


The origins of global invasions of the German wasp (*Vespula germanica*) and its infection with four honey bee viruses

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Abstract A successful control or eradication programme using biological control or genetically-mediated methods requires knowledge of the origin and the extent of wasp genetic diversity. Mitochondrial DNA variation in the native and invaded range of the social wasp *Vespula germanica* was used to examine intra-specific genetic variation and invasive source populations. We also examined wasps for the presence of four viruses found in honey bees: *Acute bee paralysis virus*, *Deformed wing virus*, *Israeli acute paralysis virus* and

Kashmir bee virus. German wasps showed reduced genetic diversity in the invaded range compared to that of their native range. Populations in the introduced range are likely to have arrived from different source populations. All four viral honey bee pathogens were found in *V. germanica*, although they varied in their distribution and strain. Multiple introductions of German wasps have occurred for most invaded regions, though some populations are genetically homogenous. The differing locations of origin will guide researchers searching for biocontrol agents and the reduced genetic diversity may make these wasps a potentially viable target for control via gene drives.

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Introduction

Many authors have lamented the difficulty of controlling well-established biological invasions (Silverman and Brightwell 2008; Hoffmann 2011; Dearden et al. 2017). Eradication of invasive species is a frequently-sought ideal but is even more difficult than control, particularly in the case of plants and animals with short generation times, high reproductive output and effective dispersal (Rejmánek and Pitcairn 2002). Current and historical approaches to control or eradication typically utilise chemical or mechanical methods such as herbicides, pesticides, lethal baits or mechanical removal. These methods are expensive as they require ongoing investment, and can result in environmental contamination as well as non-target effects (Bergstrom et al. 2009). Another approach is the implementation of classical biological control, which involves the introduction of a co-evolved natural enemy from the native range into the area that the invasive pest now occupies. Central to the efficacy of classical biological approaches is a knowledge of the home range of the pest species (Goolsby et al. 2006; Lester et al. 2014a). Many invasive species, however, have a broad geographic range. Determining the precise origin of invasive species is possible with genetic techniques, such as the use of mitochondrial DNA (mtDNA) (Corin et al. 2007; Lester et al. 2014a).

New genetic technologies, such as CRISPR/Cas9 gene drive systems, have the potential to revolutionise the control and eradication of invasive species (Esvelt

et al. 2014; Webber et al. 2015). Gene drives are genetic systems that push engineered genes through target populations far faster than possible through natural inheritance (Burt 2003). The CRISPR/Cas9 system is highly specific as the enzyme Cas9 can target precise sequences of DNA, guided by a short guide RNA (gRNA) sequence. This gRNA can be designed to target precise sequences in the genome to be edited. For the purposes of invasive species control or eradication this process could be used to drive deleterious target alleles to fixation. Deleterious mutations affecting individual fitness may be nuclear or mitochondrial in origin (Dowling et al. 2015; Hammond et al. 2016).

The German wasp (*Vespula germanica*) is a social species native to Eurasia (Archer 1998). It has become invasive in several countries around the world including Argentina, Australia, New Zealand and South Africa (Beggs et al. 2011). Along with the closely related *Vespula vulgaris*, *V. germanica* has been indicted as one of the most widespread and damaging of all invasive Vespidae globally and the species are a critical issue for entomology in New Zealand (Beggs et al. 2011; Lester et al. 2014b). New Zealand has the highest recorded *Vespula* densities in the world, with up to 370 wasps per m² of tree trunk (Moller et al. 1991). Introduced *Vespula* wasps have a variety of environmental, economic and social impacts. For instance, in New Zealand the economic costs of *Vespula* wasps are estimated to be approximately \$130 million each year (MacIntyre and Hellstrom 2015). The biomass of wasps in New Zealand honeydew beech forests is estimated to be similar to, or greater than, the entire biomasses of birds, rodents and stoats (Thomas et al. 1990). In several countries *Vespula* impacts and abundance have stimulated varying degrees of management, using chemical, mechanical and biocontrol techniques (Wood et al. 2006; Beggs et al. 2008; Edwards et al. 2017). However, currently utilised control options are untenable over large areas of many hundreds of thousands of hectares, as they are typically chemical based and require ongoing investment (Dearden et al. 2017).

The biology and distribution of *Vespula* wasps may mean they are likely to be amenable to control through systems such as CRISPR/Cas9 directed gene drives (Dearden et al. 2017). German wasps have a haplodiploid sex-determination system. Mating must be successful for workers to be produced. Any CRISPR/

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Cas9 gene drive system will be unviable if modified queens' genes are not passed to subsequent generations through the production of new queens in the following year. Therefore, male specific traits are a logical target for this class of genetically-mediated approaches. For instance, manipulating the *boule* gene for non-viable sperm production (Sekiné et al. 2015). A queen mating with infertile males will lead to nest failure, however, modified queens mating with heterozygous males would allow the CRISPR/Cas9 modification to persist and spread. Given the limited genetic diversity observed in species such as *Vespula vulgaris* (Lester et al. 2014b), it may be possible to engineer a 'precision drive' system that does not function for all genotypes in the native range (Esvelt et al. 2014). Limited genetic diversity in the introduced range would also mean any gene drive system would be less likely to encounter variation enabling resistance (Drury et al. 2017). A first step in this class of genetically-mediated approaches for control or eradication of invasive species is to identify the genetic diversity in the home and invaded range of the invasive species to be targeted (Dearden et al. 2017).

Natural enemies, such as pathogens, can have major impacts on the fitness, abundance and distribution of plants and animals. These effects may be particularly detrimental for social animals due to high host density and social interactions (Anderson and May 1979; Potts et al. 2010). In the case of social insects, pathogen effects can be even more pronounced as they often spend many generations living in fixed nesting sites. Perhaps the most well-known example of the influence of social insect pathogens is their effect on honey bees. Among pathogens, viruses appear to play a key role in causing honey bee losses (Schroeder and Martin 2012; McMahon et al. 2016). There have been a wide variety of pathogens observed in wasps (Evison et al. 2012), including *Deformed wing virus* (DWV) (Santamaria et al. 2017), which has been implicated as a key driver of honey bee colony collapse (Dainat et al. 2012; McMahon et al. 2016). *Kashmir bee virus* has recently also been observed in wasps (Dobelman et al. 2017) although its pathogenicity is uncertain. Given wasps carry a range of pathogens, one approach to genetically-mediated control could be altering or blocking wasp immune responses; as has been demonstrated in silver whitefly (*Bemisia tabaci*) (Zhang et al. 2017). However, pathogens or key pathogen strains affecting fitness may also be absent in the invaded range, as

suggested by enemy release hypothesis (Keane and Crawley 2002). An understanding of pathogen distributions could be central to both genetic and biological control methods.

Our first aim in this study was to use sequence variation in mtDNA to estimate the home range of global invasions of *V. germanica* and elucidate genetic diversity in the invaded range. Specifically, we examined invasive populations in Argentina, Australia, New Zealand and South Africa; as well as the possible home range of Europe. Our second aim was to survey wasps from throughout these ranges for the presence of *Acute bee paralysis virus* (ABPV), *Deformed wing virus* (DWV), *Israeli acute paralysis virus* (IAPV) and *Kashmir bee virus* (KBV).

Methods

Samples

Wasps used in this study were gathered by contacting researchers in the native and invaded range, or by scientists opportunistically sampling foragers while travelling. Individual worker samples were either freshly collected for this study, or were preserved specimens (Fig. 1; Table S1). In instances where wasps were collected from nests only a single worker from that nest was used in our analysis. Although we collected samples from a broad distribution, we note we will have under-sampled genetic diversity in some areas. Unfortunately, we were unable to source samples from North America where *V. germanica* has also been found. Samples were sent by individuals contacted in North America, but unfortunately were the wrong species. Samples were collected alive and immediately stored in 95% ethanol, followed by storage at -20°C as soon as possible after collection.

Wasp phylogenetic relationships

In order to elucidate the phylogenetic relationship between wasps sampled, as well as the prevalence of viral pathogens, we sequenced PCR products from DNA and RNA extracted from wasp workers sampled throughout these ranges (Fig. 1). DNA and RNA was extracted by bead-beating (BeadBeater 16, Biospec Products, USA) entire wasps in GENEzol reagent (Geneaid, Taiwan) and 5% β -mercaptoethanol

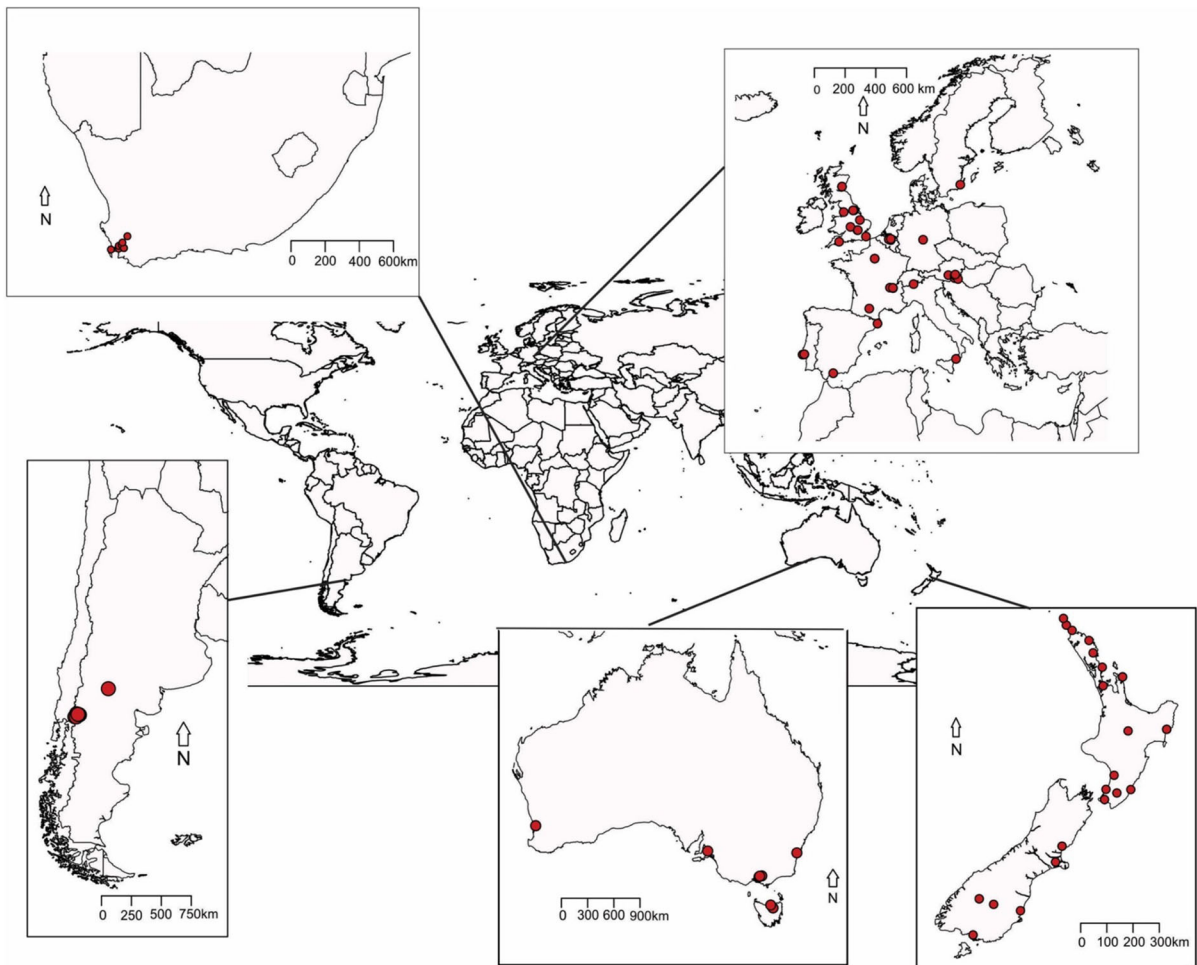


Fig. 1 Origin of the *Vespa germanica* samples used in this study

followed by chloroform and isopropanol purification. DNA concentrations were quantified with a NanoDrop spectrophotometer (NanoDrop/ThermoFisher Scientific, USA). 1 µg/sample was used for cDNA synthesis using qScript XLT SuperMix (Quantabio, USA). Extraction products were stored at -80°C .

For the phylogenetic analysis we used PCR to amplify portions of the mitochondrial loci cytochrome oxidase I (*COI*), cytochrome oxidase II (*CO2*) and cytochrome b (*cytb*). The mitochondrial primers were: *COI*. See ESM for primers and PCR protocols.

For our phylogenetic analysis we assessed the *COI* (1372 bases), *CO2* (172 bases) and *cytb* (413 bases) datasets separately and as a concatenated dataset (1957 bases; note that there was overlap between some primer pairs). To determine the most appropriate model of sequence evolution we used Bayesian

Information Criterion (BIC) scores derived in MEGA7, which also estimated base frequencies, substitution rates, the proportion of invariable sites (I) and the uniformity of substitution rates among sites (G) (Table S2). The models of evolution selected as best-fitting were slightly different across the three datasets, but the best-fitting model for the concatenated mtDNA dataset also ranked among the four best models for the *COI*, *CO2* and *cytb* datasets using BIC and Akaike information criterion scores (Table S2). Therefore, we considered the patterns of sequence evolution to be comparable and used the Hasegawa–Kishino–Yano (HKY) (Hasegawa et al. 1985) model in our phylogenetic analyses. Our selected model and its parameters were then used to build a maximum composite likelihood tree. The level of support for this tree was assessed using 1000 bootstrap replicates.

MEGA7 was also used to calculate percentage genetic distance and standard errors (SE) among groups of individuals.

The relationship between mitochondrial haplotypes and regions of origin were visualised by creating a spatially structured haplotype network in the package TempNet (Prost and Anderson 2011) using R version 3.4.4. (R Core Team 2018). We grouped samples into six geographic regions. United Kingdom (England, Scotland), Europe (Austria, Belgium, France, Germany, Italy, Portugal, Spain, Sweden, Switzerland) Argentina, Australia, New Zealand and South Africa.

The United Kingdom was separated from Europe for two reasons: First, it is geographically separated from mainland. Second, it is the most likely source of New Zealand and Australia's invasions given historical trade between the countries. To investigate the closest genetic relationships of samples from individual regions to our invaded regions (Argentina, Australia, South Africa and New Zealand), we used a maximum composite likelihood model (Tamura et al. 2004). The analysis was conducted in MEGA7.

The R package *vegan* was used to perform the rarefaction analysis to indicate whether full haplotype

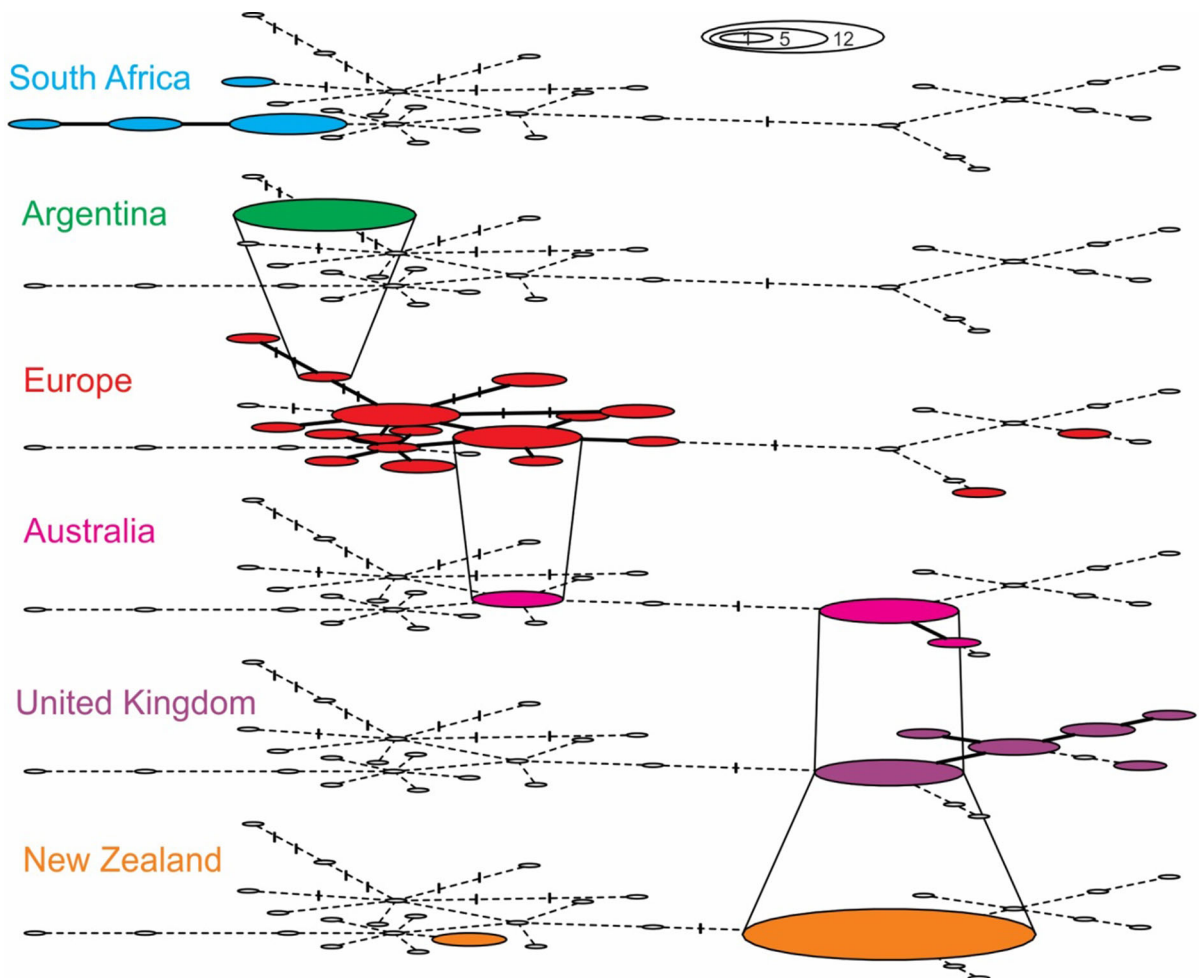


Fig. 2 Spatially structured haplotype network of *Vespa germanica* constructed in TempNet. Lines between haplotype groups in adjacent layers indicate identical haplotypes between the groups. Filled ellipses indicate a positive sample and the relative number of samples for each haplotype. Empty ellipses show the absence of a haplotype in a particular region. Each

point along the lines between haplotypes indicates base substitution. Regional groupings are: South Africa ($n = 9$), Argentina ($n = 12$), Europe (Austria, Belgium, France, Germany, Italy, Portugal, Spain, Switzerland, Sweden, $n = 31$), Australia ($n = 11$), United Kingdom (England, Scotland, $n = 16$), New Zealand ($n = 33$)

diversity in a population has been measured by sampling (Oksanen et al. 2018). As in all rarefaction analyses, if the rarefaction curve is still increasing, then the sampling is not an adequate assessment of diversity. Alternatively, if the curve has reached a horizontal asymptote, then it is reasonable to infer the haplotype diversity has been sampled adequately.

Viral infection and diversity

We used PCR to detect the presence or absence of viruses in all 112 samples used in this study. See ESM for primers and PCR protocols. For our phylogenetic analysis of viral strain diversity, we assessed a structural polyprotein gene (330 bases). We used the same process to estimate the appropriate model of sequence evolution as the wasp phylogenetic relationships (Tables S3–S4).

Results

Wasp phylogenetic relationships

Our sequencing revealed 36 unique *V. germanica* haplotypes among the 112 samples collected for this study across the native and invaded range (Fig. 2, Table 1). Our maximum composite likelihood model revealed overall genetic similarities between certain populations (Fig. S1, Table S5). In particular, the samples from New Zealand, Australia and the United Kingdom shared a number of haplotypes and the populations were most closely related to each other (Figs. 2, 3; Fig. S1, Table S5). Only two haplotypes were detected from the 33 New Zealand specimens (Fig. 2, Table 1); one was common ($n = 31$) and one

Table 1 Summary of number of *V. germanica* specimens sampled and the unique haplotypes found in each range

Region	Specimens sampled	Unique haplotypes
Europe	31	20
United Kingdom	16	6
Australia	11	3
New Zealand	33	2
Argentina	12	1
South Africa	9	4
Totals	112	36

Fig. 3 Maximum composite likelihood tree for *Vespula germanica* sampled throughout the native and introduced range. *Vespula vulgaris* was used to root the evolutionary tree. The tree was based on 1000 bootstraps of a Hasegawa–Kishino–Yano model using a concatenated dataset of *CO1*, *CO2* and *cytb* mt DNA sequences. Colours identify different regional groupings (see Fig. 2). The line connecting the outgroup *V. vulgaris* is not to scale

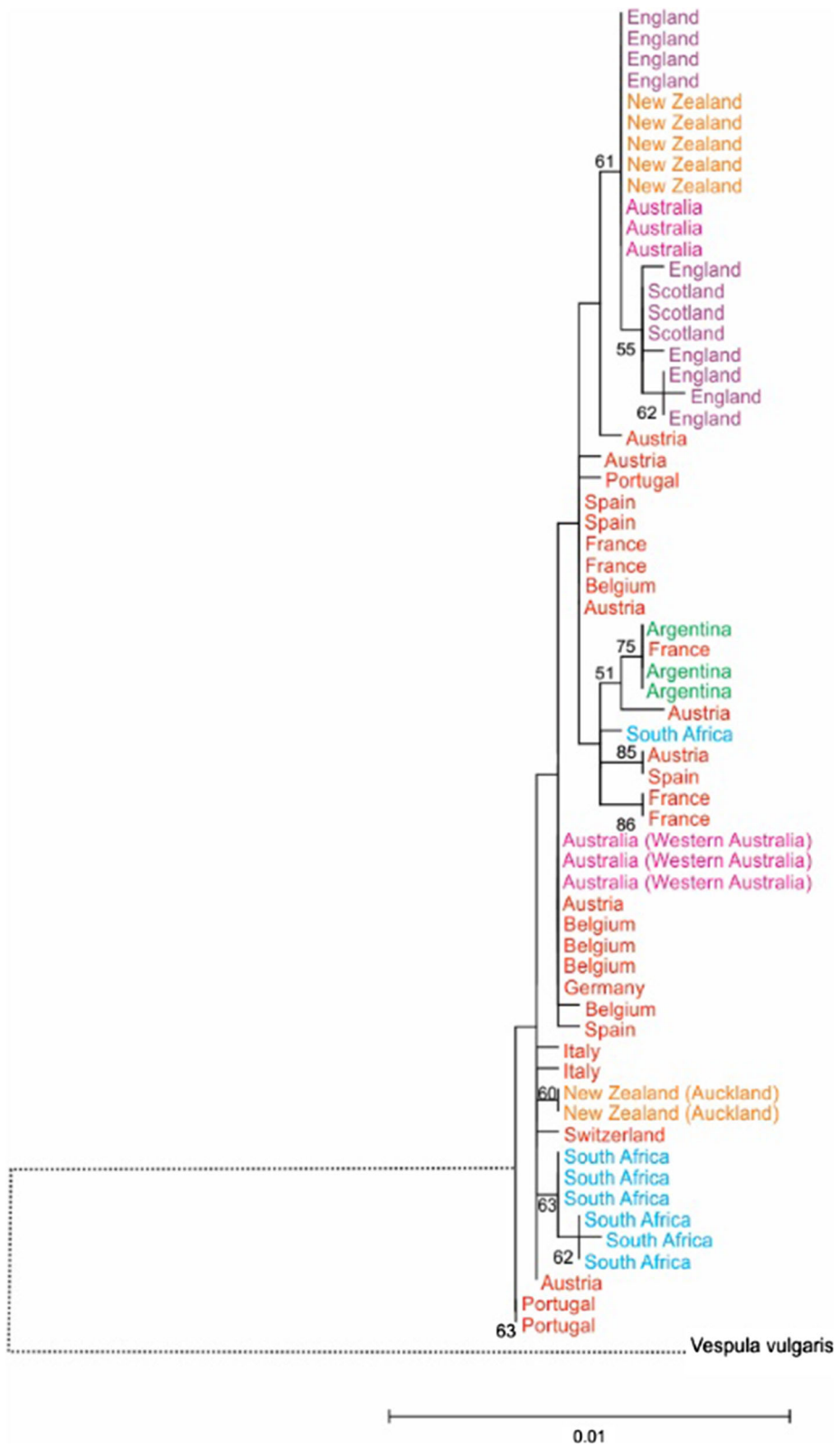
rare ($n = 2$). The common haplotype was an exact match to the most common haplotype in the United Kingdom. The rare New Zealand wasp haplotype was only found in Auckland and is genetically similar to populations from central Europe (Figs. 2, 3).

Three haplotypes were sampled from the 11 Australian specimens. The most common ($n = 7/11$) was the most frequently found haplotype in New Zealand and the United Kingdom (Fig. 2). The second haplotype ($n = 1/11$) clustered with samples from the United Kingdom in our maximum composite likelihood phylogenetic analysis (Figs. 2, 3). While the third haplotype ($n = 3/11$) was only found in Western Australia and was an exact match to samples to central Europe.

The Argentinian population sampled only contained one haplotype which was also found in a wasp from the south of France, near the border of Spain. This population therefore clustered within Europe and most closely with French samples (Figs. 2, 3; Fig. S1).

The home range of Europe was the most genetically diverse region sampled. A total of 31 specimens from nine countries revealed 20 unique haplotypes. Exact matches to European haplotypes were found in Argentina and Australia; but surprisingly not the United Kingdom (Fig. 2). We sampled nine specimens in South Africa, revealing a total of four unique haplotypes. None of these proved an exact match to our samples from Europe. However, our maximum likelihood phylogenetic analysis places them with samples from mainland Europe (Fig. 3; Fig. S1).

We performed a rarefaction analysis (Fig. 4), which suggests we have sampled much of the genetic diversity in Argentina, New Zealand and potentially Australia. Conversely, South Africa, the United Kingdom, and especially Europe likely have haplotypes that have not been sampled (Fig. 4); which may explain the lack of matches to haplotypes in South Africa and the United Kingdom. Our data suggest the native range of Eurasia harbours more genetic variability than the introduced range (Fig. 4, Table 1).



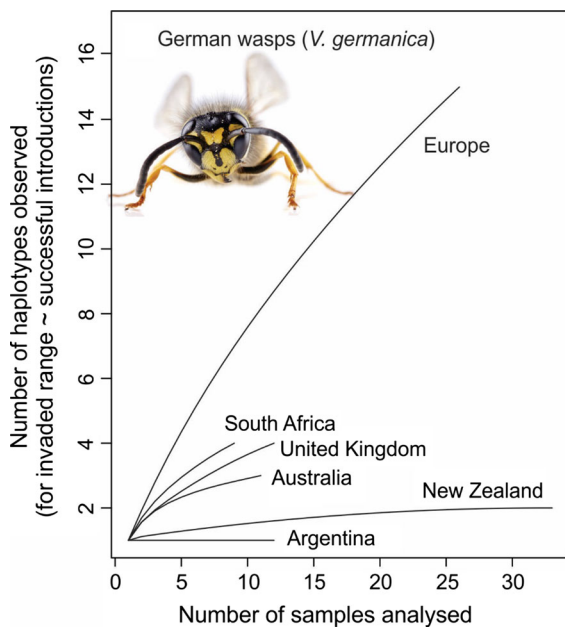


Fig. 4 Haplotype discovery curves from the six population areas sampled. These curves show that the full diversity of haplotypes was likely sampled from New Zealand and Argentina and Australia. Full haplotype diversity was not captured from the three other populations

Wasp virus pathogen presence

Three viruses in the *Dicistroviridae* family were observed: the *Acute bee paralysis virus* (ABPV), the *Kashmir bee virus* (KBV), and the *Israeli acute paralysis virus* (IAPV). ABPV presence in *V. germanica* was confirmed only in the United Kingdom ($n = 5/16$). Percentage cover of sequences was 100% with identity of 96–99%. The phylogenetic analysis demonstrated that the ABPV sequences from the United Kingdom were more closely related to each other than to global sequences from GenBank (Fig. 5). Our sampling revealed that each of the five positive samples of ABPV were distinct viral genotypes (Fig. 5). KBV presence was confirmed in New Zealand ($n = 5/33$) and England ($n = 1/16$). Percentage cover of sequences was 100% with identity of 96–99% ($n = 6$). These samples had a comparatively low strain diversity with only two KBV genotypes sampled. Four of the New Zealand samples and one English sample were the same haplotype, while the second haplotype was discovered once in Northland, New Zealand (Fig. 5). IAPV presence was only confirmed once ($n = 1/31$) in a sample from France,

which was part of our European population. Percentage cover of the sequence was 99% with identity of 98%. Our sequence formed a distinct branch relative to the ABPV and KBV samples (Fig. 5).

We detected *Deformed wing virus* (DWV) which is in the *Iflaviridae* family. DWV was present in *V. germanica* in New Zealand ($n = 8/33$) and Europe ($n = 3/31$). Percentage cover of sequences was 100% with identity of 97–99% in all cases ($n = 11$). The positive sequences from New Zealand clustered with other New Zealand samples (Fig. 6). Positive European samples clustered with other samples from central Europe; although the relationships within Europe were poorly resolved (Fig. 6). Across the two populations only two samples showed evidence of co-infection with any of these viruses. The first was a French sample, which was positive for both DWV and IAPV; the second a New Zealand sample which was positive for both DWV and KBV.

No samples testing-positive for the viruses assayed for were collected in Argentina ($n = 0/11$) Australia ($n = 0/11$) or South Africa ($n = 0/9$). Viral presence and absence data are summarised in Table 2.

Discussion

A first step in utilising biological control or technologies such as CRISPR/Cas9 gene drive systems for the control or eradication of invasive species is knowledge of the genetic diversity of populations in the native and invaded range (Dearden et al. 2017). Entrainment in an invasion pathway may lead to reduced genetic diversity in founding populations (Tsutsui et al. 2000; Corin et al. 2007; Lester et al. 2014a). Genetic diversity may be especially reduced where invasive populations are extremely geographically distant. Our first aim in this study was to use variation in mtDNA to estimate the home range of global invasions of *V. germanica* and elucidate genetic diversity in the invaded range. The three mitochondrial genes investigated suggest high levels of genetic diversity in the home range of Europe (Fig. 2, Table 1). We sampled 31 wasps from their European range and found 20 haplotypes. Indeed, it is likely that we under-sampled genetic diversity in this region (Fig. 4). Our sampling showed that, from this data, wasps in Europe are genetically diverse and appear to lack the clear haplotype boundaries found in the invaded populations.

We demonstrate reduced genetic diversity in each of the four invaded ranges (Fig. 2, Table 1). Our data have captured the likely donor regions to the invaded ranges (Table 3). However, the resolution with which we can pinpoint areas of origin varies. Our results from sampling of the invaded range indicates that the southern hemisphere invasions of this species may have potentially diverse origins (Table 3). Ultimately, however, they largely match haplotypes found in western Europe (Figs. 2, 3; Fig. S1, Table S5). As German wasp nests are founded by a single queen and mtDNA is maternally inherited, the presence of several mitochondrial haplotypes in a region indicates the successful introduction and establishment of

multiple queens. Vespidae wasp queens have been found to hibernate in an array of human commodities and structures such as, under roofing eaves, within wood piles and in-between books in shelves (personal observations). Given findings of long-range intra-country movement of Vespidae wasps by humans (Crosland 1991; Masciocchi and Corley 2013; Masciocchi et al. 2016) it appears likely that fertilised queens are probably moved internationally subsequent to finding a suitable overwintering habitat.

Our work highlighting the likely region of origin of invasive propagules of *V. germanica* could aid in the discovery of biological control agents. Biological control agents such as parasitoids coevolve with their

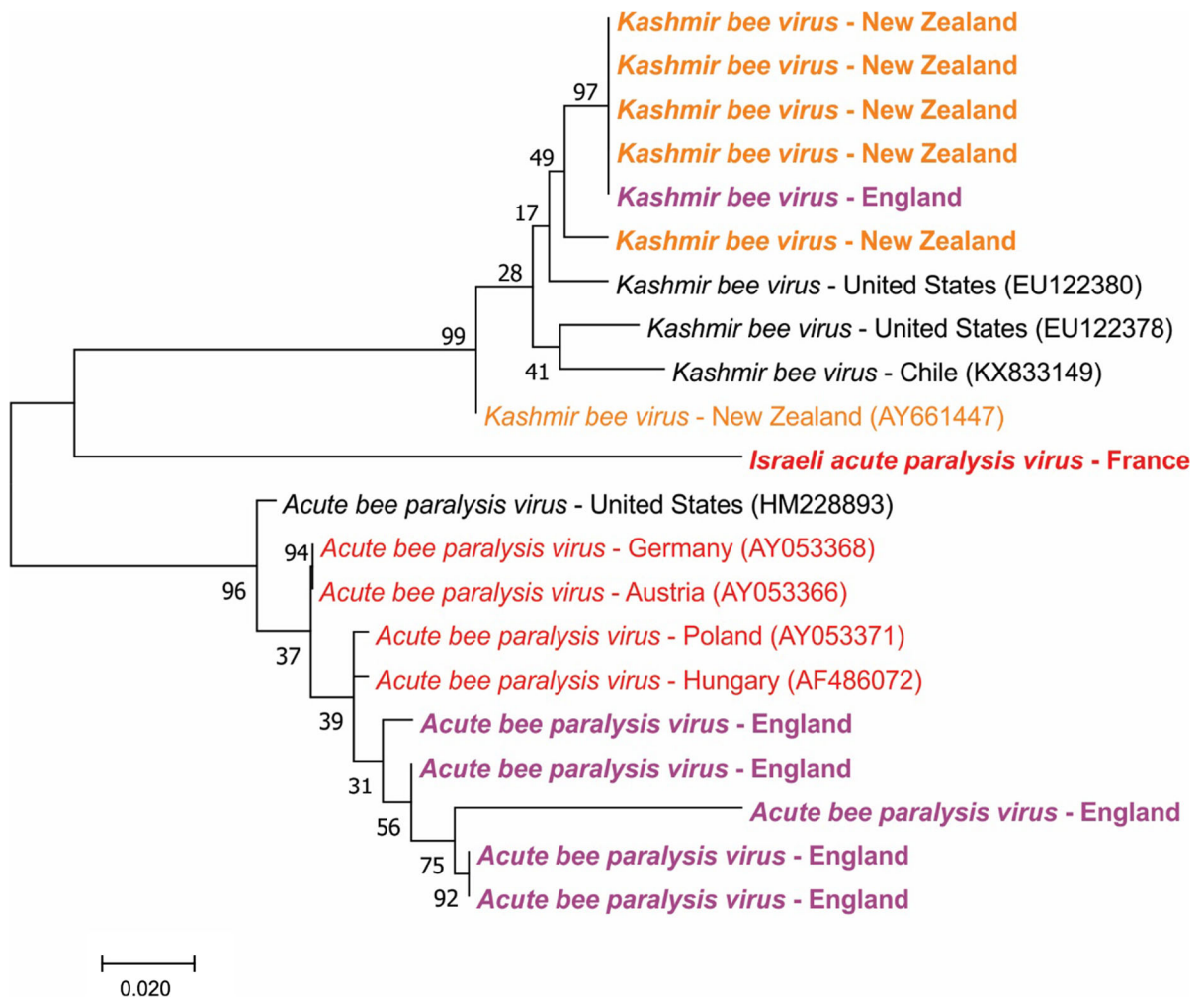


Fig. 5 Maximum composite likelihood tree for Dicistroviridae sequences sampled, as well as samples from utilised from GenBank. The tree was based on 1000 bootstraps of a Kimura-2

parameter model. Samples in bold are from this study. Colours indicate geographic origin (see Fig. 3)

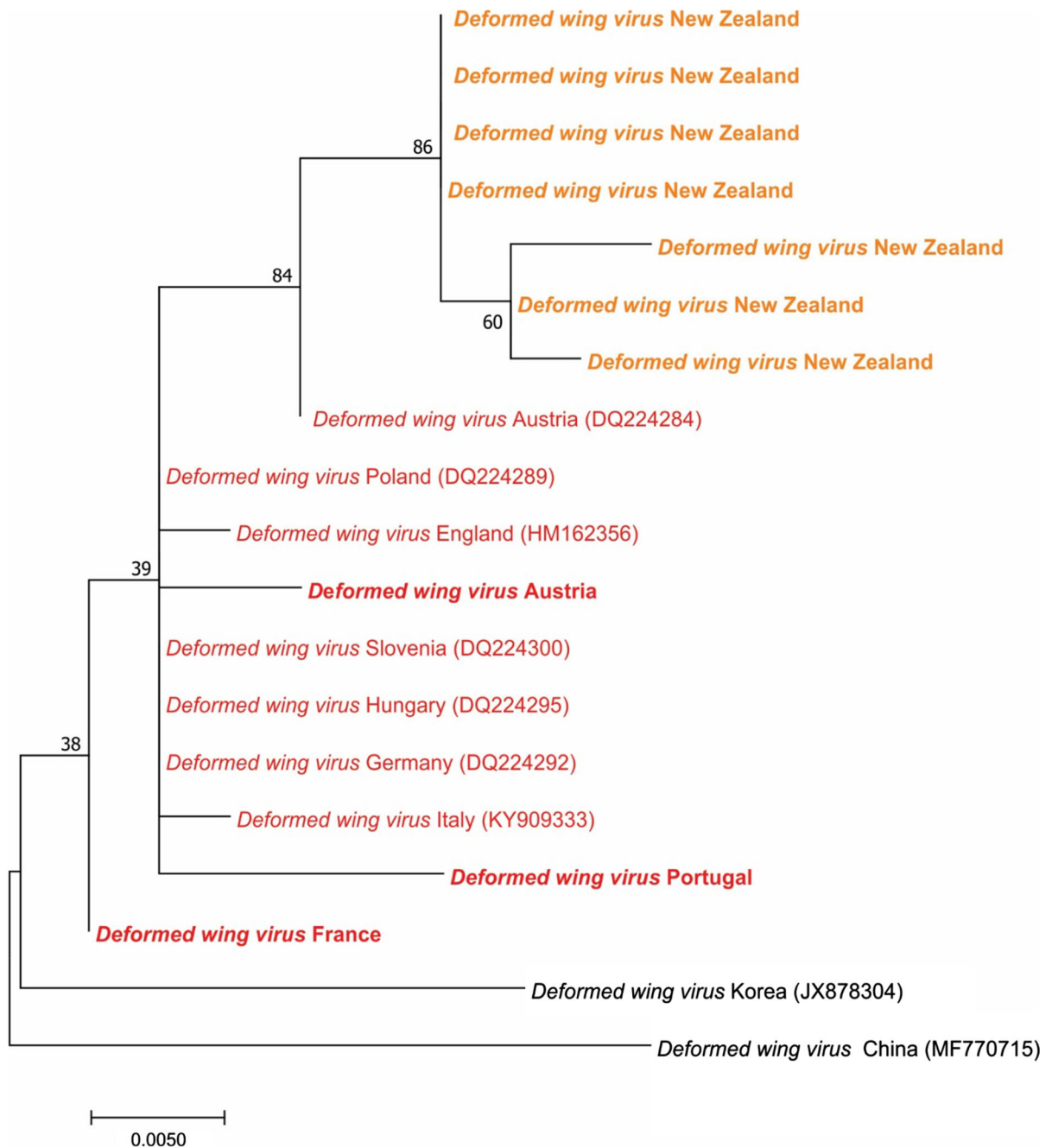


Fig. 6 Maximum composite likelihood tree for DWV sequences sampled, as well as samples from utilised from GenBank. The tree was based on 1000 bootstraps of a Kimura-2

parameter model. Samples in bold are from this study. Colours indicate geographic origin (see Fig. 3)

host prey, which can lead to distinct host-strain relationships that vary geographically (Dupas et al. 2003). The failure of biological control agents to affect prey densities has previously been hypothesised as due to a mismatch between prey strain and predator

(Grodowitz et al. 1997). Wasp biological control has previously been attempted in New Zealand by the importation of the Holarctic parasitoid *Sphex vesparum* (Hymenoptera: Ichneumonidae). Populations of *S. vesparum burra* (Cresson) were sourced

Table 2 Viral presence in German wasps in the native and invaded range

Virus	Region					
	Argentina	Australia	Europe	New Zealand	South Africa	United Kingdom
ABPV	–	–	–	–	–	31%
DWV	–	–	10%	24%	–	–
IAPV	–	–	3%	–	–	–
KBV	–	–	–	15%	–	6%

The data shown are the mean prevalence rate. Dashes indicate the pathogen was not detected

from Washington State in the USA, and *S. vesparum vesparum* were sourced from Austria, Germany and Switzerland (Donovan and Read 1987). Populations of *S. vesparum* have established in New Zealand but have offered no evidence of social wasp control (Beggs et al. 2008). There is no evidence of *S. vesparum burra* establishment (Beggs et al. 2002). Part of the reason for the failure of these biological control attempts may be a mismatch between the geographically distinct social wasp and parasitoid strain. Our results should guide future searches for biological control agents. For example, we would recommend the UK or western Europe as a source of coevolved natural enemies for strains of *V. germanica* found in Australia and New Zealand. Similarly, central Europe appears to be the location for invasive strains of German wasps in South Africa. Natural enemies that might be effective for New Zealand strains may not have similar results in South Africa.

Current techniques used to control invasive species such as wasps rely heavily on chemical and mechanical treatment, as well as requiring ongoing investment. Traditional methods of invasive species control across large land areas of many thousands of hectares is untenable. Biological control is one potential method to reduce wasp densities at a large scale. Genetically-mediated pest-management technologies such as gene drives also have the potential for large-scale control, and may revolutionise the control or eradication of invasive species (Esvelt et al. 2014; Webber et al. 2015).

The utilisation of this class of control techniques requires knowledge of the genetic diversity within native and invaded populations (Dearden et al. 2017). Capture of an invasive species in an invasion pathway may lead to reduced genetic diversity in the founding population (Tsutsui et al. 2000; Sakai et al. 2001; Corin et al. 2007; Lester et al. 2014a). Pest populations

with high levels of genetic diversity can contain genetic variants that render certain genotypes immune to CRISPR/Cas9 gene drive systems (Drury et al. 2017). Our data have demonstrated that populations of the invasive *V. germanica* appear to harbour reduced mitochondrial genetic diversity relative to the home range of Eurasia (Fig. 2, Table 1). This reduced genetic diversity in the invaded range may act as a safeguard for the use of a gene drive system in these populations, because if any genetically modified individuals were re-introduced back to their native range, it would be likely that most haplotypes will be unaffected (Dearden et al. 2017). Further sequencing of genes proposed as targets in a gene drive would need to be undertaken to confirm this hypothesis. The likelihood of gene drive ‘escape’ is further reduced by the geographical isolation of New Zealand relative to the United Kingdom where a matching mitochondrial haplotype is found. However, genetically-mediated pest control technologies are nascent and are of concern to regulators and the public. For a discussion of the issues relating to the use of genetically-mediated pest-control internationally and within New Zealand see Esvelt et al. (2014) and Dearden et al. (2017), respectively.

Our second aim in this study was to survey wasps from throughout the native and invaded range for the presence of viruses commonly found in honey bees, as these pathogens have been implicated as key contributors to the fitness of honey bees and even colony collapse disorder (Schroeder and Martin 2012; McMahon et al. 2016). Other pollinators such as bumble bees have been shown to be deleteriously impacted by ‘honey bee’ diseases (Gegear et al. 2006; Genersch et al. 2006; Cameron et al. 2011; Meeus et al. 2011, 2014). We confirmed the presence of each virus tested for (Table 2) and that there was evidence for viral replication within wasps (i.e. that wasps were

Table 3 Summary of previous literature and findings of this study on the origin of invasions of *V. germanica* populations around the world

Invaded range	Date of first report and location of origin previous literature	Closest haplotypes (this study)
Argentina	Adults first reported during 1980s (Willink 1980). Hypothesised donor region of Chile (Beggs et al. 2011). Present across much of central Argentina (Masciocchi and Corley 2013)	We discovered one haplotype in Argentina. It was most similar to a sample from the south of France on the border with Spain (Fig. 2). Given this finding the Argentinian population clusters with French and Spanish haplotypes (Fig. 3). Although our haplotype discovery curve suggests we have sampled the full mitochondrial diversity in Argentina (Fig. 4); our geographic sampling area does not encompass the entire range of the species in South America. We cannot therefore exclude incursions from elsewhere
Australia	Reported in Tasmania 1959 (Crosland 1991). Recorded on mainland in 1975 (Spradbery and Maywald 1992). Hypothesised donor pathway and region of origin: Cargo from New Zealand (Spradbery and Maywald 1992)	Three haplotypes were sampled in Australia. The most common ($n = 7/11$) was the most frequently found haplotype in New Zealand and the United Kingdom (Fig. 2). The second haplotype ($n = 1/11$) clustered with samples from the United Kingdom (Figs. 2, 3). While the third haplotype, found in Western Australia ($n = 3/11$), was an exact match to samples from central Europe (Figs. 2, 3); and this haplotype was not found elsewhere in Australia. There have likely been multiple introduction events in Australia. These have likely been from New Zealand, the United Kingdom and central Europe. Our haplotype discovery curve (Fig. 4) suggests we have sampled most of the diversity present in Australia
New Zealand	First reported 1945 (Thomas 1960). Hypothesised to have arrived with crates of equipment flown from Europe to New Zealand (Thomas 1960)	The majority of New Zealand's samples ($n = 31/33$) belonged to a single haplotype, identical to the most common haplotype in the United Kingdom and Australia (Fig. 2). This haplotype was spread across the country. A second rare haplotype was discovered in Auckland and is most similar to central European haplotypes (Fig. 3). There appear to have been at least two introduction events to New Zealand. The 1945 introduction from the United Kingdom, which became widespread; and a more recent introduction, potentially from central Europe, which at present only found in Auckland. Our haplotype discovery curve suggests we have sampled most of the diversity present in New Zealand (Fig. 4)
South Africa	First reported 1974 (Whitehead and Prins 1975) at a container depot (Tribe and Richardson 1994)	We detected the presence of four haplotypes in South Africa and sampled across the full invaded range (Veldtman et al. 2012). None of these proved an exact match to our samples from Europe. However, our maximum likelihood phylogenetic analysis places them within samples from mainland Europe (Fig. 3). The presence of four haplotypes may indicate one introduction event with several queens, or alternatively, multiple introductions. Our haplotype discovery curve suggests we have not sampled the full mitochondrial diversity in South Africa (Fig. 4)

A more detailed description may be found in the supplementary materials

being parasitised by the viruses). No wasps that we sampled exhibited phenotypic evidence of infection such as deformed wings. This result is unsurprising as all samples collected were foragers and were therefore

able to move, fly and perform tasks. The presence and infection rates appeared to vary between ranges, although we recognise the limited amount of samples for such a comparative analysis. Each virus detected

showed distinct geographic clustering based on our maximum composite likelihood trees (Figs. 5, 6). This finding can potentially be explained by the large geographic distances between populations and the high mutation rates of viruses. If these results are, in fact, indicative of a low pathogen infection rate any biological control or modification of immune systems to increase the effects of viruses on wasps may have low efficacy.

Previous work has found that *Vespula* wasps carry a range of honey bee pathogens (Evison et al. 2012; Lester et al. 2014a, 2015; Dobelmann et al. 2017). Wasps forage in the same habitat as honey and bumble bees, as well as raiding honey bee hives (Clapperton et al. 1989), increasing opportunities for pathogen sharing. Honey bee viruses have been found actively replicating in a range of arthropods (Levitt et al. 2013). From our data we cannot conclude that any viruses tested for are definitively absent from the populations in which they were not found, though the absence of viruses such as IAPV from New Zealand is in agreement with extensive sampling from honey bees (McFadden et al. 2014). Our viral pathogen assays instead indicate that *V. germanica* is a globally widespread invasive species capable of carrying honey bee viruses. However, the biological relevance of these pathogens in wasps and other arthropods is unknown and is an important avenue for future research. An increasing number of studies reveal that ostensible ‘honey bee’ pathogens may in fact be generalist pathogens found in a wide number of arthropods (Evison et al. 2012; Levitt et al. 2013; Lester et al. 2014a, 2015; Sébastien et al. 2015; Gruber et al. 2017).

The functional impacts of these pathogens in hymenopteran arthropods besides honey and bumble bees are not well elucidated. If these pathogens were to be used as biological control agents, it would need to be shown they have an impact on wasp fitness. To our knowledge there are no data demonstrating a causal link between *V. germanica* death and viral pathogens of honey bees. However, at least in the case of KBV infection in *V. vulgaris*, increasing viral infections are negatively correlated with wasp fitness and expression of wasp antiviral immune genes (Dobelmann et al. 2017). This result suggests pathogens can have negative impacts in wasps. Latent infections in asymptomatic hosts can eventually result in deleterious impacts (Speck and Ganem 2010). Apparently mild illnesses can reduce fitness by influencing

interspecific interactions and community composition (Vincent et al. 2014; Jones and Coutts 2015).

If fitness impacts were demonstrated on *V. germanica*, one potential avenue of control could be the use of a gene silencing approach to alter wasp immune responses to these pathogens—increasing pathogen efficacy as a biocontrol. Such an approach has been demonstrated in a major agricultural pest, the silver whitefly (*Bemisia tabaci*) (Zhang et al. 2017). Further, differential immune gene expression has been demonstrated in other Hymenoptera in response to virulent and avirulent virus infections (Niu et al. 2016), providing potential target genes for such approaches. This approach may, however, differ in its efficacy between countries based on the distribution and prevalence of viral pathogens. Immune gene silencing in invasive wasps may also be of concern if it significantly increases the local abundance of viruses causing spillover to other species including honey bees.

Controlling well-established biological invasions is extremely difficult (Silverman and Brightwell 2008; Hoffmann 2011; Dearden et al. 2017). An understanding of the intra-specific genetic diversity is critical for successful control programmes involving biological control or emerging genetic methods such as gene drives or gene silencing. We have demonstrated that the Southern hemisphere populations of invasive wasps are likely to have arrived from a small number of introductions from European source populations. This is a first and important step in developing novel ways of reducing populations of German wasp in the Southern hemisphere.

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Author contributions ECB-R, and PJJ conceived the project; ECB-R, JD, RLB, LD, JG, MM, CM, CRS, JS, CVZ, and RV collected the samples, ECB-R, JD, JWB analysed the samples; ECB-R led the writing and interpretation with input from all authors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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