The role of Cytochrome P450s towards the control of ticks and other arthropods

Kirsty Graham, Robert Finn, Seth Racey and Olivier Sparagano

Northumbria University, School of Life Sciences, Department of Biomedical Sciences, BioReC Research Centre, Newcastle Upon Tyne, NE1 8ST, UK

Pesticide resistance is not a new problem but it is increasing in severity as more and more arthropods that are of economic, medical and agricultural importance (such as ticks, vectors of many diseases) are developing resistance. In particular over the last 10 years, there is an increasing amount of research that shows ticks species are becoming increasingly resistant to acaricides, (Li *et al.*, 2003; Miller, Davey & George, 2005; Barre *et al.*, 2008). The pesticides that are most commonly used throughout the world are commonly made from the following, organophosphates, dieldrin (used more now instead of DDT) or pyrethroids. Although research shows that ticks most notably *Rhipicephalus microplus* (also known as *Boophilus microplus*) are becoming increasingly resistant, the mechanisms behind this resistance have not been explored in any great detail with the most common method of research being synergism studies, (Li *et al.*, 2003) and those looking solely at the mortality rates of populations within the laboratory, (Miller *et al.*, 2007). Research into pesticide resistance however is often broken down into the 3 main mechanisms of detoxification involving 3 groups of enzymes. These 3 main groups of detoxification mechanisms (enzymes) are the Cytochrome P450s (monooxygenases), Glutathione-S-transferase's (GST) or Esterase's (ESTor carboxylesterases) (Hemingway *et al.*, 2004) and these mechanisms are known to be similar amongst all taxa (Brogdon & McAllister, 1998). This is beneficial when researching an area that has little current research available such as CYPs in tick species as established and similar research techniques can be used to provide a greater insight.

Research into resistance mechanisms has previously focused on these ESTs and GSTs until recently. It has been acknowledged for some years that CYPs are important in terms of resistance development in insects but little research has focused on these until quite recently as interests have been directed at ESTs and GSTs not just in insects but in tick and mite research as well.

Cytochrome P450 monooxygenases (CYP or P450) are a highly diverse group of proteins that are found in all the forms of life. Due to the fact that the P450s are a hugely diverse superfamily, a variety of functions are attributed to them most notably, detoxification. In humans, P450s are involved in hormone synthesis and breakdown, cholesterol synthesis, and the metabolism of xenobiotics and in arthropods, they are also important in ecdysis. They get the name cytochrome P450 monooxygenases because this is the most common reaction that they take part in (monooxygenase reaction). Most CYPs are hydrophobic but they all contain a heme-binding region and this is characteristic for all P450s in all organisms (Hemingway *et al.*, 2004).

Research suggests that resistance is evolving because of overexpression of genes due to mutations and in some instances, gene duplications have been reported which all helps in the resistance to pesticides (Kwon, Clark & Lee, 2010).

Materials & Methods

To date the most common methods of studying CYPs is to identify novel P450s. This is most commonly done starting with degenerate primers to identify a novel CYP using conserved regions such as the heme-binding region. Numerous cytochromes have been identified in arthropods and many of them have been characterised in some detail to link them with pesticide resistance. In particular there has been a lot of focus on mosquito species such as *Culex spp, Aedes spp* and *Anopheles spp*.

In terms of tick and mite pesticide resistance, research to date has focused on identifying which acaricides are becoming redundant in terms of effectiveness to kill the organism. Much of this research uses mortality based experimental procedures with little work looking at the molecular/enzymatic basis of resistance (Li *et al.*, 2003) and (Miller, Davey & George, 2005). That research that has looked at the molecular basis of resistance has focused more on GSTs and ESTs in ticks and mites.

Another possible method of investigation of CYPs in ticks and mites may be to use tick cell cultures. These could provide an ideal way to monitor CYP expression before, during and after treatments such as pesticides. Moreover, the cell cultures could be used to identify what else has an effect on the expression of P450s for example, viral or bacterial infection, again before, during and after this treatment. In *Anopheles gambiae*, expression levels of CYPs, GSTs and ESTs were monitored using a microarray following infection of the mosquito by *Plasmodium (Felix et al., 2010).* Numerous genes were overexpressed including CYP6M2 that is associated with xenobiotic detoxification in the same species (Djouaka *et al.*, 2008).

Table 1: Table showing the Cytochrome P450 genes identified in ticks and mites as well as methods used and primers if given.

Species	Gene Identified/Studied	Methods Used	Primers Used	
			Forward	Reverse
Boophilus microplus	CYP4W1	Phylogenetics, no expression data		
Boophilus microplus	CYP41	Phylogenetics, no sequence data		
Boophilus microplus	CYP319A1	Semi quantitative PCR showed no difference in expression levels	ACGAATCACCGACC GAATCAAGGAC	GAATCGCAGACACGTTCTT TTGG
Boophilus microplus	'Cytochrome P450'	Serial analysis of gene expression (SAGE) followed by relative quantitative RT-PCR	AACAAAGGTTCCCCTCGA GT	CAGATAACCAACACCAGCA CA
Amblyseius womersleyi	CYP4	RT-PCR of 4 groups of CYP4 proteins, a, b, c and d. CYP4-d showed greater expression after acaricide treatment.	a –GAAGTTGATACCTTGA TGTTCG b –AATCGCCTGGTCCCTG TATCA c –GGCGTTGACATATGTT CTGTAC d – TGGCTCAGAACCCCG AAGCTCA	a – CCTATACAGTTTCTGG GTCC b – CGGAATGTAAGAGTAA GGATGC c - GGATTCCTGTTCTTCGA CTGC d –ATGCAGTTCCTCGGGC CAGCCGAG

Table 1 shows the Cytochrome P450 genes that have been identified and studied in ticks and mites. It illustrates the gaps in the research and shows that there is more to be discovered in these arthropods. Some of the primers are given for those genes that have been studied using (relative quantitative) RT-PCR as well as semi quantitative PCR (He *et al.*, 2002; Guerrero *et al.*, 2007; Sato, Tanaka & Miyata, 2007).

Results

In 1998 the insect P450s could be assigned to six CYP families. Five of these were insect-specific (CYPs 6, 9, 12, 18 and 28) and one CYP4 which was shared with sequences from other organisms (Berge, Feyereisen & Amichot, 1998). However as of August 2009, it was reported that there were a total of 3282 CYPs in the Animalia kingdom, in approximately 253 species. 1675 of these have been identified in the insects and 1607 are not insect sequences. Of these 1675 insect CYPs, they are split across 59 families and 338 subfamilies (Nelson, 2009), a dramatic increase from the 6 CYP families described in 1998 (Berge, Feyereisen & Amichot, 1998). Interestingly, these figures are from only 89 species of insects so there is great scope to find and characterise more CYPs, not just in insects but in other arthropods. This shows how as more information becomes available, due to the likes

of more genome sequencing, knowledge on CYPs is improving however there is a great deal of scope for research in CYPs in ticks and mites as well as many other arthropods.

To date, there are 3 key papers published that have identified and isolated CYP genes in the agriculturally important cattle tick, *Boophilus microplus*. (Crampton, Baxter & Barker, 1999b) identified the first known CYP gene from any arachnid species. The gene was identified as being from the CYP4 family and so was given the name CYP4W1. This is a great step towards further identification of other genes both within this family as well as within other CYP families. Subsequently, (Crampton, Baxter & Barker, 1999a), published a second paper in this year identifying a CYP gene in a different family, CYP41. This was shown to be similar to CYP3 but due to low homology, a new gene family was created, CYP41. This gene is related to the members of the CYP3 family which suggests that this CYP might be important for detoxification however no further experiments were done to confirm or reject this hypothesis. Most recently, (He *et al.*, 2002) identified and isolated another CYP gene CYP319A1, although there is uncertainty as to whether this gene is involved in resistance to pesticides due to expression levels being consistent across different strains of tick used when exposed to levels of organophosphates and pyrethroids.

Discussion & Conclusions

Among the insects, research strongly suggests that the CYP6 (Hemingway *et al.*, 2004) and CYP9 family are most highly associated with xenobiotic resistance and a lot of research has been carried out looking at these CYP families in various insect species. In addition to this, a lot of phylogenetic evidence suggests that the CYP6 and CYP9 families are closely related to the CYP3 family in humans (Feyereisen, 2006). This is an important association as the CYP3 clade in humans has some of the most important CYP genes in terms of xenobiotic metabolism including the CYP3A subfamily of genes. However, to date there has been little research carried out on members of these families in ticks or mites despite the information available in other arthropods. For example, a lot of research is available to show that mosquito species are becoming resistant to pesticides such as pyrethrine due to their P450 enzymes evolving to detoxify these chemicals at increasing concentrations (Nikou, Ranson & Hemingway, 2003) showed that a CYP6 gene (CYP6Z1) is overexpressed in a pyrethroid-resistant strain of *A. gambiae*. The overexpression was found not to be due to gene duplication but rather a mutation that in turn confers increased resistance. Unlike the 2 duplicated P450 genes that were found to be associated with insecticide resistance in the mosquito *An. funestus*, (Wondji *et al.*, 2009). This is important as it shows the significance of identifying the CYPs as well as understanding how they are affected so they can be monitored in populations, for example to identify future mutations.

As more CYPs are being identified, several papers have been written that use microarray technology to look at the expression levels of numerous genes from the same (or similar species as is often the case for mosquitoes). This is useful as numerous genes can be investigated and compared at the same time whilst comparing different susceptible and resistant strains (David *et al.*, 2005)

Also, as technology improves, the shape of the CYP protein can be identified and this in turn can be used to ascertain whether particular pesticide molecules could in theory be detoxified as they would fit into the active site. This has been shown used when looking at closely related genes such as CYP6Z1 and CYP6Z2 (Chiu *et al.*, 2008) This is useful for novel CYPs that are not characterised and also if little is known about the population or pesticides that the organism has been in contact with.

The three key papers (Crampton, Baxter & Barker, 1999a; Crampton, Baxter & Barker, 1999b; He *et al.*, 2002), illustrate the need to identify more novel CYPs in ticks and mites, ideally from the CYP6 or CYP9 families but also the need to characterise these identified CYPs in more detail. Determine if the CYP is overexpressed for example when the tick is subjected to high concentrations of pesticides. In addition to this, as stated above, the use of tick cell cultures is to be considered as this may provide more information allowing more detailed characterisation of cytochromes.

The work carried out by Crampton *et al.,* (1999) on the new CYP family, CYP41, has some interesting results. This new family of CYPs was created due to the lack of homology between this new family (CYP41) and currently known CYP families. This new CYP is closest to CYP3A2, however there is only 36% homology, which is low when considering that this is the closest of the CYPs. Therefore, it is little surprise the phylogeny of the CYP family CYP41 could not be resolved. One of the problems when looking at tick and mite P450s is the lack of published work on this subject and the lack of information available on databases such as NCBI. However thanks to the sequencing of *Ixodes scapularis* genome, the identification and subsequent characterisation of novel genes should become easier and more enhanced.

Our group is now monitoring cytochrome P450 expression levels after tick-cell lines have been exposed to pathogens or chemicals. We also plan to work on phylogenetic links for those genes between different arthropod groups.

References

Barre, N., Li, A. Y., Miller, R. J., Gaia, H., Delathiere, J. M., Davey, R. B. & George, J. E. (2008) 'In vitro and in vivo evaluation of deltamethrin and amitraz mixtures for the control of Rhipicephalus (Boophilus) microplus (Acari : Ixodidae) in New Caledonia', *Veterinary Parasitology,* 155 (1-2), pp. 110-119.

Berge, J. B., Feyereisen, R. & Amichot, M. (1998) 'Cytochrome P450 monooxygenases and insecticide resistance in insects', *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 353 (1376), pp. 1701-1705.

Brogdon, W. G. & McAllister, J. C. (1998) 'Insecticide resistance and vector control', *Emerging Infectious Diseases,* 4 (4), pp. 605-613.

Chiu, T. L., Wen, Z. M., Rupasinghe, S. G. & Schuler, M. A. (2008) 'Comparative molecular modeling of Anopheles gambiae CYP6Z1, a mosquito P450 capable of metabolizing DDT', *Proceedings of the National Academy of Sciences of the United States of America,* 105 (26), pp. 8855-8860.

Crampton, A. L., Baxter, C. D. & Barker, S. C. (1999a) 'A new family of cytochrome P450 genes (CYP41) from the cattle tick, Boophilus microplus', *Insect Biochemistry and Molecular Biology,* 29 (9), pp. 829-834.

Crampton, A. L., Baxter, G. D. & Barker, S. C. (1999b) 'Identification and characterisation of a cytochrome P450 gene and processed pseudogene from an arachnid: the cattle tick, Boophilus microplus', *Insect Biochemistry and Molecular Biology,* 29 (4), pp. 377-384.

David, J. P., Strode, C., Vontas, J., Nikou, D., Vaughan, A., Pignatelli, P. M., Louis, C., Hemingway, J. & Ranson, H. (2005) 'The Anopheles gambiae detoxification chip: A highly specific microarray to study metabolic-based insecticide resistance in malaria vectors', *Proceedings of the National Academy of Sciences of the United States of America,* 102 (11), pp. 4080-4084.

Djouaka, R. F., Bakare, A. A., Coulibaly, O. N., Akogbeto, M. C., Ranson, H., Hemingway, J. & Strode, C. (2008) 'Expression of the cytochrome P450s, CYP6P3 and CYP6M2 are significantly elevated in multiple pyrethroid resistant populations of Anopheles gambiae s.s. from Southern Benin and Nigeria', *Bmc Genomics,* 9, pp. -.

Felix, R. C., Muller, P., Ribeiro, V., Ranson, H. & Silveira, H. (2010) 'Plasmodium infection alters Anopheles gambiae detoxification gene expression', *Bmc Genomics,* 11, pp. -.

Guerrero, F. D., Bendele, K. G., Chen, A. C., Li, A. Y., Miller, R. J., Pleasance, E., Varhol, R., Rousseau, M. E. & Nene, V. M. (2007) 'Serial analysis of gene expression in the southern cattle tick following acaricide treatment of larvae from organophosphate resistant and susceptible strains', *Insect Molecular Biology,* 16 (1), pp. 49-60.

He, H., Chen, A. C., Davey, R. B. & Ivie, G. W. (2002) 'Molecular cloning and nucleotide sequence of a new P450 gene, CYP319A1, from the cattle tick, Boophilus microplus', *Insect Biochemistry and Molecular Biology,* 32 (3), pp. 303-309.

Hemingway, J., Hawkes, N. J., McCarroll, L. & Ranson, H. (2004) 'The molecular basis of insecticide resistance in mosquitoes', *Insect Biochemistry and Molecular Biology,* 34 (7), pp. 653-665.

Kwon, D. H., Clark, J. M. & Lee, S. H. (2010) 'Extensive gene duplication of acetylcholinesterase associated with organophosphate resistance in the two-spotted spider mite', *Insect Molecular Biology,* 19 (2), pp. 195-204.

Li, A. Y., Davey, R. B., Miller, R. J. & George, J. E. (2003) 'Resistance to Coumaphos and Diazinon in Boophilus microplus (Acari : Ixodidae) and evidence for the involvement of an oxidative detoxification mechanism', *Journal of Medical Entomology,* 40 (4), pp. 482-490.

Miller, R. J., Davey, R. B. & George, J. E. (2005) 'First report of organophosphate-resistant Boophilus microplus (Acari : Ixodidae) within the United States', *Journal of Medical Entomology,* 42 (5), pp. 912-917.

Miller, R. J., Rentaria, J. A. E., Martinez, H. Q. & George, J. E. (2007) 'Characterization of permethrin-resistant Boophilus microplus (Acari : Ixodidae) collected from the state of coahuila, Mexico', *Journal of Medical Entomology,* 44 (5), pp. 895-897.

Nelson, D. R. (2009) *The Cytochrome P450 Homepage. Human Genomics 4, 59-65.* [Online]. Available at: http://drnelson.uthsc.edu/cytochromep450.html (Accessed: Last accessed 11/04/2011).

Nikou, D., Ranson, H. & Hemingway, J. (2003) 'An adult-specific CYP6P450 gene is overexpressed in a pyrethroid-resistant strain of the malaria vector, Anopheles gambiae', *Gene,* 318, pp. 91-102.

Sato, M. E., Tanaka, T. & Miyata, T. (2007) 'A cytochrome P450 gene involved in methidathion resistance in Amblyseius womersleyi Schicha (Acari : Phytoseiidae)', *Pesticide Biochemistry and Physiology,* 88 (3), pp. 337-345.

Wondji, C. S., Irving, H., Morgan, J., Lobo, N. F., Collins, F. H., Hunt, R. H., Coetzee, M., Hemingway, J. & Ranson, H. (2009) 'Two duplicated P450 genes are associated with pyrethroid resistance in Anopheles funestus, a major malaria vector', *Genome Research,* 19 (3), pp. 452-459.