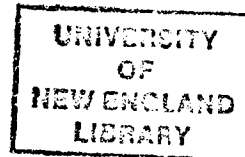


Identification and Characterisation of Dominant Ivermectin  
Resistant Genes in *Caenorhabditis elegans*.

Peter William Hunt B. Rur. Sci. (Hons)  
(University of New England).



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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  $1.7 \times 10^{-5}$  (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  $7.9 \times 10^{-7}$ , 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 than 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*.