Identification and Characterisation of Dominant Ivermectin Resistant Genes in *Caenorhabditis elegans.*

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Submitted: September, 1995

A thesis submitted for the degree of Doctor of Philosophy of the University of New England.

ACKNOWLEDGMENTS.

During the three and a half years in which this thesis, and the experimental work described within it, have been undertaken, many people have provided assistance, guidance, comments and moral support.

Firstly, I would like to thank Dr. Warwick Grant as my principal supervisor for expert guidance, comments and assistance. Special thanks must be awarded to Warwick for having the foresight to re-introduce *C. elegans* research to Australia, thus providing me with an opportunity to work with this noble creature. My other supervisors, Dr. Margaret Katz and Dr. Brian Cheetham have also provided me with helpful comments and advice over the period of my candidature and have provided invaluable help during thesis preparation.

Invaluable assistance was also provided by Dr. Leo LeJambre and Dr. Carl Johnson, who made important contributions to my understanding of parasitic nematodes and ivermectin resistance respectively. I would also like to thank all workers in the molecular parasitology laboratory, Warwick Grant, Leo LeJambre, Ian Lenane, Lisa Mascord, Gill Whitington and Michelle Wooster for helpful discussions, advice, technical help and comradeship.

Thanks also to the *C. elegans* research community for the support network that is provided for all workers in the field. Especially I would like to thank Drs L. Avery, D. Baillie, A. Coulson, R. Herman, J. Hodgkin, C. Johnson, D. Riddle, T Stiernagle and J. Thomas for helpful communications with myself (or with Warwick on my behalf) and/or for supplying strains.

I also acknowledge the support of CSIRO Division of Animal Health, the Australian Wool Corporation and the University of New England, who provided facilities and financial support.

Finally I would like to thank my friends and family for being understanding and supportive throughout my PhD, and especially my wife Raelene who has endured the last three and a half years, providing sympathy, encouragement and love.

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ABSTRACT.

Ivermectin (IVM) is a potent anthelmintic which is lethal to *Caenorhabditis elegans* when included in nutrient agar growth medium at concentrations above 2 ng/mL. Recessive mutations imparting resistance to 5 ng/mL are obtainable in "1 out of 204 mutagenised genomes" (0.05 M ethyl methanesulfonate) and arise spontaneously at $1.7x10^{-5}$ (Kim and Johnson, 1991). These "low-level" resistance mutations (resistant to 5-30 ng/mL IVM) occur at about thirty loci (Kim and Johnson, 1991). Dominant IVM resistant alleles have been observed under field conditions in *Haemonchus contortus* and these are resistant to low levels of IVM in *in vitro* larval development assays (Lacey, Redwin *et. al.*, 1990; Martin and Turney, 1992; LeJambre, 1993; LeJambre, Gill et *al.*, in press). *C. elegans* was used as a model to study IVM resistance in parasitic nematodes. To identify loci at which dominant IVM resistance alleles occur, a *C. elegans* mutagenesis screen was undertaken to obtain dominant IVM resistance (5 ng/mL) alleles. The rate of mutagenesis for dominant IVM resistance was found to be $7.9 x10^{-7}$, indicating that these alleles are much rarer than recessive alleles.

Characterisation of the dominant IVM resistance alleles revealed that they have varying levels of IVM resistance as homozygotes and heterozygotes. Three of four alleles isolated (nr272, nr2389 & nr2477) are incompletely dominant for IVM resistance and the IVM lethal dose for heterozygotes and homozygotes of the fourth allele (nr2344) were identical. IVM resistance of heterozygotes was greatest when the resistance allele was inherited paternally for nr272 and nr2389 and heterozygote resistance was greatest when the resistance allele was inherited maternally for nr2477. Finally, IVM resistance is observed in nr2389/+ hermaphrodites but not in nr2389/+ males (*ie.* the dominance is sex limited).

The four dominant IVM resistant alleles have a Dyf (dye filling defective) phenotype as homozygotes, indicating that the mutations may cause structural or functional abnormalities in the amphids. The Dyf phenotype is recessive for nr2389 and incompletely dominant for nr272, nr2344 and nr2477; the number of Dyf heterozygotes is low and the staining in the remainder is fainter then for wild type worms. Subsequent to these findings a large number of previously characterised mutant strains were analysed for IVM resistance, and it was found that mutations at 27 loci which are Dyf are also resistant to 5 ng/mL IVM. Alleles of three *unc* loci also are IVM resistant and have faint amphid staining. nr2389 fails to complement the Dyf phenotype of *dyf-10*(e1387) and nr272, nr2344 and nr2477 fail to complement the Dyf phenotype of one another and *dyf-12*(sa127). Three factor interval mapping using the IVM resistance phenotype placed nr2389 on linkage group I near *dyf-10* and nr272, nr2344 and nr2477 on linkage group X near *dyf-12*. The complementation and mapping data support nr2389 being an allele of *dyf-10* and nr272, nr2344 and nr2477 being alleles of *dyf-12*. The dominant IVM resistant alleles of *dyf-10* and *dyf-12* isolated here fail to complement one another for the Dyf phenotype, which suggests an interaction between the two genes in amphid neuron formation or function.

Some mutations which impart resistance to very low levels of IVM (between 2 and 5 ng/mL) have also been identified, these mutations have Tax, Bli, Notch or Rounded nose phenotypes and may result in slight alterations to amphid morphology or function. A group of mutations which are supersensitive to IVM were also identified, most notable among these were alleles of six *eat* loci. The super sensitivity of Eat mutants, along with the findings of a number of other studies implicate the pharynx as a major site of action of IVM. *unc-104*(e1265) suppresses IVM resistance (but not Dyf) in a range of Dyf strains (this study) and *unc-116*(e2310), *snt-1*(md290) and *snt-1*(md325) also suppress IVM resistance (but not Dyf) in some Dyf strains (Grant Pers. Comm., 1995). These mutations which suppress IVM resistance in Dyf worms are involved in synapse function, suggesting that proper synapse function is necessary for IVM resistance.

Information provided by the experiments described here and from published studies of the mode of action of IVM, suggest a mechanism for IVM resistance in *dyf* mutants of *C. elegans*. I propose that IVM resistance in *C. elegans* is dependent on a

constitutive neuronal (or humoral) signal from the amphids which stimulates the pharynx decreasing the pharyngeal response to IVM. This hypothesis can be readily tested in the laboratory, and a number of experiments are discussed which might reveal more about amphid-mediated control of the pharynx and IVM resistance in Dyf worms.

AIMS

1. To isolate and characterise mutants carrying dominant ivermectin resistant mutations in *Caenorhabditis elegans* comparable to dominant IVM resistant mutations in *Haemonchus contortus*.

2. To investigate the nature of ivermectin resistance in *Caenorhabditis elegans*.