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Leigh Alden Nelson
University of Wollongong

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The molecular identification and thermal
attributes of forensically important blowflies
(Diptera: Calliphoridae: *Chrysomya*)

A thesis submitted in fulfilment of the requirements for the award of the degree of

Doctor of Philosophy

from

University of Wollongong

by

Leigh Alden Nelson

BBiotech (Hons), GradCertBus

School of Biological Sciences

2008



Declaration

I, Leigh Alden Nelson, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced and acknowledged. This document has not been submitted for qualifications at any other academic institution.

Leigh Alden Nelson

November, 2008

The fly heeds not death; eating is all to him.

African proverb



The forensically important *Chrysomya rufifacies* female (left) and male (right) on pig carrion.
Photo: L.A. Nelson

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Abstract

Forensic entomology applies the study of arthropods associated with carrion, in terms of species succession and development rates, to determine the minimum time since death, or postmortem interval (PMI). Correct species identification is crucial, as the rate of larval development can vary substantially between species. The identification of forensically important blowflies of the genus *Chrysomya* (Diptera: Calliphoridae) may be hampered by their close morphological similarities, especially as immatures. DNA-based approaches, such as those investigated here, have the capacity to be useful for the identification of forensic entomological evidence in cases where morphological characters are unreliable.

In this study, two DNA regions were investigated as potential candidates for the identification of the nine *Chrysomya* species in Australia: (1) the second internal transcribed spacer (ITS2) of ribosomal DNA (rDNA) and (2) the cytochrome oxidase I (COI) DNA ‘barcode’ region. The ITS2 region was assessed by sequence comparison, which identified five restriction enzymes (DraI, BsaXI, BciVI, AseI and HinfI) that were able to differentiate most members of the genus by polymerase chain reaction (PCR) restriction fragment length polymorphism (PCR-RFLP). The closely related species pairs *Chrysomya latifrons* + *Chrysomya semimetallica* and *Chrysomya incisuralis* + *Chrysomya rufifacies* could not be separated by restriction profile analysis, but the latter could be separated using the size differences resulting from amplification of the entire ITS region. Identical restriction profiles were generated from eight *Ch. incisuralis* specimens, suggesting low intraspecific ITS2 variation within this species. Phylogenetic analysis of the ITS2 sequence, which is a possible means by which species could be identified, proved successful for the identification of the majority of *Chrysomya* species.

The COI ‘barcode’ region resolved all nine *Chrysomya* species as reciprocally monophyletic, following a neighbour-joining (NJ) analysis of the Kimura two-parameter distances. Mean intraspecific and interspecific sequence divergences were 0.097% and 6.499%, respectively. The hybrid status of one specimen was confirmed

following subsequent ITS2 sequence analysis. In another instance, this nuclear region was used to verify four cases of specimen misidentification that had been highlighted by the COI analysis. The COI DNA barcode was successful in identifying *Chrysomya* species from the east coast of Australia. The ability of the barcode to identify two *Chrysomya nigripes* specimens from Thailand shows potential for this method to be expanded to other blowfly genera and continents. This result confirmed previous successes with COI as a genetic barcode for species identification and comparisons at the intra- and interspecies levels.

Together with correct species identification, thermodevelopment data of blowfly species are vital for the estimation of the PMI. The close morphological and molecular similarities among *Chrysomya* species led to speculation as to whether members of this genus shared similar developmental profiles. The aim was to establish whether genetically closely related species would share similar developmental profiles. This would permit the application of developmental data to a number of closely related species, including those for which thermodevelopmental studies are lacking. If Australian *Chrysomya* were found to share developmental profiles, identification of the blowfly specimen to a level beyond genus may not be necessary, or at least it may not be necessary to distinguish morphologically similar sister species. The experimental design employed in this study sets it apart, to date, from other published larval development studies. Nowhere else have the developments of such closely related blowfly species been compared. As the species were collected from the same geographical location, the effects of acclimation and population-level genetic variation were not variables in this study. The experimental conditions in this study were virtually identical, which enabled direct comparisons to be made among the species. This study established that the sister species *Ch. megacephala* and *Ch. saffrana* differed significantly in their developmental profiles, as well as compared with the more distantly related *Ch. rufifacies*. Because of this, genetic distance was not considered to be a useful factor for predicting thermodevelopment profiles of closely related species within a genus, and highlighted the necessity for correct species identification.

List of Abbreviations

%	percent
±	plus or minus
ACT	Australian Capital Territory
ANOVA	analysis of variance
approx.	approximately
<i>C.</i>	<i>Calliphora</i>
<i>Ch.</i>	<i>Chrysomya</i>
cm	centimetre
COI	cytochrome oxidase subunit I
COII	cytochrome oxidase subunit II
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
E	east
e.g.	for example
EDTA	ethylenediaminetetraacetic acid
EtOH	ethanol
g	gram
GTR + I + Γ	general time reversible model with some sites assumed to be invariable and with variable sites assumed to follow a discrete gamma distribution
GTR	general time-reversible model
h	hour
i.e.	that is
ITS	internal transcribed spacer
kb	kilobase
km	kilometre
L	litre
ml	millilitre
mM	millimolar

mtDNA	mitochondrial DNA
N	number
ng	nanogram
NSW	New South Wales
°C	degrees Celcius
P	probability
PCR	polymerase chain reaction
PEG	polyethylene glycol
pers. comm.	personal communication
<i>Pfu</i>	<i>Pyrococcus furiosus</i>
PMI	post-mortem interval
Qld	Queensland
rDNA	ribosomal DNA
S	south
SE	standard error
sp.	Species (singular)
spp.	Species (plural)
UV	ultraviolet
V	Volts
Vic.	Victoria
vs.	versus
V/V	on a volume per volume basis
µl	microlitre
µM	micromolar

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Female *Chrysomya rufifacies* resting on foliage above carrion.
Photo: L.A. Nelson