039	34	71%
Base + BC	0.456 (0.456)	672.8	5.24	0.037	42	86%
Base + DD	-1.491 (1.437)	672.9	5.34	0.035	2	50%
Base + CE	-0.942 (0.932)	673.0	5.40	0.034	5	60%
Base + AC	-0.272 (0.278)	673.0	5.40	0.034	81	74%
Base + CC	-0.847 (0.889)	673.1	5.53	0.032	6	67%
Base + BB	-0.230 (0.283)	673.3	5.69	0.030	79	75%
Base + EE	-1.025 (1.449)	673.4	5.85	0.027	2	50%
Base + AD	-0.236 (0.352)	673.5	5.90	0.027	48	75%
Base + AE	-0.239 (0.428)	673.6	6.03	0.025	31	74%
Base + AB	-0.078 (0.234)	673.8	6.22	0.023	137	77%
Base + CD	-0.079 (0.589)	673.9	6.31	0.022	17	76%
Base + DE	-	-	-	-	6	100%

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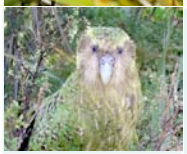
Grueber, Wallis & Jamieson *Molecular Ecology* (in press)

TLR diversity in threatened NZ birds

	1LA	1LB	2A	2B	3	4	5	7	15	21
	✓	✓			✓	✓	✓	✓	✓	✓
	✓	✓		✓	✓	✓	✓	✓	✓	
	✓			✓	✓	✓	✓	✓		
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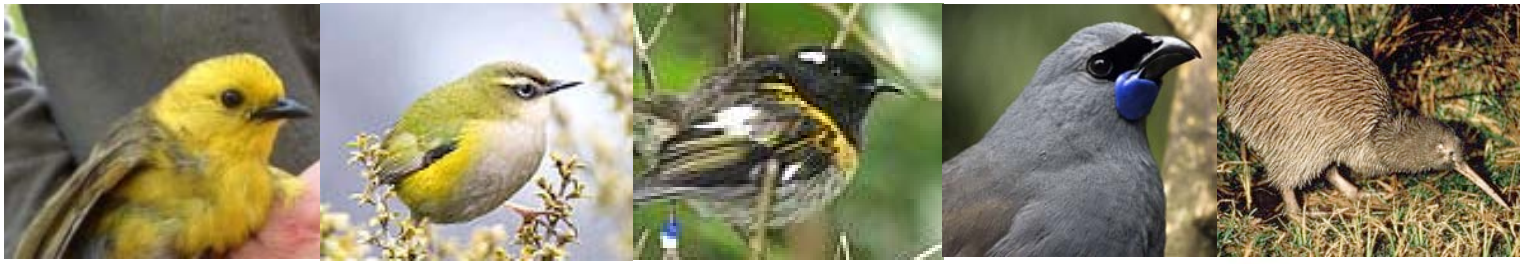

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Grueber & Jamieson *Conserv. Genet. Resour.* (in press)

Next steps

Can we learn more about how TLRs evolve? Phylogenetic approach.

Initial surveys of TLR diversity (modest sample sizes – more to come…)

- Takahe: 2/8 TLR loci variable
- Saddleback: 2/8 TLR loci variable
- Kakapo: 1/6 TLR loci variable
- Kakariki: 6/7 TLR loci variable

MHC work is underway (Gabby Knafler)

Can genetics aid in identification of kakariki that are susceptible to, or carriers of, beak and feather disease virus (BFDV)?

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Thank you

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Graham Wallis, Tania King, Stefanie Großer, Chris Harris, Sheena Townsend, Jolene Sutton