Genetic diversity of the dengue vector *Aedes aegypti* in Australia and implications for future surveillance and mainland incursion monitoring

*Nigel W Beebe,*1 *Peter I Whelan,*2 *Andrew van den Hurk,*3 *Scott A Ritchie,*4 *Robert D Cooper*5

**Abstract**

In February 2004, the discovery of an incursion of the dengue vector *Aedes aegypti* into the town of Tennant Creek in the Northern Territory caused concern for the Northern Territory health authorities who proceeded to implement a Commonwealth-funded eradication program. To determine the origin of the incursion, we performed a genetic analysis on *Ae*. *aegypti* from several Queensland and overseas localities. A comparison of DNA sequences from the mitochondrial cytochrome oxidase 1 gene indicated that the incursion was probably from Cairns or Camooweal. This genetic marker was also useful in identifying a separate Townsville haplotype population and another population on Thursday Island in the Torres Strait that was genetically divergent to the mainland populations. The possible use of this marker as a surveillance tool for identifying the origins of local and overseas incursions is discussed. *Commun Dis Intell* 2005;29:299–304.

**Keywords:** *Aedes aegypti*, mtDNA, cytochrome oxidase 1 gene, dengue, surveillance

**Introduction**

*Aedes aegypti* is the primary vector of dengue virus. It is the only dengue vector in mainland Australia and has been responsible for outbreaks of dengue fever that reappeared in northern Queensland in the early 1980s and have continued until the present.1,2 Historically, the distribution of *Ae*. *aegypti* included all mainland states and territories except Victoria and South Australia. However, in the 1950s it disappeared from Western Australia, New South Wales and the Northern Territory.3 It maintains a strong hold in Queensland where its southern limit is Dirranbandi to Roma and west to Cloncurry and Mount Isa.4 In February 2004, specimens of *Ae*. *aegypti* were identified in Tennant Creek in the Northern Territory.5 This town is located on the main road links to Queensland (via the Barkly Highway) and Darwin (via the Stuart Highway) and is 670 km from Mount Isa—the nearest previously known source of *Ae*. *aegypti*.

Apart from the potential for this species to spread from Queensland into other states or territories, there is the continual threat of its introduction to Australia from overseas via international ports. Darwin alone had 13 importations of *Ae*. *aegypti* between 1998–2000,6 and there have been numerous other detections by the Australian Quarantine Inspection Service (AQIS) since then, including the recent detection of an importation in February 2005 from an Indonesian fishing vessel (Whelan, unpublished data). *Aedes aegypti* is a competent traveller with three attributes that contribute to its dispersal: 1) it has a very close association with humans; 2) it readily breeds in artificial receptacles; and 3) its eggs can withstand desiccation for many months.

1. Senior Research Fellow, Institute for the Biotechnology of Infectious Diseases, University of Technology Sydney, St Leonards, New South Wales
2. Senior Medical Entomologist, Medical Entomology, Centre for Disease Control, Department of Health and Community Services, Northern Territory
3. NHMRC Research Officer, Department of Microbiology and Parasitology, School of Molecular and Microbial Sciences, University of Queensland, Brisbane, Queensland
4. Senior Medical Entomologist, Tropical Public Health Unit, Queensland Health, Cairns, Queensland
5. Senior Entomologist, Australian Army Malaria Institute, Enoggera, Queensland

Corresponding author: Dr Nigel Beebe, Institute for the Biotechnology of Infectious Diseases, University of Technology Sydney, Westbourne Street, St Leonards, NSW 2065. Telephone: +61 2 9514 4127. Facsimile: +61 2 9514 4003.

Email: Nigel.Beebe@uts.edu.au
The movement of this species, either within or from outside Australia, is of great concern to public health authorities and AQIS. From a surveillance and control perspective, it would be useful to know if the recent infestation at Tennant Creek originated from Queensland, or from Darwin after being imported from overseas. If it is the former, then inspections of towns along the main road, working back to Mount Isa, as the nearest probable source, will be required. If the latter, then increased surveillance and trapping in the towns from Darwin to Tennant Creek will be required. With incursions from outside of Australia, it would be relevant to know in which country the strain originated, as different geographic strains can have different colonising abilities and different competencies with regards to transmitting the dengue virus.7–9 This situation is complicated by the fact that vessels coming to Australia may have stopped at several Asian ports where Ae. aegypti is endemic.

Identifying differences in mosquito strains or populations requires a DNA-based genetic marker that will be informative, will deliver an unambiguous result, will be relatively straightforward to use, and ideally, be useful in later studies of evolution or population genetics. As Ae. aegypti is an exotic mosquito that probably arrived in Australia during the mid-19th century,10 a rapidly evolving genetic marker would be required to identify population variation within this species. Genetic markers based on the mitochondrial DNA (mtDNA) have been to be useful for genetic studies of other species and populations.11,12

The aim of this study was to assess the use of the mtDNA cytochrome oxidase 1 (CO1) gene as a genetic marker to evaluate the origin of the Ae. aegypti incursion into Tennant Creek. We also evaluated this marker as a potential surveillance tool for identifying populations of Ae. aegypti that originated from locations outside of Australia.

**Method**

Australian specimens of Aedes aegypti were collected as larvae from three different breeding sites in Tennant Creek in the Northern Territory, and from breeding sites in Cairns, Townsville and Thursday Island in Queensland. Following the discovery of Ae. aegypti in Tennant Creek, a container breeding survey was conducted at Camooweal located on the Barkly Highway at the Queensland-Northern Territory border, 188 km west of Mount Isa. Specimens collected during this survey were also included in this study. Specimens were also obtained from an Indonesian fishing vessel that was intercepted and inspected by AQIS approximately 1.5 km outside Melville Bay near Nhulunbuy on the north-east coast of the Northern Territory in February 2005. The ship contained Ae. aegypti larvae and pupal skins categorising it as a risk importation that had a potential for live adults to disperse to shore, had it not been intercepted and appropriately treated. Collection sites from within Australia are indicated in Figure 1. Specimens, collected as immature stages or from established colony material, from outside Australia were obtained from South East Asia and the south-west Pacific: Burma, Viet Nam, Thailand, Timor Leste, Papua New Guinea (PNG) and Vanuatu.

Figure 1. Northern Australia indicating Aedes aegypti collection sites

Mosquito DNA extraction, polymerase chain reaction amplification and DNA sequencing

Mosquitoes (partial or whole adults and larvae) were thoroughly ground in a 1.5 ml microfuge tube containing 50 μl of lysis buffer (1.0M NaCl, 0.2M sucrose, 0.1M Tris-HCl (pH 9.0), 0.05M EDTA and 0.5% SDS). Tubes were pulse microfuged to concentrate the homogenate in the bottom of the tube prior to incubation at 65°C for 30 minutes. Then 7 μl of 8.0M KAc was added to each tube; these were mixed, placed on ice for 15–30 minutes and microfuged for 15 minutes at 14,000 rpm. Supernatants were placed in a new tube to which 100 μl of 100 per cent EtOH was added and microfuged at 14,000 rpm for 15 minutes. Supernatants were removed, 100 μl of 70 per cent EtOH was added, and tubes were centrifuged again at 14,000 rpm for 5 minutes. Supernatants were again removed, tubes were air dried and resuspended in 50 μl TE containing RNase (5 μg/ml).

A 5’ segment of the mtDNA CO1 gene was amplified in 25 μl volumes using a thermal cycler (DNA Engine, MJ Research Inc.). The forward primer (5’-TAGTTCCCTTTAATATTAGGAGC-3’) was designed to start approximately 245 bp into the CO1 5’ region and the reverse primer (5’-TAATAGCATAAATTATCC-3’) was designed back from 813 bp into the CO1 gene. The final polymerase chain reaction (PCR) mixture contained 1x Taq buffer II (Fisher Biotech Australia), 2.5 mM MgCl, 0.125 mM of each dNTP, 0.4 μM of
each primer, 0.5–1.0 unit of *Taq* polymerase and 5.0–
10.0 ng of extracted genomic DNA (1 μl of extraction). The cycling involved an initial denaturation of 94° C
for three minutes, then 35 cycles of 94° C for one
minute, 50° C for one minute and 72° C for one minute
with minimal transition times. The PCR products were
separated by agarose gel electrophoresis (1.0%) at
100 V for 40 minutes, then visualised by staining with ethidium bromide (0.3 μg/ml) at 312 nm.

**DNA sequencing and genetic analysis**

Amplified products were purified using the Qiagen QiAquick PCR purification kit following their set pro-
tocol. Sequencing was performed using an ABI Big Dye™ Terminator kit (PE Biosystems) according to
the manufacturer’s recommendations and the same
forward and reverse primers described above were
used for sequencing.

The sequence alignment was performed using the PILEUP algorithm in the GCG package using default
settings (Genetics Computer Group, Version 8,
1994). Genetic analyses using traditional tree-build-
ing phylogenetic methods can be inappropriate for
these types of studies because they make assump-
tions that are invalid at the intraspecific population
level. Thus the analysis was performed using the TCS algorithm which estimates genealogical rela-
tionships and generates a parsimonious network.

**Results**

*Aedes aegypti* genomic DNA was extracted from
46 individual specimens from Australia and various
countries of South East Asia and the south-west
Pacific. From these, 46 CO1 sequences were derived
and aligned together and with two other *Ae. aegypti*
sequences (laboratory strains originating from East
and West Africa) obtained from Genbank (Table). After editing, the sequence alignment length was 503
bp and showed eight separate sequence haplotypes.
All nucleotide changes occur at the third codon posi-
tion. A summary of the DNA sequence variation for
each haplotype (relative to haplotype 1: Tennant
Creek and Cairns population, Genbank accession
DQ026284) is presented in the Table along with the
haplotype distributions and their frequency. Figure 2
shows a minimum parsimony network of the eight
haplotypes.

The CO1 haplotypes obtained from the three
separate breeding sites in Tennant Creek were the
same as those found in Cairns but different to those
identified from Townsville. It appears that the Tennant
Creek population represents a single haplotype pop-
ulation (H1). The H1 haplotype from Cairns appears
well dispersed as it was also found from mosquitoes
collected in Viet Nam and Thailand. Haplotype H1
is one mutational step (1 nucleotide) from another
well-dispersed haplotype H4, which was found in

**Table. Collection sites, haplotype distribution and haplotype diversity of Aedes aegypti populations used in this study**

<table>
<thead>
<tr>
<th>Collection site</th>
<th>n</th>
<th>CO1 haplotype</th>
<th>Haplotype diversity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairns Qld (2 sites)†</td>
<td>5</td>
<td>H1</td>
<td>bp</td>
</tr>
<tr>
<td>Townsville Qld†</td>
<td>3</td>
<td>H3</td>
<td>11222233333334</td>
</tr>
<tr>
<td>Tennant Creek NT (3 sites)†</td>
<td>9</td>
<td>H1</td>
<td>46890129044568903</td>
</tr>
<tr>
<td>Camooweal Qld†</td>
<td>2</td>
<td>H1</td>
<td>56657984625737928</td>
</tr>
<tr>
<td>Thursday Is. Torres Strait†</td>
<td>3</td>
<td>H8</td>
<td>H1 GTAAACTAGTTACACA</td>
</tr>
<tr>
<td>Indonesian fishing vessel†</td>
<td>5</td>
<td>3xH7, 2xH4</td>
<td>H2 ............C....</td>
</tr>
<tr>
<td>Timor Leste (3 sites)†</td>
<td>7</td>
<td>H2</td>
<td>H3 A. .............</td>
</tr>
<tr>
<td>Thailand (Bangkok)‡</td>
<td>2</td>
<td>H1</td>
<td>H4 .....T.........</td>
</tr>
<tr>
<td>Viet Nam (Hanoi)‡</td>
<td>3</td>
<td>H1, H7, H6</td>
<td>H5 A. ............</td>
</tr>
<tr>
<td>Burma‡</td>
<td>3</td>
<td>H4</td>
<td>H6 .G...C...C....</td>
</tr>
<tr>
<td>Vanuatu†</td>
<td>1</td>
<td>H4</td>
<td>H7 .....C.A...CGCTG.G</td>
</tr>
<tr>
<td>Papua New Guinea†</td>
<td>3</td>
<td>H4</td>
<td>H8 .GGG.C.A..GCT...</td>
</tr>
</tbody>
</table>

* Nucleotide changes relative to H1 (Genbank accession number DQ026284).
† Specimens collected as immature stages from breeding sites.
‡ Specimens from established colonies.
Figure 2. Mitochondrial CO1 haplotype network showing genealogical relationships

Legend: Circles represent the different CO1 sequence haplotypes with geographic regions of specimens listed. Connecting nodes represent single mutational steps between haplotypes and may be unidentified extant haplotypes.

PNG, Timor Leste, Burma, Viet Nam and Vanuatu. Haplotype H3, identified from Townsville, is also a single mutational step from the H4 haplotype, but H3 appears restricted to Townsville. The specimens from Thursday Island were all H8 and the same sequence as the Liverpool laboratory strain that was originally collected from West Africa. This Thursday Island material was considered quite divergent to the Australian mainland material (H1 from Tennant Creek, Cairns and Camooweal, and H3 from Townsville). This study suggests that the incursion into Tennant Creek was not from the military and industrial centre of Townsville, but from Cairns or Camooweal. The most likely spread was by the carriage of eggs in dry receptacles by vehicle traffic. The presence of *Ae. aegypti* at Camooweal moves the western distribution of *Ae. aegypti* in Queensland to the Northern Territory border. However, these conclusions should be viewed with caution as further sampling and analysis of sites within these towns will be required to determine if additional haplotypes are present.

Within Australia, the haplotype population identified on Thursday Island in the Torres Strait (H8), shows considerable genetic distance to the Australian mainland haplotypes (10 mutational steps). It is interesting to note that *Ae. aegypti* populations from Thursday Island have displayed enhanced vector competence to the dengue 2 and 4 serotypes compared to the mainland populations from Cairns and Townsville. The substantial genetic distinction between the Thursday Island H8 population and the mainland Australia H1 and H3 populations may help in the understanding of the observed difference in vector competence between these different populations. It also highlights the need for state authorities and AQIS to prevent the movement of *Ae. aegypti* from the Torres Strait to mainland Australia.

Specimens of *Ae. aegypti* collected from the Indonesian fishing vessel revealed two separate haplotypes (H4 and H7). The maternal inheritance of the mitochondrial genome means that each female mosquito will only produce her own haplotype, and indicates that at least two separate egg batches were laid in the receptacle on this vessel by different CO1 haplotype *Ae. aegypti* females. The origin of these haplotype populations could not be determined, as we have no samples from Indonesia for comparison. However, it is likely that these haplotypes represent Indonesian populations of *Ae. aegypti*.

The appearance of a divergent haplotype or lineage in the Torres Strait population may reflect the successful dispersal capabilities of this species. No one has looked at the movement of these haplotypes on a global scale. However such movement appears to be considerable, this small study has revealed, for
example, that haplotypes are shared by populations as widely dispersed as Burma and Vanuatu (H4) and Viet Nam and Australia (H1).

Each node in the network in Figure 2 may represent an extant haplotype sequence, and this study suggests that there could be 11 unidentified haplotypes that exist within this network. If we view this haplotype network, bearing in mind it is a small sampling regime, haplotypes H1 and H4 were found most frequently, were well dispersed geographically and appear embedded within the haplotype network. These factors suggest H1 and H4 may be the original (ancestral) haplotypes introduced into the Asia-Pacific region. It is also interesting that the laboratory strains found in Genbank that had origins in West Africa (H8, Liverpool) and in East Africa (H5, Moyo-R, Kenya) are at the ends or tips of the network. Their positioning may indicate the breadth of genetic diversity of this species within Africa.

The dispersal and colonising ability of this species makes it a continual threat to ports in Australia and highlights the need to prevent the further westward spread from Queensland into the Northern Territory and Western Australia. We suggest it should now be a priority to screen Ae. aegypti populations in Australia and around our region to record and monitor the possible spread of the endemic and exotic genetic diversity of this species.

In summary, the partial sequence of the mtDNA CO1 gene from a small number of Ae. aegypti has enabled the identification of different genetic populations within Australia, as well as the origin of an incursion into the Northern Territory from Queensland. There was also considerable genetic difference between the mainland Australian and Thursday Island populations, which have been shown to display different vector competencies to dengue viruses. Though further extensive sampling and analysis will be required to verify the robustness of this potentially useful genetic marker, this study suggests that the CO1 gene will be a practical tool to study the genetic diversity and spread of Ae. aegypti in Australia, as well as to monitor foreign incursions. It has a potential application in studying other species of quarantine and public health importance in Australasia such as the recent establishment of Ochlerotatus camptorhynchus in New Zealand, or the dispersal of Aedes albopictus into the Torres Strait and other areas of northern Australia.

Acknowledgements

The authors would like to thank Mr Geoff Kumjew of AQIS for specimens from the Indonesian fishing vessel, Mr Bill Pettit and Mr Jeffery Kennedy for specimens of Ae. aegypti from Tennant Creek, Mr Bill Pettit and Matthew Shortus for the Camooweal specimens and Dr Bart Currie of the Menzies School of Health Research and the Northern Territory Department of Health and Community Services for comments on the origin of the Tennant Creek incursion. The authors also acknowledge the following for providing specimens used in this study: Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand; Military Institute of Hygiene and Epidemiology, Hanoi, Viet Nam and Department of Medical Research, Yangon, Burma.

References


Erratum


The rates shown in the map ‘Notification rates of invasive pneumococcal disease, Australia, 2003 by statistical division of residence’ were incorrect.

Table 16: ‘Details of cases of invasive pneumococcal disease that occurred in those fully vaccinated for age with 23-valent pneumococcal vaccine, by jurisdiction, Australia, 2003’ contains incorrect data for New South Wales and Victoria and the totals are consequently incorrect.

A revised version of the report with correct map and Table 16 are available on the CDA website in HTML and PDF formats.
<table>
<thead>
<tr>
<th>Journal Name</th>
<th>ISSN</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Neuropsychiatry</td>
<td>1354-6805</td>
<td>Only articles that have been indicated as being refereed are acceptable</td>
</tr>
<tr>
<td>Collegian</td>
<td>1322-7696</td>
<td></td>
</tr>
<tr>
<td>Colloquium – The Australian and New Zealand Theological Review</td>
<td>0588-3237</td>
<td></td>
</tr>
<tr>
<td>Colloquy: text theory critique</td>
<td>1325-9490</td>
<td></td>
</tr>
<tr>
<td>Common Law World Review</td>
<td>1473-7795</td>
<td></td>
</tr>
<tr>
<td>Communal Plural: Journal of Trans-national and Cross-cultural Studies</td>
<td>1320-7873</td>
<td></td>
</tr>
<tr>
<td>Communal Societies</td>
<td>0739-1250</td>
<td></td>
</tr>
<tr>
<td>Communicable Disease Intelligence</td>
<td>0725-3141</td>
<td></td>
</tr>
<tr>
<td>Communication Management, Journal of</td>
<td>1363-254X</td>
<td></td>
</tr>
<tr>
<td>Community Work and Development, Journal of</td>
<td>0947919.732</td>
<td></td>
</tr>
<tr>
<td>Company and Securities Law Journal</td>
<td>0729-2775</td>
<td></td>
</tr>
<tr>
<td>Company Financial and Insolvency Law Review, the</td>
<td>1368-051X</td>
<td></td>
</tr>
<tr>
<td>Comparative American Studies</td>
<td>1477-5700</td>
<td></td>
</tr>
<tr>
<td>Compass</td>
<td>0819-4602</td>
<td></td>
</tr>
<tr>
<td>Competition and Consumer Law Journal</td>
<td>1039-5598</td>
<td></td>
</tr>
<tr>
<td>Complimentary Medicine</td>
<td>1446-8263</td>
<td></td>
</tr>
<tr>
<td>Computer, the Internet and Management, International Journal of the</td>
<td>0858-7027</td>
<td></td>
</tr>
<tr>
<td>Computers and Industrial Engineering, International Journal of</td>
<td>0360-8352</td>
<td></td>
</tr>
<tr>
<td>Computers and Security</td>
<td>0167-4048</td>
<td></td>
</tr>
<tr>
<td>Computers in Mathematics and Science Technology, Journal of</td>
<td>0713-9258</td>
<td></td>
</tr>
<tr>
<td>Concrete in Australia</td>
<td>1440-656X</td>
<td>Articles must be identified as being peer-reviewed</td>
</tr>
<tr>
<td>Construction Management and Economics</td>
<td>0144-6193</td>
<td></td>
</tr>
<tr>
<td>Construction Marketing, International Journal of</td>
<td>1463-7189</td>
<td></td>
</tr>
<tr>
<td>Consumer Marketing, the Journal of</td>
<td>0736-3761</td>
<td></td>
</tr>
<tr>
<td>Consumer Studies, International Journal of</td>
<td>1470-6423</td>
<td></td>
</tr>
<tr>
<td>Construction Research, Journal of</td>
<td>1609-9451</td>
<td></td>
</tr>
<tr>
<td>Contemporary Issues in Communication, Science and Disorders</td>
<td>0736-0312</td>
<td></td>
</tr>
<tr>
<td>Contemporary Issues in Early Childhood</td>
<td>1463-9491</td>
<td></td>
</tr>
<tr>
<td>Contemporary Justice Review</td>
<td>1028-2580</td>
<td></td>
</tr>
<tr>
<td>Contemporary Nurse</td>
<td>1037-6178</td>
<td></td>
</tr>
<tr>
<td>Contemporary Perspectives on Management Accounting</td>
<td>1442-0767</td>
<td></td>
</tr>
<tr>
<td>Contemporary Politics</td>
<td>1356-9775</td>
<td></td>
</tr>
<tr>
<td>Context: A Journal of Music Research</td>
<td>1038-4006</td>
<td></td>
</tr>
</tbody>
</table>
Communicable Diseases Intelligence

News and Notes

Communicable Diseases Intelligence

Download Article

Communicable Diseases Intelligence (CDI) is a fortnightly publication of the Australian Department of Human Services and Health and the Communicable Diseases Network of Australia and New Zealand. The Network comprises representatives of the Australian Department of Human Services and Health, the State and Territory health authorities, and other organizations involved in communicable disease surveillance and control from throughout the country. In addition, there is a representative from New Zealand. It has fortnightly teleconferences and other meetings to exchange information on emerging communicable disease activity and to coordinate surveillance and control activities.

Each issue of CDI incorporates reports from Australia's national communicable diseases surveillance systems, including the National Notifiable Diseases Surveillance System, the CDI Laboratory Reporting Schemes, and the Australian Sentinel General Practitioner Surveillance Network. Reports from the National Salmonella Surveillance Scheme, the Australian Gonococcal Surveillance Programme and the National HIV, AIDS, and Tuberculosis Reporting Systems are also regularly included.

CDI also publishes timely reports of communicable disease outbreaks and other articles dealing with a wide range of subjects relevant to the surveillance and control of communicable diseases in Australia. Recently published items have reported, for example, the first identification of endemically acquired hepatitis E in the Northern Territory of Australia, an outbreak of influenza in a nursing home, the epidemiology of hepatitis A in South Australia, the epidemiology of Barmah Forest virus disease in Western Australia, and the outbreak of respiratory disease in humans and horses due to a previously unrecognized paramyxovirus.

CDI is available from:
The Editor
Communicable Diseases Intelligence
AIDS and Communicable Diseases Branch
Department of Human Services and Health
GPO Box 9848
Canberra ACT 2601
Australia

Helen Longbottom
Department of Human Services and Health
Canberra ACT Australia

Return to Contents

Return to EID Home Page