

# Causes of ant sting anaphylaxis in Australia: the Australian Ant Venom Allergy Study

Simon G A Brown, Pauline van Eeden, Michael D Wiese, Raymond J Mullins, Graham O Solley, Robert Puy, Robert W Taylor and Robert J Heddle

The prevalence of systemic allergy to native ant stings in Australia is as high as 3% in areas where these insects are commonly encountered, such as Tasmania and regional Victoria.<sup>1,2</sup> In one large Tasmanian emergency department study, ant sting allergy was the most common cause of anaphylaxis (30%), exceeding cases attributed to bees, wasps, antibiotics or food.<sup>3</sup>

*Myrmecia pilosula* (jack jumper ant [JJA]) is the major cause of ant sting anaphylaxis in Tasmania.<sup>2</sup> A double-blind, randomised placebo-controlled trial has demonstrated the effectiveness of JJA venom immunotherapy (VIT) to reduce the risk of sting anaphylaxis, and an ongoing treatment and research program has been established.<sup>4,5</sup> Access to treatment outside Tasmania is limited by inadequate knowledge of the causative species in other regions and the absence of diagnostic tests for other ant species. Accurate diagnosis is further complicated because the JJA is a “species complex”, comprising seven closely related species with almost identical morphology. These were first recognised by chromosomal differences but can now be distinguished using subtle differences in morphological characteristics.<sup>6</sup>

The objectives of the Australian Ant Venom Allergy Study were to determine the geographical distribution of the major ant species associated with anaphylaxis throughout Australia, and to examine the feasibility of newly developed diagnostic testing to confirm the diagnosis of allergy to non-JJA ant species.

## METHODS

### Study population

In 2006 and 2007, we requested case referrals from Australian doctors with allergy practices by emailing all members of the Australasian Society of Clinical Immunology and Allergy, and from emergency medicine specialists by emailing all Fellows of the Australasian College for Emergency Medicine. We also used press releases and stories in regional newspapers and on radio and television, and web search indexing with a

## ABSTRACT

**Objective:** To determine the Australian native ant species associated with ant sting anaphylaxis, geographical distribution of allergic reactions, and feasibility of diagnostic venom-specific IgE (sIgE) testing.

**Design, setting and participants:** Descriptive clinical, entomological and immunological study of Australians with a history of ant sting anaphylaxis, recruited in 2006–2007 through media exposure and referrals from allergy practices and emergency physicians nationwide. We interviewed participants, collected entomological specimens, prepared reference venom extracts, and conducted serum sIgE testing against ant venom panels relevant to the species found in each geographical region.

**Main outcome measures:** Reaction causation attributed using a combination of ant identification and sIgE testing.

**Results:** 376 participants reported 735 systemic reactions. Of 299 participants for whom a cause was determined, 265 (89%; 95% CI, 84%–92%) had reacted clinically to *Myrmecia* species and 34 (11%; 95% CI, 8%–16%) to green-head ant (*Rhytidoponera metallica*). Of those with reactions to *Myrmecia* species, 176 reacted to jack jumper ant (*Myrmecia pilosula* species complex), 18 to other jumper ants (15 to *Myrmecia nigrocincta*, three to *Myrmecia ludlowi*) and 56 to a variety of bulldog ants, with some participants reacting to more than one type of bulldog ant. Variable serological cross-reactivity between bulldog ant species was observed, and sera from patients with bulldog ant allergy were all positive to one or more venoms extracted from *Myrmecia forficata*, *Myrmecia pyriformis* and *Myrmecia nigriceps*.

**Conclusion:** Four main groups of Australian ants cause anaphylaxis. Serum sIgE testing enhances the accuracy of diagnosis and is a prerequisite for administering species-specific venom immunotherapy.

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study home page, to encourage people with ant sting allergy to contact us directly.

Inclusion criteria were a history of a generalised systemic allergic reaction to an identified or suspected ant sting. Cases were excluded if investigation indicated that a systemic reaction did not occur or that an ant sting was not the cause. The human research ethics committees of each investigator's institution granted ethics approval. Participants gave written informed consent.

### Clinical data

Participants identified the responsible ant (where possible) from colour illustrations of common species and completed a questionnaire, followed by a structured telephone or face-to-face interview. We recorded participants' age and sex, the geographical location where each reaction occurred, reaction features, a description of the insect and whether it was clearly seen to sting or

implicated by circumstance (eg, seen nearby), and a reaction severity grade of mild (skin only), moderate (involvement of additional organ systems) or severe (hypotension or hypoxaemia).<sup>3</sup> Serum samples were obtained and stored at –80°C until analysis.

### Entomological specimens, collection and identification

With the assistance of participants' non-allergic family or friends, 2–4 specimens of ant(s) were provided from each location where systemic reactions had occurred. Ants were not collected from the Northern Territory, northern Queensland or northern Western Australia because few participants came from these areas, nor from Tasmania, as ants in that region are already well characterised. Wherever possible, the investigators made field trips to collect additional specimens for identification and whole ant

nests (colonies) for venom extraction from areas where stings had occurred. Ant colonies were transported on dry ice, then stored at  $-80^{\circ}\text{C}$  until venom sac dissection and processing, as previously described.<sup>4,7,8</sup> Specimens were identified by one of us (RWT) and deposited in the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian National Insect Collection.

### Venom extracts

After morphological identification, venoms extracted from different sibling species of the JJA species complex were analysed by polyacrylamide gel electrophoresis according to our previously established methods.<sup>9</sup> Once homology of venoms from sibling species was confirmed, we used a standardised JJA extract produced by the Tasmanian Jack Jumper Allergy Program for our venom-specific IgE (sIgE) assays.<sup>7</sup>

For all other species, venom extracts from the same species were pooled to create

reference venoms for use in sIgE assays, only after both formal entomological identification and confirmation of the presence of identical bands on polyacrylamide gel electrophoresis in venom samples from each component colony.

### Venom-specific IgE assays and determining reaction causation

A time-resolved fluorescence method, dissociation-enhanced lanthanide fluoroimmunoassay (DELFI; Wallac, Turku, Finland),<sup>10</sup> was used to detect sIgE against a panel of ant venoms relevant to each geographical region where sting reactions had occurred. Venom panels for sIgE testing were chosen for each region based on our collected specimens and known distributions.<sup>11,12</sup> We were unable to include species if they were rarely encountered and we could not obtain sufficient venom.

The cause of each reaction was attributed using a combination of ant identification and sIgE testing, as outlined in Box 1. A single

positive sIgE result allowed us to confirm a clear ant description or, if there was some uncertainty about the ant(s) described, allowed us to decide between several possible causes. However, multiple positive sIgE results (representing either cross-reactivity or multiple sensitisations) required a high degree of clinical certainty (visual identification) before attributing causation.

### Statistical analysis

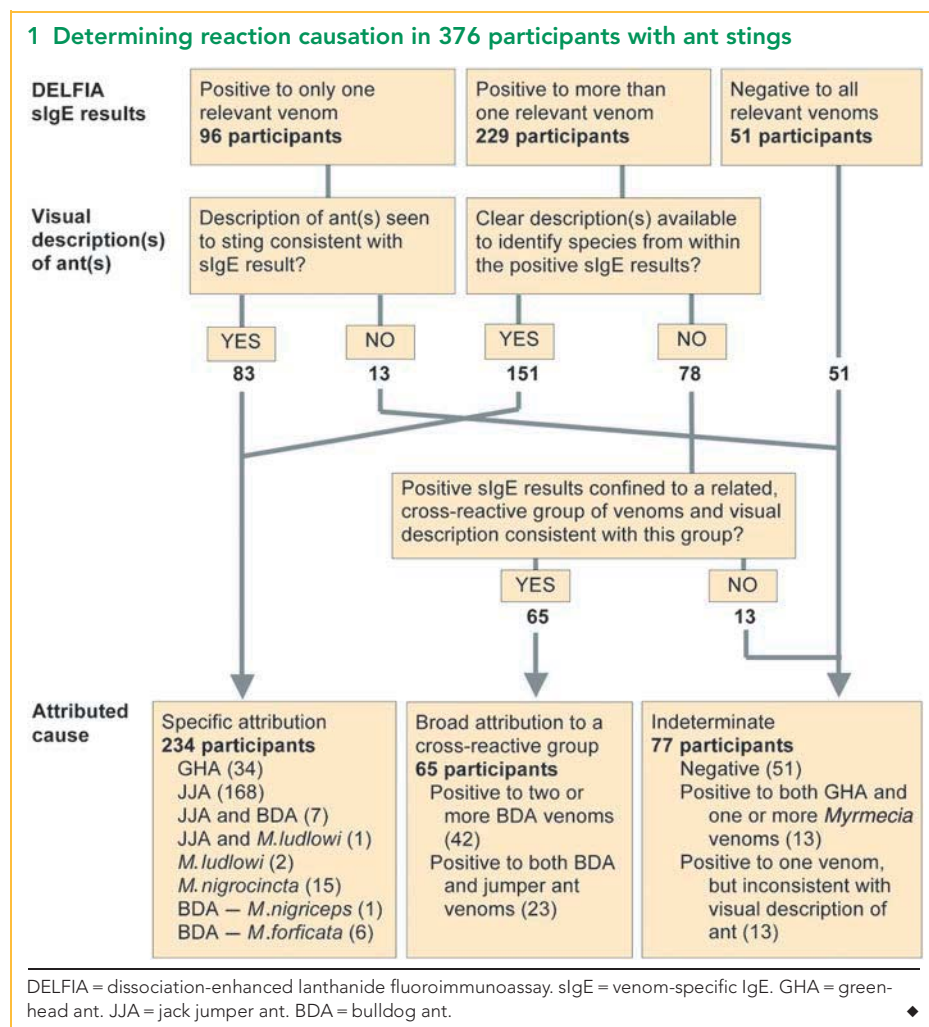
Proportions were calculated with 95% confidence intervals (binomial exact) (Stata, release 11; StataCorp, College Station, Tex, USA).

## RESULTS

Three hundred and seventy-six participants reported 735 systemic reactions. Basic demographic and reaction data are shown in Box 2. We identified 283 specimens of stinging ants collected from locations where reactions had occurred (Box 3). There were four dominant ant species or groups, each with characteristic morphology: (i) JJA species complex; (ii) other jumper ants (*Myrmecia nigrocincta* in New South Wales and Queensland, *Myrmecia ludlowi* in WA); (iii) bulldog ants (BDA) of the *Myrmecia gulosa* species group; and (iv) *Rhytidoponera metallica* (green-head ant [GHA]) (Box 4).

### Venom-specific IgE results and reaction causation

Venoms used for sIgE testing for each region are shown in Box 3. Serum samples from 325 participants (86%; 95% CI, 83%–90%) were sIgE-positive to one or more venoms relevant to the geographical regions where the stings occurred. Reaction causes were



### 2 Participant demographics and reaction characteristics

Characteristic	
Number of participants	376
Age in years, median (IQR)	46 (33–64)
Male	158 (42%)
Age in years at first reaction, median (IQR)	46 (19–58)
Years since last reaction, median (IQR)	1.7 (0.4–5.0)
Severity of worst reaction	
Mild	22 (6%)
Moderate	150 (40%)
Severe	204 (54%)

IQR = interquartile range.

### 3 Ant species implicated in sting reactions, and venoms used for sIgE testing\*

Species	No. of specimens collected						Total (%; 95% CI)
	Tas <sup>†</sup>	SA	Vic	NSW/ACT	Qld	WA	
<b>Jumper ants</b>							
Jack jumper ant		102	44	14		1	161
<i>Myrmecia nigrocincta</i>				5	13		18
<i>Myrmecia chasei</i>						1	1
<i>Myrmecia swalei</i>						1	1
<i>Myrmecia ludlowi</i>						2	2
Total jumper ants	—	102	44	19	13	5	183 (65%, 59%–70%)
<b>Bulldog ants</b>							
<i>Myrmecia forficata</i>		2	2				4
<i>Myrmecia pyriformis</i>		8	4				12
<i>Myrmecia brevinoda</i>				1			1
<i>Myrmecia nigriceps</i>				1			1
<i>Myrmecia tarsata</i>				1			1
<i>Myrmecia simillima</i>	1 <sup>‡</sup>						1
<i>Myrmecia gulosa</i>				3			3
<i>Myrmecia gratioiosa</i>						12	12
<i>Myrmecia pavida</i>						4	4
<i>Myrmecia nigriscapa</i>						1	1
<i>Myrmecia regularis</i>						1	1
Total bulldog ants	—	11	6	6	0	18	41 (14%, 11%–19%)
Green-head ants	—	1	0	12	32	0	45 (16%, 12%–21%)
Other stinging ants	—	2	2	4	3	1	14 <sup>§</sup> (5%, 3%–8%)
<b>Total</b>	—	<b>116</b>	<b>52</b>	<b>41</b>	<b>48</b>	<b>24</b>	<b>283</b>

sIgE = venom-specific IgE. Tas = Tasmania. SA = South Australia. Vic = Victoria. NSW = New South Wales. ACT = Australian Capital Territory. Qld = Queensland. WA = Western Australia. \* Shaded cells indicate venoms used for sIgE testing. Sera were tested according to the location of participants' reported reactions (ie, multiple regions if relevant). † Specimens were not collected from Tasmania, as ants in that region are already well characterised. ‡ This specimen was returned from an area close to the Victorian border; *M. simillima* was not encountered on field trips to any other regions of SA. § *Rhytidoponera* sp. (not *R. metallica*), 8; *Odontomachus* sp., 4 (includes 2 in Northern Territory); *Aphaenogaster*, 1; and *Tetramorium*, 1. ◆

designated for 299 participants (80%; 95% CI, 75%–83%) (Box 1). For the remaining 77 participants (20%), 38 of whom were stung in northern Australia, a reaction cause could not be attributed.

Of the 299 participants for whom a cause could be determined, 265 (89%; 95% CI, 84%–92%) had reacted to *Myrmecia* species and 34 (11%; 95% CI, 8%–16%) to GHA. No participant was judged as reacting clinically to both GHA and *Myrmecia* species. Of the 265 participants clinically reactive to *Myrmecia* species, 186 (70%; 95% CI, 64%–76%) reacted to jumper ants alone, 49 (18%; 95% CI, 14%–24%) to BDA alone and seven (3%; 95% CI, 1%–5%) to both BDA and jumper ants. For 23 (9%; 95% CI, 6%–13%), the species or species group of *Myrmecia* responsible could not be determined. Of the 193 who had reacted clinically

to jumper ants, this was JJA in 175 cases (91%; 95% CI, 86%–94%), *M. nigrocincta* in 15 (8%; 95% CI, 4%–12%), *M. ludlowi* in two, and both *M. ludlowi* and JJA in one case.

#### Geographical distribution of reactions

The geographical locations of reactions to causative ants are mapped in Box 5. JJA reactions occurred in Tasmania, southern coastal WA, South Australia, Victoria and southern coastal and mountainous regions of NSW and the Australian Capital Territory. The distribution of JJA sting reactions closely mirrors that for entomological collection records of specimens of the *M. pilosula* species complex.<sup>12</sup> Reactions to BDA occurred in the same areas as JJA reactions and also extended to more inland and northern parts of NSW and further north in WA, as far as Geraldton. *M. ludlowi* was also a cause of

reactions in the areas where BDA were found in WA. *M. nigrocincta* and GHA reactions were largely clustered around northern coastal NSW and south-east Queensland.

#### Venom-specific IgE cross-reactivity and multiple sensitisations

We were unable to distinguish between sensitisation to multiple ant species and true cross-reactivity. However, some patients were sIgE-positive to one venom or venom group alone without positive results to other venoms tested, indicating the presence of unique allergens in these venoms — this applied to 15 of 34 patients reacting to GHA (44%), 55 of 176 reacting to JJA (31%), 5 of 15 reacting to *M. nigrocincta* (33%), one of three reacting to *M. ludlowi* (33%), and 47 of 56 reacting to venom(s) of BDA (84%).

In sera tested for sIgE against multiple venoms from BDA, the following patterns were observed.

**WA:** With the exception of one sample negative to *Myrmecia nigriceps*, all 18 serum samples tested for reactions occurring in WA were positive to all of *Myrmecia gratioiosa*, *Myrmecia pavida* and *M. nigriceps* venoms with very similar quantitative titres. These three venoms also had identical protein bands on gel electrophoresis. Nine serum samples were also sIgE-positive to *Myrmecia regularis*.

**SA and Victoria:** Of 26 serum samples tested for reactions in these states, 22 were sIgE-positive to *Myrmecia forficata* venom, of which 14 were also positive to *Myrmecia pyriformis*, and 10 to *M. nigriceps*. Of the remaining four sera, one was sIgE-positive to *M. nigriceps* venom alone, one to *M. pyriformis* venom alone, and two were positive to both these venoms. Five of eight sera tested for reactions in Victoria were sIgE-positive to *Myrmecia simillima*, but never to this venom alone.

**NSW/ACT/Queensland:** All 13 serum samples tested for reactions occurring in these regions were sIgE-positive to *M. forficata* and/or *M. nigriceps* venoms. Ten were also positive to one or more of the other venoms of BDA for these regions.

#### DISCUSSION

We found *Myrmecia* species to be the predominant cause of ant sting anaphylaxis in Australia. JJA stings were the most common cause, followed by stings from species of BDA, the GHA, and then the jumper ants *M. nigrocincta* in northern NSW and Queens-



land and *M. ludlowi* in WA. While our findings are broadly consistent with a number of previous reports,<sup>1,2,13-16</sup> this is the first time that it has been possible to confirm the causative species using sIgE testing against an extended panel of relevant venoms.

Our study has some limitations. Recruitment into the study was potentially biased because the doctors we targeted (allergists, clinical immunologists and emergency physicians) tend to be in or near population centres, and because of potentially variable interest by regional media outlets. For practical reasons, we also did not obtain venom extracts from far northern areas of Australia.

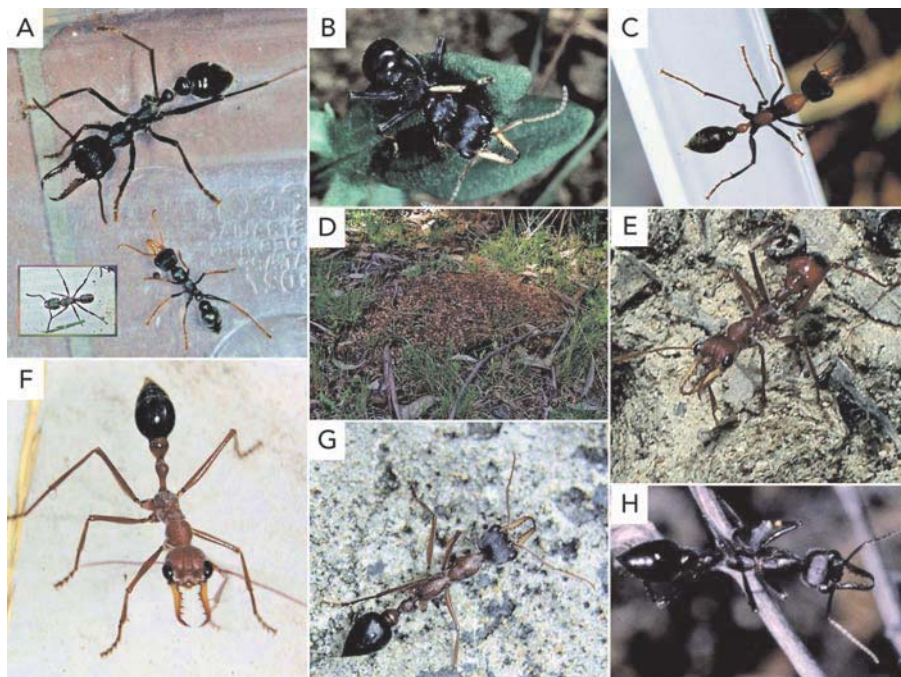
The causative species group could not be confirmed in 20% of cases, mostly due to negative sIgE results. Possible causes of this include allergy to less common species for which venom extracts were unavailable, and false negative results because of relatively poor sensitivity of serum sIgE assays compared with intradermal skin testing (IDT). The multicentre nature of our study precluded IDT, although it should be noted that this is also an imperfect test.<sup>17</sup>

While substantial antigenic cross-reactivity and/or multiple sensitisations to different venoms was observed,<sup>18</sup> the frequency with which sera were positive to only one of the venoms or venom groups indicated the presence of venom allergens unique to each species. In particular, it should be noted that while the venoms of the various sibling species of the *M. pilosula* species complex appear to be homologous by gel electrophoresis, there are other jumper ants (*M. nigrocincta* and *M. ludlowi*) with very different venoms for which the currently available JJA venom extract will not be useful for diagnosis or VIT.

By contrast, the venoms of the *M. gratiosa*, *M. pavidata* and *M. nigriceps* BDA appear to be practically identical. While the venom recognition patterns are more complex for other BDA, all sera in our study were positive to one or more of *M. forficata*, *M. pyriformis* and *M. nigriceps*. These three venoms are therefore likely to include most or all of the major BDA allergens.

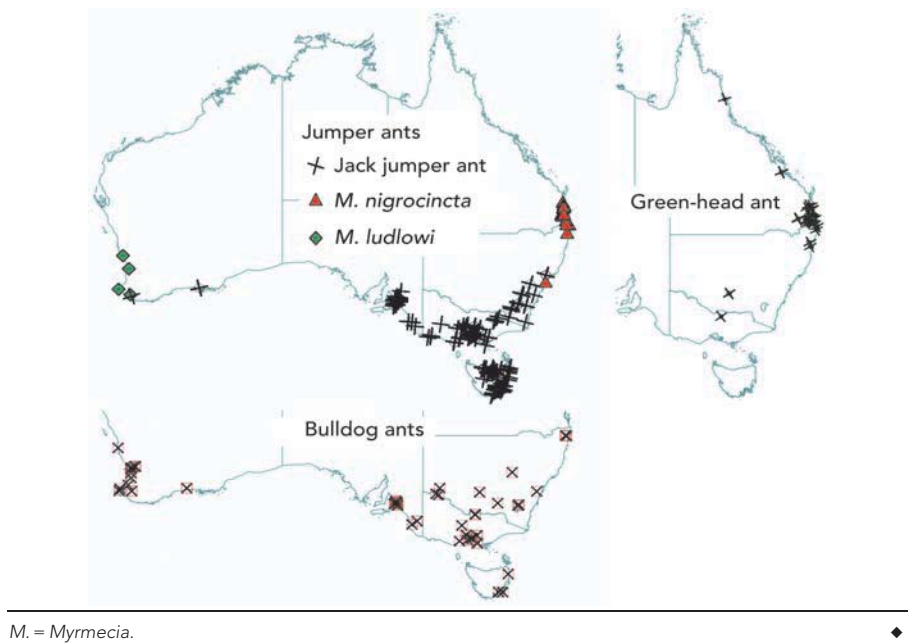
A major challenge we encountered was the large number of potentially allergenic venoms, allergenic cross-reactivity between venoms, and the potential for multiple sensitisations from stings by different species experienced by any one individual. This is not uncommon when assessing patients with insect venom allergy. Examining the ability of different venoms to inhibit sIgE binding to each other in each serum sample

#### 4 Australian ant species that cause allergic reactions



A: A large bulldog ant (*Myrmecia pyriformis*) and a smaller jumper ant and (inset) a green-head ant (~6 mm long, dark black-metallic green). Jumper ants can be further divided on the basis of colouration as either a jack jumper ant (JJA) or another jumper ant. B: JJA, usually 10–12 mm long, black body with orange–yellow mandibles, and moves with short jerks and jumps. C: Another jumper ant, *Myrmecia nigrocincta*, which is similar in size and behaviour to the JJA but with bright red body segment(s). The jumper ants *Myrmecia ludlowi*, *Myrmecia swalei* and *Myrmecia chasei* have similar body colouration. D: A typical jumper ant nest, covered with small stones. E–H: Bulldog ants of the *Myrmecia gulosa* species group, 20–30 mm long and a variety of colours. E: *Myrmecia gulosa*, the prototype for the group; F: *Myrmecia gratiosa*, which predominates in the Darling Scarp area east of Perth, Western Australia; G: *Myrmecia nigriceps*; and H: *Myrmecia forficata*, which predominates in Tasmania. ◆

#### 5 Geographical distribution of ant sting allergic reactions



can distinguish the primary sensitising venom, identify allergenically identical venoms or confirm the presence of sensitisations to multiple venoms.<sup>19</sup> However, sensitisation with demonstrable sIgE does not necessarily result in clinical reactivity.<sup>20</sup> Thus, the presence of sIgE is only used to confirm a diagnosis that has been made from a clinical history including a description of the insect (if seen), circumstances of the sting and a detailed knowledge of local insect species.<sup>21</sup>

Management of sting anaphylaxis centres on identification of the causative insect, avoidance strategies where possible (eg, nest removal, moving to a location where the species is absent or less common), provision of an emergency action plan, and VIT where available. Although economic factors may preclude the production of therapeutic ant venom extracts for all species when only small numbers of patients are affected, the development of diagnostic sIgE assays will help distinguish between allergy to native ant species and allergy to other insect species, and thus facilitate the accurate application of VIT.

VIT is currently subsidised by the Pharmaceutical Benefits Scheme (PBS) in Australia for the treatment of honeybee and wasp (*Polistes* and yellowjacket) allergy. JJA VIT is currently funded in Tasmania by the state government; the venom extract can be supplied to interstate hospitals as an active pharmaceutical ingredient for on-site formulation and dispensing,<sup>22</sup> but is not subsidised by the PBS and the cost must be covered in full by the hospitals and/or patients. No venom extracts suitable for human use are available for other Australian ant species at this time. Future work in this area should focus on confirming the apparent antigenic homology of closely related venoms and developing standardised venom extracts for diagnostic and therapeutic use.

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## COMPETING INTERESTS

Raymond Mullins received unrestricted investigator-initiated grants for data purchase from CSL Limited and Alphapharm Australia (the past and current Australian distributors of EpiPen) and the Ilhan Food Allergy Foundation, Melbourne. Robert Heddle is employed by SA Pathology, which offers in-vitro diagnostic testing for specific IgE to jumper ant venom. Robert Taylor has received honoraria and travel support from the NHMRC.

## AUTHOR DETAILS

**Simon G A Brown**, MB BS, PhD, FACEM, Professor,<sup>1</sup> and Consultant<sup>2</sup>  
**Pauline van Eeden**, BAppSci(MLS), PhD, Research Associate<sup>1</sup>  
**Michael D Wiese**, BPharm, MCLinPharm, PhD, Senior Lecturer,<sup>3</sup> and Consultant<sup>2</sup>  
**Raymond J Mullins**, PhD, FRACP, FRCPA, Immunology and Allergy Physician and Adjunct Professor<sup>4</sup>  
**Graham O Solley**, MB BS, FACP, Allergist<sup>5</sup>  
**Robert Puy**, MB BS, FRACP, Immunology and Allergy Physician<sup>6</sup>  
**Robert W Taylor**, PhD, Adjunct Professor,<sup>7</sup> and Honorary Fellow<sup>8</sup>  
**Robert J Heddle**, PhD, FRACP, FRCPA, Head of Clinical Immunology, Chief Pathologist and Associate Professor<sup>9</sup>

- 1 Centre for Clinical Research in Emergency Medicine, Western Australian Institute for Medical Research, Royal Perth Hospital, University of Western Australia, Perth, WA.
- 2 Jack Jumper Allergy Program, Royal Hobart Hospital, Hobart, TAS.
- 3 Sansom Institute, University of South Australia, Adelaide, SA.
- 4 Faculty of Health, University of Canberra, Canberra, ACT.
- 5 Watkins Medical Centre, Brisbane, QLD.
- 6 Department of Allergy, Immunology and Respiratory Medicine, Alfred Hospital, Melbourne, VIC.
- 7 Research School of Biology, Australian National University, Canberra, ACT.
- 8 CSIRO Division of Sustainable Ecosystems, Canberra, ACT.
- 9 Immunology Directorate, SA Pathology, Royal Adelaide Hospital and Flinders University, Adelaide, SA.

Correspondence: [simon.brown@uwa.edu.au](mailto:simon.brown@uwa.edu.au)

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