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BIODIVERSITY RESEARCH

Determining the origin of invasions and demonstrating a lack of enemy release from microsporidian pathogens in common wasps (*Vespula vulgaris*)

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ABSTRACT

Aim Understanding the role of enemy release in biological invasions requires an assessment of the invader's home range, the number of invasion events and enemy prevalence. The common wasp (*Vespula vulgaris*) is a widespread invader. We sought to determine the Eurasian origin of this wasp and examined world-wide populations for microsporidian pathogen infections to investigate enemy release.

Location Argentina, Eurasia, New Zealand.

Methods A haplotype network and phylogenetic tree were constructed from combined wasp *COI* and *cytb* mitochondrial markers. A morphometric study using canonical discriminant analysis was conducted on wing venation patterns. Microsporidian pathogens prevalence was also examined using small subunit rRNA microsporidia-specific primers.

Results Our spatially structured haplotype network from the native range suggested a longitudinal cline of wasp haplotypes along an east to west gradient. Six haplotypes were detected from New Zealand, and two from Argentina. The populations from the introduced range were genetically similar to the western European, United Kingdom and Ireland. The morphometric analysis showed significant morphological variation between countries and supported the Western European origin for New Zealand populations, although not for Argentine samples. Microsporidian infection rates were highest in New Zealand samples (54%), but no significant differences in infection rates were observed between the invaded and native range. *Nosema* species included matches to *N. apis* (a pathogen from honey bees) and *N. bombi* (from bumble bees).

Main conclusions Multiple introductions of the common wasp have occurred in the invaded range. A high microsporidian infection rate within the native range, combined with multiple introductions and a reservoir of pathogens in other social insects such as bees, likely contributes to the high microsporidian infection rates in the invaded range. Enemy release is likely to be more frequent when pathogens are rare in the home range, or are host specific and rare in reservoir populations of the introduced range.

Keywords
Biological invasions, enemy release, *Nosema*, pathogen, social wasp, *Vespula vulgaris*.

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INTRODUCTION

The enemy release hypothesis proposes that invasive species can become abundant because of the absence of co-evolved natural enemies such as pathogens and parasites (Keane & Crawley, 2002; Torchin *et al.*, 2003). Reduced enemy abundances are, however, likely to occur only under certain circumstances. The more often an invader is introduced, the more likely natural enemies will also be introduced. The probability of the introduction of enemies must also be related to their prevalence in host populations within the native range, as a high pathogen infection rate of an invasive species within their native range must correspond to a high probability of any invasive propagules being infected. The host specificity of natural enemies likewise must influence the prevalence and effects of natural enemies on invaders. Should existing exotic or native natural reservoirs of natural enemies occur in a new environment, they may spillback to arriving invasive species (Flory & Clay, 2013). The potential role of alternative hosts for pathogens in maintaining, or in some situations reducing, the presence and abundance of pathogens in an environment has been clearly demonstrated with Lyme disease (Ostfeld & Keesing, 2000).

The substantial influence that natural enemies such as pathogens can have on populations of social insects is exemplified in the global decline of bee populations, which is widely referred to as colony collapse disorder. The exact causes of this disorder are currently unknown, but likely involve a combination of several pathogens or parasites and perhaps other factors such as pesticide exposure (Bromenshenk *et al.*, 2010; Evans & Schwarz, 2011). Bumble bees are also experiencing a substantial population decline associated with pathogens and low genetic diversity (Cameron *et al.*, 2011). Pathogens thought to be responsible for bumble bee and honey bee declines include microsporidians in the genus *Nosema*. These studies suggest that pathogens can have a major effect on social insects, which may be compounded by factors such as low genetic diversity. Invasive social insects typically have low mean population genetic diversity as a result of a limited number of invasive propagules (e.g. Corin *et al.*, 2007; Gruber *et al.*, 2012).

The common wasp (*Vespula vulgaris*) is an invasive species of major biodiversity and conservation importance. Their high densities in the invaded range of countries such as New Zealand and Argentina are the driver of their substantial ecological impacts. In New Zealand, for example, densities of up to 370 wasps m^{-2} of tree trunk (Moller *et al.*, 1991) and 34 nests ha^{-1} (Beggs *et al.*, 1998) have been observed. Under such conditions, the probability of an orb-web spider surviving to the end of a wasp season has been estimated at virtually zero (Toft & Rees, 1998). Common wasps also compete with native birds and have been recorded attacking and killing chicks (Moller, 1990). These wasps are native to and widespread in Eurasia (Archer, 1989; Dvořák, 2007). Within this native range, population densities appear to fluctuate dramatically. Exceptional years of high abundance in the native range are frequently followed by years of scarcity, with queen productivity varying by a factor of 100 between nests and years (Archer, 1981). These results indicate some form of endogenous density dependence, and additional exogenous factors such as climate may promote variation in abundance (Archer, 1981, 1985). Alternatively, fluctuations in wasp abundance may be driven by pathogens and parasites. Such natural enemies are prevalent in *Vespula* spp. wasp populations within their native range (Rose *et al.*, 1999; Evison *et al.*, 2012), although their abundance and influence in the introduced range are unknown.

A critical first step towards identifying co-evolved pathogens, and assessing whether enemy release has occurred, is to identify the home range. In the absence of the knowledge of a specific home range, the degree of enemy release may be overestimated (Colautti *et al.*, 2005). The matching of an invasive species to their specific origin can be essential for the successful selection of a haplotype or genotype for biological control (Goolsby *et al.*, 2006). Molecular methods have been particularly useful for determining the home range of invasive plants and animals, indicating invasion pathways and origins for a range of species (e.g. Goolsby *et al.*, 2006; Corin *et al.*, 2007). Morphological variation may also be useful for identifying intraspecific genetic variation and areas of origin (Nielsen *et al.*, 1999; Abramoff *et al.*, 2004; Al-Ghamdi *et al.*, 2013). Our first aim in this study was to estimate the specific area within the Eurasian host range from which the New Zealand and Argentinian populations of the common wasp originated, using variation in mitochondrial DNA and morphology within the home range. We examined wasps from throughout the invaded range in New Zealand and Argentina to search for evidence of multiple successful introductions. Our second aim was to assess whether enemy release occurs in common wasp populations outside the home range by comparing the prevalence of *Nosema* microsporidian pathogens in the invaded and native range. Microsporidian pathogens may be important in social insect population dynamics, such as with honey bee colony collapse disorder (Bromenshenk *et al.*, 2010; Evans & Schwarz, 2011). Moreover, these pathogens have also been demonstrated to infect and multiply in *Vespula* wasps, with the potential to kill entire wasp nests (Fantham & Porter, 1913).

METHODS

Samples

Wasps were obtained by contacting entomologists located throughout the native and invaded range. Samples of foraging workers or workers from nests were either freshly collected for this study or were from preserved samples (Fig. 1; Table S1 in Supporting Information). If wasps were collected from nests, only a single individual from the nest was subsequently used in the analysis. We note that while we achieved collection of samples from a representative distribution of these wasps, given their wide distribution, it is likely that we

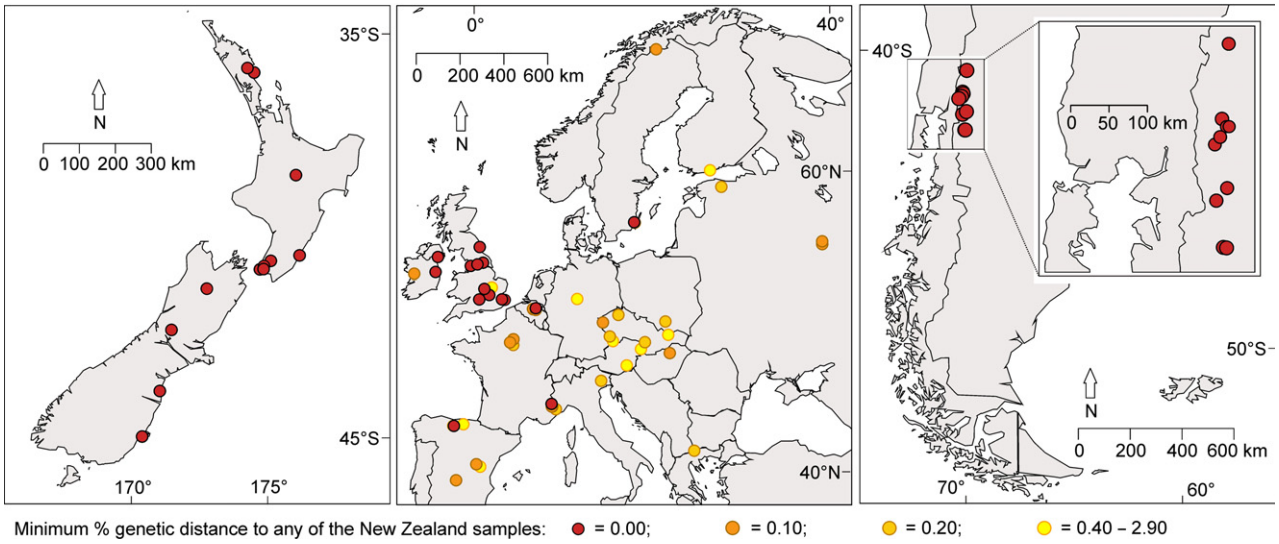

Figure 1 Origins of the *Vespula vulgaris* samples. The samples are coloured according to their percentage genetic similarity to one or more of the specimens from New Zealand. The Chinese specimen showed the highest genetic difference (2.9% base pairs difference) compared to the New Zealand samples (Chinese sample location not shown on the map).

have missed genetic diversity occurring in under-sampled regions such as Asia and Russia. Common wasps were only recently detected in Argentina (Masciocchi *et al.*, 2010), and the samples from this country spanned their entire known distribution at the time of collection (February and March 2013). Australia has been invaded by the common wasp (Richards, 1978), but despite attempts to collect fresh samples, no Australian specimens were obtained, and only two historic samples from the 1970s were sourced. DNA extractions from these specimens were not successful. Australian entomologists we contacted suggested that common wasp populations had been superseded by the more recently arrived German wasp (*V. germanica*). For the phylogenetic analysis, we used an individual German wasp (*V. germanica*) as an outgroup, which was collected in Auckland, New Zealand. Note that the range of the common wasp was previously thought to extend into North America, although recent work has demonstrated '*V. vulgaris*' in this region are actually *V. alascensis* (Carpenter & Glare, 2010).

Wasp phylogenetic relationships

To ascertain the phylogenetic relationships and prevalence of microsporidian infections among the *V. vulgaris* samples from the native and introduced ranges, we sequenced DNA from wasp workers sampled throughout these ranges (Fig. 1). Wasps were dissected, and the gut used for both wasp DNA and microsporidian extractions (these are primarily gut parasites). We extracted DNA using a standard digestion with 0.2% SDS and 0.5 mg/mL proteinase-k until the tissue dissolved (2–4 h), followed by phenol/chloroform purification, ethanol precipitation and re-suspension in Tris-EDTA buffer.

We used PCR to amplify portions of the mitochondrial loci *COI* (cytochrome oxidase I) and *cytb* (cytochrome b) for phylogenetic analysis. The mitochondrial primers were C1-J-1718(Sid) 5′-GGA GGA TTT GGA AAT TGG CTT ATT CC-3′ and C1-N-2191(Nancy) 5′-CCC GGT AAA ATT AAA ATA TAA ACT TC-3′ for *COI*, and CB1 5′- TAT GTA CTA CCA TGA GGA CAA ATA TC-3′ and CB2 5′- ATT ACA CCT CCT AAT TTA TTA GGA AT-3′ for *cytb* (Simon *et al.*, 1994). Each 15-μL PCR consisted of 1 μL of template DNA (~20 ng DNA), 1 X PCR Buffer, 0.4 mg/mL of bovine serum albumin (BSA), 1.5 mM MgCl2, 200 μM of each dNTP, 0.4 μM of each primer and 0.1 Unit of Taq DNA Polymerase (Fisher). Thermal cycling conditions for *COI* and *cytb* consisted of initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturing at 94 °C for 30 s, annealing for 40 s at 45 °C and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Amplified products were purified using ExoSAP-IT (US Biochemicals, Cleveland, Ohio, USA) and sequenced directly with an ABI 3730XL Genetic Analyser (Applied Biosystems) by Macrogen Inc., Seoul, Korea and the Massey Genome Service, Palmerston North, New Zealand. Genomic DNA was sequenced from 103 samples. We manually checked for quality, edited and aligned the DNA sequences using MEGA 5.1 (Tamura *et al.*, 2011). The sequence of the sample from Mongolia was of poor quality and was discarded from further analysis. We used BLASTn searches of the NCBI (GenBank) nucleotide (nr) database to confirm the authenticity of our 105 clean sequences as *V. vulgaris* (and the outgroup *V. germanica*). For our phylogenetic analysis, we assessed the *cytb* (420 bases) and *COI* datasets (432 bases) separately, and both sets of sequences as a concatenated dataset (852 bases).

To determine the most appropriate model of sequence evolution for our datasets, we used Bayesian information criterion (BIC) scores derived in MEGA, which also estimated base frequencies, substitution rates, the proportion of

invariable sites (I) and the uniformity of substitution rates among sites (G). The models of evolution selected as best-fitting differed slightly for the three datasets, but the best-fitting model for the concatenated dataset also ranked among the three best models for the cytb and *COI* datasets using BIC scores (Table S2). In addition, the Hasegawa–Kishino–Yano model (Hasegawa *et al.*, 1985) with a gamma distribution parameter (HKY + G model) ranked among the four best-fitting models for all three datasets using Akaike information criterion (AICc) and maximum likelihood (lnL) values. We therefore considered the models of evolution to be comparable and used the model selected for the concatenated dataset (HKY + G = 0.16) for tree building. The estimated model and parameters were then used to generate a maximum composite likelihood (MCL) tree, and the level of support was assessed with 2000 bootstrap replicates in MEGA. We also used MEGA to calculate percentage genetic distances and standard errors (S.E.) among groups of individuals. The HKY model is not implemented in MEGA for genetic distance calculation, so we used the Tamura-Nei model (TN93; Tamura & Nei, 1993) with a gamma parameter of 0.16 as this was among the best-fitting models for the concatenated dataset.

We visualized the relationships between mitochondrial haplotypes and regions by creating a spatially structured haplotype network in TempNet (Prost & Anderson, 2011). We grouped samples into seven geographic regions: Asia (China, Mongolia); Eastern Europe (Poland, Hungary, Austria, Czech Republic, Greece, Slovakia); Northern Europe (Russia, Estonia, Finland, Sweden); Western Europe (France, Belgium, Germany, Italy, Spain); UK & Ireland (England, Ireland, Northern Ireland).

To test the closest genetic relationships of samples from individual regions to our New Zealand and Argentine samples, we used generalized linear models (GLM) with a negative binomial distribution and log-link function using the *MASS* package (Venables & Ripley, 2002) in R version 2.15.1 (Ihaka & Gentleman, 1996; R Development Core Team, 2012). Genetic distance to the New Zealand or Argentinian samples was modelled as the response variable and region as the predictor variable. To assess correlations between geographic and genetic distance (i.e. isolation by distance), we conducted a Mantel test using the *ade4* package (Dray & Dufour, 2007) in R, using 9999 replicates.

Wasp wing morphology

In addition to genetic markers, we examined variation in wing morphology as another potential character that may be useful to derive the area of origin for the invasive populations. Wing morphology has been utilized to successfully derive subspecies status in other hymenopteran populations, such as honey bees (e.g. Ruttner *et al.*, 1978). The right forewing of adult worker wasps was removed and placed on a microscope slide, with an additional slide placed on top to flatten the wing. A digital photograph was taken of the wing, and the distances between 25 wing nodes were measured using ImageJ (Abramoff *et al.*, 2004; Al-Ghamdi *et al.*, 2013). To account for variation in wasp size, data were standardized by dividing the distance between two nodes by the distance between the nodes b and i (Fig. 2a). We only analysed samples from countries from which we had ≥ 10 individual wasps.

To examine variation in wing morphology between wasp populations in the native and invaded range of *V. vulgaris*, we used canonical discriminant analysis in the *candisc* package (Friendly & Fox, 2013) in R. We first utilized stepwise discriminant function analysis to reduce the number of variables in an attempt to avoid issues associated with colinearity. Country was used as the discriminator variable and 'unexplained variance' used as the stepwise method. The F-value to enter variables was set at $\alpha < 0.05$ and minimum significance to remove variables at $\alpha > 0.10$. The *candisc* output includes a Type II MANOVA, which tested the hypothesis that there is morphological differentiation among the populations.

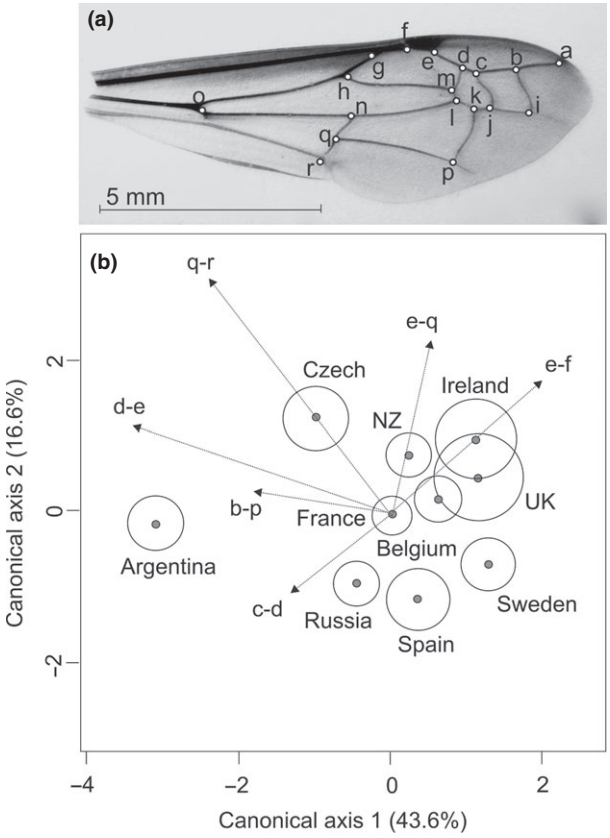

Figure 2 Canonical discriminant analysis examining variation in wasp wing morphology within and between different countries. (i) Distances measured between nodes on wing veins and (ii) Dots represent centroids of the group, while circles are 95% confidence intervals. The alphabetical script (a–b, etc.) represents axes related to individual distance measurements. We included wasps from countries from which we had 10–22 wasp samples.

Microsporidian pathogen prevalence

Small subunit rRNA (SSU rRNA) general microsporidia-specific primers, which amplify the V1–V3 regions of the 16S (SSU) gene, were used for the microsporidian assay. The primers were V1f 5′-CAC CAG GTT GAT TCT GCC TGA C-3′ and 530r 5′-CCG CGG CTG CTG GCA C-3′ (Baker *et al.*, 1995) used the same DNA extractions as for the phylogenetic analysis, together with additional extractions, and analysed all samples for microsporidian infection. Using samples which produced *cytb* and *COI* amplification products allowed us to confirm the DNA extraction quality for the microsporidian assay. Thermal cycling conditions included initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturing at 95 °C for 40 s, annealing at 60 °C for 40 s and extension at 72 °C for 40 s, followed by a final extension at 72 °C for 10 min. Triplicates of the microsporidian pathogen assays were run to estimate false-negative rates using the equation $1-(a/(b*3))$, where a was the total number of positive amplifications and b was the number of samples that resulted in at least one positive amplification. Amplified products of microsporidian-positive PCR products were purified and sequenced as for the wasp samples ($n = 51$). Although the resulting sequences (186 bases) were of sufficient resolution to enable identification to genus level (and species in some cases), low electropherogram peaks prevented a robust examination of phylogenetic similarity or sequence diversity. We used BLASTn searches of the NCBI (GenBank) nucleotide (nr) database to identify the closest matching species to our sequences.

Estimates of mean microsporidian pathogen infection rates (± 95% confidence intervals) were calculated using 10,000 bootstraps in IBM SPSS v 20.0 (IBM Corp, 2011). Infection rates were considered significantly different if confidence intervals did not overlap.

RESULTS

Wasp phylogenetic relationships

Our phylogenetic analysis revealed 33 unique *V. vulgaris* haplotypes among our samples (Fig. 3). The New Zealand samples were closest in genetic distance to those from Argentina, the United Kingdom, France and Belgium (Fig. 1, Table S3). Six haplotypes were detected among the New Zealand samples (Fig. 4), which grouped closely with Argentine haplotypes (Figs 3 & 4). Our spatially structured haplotype network suggested a longitudinal cline of haplotype diversity in the native range, with the eastern European populations more similar to the Asian populations, and UK & Ireland populations more similar to western European populations. The populations from the invaded range were more similar to the western European, UK and Ireland haplotypes (Fig. 4 & Fig. S1 in Supporting Information).

The genetic distance between the New Zealand samples and all other regions except Argentina was significantly greater than within New Zealand samples (Fig. S1, Table S3). The genetic distance between the Argentine samples and other regions compared to the genetic distance between the Argentine samples followed a similar pattern to that of New Zealand. Again, differences were not significant between Argentina and New Zealand (Fig. S1, Table S4). The results of these tests were consistent with the spatial structure observed in haplotype diversity and genetic distance, with the closest relationships between the native range and New Zealand and Argentinian samples being those from the UK and Ireland. Our Mantel test found a significant correlation between genetic and geographic distance in the native range (Fig. S2, $r = 0.838$, $P < 0.001$) consistent with a longitudinal cline in genetic variation.

Wasp wing morphology

We analysed for morphological differences between the wasp populations from different countries or regions. Using these standardized measurements, the canonical discriminant analysis indicated significant population differentiation (MANOVA $F_{9,117} = 3.827$, $P < 0.001$). The stepwise function of the analysis used 13 of the 25 possible measurements (Fig. 2b: relative distances between a–b, c–d, d–e, e–f, j–k, k–l, n–o, q–r, b–p, e–q, h–r). New Zealand samples grouped near the UK, Ireland, Belgium and France (Fig. 2b). Argentinian samples were the most differentiated based on the canonical axis 1, which explained 43.6% of the morphological variation. The measurement giving the highest proportionate differentiation between the New Zealand and Argentinian samples was e-q (Fig. 2a), which indicated this measurement was on average 9.3% smaller in Argentine samples relative to New Zealand specimens.

Microsporidian pathogen prevalence

Our microsporidian pathogen assays revealed that the closest matching sequences on GenBank were typically *Nosema* spp. with percentage cover of 97–100% and identity of 97–100% in the majority of cases ($n = 57/67$). Some sample sequences ($n = 6$) were less well resolved (70–97% cover and 79–92% identity), and four sequences were unresolved. Specific *Nosema* species matches on GenBank were to *N. apis* (two samples from Belgium and two samples from Slovakia with 100% cover and 100% identity) and *N. bombi* (four samples from New Zealand with 100% cover and 98% identity). Nucleotide signals in the electropherograms for a number of sample sequences were weak and were occasionally given as false negatives in the PCR analysis (Table S5). Given these weak signals, we were not confident in undertaking any further analysis of microsporidian diversity.

The false-negative rate among all samples was 56.86%, which may account for the higher microsporidian infection observed here relative to studies such as Evison *et al.* (2012). The prevalence of microsporidian pathogen infection among regions ranged from 0% (Asia, $n = 2$) to 54% (New Zealand,

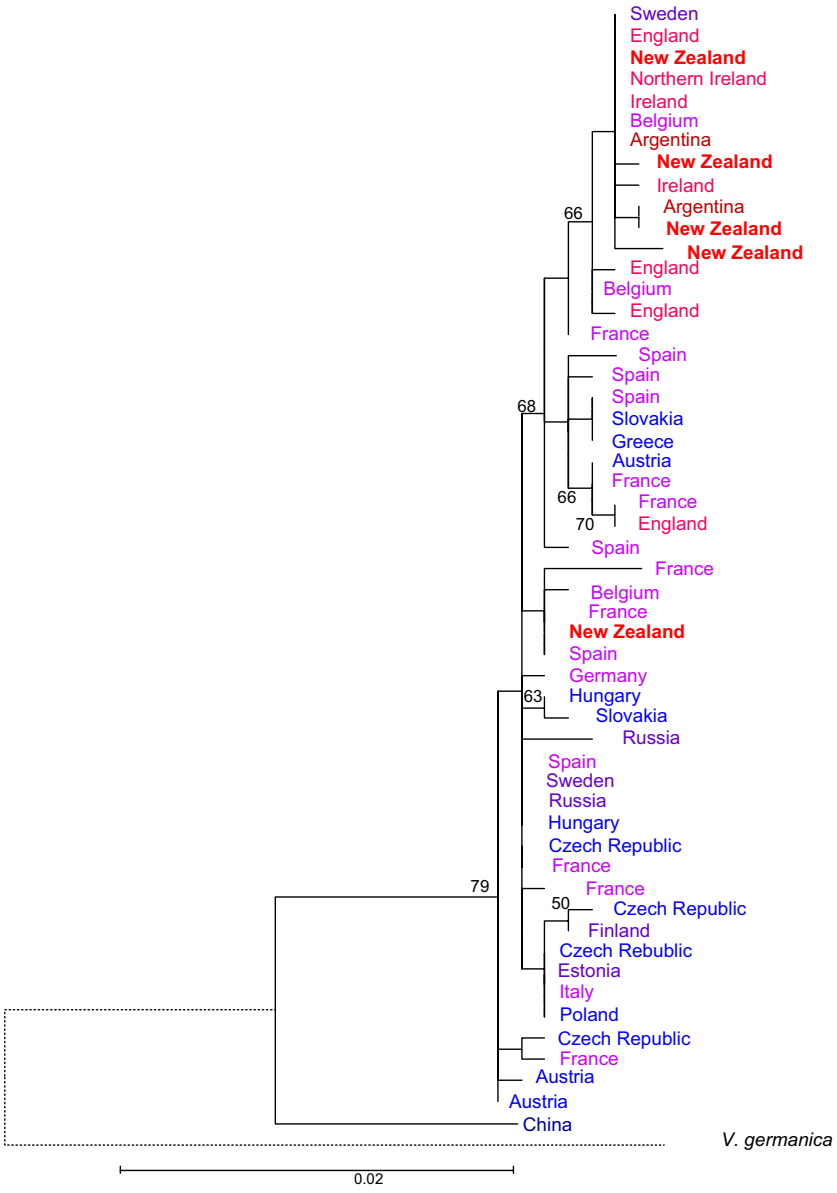

Figure 3 Maximum composite likelihood tree for *Vespula vulgaris* sampled throughout the native and introduced range, with *V. germanica* used to root the evolutionary tree. The tree was based on 2000 bootstraps of a Hasegawa–Kishino–Yano (HKY + G) model with gamma parameter (0.16), using a concatenated dataset of *COI* and *cytb* mtDNA sequences. The estimates of levels of support shown are bootstrap values greater than 50%. Colours identify different regional groupings (see Fig. 4). The dashed line connecting *V. vulgaris* is not to scale.

n = 39; Table 1); however, the variation in infection rates among regions was not significant given that the 95% confidence intervals for the infection rates of all regions overlapped (Table 1).

DISCUSSION

Understanding the role of enemy release in biological invasions requires an accurate assessment of the invader's home range, the number of invasion events and pathogen infection rates. Our first aim in this study was to estimate the specific area within the Eurasian host range from which the New Zealand and Argentinian populations originated. The two mitochondrial DNA genes produced a distinct cline in the geographic structuring in the native range of the common wasp. This cline was related to geographic distance, primarily along an east to west gradient (Fig. 4). Clines may result from geographic variation in selection pressure (e.g. Wunderle, 1981), from introgression following secondary contact (e.g. Cooke *et al.*, 1985) or simply from isolation with increasing geographic distance or other environmental variables (e.g. Brazeau *et al.*, 2013). While a clear geographic cline was observed, some haplotypes were shared in multiple geographic locations. For example, haplotypes occurring in the UK and Ireland were present in Western and Eastern Europe. The wasp collected from China was the only one to have a unique haplotype not shared with any other region. The lack of distinct haplotype boundaries within the native range suggests that mixing or dispersal within these wasp populations might have occurred, which could have been caused by either natural or human-aided means. Estimates of natural dispersal for Vespid queen wasps range in the hundreds of metres per year, while human-aided transport of overwintering or hibernating queens likely accounts for the

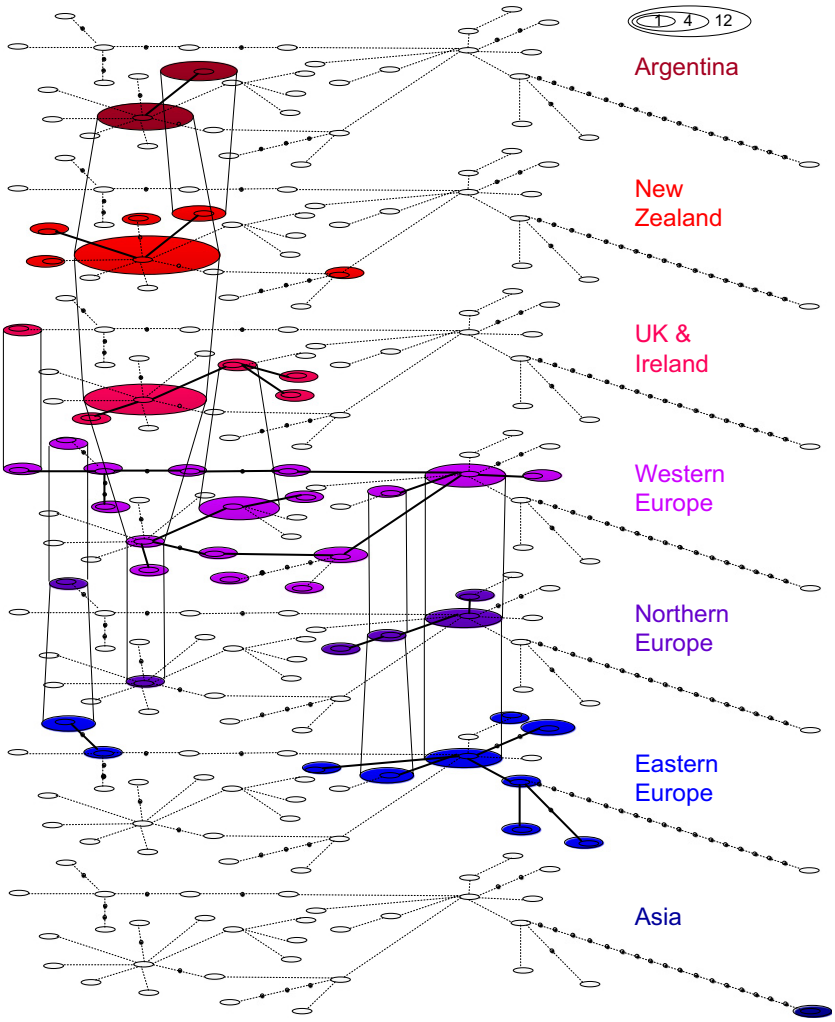

Figure 4 Spatially structured haplotype network of *Vespula vulgaris* constructed in TempNet. Lines between haplotype groups in adjacent layers indicate relationships between the groups. Filled ellipses denote a positive sample and the relative number of samples for each haplotype. Empty ellipses represent the absence of a haplotype in a particular region. Each point along the lines between haplotypes indicates a base substitution. Regional groupings are: Asia (China, n = 1); Eastern Europe (Poland, Hungary, Austria, Czech Republic, Greece, Slovakia, n = 16); Northern Europe (Russia, Estonia, Finland, Sweden, n = 9); Western Europe (France, Belgium, Germany, Italy, Spain, n = 26); UK & Ireland (England, Ireland, Northern Ireland, n = 17), New Zealand (n = 23) and Argentina (n = 11).

Table 1 Prevalence of microsporidian pathogen infection in the common wasp samples from the study regions

Region	n	Positive	Prevalence	95% CI range
Argentina	19	7	0.368	0.158–0.579
Asia	2	0	0.000	0.000–0.000
Eastern Europe	14	5	0.357	0.143–0.643
New Zealand	39	21	0.538	0.385–0.692
Northern Europe	7	1	0.143	0.000–0.429
UK & Ireland	25	10	0.400	0.200–0.600
Western Europe	27	7	0.259	0.111–0.444

The 95% confidence intervals (CI) were obtained from a boot-strap analysis.

their movement over tens of kilometres per year (Donovan, 1991; Goodisman *et al.*, 2001; Masciocchi & Corley, 2012). Human-aided transport of queens within the native range has likely resulted in the mixing of haplotypes and could have contributed to the observed clinal pattern.

Six haplotypes were detected among the New Zealand samples, and two haplotypes were observed in the Argentinian samples. Given the geographic cline in the mitochondrial DNA, these haplotypes indicate that the introduced populations in New Zealand and Argentina both originated from Western Europe. In addition, the observation of several haplotypes in Argentina and New Zealand indicates multiple successful incursions of the common wasp into both countries. Common wasp nests are founded by a single queen (Archer, 2012), and mitochondrial DNA is maternally inherited. Hence, the occurrence of multiple haplotypes within a country indicates the introduction of multiple queens into that country. Established nests with a queen and hundreds or thousands of workers are readily apparent and are thus unlikely to have been moved. It is much more likely that fertilized queens have been moved after they have sought refuge for overwintering shelter (Beggs *et al.*, 2011). Vespine queens have long been observed to hibernate or overwinter in human goods and produce, including between books in a case, in beetle holes in wood, under corrugated iron sheeting, clinging to curtains or sacking (Duncan, 1939; Thomas, 1960). Quarantine records spanning several decades in New Zealand indicate multiple interceptions of Vespid wasps from a variety countries within Europe (e.g. France, Germany, Switzerland) and also from

their introduced range (e.g. Australia) (Keall, 1981; Townsend, 1984; Richardson, 1979).

Within New Zealand, isolated individual specimens of the common wasp were observed since 1921 in several widely separated locations, supporting the theory of multiple introductions (Donovan, 1983). These New Zealand incursions may have come directly from Europe, or populations established during the 1950s in nearby Australia (Anonymous, 1962) may have also contributed propagules. The haplotype discovery curve (Fig. S3) indicates there are more haplotypes in New Zealand than we observed, implying that there have been additional introductions. We thus cannot exclude incursions from regions such as Asia into New Zealand or Argentina, but our data are indicative that the predominant haplotypes established have their ultimate origin from Western Europe. The pathway of these populations into Argentina is less clear. Given the genetic similarity between New Zealand and Argentina, our results cannot discount the possibility of the Argentine populations arriving via New Zealand. Chile was the likely source of the German wasp (*Vespula germanica*) in Argentina (Beggs *et al.*, 2011; Masciocchi & Corley, 2012), and the common wasp may have arrived in Argentina via a similar route. Given the highly invasive nature of these wasps, the global propagule pressure from populations is likely to increase in an exponential fashion due to an increasing number of potential propagule sources.

Wing morphology has been used as a character for differentiating intraspecific variation in related insects including honey bees (Al-Ghamdi *et al.*, 2013), with strong support from mitochondrial DNA (Nielsen *et al.*, 1999). With our wasps, the morphometric analysis showed significant morphological variation between countries and supported the Western European origin for New Zealand populations, although not for Argentine samples. We expected considerable overlap between the New Zealand population and that of Argentina given the genetic similarity between the populations. Perhaps, some environmental feature can influence wasp wing morphology, as it can for damselfies (Taylor & Marriam, 1995). The lack of overlap between these samples suggests that variation wing morphology may not be a good character for determining the origin of common wasp propagules.

The enemy release hypothesis predicts that the abundance or diversity of pathogens, parasites and predators of invasive species is reduced in the introduced range, relative to the native range, largely due to population bottlenecks during the colonization process (Keane & Crawley, 2002; Torchin *et al.*, 2003). In the present study, we assessed the prevalence of the microsporidian pathogens within both the native and the introduced range of the common wasp. Microsporidian pathogens such as *Nosema* spp. have been associated with colony collapse in honey bees (Bromenshenk *et al.*, 2010; Evans & Schwarz, 2011), and the synergistic effects of low genetic diversity and *Nosema* spp. infection are thought to cause declines in bumble bee populations of North America (Cameron *et al.*, 2011).

We observed no significant differences in the prevalence of microsporodian infections between populations of the native and introduced ranges, and thus no evidence to support the enemy release hypothesis. Wattier *et al.* (2007) similarly found no microsporidian parasite loss after invasion by an exotic amphipod. They concluded that the amphipod invasion was either massive or recurrent, enabling the microsporidian pathogen to follow its host. Multiple introductions of the common wasp have clearly occurred in New Zealand and Argentina. The multiple incursions have likely facilitated multiple microsporidian introductions, especially given the high infection rate of these pathogens within the home range of the wasps. Microsporidia may also be acquired in the new range as exemplified in our study with apparent infection *N. bombi* in the New Zealand samples, which is a pathogen of bumble bees. Recent work has demonstrated that the individual *Nosema* species are capable of infecting multiple host species and genera (Graystock *et al.*, 2013; Fürst *et al.*, 2014). Thus, pathogens already present in an invaded zone may contribute to the pathogen loading for a new invader. Furthermore, the duration since arrival into an area may be positively correlated with increasing pathogen accumulation, because there has been more time to acquire and accumulate infections. This phenomenon has been observed in plants (Flory & Clay, 2013) and may apply to microsporidian infections in common wasp populations as well.

Wasps share the same habitat and compete for the same resources as honey bees and bumble bees (Moller & Tilley, 1989), and they also raid honey bee hives (Clapperton *et al.*, 1989). Together, these behaviours could increase the exposure of wasps to bee pathogens such as *N. apis* and *N. bombi*. Evidence of such microsporidian pathogen spillover has been recently observed between honey and bumble bees (Fürst *et al.*, 2014). The rate of microsporidian infection in wasps of up to 53% that we observed is much higher than those previously noted in wasps or bees (e.g. 7–9% in honey and bumble bees; Fürst *et al.*, 2014). Our results are of concern to apiarists and growers reliant on bumble bee pollination, as the high infection rate in wasps may result in pathogen spillback. The management of wasp populations may be a requirement to manage disease in bees.

The common wasp may thus be subject to pathogen acquisition, but the pathogenicity of these different microsporidian species remains unknown. Micosporidian pathogens, including *Nosema* sp., do infect and multiply in *Vespula germanica* (F.), with the potential to kill entire wasp nests (Fantham & Porter, 1913). Our work has similarly demonstrated that *N. apis* can infect and multiply in common wasps (unpublished data). Evison *et al.* (2012) also observed microsporidian infection in common wasps. However, the presence of 'pathogens' may thus not always be deleterious and perhaps can even be advantageous for their hosts. For example, one microsporidian species is thought to have little effect on its primary invasive ladybeetle host, but the pathogen has lethal effects on native ladybeetles (Vilcinskas *et al.*, 2013). This microsporidian thus appears to facilitate the invasion and spread of its

host. Further, even if enemy release does occur, it may not correlate with increased demographic success, for example if enemies do not limit species in the native range (Prior & Hellmann, 2013). Our work with microsporidian infections in the common wasp does not support the enemy release hypothesis, but neither can we reject the hypothesis. Perhaps, other key pathogens that are actually pathogenic, and are rarer in wasps than microsporidian infections, are absent from their invaded range.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Comparison of genetic distance between *V. vulgaris* wasps from New Zealand, Argentina and other regions sampled.

Figure S2 Relationship between genetic and geographic distance among all samples from the native range.

Figure S3 Haplotype discovery curve for *V. vulgaris* sampled in New Zealand.

Table S1 Sampling origins and approximate collection date for *V. vulgaris* and the single *V. germanica* used in this study.

Table S2 The maximum likelihood fits for the five best-fitting models of the 24 different nucleotide substitution models calculated in MEGA 5.1 for the separate *cytb* and *COI* datasets and concatenated dataset (852 bases).

Table S3 Matrix of pairwise genetic distances between individuals using the Tamura-Nei model (Tamura & Nei, 1993).

Table S4 Results of GLM analysis comparing: (a) the genetic distance between the New Zealand samples and all other regions; and (b) the genetic distance between the Argentina samples and all other regions.

Table S5 PCR results from triplicate PCRs including positives and negatives.

BIOSKETCH

The general research interests of the authors lie in biological invasions of social insects, or wasp biology and population ecology. Major goals of the group are to understand why dramatically higher populations of wasps occur in the native compared to introduce range and to find mitigation options.

Author contributions: P.J.L conceived the project; P.J.L, E.C.B-R, M.A., J.C.C., L.D., M.M and A.VO collected the data; M.A.M.G., E.C.B-R and P.J.L. analysed the data; and P.J.L and M.A.M.G led the writing.

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