Reproductive isolation associated with the copper tolerance locus in *Mimulus guttatus*

Submitted by Deborah L. Lloyd to the University of Exeter
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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Acknowledgements

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Abstract

The evolution of reproductive isolating barriers that prevent gene flow between species is essential to the process of speciation. One such barrier is intrinsic postzygotic isolation, which proceeds as hybrid sterility or inviability, and is commonly attributed to Dobzhansky-Muller genic incompatibilities. Here, deleterious interlocus interactions occur between incompatible alleles of complementary genes when brought together in the genome of a hybrid. Although these hybrid incompatibilities are widespread, having been identified in mammals, fish, plants and fungi, still relatively little is known about the nature of the genes involved.

In the model plant species Mimulus, a Dobzhansky-Muller incompatibility exists between two populations of the yellow monkey flower, *Mimulus guttatus*, in which the interaction between a single gene from a copper tolerant population, Copperopolis, and a small number of polymorphic genes from a second non-tolerant population, Cerig-y-drudion, results in hybrid necrosis in the F_1. Hybrid necrosis, a form of hybrid inviability with phenotypic characteristics strongly similar to those of plants responding to pathogen attack, is a common barrier preventing hybridization in plants. As well as being of interest in terms of evolution, hybrid necrosis has practical implications in plant breeding as it prevents the combining of desirable traits from related species in commercial cultivars.

In the cross between Copperopolis and Cerig-y-drudion, copper tolerance, conferred by a single major gene, and hybrid necrosis are tightly linked but the independent or synonymous nature of the gene(s) in the Copperopolis population that contribute to these two characteristics is unknown.

A key aim of this thesis was to establish the nature of the single gene in Copperopolis that contributes to hybrid necrosis with regards to its linkage to copper tolerance. The gene for hybrid necrosis was found to be tightly linked to, but discrete from, the gene controlling copper tolerance. Three candidate genes for this hybrid necrosis locus were indentified: a Jumonji-domain containing protein with probable function as a methyltransferase, a glycosyltransferase and a possible phosphatase. Interestingly, the latter two have potential functional roles in the plant immune system.

The second key aim of this thesis was to perform the first investigation into the small number of genes in the Cerig-y-drudion population that contribute to the crossing barrier. Two QTLs for hybrid necrosis were identified. One QTL on Chromosome 9 is responsible for around 20% of the hybrid necrosis whilst the second QTL on Chromosome 12 acts as an enhancer of the first QTL causing an additional 10% of necrosis. Interestingly both these QTLs contain R genes, further implicating the possible involvement of the plant immune system in this crossing barrier.

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Author's Declaration

Aspects of this thesis are based on a collaborative project between Kevin Wright, a PhD student based at Duke University, North Carolina, and myself. Provided below is a summary of the individual contribution each of us to the research reported in this thesis. I certify that all writing and data analysis included in this thesis is my own work.

Work	Performed By
Chapter 3	
Growing plants	Deborah Lloyd
Phenotyping for copper tolerance	Deborah Lloyd
Tissue sampling	Deborah Lloyd
Identifying Cerigs to use in crosses	Deborah Lloyd
Conducting crosses	Deborah Lloyd
Phenotyping for hybrid necrosis	Deborah Lloyd
DNA Extraction	Kevin Wright
Genotyping to identify recombinants	Kevin Wright
Chapter 4	
Growing plants	Deborah Lloyd
Phenotyping for copper tolerance	Deborah Lloyd
Tissue sampling	Deborah Lloyd
Conducting crosses	Deborah Lloyd
Phenotyping for hybrid necrosis	Deborah Lloyd
DNA Extraction	Kevin Wright
Genotyping to identifying recombinants	Kevin Wright
Chapter 5	
Growing plants	Deborah Lloyd
Conducting crosses	Deborah Lloyd
Scoring for hybrid necrosis	Deborah Lloyd
Design of new primers	Deborah Lloyd
DNA extraction	Deborah Lloyd
Screening markers	Deborah Lloyd
Bioinformatics	Deborah Lloyd
Screening SNP markers	Kevin Wright

Work	Performed By
Chapter 6	
Growing plants	Deborah Lloyd
Conducting crosses	Deborah Lloyd
Phenotyping for hybrid necrosis	Deborah Lloyd
Tissue sampling	Deborah Lloyd
Designing primers	Deborah Lloyd
DNA extraction	Deborah Lloyd
Marker Genotyping in BSA	Deborah Lloyd
Marker Genotyping of linked markers in all F_2	Deborah Lloyd

Abbreviations

'A' plant	Plant 'A' used in crosses (bred by Professor Macnair, a copper non- tolerant plant with the SB genetic background)
A. thaliana	Arabidopsis thaliana
ANOVA	Analysis of Variance
Avr	Avirulence protein
bp	basepairs
BSA	Bulked Segregant Analysis
BSC	Biological Species Concept
Cerig/Cer	'Cerig-y-drudion'
Cf-	*Cladosporium fulvum* resistance gene
cM	Centimorgans
Cop	'Copperopolis'
'D' plant	Plant 'D' used in crosses (bred by Professor Macnair, a copper tolerant plant with the SB genetic background and a section of the Cop genome)
D. melanogaster	*Drosophila melanogaster*
D. simulans	*Drosophila simulans*
DM	Dobzhansky-Muller
DM1	*Dangerous Mix 1*
DM2	*Dangerous Mix 2*
EMBOSS	European Molecular Biology Open Software Suite
EST	Expressed Sequence Tag
F_1	First filial generation
F_2	Second filial generation
Hdb2	*Hybrid breakdown 2*
Hdb3	*Hybrid breakdown 3*
Hmr	*Hybrid male lethality*
HR	Hypersensitive Response
Hw (a/b/c/d/e/f/g/h)1	*Hybrid weakness gene (a* to *h)*
Hw (a/b/c/d/e/f/g/h)2	*Hybrid weakness gene (a* to *h)*
JGI	Joint Genome Institute
JmjC	Jumonji domain
kb	kilobases
KEGG	Kyoto Encyclopaedia of gene and genomes
KOG	Eukaryotic Orthologous Groups
L. esculentum	*Lycopersicon esculentum*

L. pimpinellifolium	*Lycopersicon pimpinellifoliu*
L. saligna	*Lactuca saligna*
LG	Linkage Group
Lhr	*Lethal hybrid rescue*
M. guttatus	*Mimulus guttatus*
Mhr	*Maternal hybrid rescue*
mRNA	messenger RNA
N. longiflora	*Nicotiana longiflora*
N. suaveolens	*Nicotiana suaveolens*
N. tabacum	*Nicotiana tabacum*
NBS-LRR	Nucleotide-binding Leucine-Rich-Repeat
NCBI	National Centre for Biotechnology Information
Ne1	*Necrosis gene 1*
Ne2	*Necrosis gene 2*
***NEC* (locus)**	*Hybrid necrosis locus*
NPC	Nuclear pore complex
NT	Copper non-tolerant
NTR	Copper non-tolerant recombinant
O. sativa	*Oryza sativa*
Ovd	*Overdrive*
P. syringae	*Pseudomonas syringae*
PANTHER	Protein Analysis Through Evolutionary Relationships
PCD	Programmed Cell Death
PCR	Polymerase Chain Reaction
PFAM	Protein Family Database
Prdm1	*PR domain containing 1*
QTL	Quantitative Trait Loci
***R* gene**	Disease Resistance Gene
RF	Recombination Frequency
RI	Reproductive Isolation
RIN4	RPM1 Interacting Protein 4
RPM1	Resistance to *Pseudomonas syringae PV Maculicola*
RPS2	Resistance to *Pseudomonas syringae* 2
S. cerevisiae	*Saccahromyces cerevisiae*
SA	Salicylic acid
SB	Stinson Beach
Sc_	Scaffold (of *Mimulus* genome sequence)

SGE	Selfish Genetic Element
SNP	Single Nucleotide Polymorphism
***T* (locus)**	Copper tolerance locus
T	Copper tolerant
TAIR	Arabidopsis Information Resource
TR	Copper tolerant recombinant
X. maculates	*Xiphophorus maculates*
Xmrk2	*Xiphophorus melanoma receptor tyrosine kinase*
Zhr	*Zygotic hybrid rescue*