**Oikos 000: 001–011, 2017**
doi: 10.1111/oik.04117

Subject Editor: Marjo Saastamoinen. Editor-in-Chief: Dries Bonte. Accepted 23 January 2017

# Fitness in invasive social wasps: the role of variation in viral load, immune response and paternity in predicting nest size and reproductive output

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Within any one habitat, the relative fitness of organisms in a population can vary substantially. Social insects like the common wasp are among the most successful invasive animals, but show enormous variation in nest size and other fitness-related traits. Some of this variation may be caused by pathogens such as viruses that can have serious consequences in social insects, which range from reduced productivity to colony death. Both individual immune responses and colony-level traits such as genetic diversity are likely to influence effects of pathogen infections on colony fitness. Here we investigate how infections with *Kashmir Bee Virus* (KBV), immune response and intracolony genetic diversity (due to queen polyandry) affect nest size in the invasive common wasp *Vespula vulgaris*. We show that KBV is highly prevalent in wasps and expression of antiviral immune genes is significantly increased with higher viral loads across individuals. Patriline membership within a nest did not influence KBV susceptibility or immune response. A permutational MANCOVA revealed that polyandry, viral load and expression of the immune gene *Dicer* were significant predictors of variation in nest size. High intracolony genetic diversity due to polyandry has previously been hypothesized to improve colony-level resistance to parasites and pathogens. Consistent with this hypothesis, we observed genetically diverse colonies to be significantly larger and to produce more queens, although this effect was not driven by the pathogen we investigated. Invasive wasps clearly suffer from pathogens and expend resources, as indicated here by elevated immune gene expression, toward reducing pathogen-impact on colony fitness.

Considerable variation is frequently observed in the reproductive output and relative fitness of organisms within populations. Organisms might survive to maturity but produce few or no offspring, while the reproductive output of other population members can be many orders of magnitude higher. Environmental variation, including temperature or nutrient variation, as well as pathogens (Lazzaro and Little 2009), can be a key driver in these disparate fitness outcomes, which is especially apparent in ectothermic animals such as insects (Kingsolver and Huey 2008). The distribution of pathogens is one key environmental factor that influences fitness (Lachish et al. 2011). In order to reduce fitness costs of pathogens and parasites, immune defence, which is costly, may be traded off against other fitness components (Sheldon and Verhulst 1996). In addition, within-population genetic variation in immune traits can dramatically influence reproductive success (Lazzaro and Little 2009). Determining the relative roles of pathogen infection and immune response on fitness is important for understanding population dynamics, and can inform the management of invasive species.

Pathogens such as viruses can be extremely important for health in insect communities. The social behaviour of eusocial Hymenoptera (ants, bees and wasps) facilitates the transmission of pathogens and pathogen associations, which has been best studied in the honey bee (Evans and Schwarz 2011) but remains poorly understood in non-model organisms. Increased pathogen loads and the presence of RNA viruses such as *Deformed Wing Virus* (DWV), *Israeli Acute Paralysis Virus* (IAPV) and *Kashmir Bee Virus* (KBV) are emerging as key factors associated with colony collapse in honey bees (Cornman et al. 2012, McMenamin and Genersch 2015). KBV becomes extremely virulent when in high titres in bees, but is frequently observed only at low abundance, and with bees showing no obvious symptoms on infection (de Miranda et al. 2010). Viruses like KBV can be induced to change from symptomless or covert infections, to pathogenic or overt infections in bees by the acquisition of biologically active substances (Dall 1985, 1987). Once introduced, viruses can quickly spread throughout entire hives or nests. For example, KBV in *Apis mellifera* colonies was found

to be transmitted both horizontally between workers or from workers to larvae, and vertically from queens to eggs (Shen et al. 2005). Additional horizontal transmission routes for viruses are likely, such as through interactions while foraging in the same area (Singh et al. 2010). A wide variety of insects can host these viruses (Levitt et al. 2013), including social wasps (Singh et al. 2010, Lester et al. 2015).

The RNA interference (RNAi) pathway provides a key antiviral immune response in insects including hymenopterans. Individual insect immune defence includes humoral responses through the activation of immune pathways that result in the production of antimicrobial peptides and cellular immune responses (Lemaitre and Hoffmann 2007). The best studied antiviral response is within the small interfering RNA (siRNA) pathway (Ding 2010). Double-stranded RNA is recognised by endoribonuclease dicer proteins and processed into siRNA that can guide a specific antiviral immune response (Aliyari and Ding 2009, Brutscher et al. 2015). Another recently discovered potential antiviral immune response gene in wasps is the RNA helicase *Skiv2l2* (Superkiller Viralicidic Activity 2-Like 2), that is paralogue to yeast antiviral *Ski2p* and may be involved in regulation of viral RNA (Dangel et al. 1995). The immune response via the siRNA pathway was found to increase with viral load in honey bees (Niu et al. 2014). Comparative genomics have revealed immune pathways in honey bees that are orthologous to fruit flies and mosquitoes, suggesting these responses may be conserved across a range of social hymenopteran species (Evans et al. 2006).

In addition to individual immunity, colony-level properties such as genetic diversity and colony size are predicted to mediate the degree to which colonies are affected by pathogens (Cremer et al. 2007). High intracolony genetic diversity has been hypothesized to improve colony-level resistance to parasites and pathogens by reducing transmission among colony members (Schmid-Hempel 1994). This increase in resistance could occur if pathogens or parasites adapt to host genotypes, resulting in variable infection susceptibility within a genetically diverse colony (Sherman et al. 1988, Schmid-Hempel 1994, Crozier and Fjerdingstad 2001, van Baalen and Beekman 2006). In many social insects with single queens, including social vespine wasps, intracolony genetic diversity is determined by polyandry (i.e. the degree to which queens mate multiply; Strassmann 2001). In *Vespula* wasps, polyandry has been positively associated with colony fitness (Goodisman et al. 2007) and average intracolony genetic diversity is positively correlated with average colony size across species of Vespinae wasps (Loope et al. 2014). More direct support for the link between genetic diversity and pathogen resistance comes from studies in honey bees, where experimentally enhanced polyandry was associated with lower disease rates (Tarpy 2003, Seeley and Tarpy 2007). While the benefits of genetic diversity in social insect colonies extend beyond disease resistance and are well demonstrated (Mattila and Seeley 2007), few studies have explored the mechanisms by which colonies gain immune benefits from genetic diversity (but see Hughes and Boomsma 2004).

The common wasp *Vespula vulgaris* is an invasive species that was first observed in New Zealand in 1921 and became widely established in the 1980s (Donovan 1984). This wasp species is an abundant pest in New Zealand that harms native birds and insects through predation and competition for food sources (Moller 1990, Moller et al. 1991, Toft and Rees 1998, Burne et al. 2014). Wasps are observed in extremely high densities in South Island beech forests where they can dominate honeydew resources (Harris et al. 1994, Beggs and Rees 1999). Densities of up to 370 wasps per $m^2$ of tree trunk have been observed (Moller et al. 1991). Colonies are founded by a single hibernating queen in early spring. The annual nest is extended throughout the season by workers, and at the end of the season colonies produce males and new reproductive queens (Archer 1981). These nests can vary substantially in size and queen productivity (Barlow et al. 2002). The expansion of a species' range has been associated with changes in immunocompetence (Cornet et al. 2016) and it has been hypothesized that low genetic diversity in social invasive species may affect immune responses on colony and individual level (Ugelvig and Cremer 2012). Recently, the common wasp has been shown to carry viral pathogens. The honey bee viruses KBV and DWV were found to be present in the common wasp populations in New Zealand, with KBV sequences closely matching those from local honey bees (Lester et al. 2015).

In this study, we examined the evidence that viral load, immune gene expression and intracolony genetic diversity interact to affect nest size and productivity in the common wasp. In order to test this hypothesis, we examined a large number of individual wasps from randomly chosen nests that varied considerably in size and productivity. Preliminary work showed KBV but not DWV presence in the studied location. We first determined KBV infection prevalence and load variation between and within nests. Secondly, we looked at the relationship between KBV infection rates and immune responses of individual wasps. Thirdly, we determined the variation in polyandry among nests and looked at associations between KBV infections and immune responses with patriline membership (father identity) within nests.

## Methods

### Sampling

To limit environmental variation and better estimate intracolony variation, we examined a large number of individual *Vespula vulgaris* workers (n = 182) in six spatially aggregated nests. All nests were obtained from an environmentally homogenous habitat and were separated by less than 150 m in *Fuscospora* beech forest of Nelson Lakes National Park (41°46′S, 172°57′E), New Zealand. Sampling was undertaken in late March 2016. The presence of new queens and drones, which are annually produced by this time, represents the whole reproductive output of a colony. Nests were located by observing foraging workers and anaesthetised with diethyl ether before being excavated and transferred into a sealed bucket. Buckets were then chilled at –20°C for ~2 h. After chilling, all wasps were still alive, but were able to be handled without stinging. A subset of ~30 live adult workers was randomly collected from every nest and snap frozen in liquid nitrogen at –196°C in a dry-shipper. Samples were

then stored at –80°C for three weeks before RNA extraction and dissection. One hind leg was removed on dry ice for genotyping and heads were removed for another study. The remaining body was used to determine immune gene expression and viral loads.

To assess colony productivity, nest paper was removed and combs were cut out and placed flat on a table. Photos were taken to analyse the number of worker and queen cells. Worker cells are used to rear workers and the majority of males, whereas the larger queen cells are used to rear new queens (Spradbery 1971) and therefore used as an indicator of colony fitness. We used ImageJ2 software (Schindelin et al. 2015) to measure the size of a minimum of 10 large queen cells and 10 small worker cells from photographs of each nest. Then total areas of worker and queen cells were divided by the mean cell size to calculate cell number for each nest.

### RNA extraction and cDNA synthesis

We extracted RNA from 142 individual wasps (24 workers per colony, with 22 in colony A) to examine viral loads and immune gene expression. Samples were homogenized by bead beating (Mini-Beadbeater-16) in a mix of 950 µl GENEzol and 50 µl β-mercaptoethanol followed by incubation at 65°C for 15 min. The product was purified with chloroform and RNA was precipitated with isopropanol followed by 70% ethanol. RNA pellets were then resuspended in 100 µl nuclease-free water and quantified on a NanoDrop ND-1000 spectrophometer. Residual DNA was removed from 100 ng RNA using 0.5 units PerFecta DNaseI in a 10 µl reaction carried out according to manufacturer's instructions. cDNA was prepared by reverse transcription of 8 µl RNA (80 ng) with random hexamers and oligo(dT) primers using qScript cDNA SuperMix in a 10 µl reaction. The thermal incubation consisted of 5 min at 25°C, 60 min at 42°C and 5 min at 85°C. The resulting cDNA was diluted 1:10 with nuclease-free water (final concentration approx. 0.8 ng $\mu l^{-1}$).

### Immune gene expression and virus quantification

Quantitative real-time PCR (qPCR) was performed to detect mRNA expression of Dicer and Superkiller genes, and the RNA virus KBV. We used a primer pair specific to KBV, targeting the virus particle protein VP3, making cross-amplification of other Dicistroviridae viruses unlikely (Chantawannakul et al. 2006, de Miranda et al. 2010). Immune genes were selected from the *Polistes canadensis* and *P. dominula* genomes, and orthologs identified using a BLAST search on a common wasp draft transcriptome assembly. BLAST searches were run against the Victoria University of Wellington's Science Faculty High Performance Computing (Sci Fac HPC) Facility's BLAST ver. 2.2.25 installation. Two reference gene orthologs were also identified in this search: *eukaryotic translation initiation factor 3 subunit C* (*eIF3-S8*) and *Proteasome 54kD subunit* (*Pros54*). These genes were previously shown to be stably expressed in the honey bee (Cameron et al. 2013, Vannette et al. 2015) and were included to normalize gene expression, as the use of multiple references is recommended by the MIQE guidelines (Bustin et al. 2009). Primers were designed using PrimerBLAST software (<www.ncbi.nlm.nih.gov/tools/primer-blast>) (Supplementary material Appendix 1 Table A1). Gene expression was analysed in two technical replicates run in parallel on a qPCR cycler (mic qPCR, Bio Molecular Systems). Reaction mixes of 12.5 µl contained 5 µl Perfecta SYBR Green FastMix, 4 ng template and 250 nM of each forward and reverse primer. The thermal protocol for the KBV assay consisted of 2 min at 95°C, followed by 40 cycles of a three-step protocol with 30 s denaturation at 95°C, 15 s annealing at 54°C and 30 s extension at 72°C. All other assays were carried out with 30 s initial denaturation at 95°C, 40 cycles of 5 s at 95°C, 15 s at 60°C and 10 s at 68°C. Fluorescence was measured during the extension step. A final slow increase in temperature to 95°C for the melt analysis was performed to verify expected target amplification. Two no-template controls were run in parallel for every primer pair to ensure absence of contamination and primer–dimer artefacts. Mean of duplicates was taken as quantification cycle (Cq) values. ΔCq values normalised against the average reference gene expression were calculated for further analysis. Primer specificity was confirmed by 2% agarose gel electrophoresis with SYBR safe stain and amplicons were purified using ExoSAP-IT and sequenced by the Massey Genome Service, Palmerston North, New Zealand. We confirmed active replication of positive-sense KBV by detecting the negative viral strand using reverse transcription (SuperScript IV) with tagged primer tag-KBV_F followed by PCR using the primers KBV_R and Tag (Supplementary material Appendix 1 Table A1).

### Phylogenetic analysis

For phylogenetic relationship analysis, we amplified sequences of KBV, *Dicer* and *Superkiller.* Reverse transcribed template cDNA was amplified in 15 µl PCR reaction using the same primers and cycling conditions used in the qPCR assays. Reactions consisted of 10 ng cDNA, 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 nM of each dNTP, 0.4 nM of each primer and 0.5 units of Taq DNA Polymerase (Invitrogen). Samples from every nest were analysed in MEGA7 (Kumar et al. 2016). We used the ClustalW alignment and the Bayesian information criterion to choose evolutionary models. Trees were built using the Kimura 2-parameter model (Kimura 1980) for *Superkiller*, and the Jukes Cantor model (Jukes and Cantor 1969) for KBV and *Dicer* with 500 bootstrap replicates.

### Microsatellite analysis of polyandry

We obtained genotypes for 30 workers from each colony at seven microsatellite loci, and used these genotypes to assign workers to patrilines. Methods followed a previous study of paternity in *Vespula* (Loope et al. 2014). Briefly, DNA was extracted from frozen legs using a Chelex protocol (Walsh et al. 1991), and 1 µl of extracted DNA (~100 ng $\mu l^{-1}$) was used in each of two 5.5 µl multiplex PCR reactions. Mix 1 contained FAM, HEX, NED and PET dye-labelled primers for *List2018*, *List2001*, *Rufa15*, *Rufa19* (Thorén et al. 1995, Daly et al 2002), and Mix 2 contained primers for *VMA3*, *VMA6*, *Rufa18* (Thorén et al. 1995, Hasegawa and

Takahashi 2002). We used Qiagen Type-It Kits for Mix 1, and Kapa2G multiplex PCR kits for Mix 2. Both reactions were improved by the addition of SolutionQ from the Qiagen kit. PCR reactions for Mix 1 were 15 min at 95°C, 33 cycles of 30 s at 95°C, 45 s at 55°C, 60 s at 72°C, 30 min at 60°C. Mix 2 reaction conditions were identical, except for an annealing temperature of 51°C and a total of 36 cycles. One microliter of each PCR product was combined with 10 µl of diluted MCLabs Orange 500 size standard in SuperDi Formamide, and fragment analysis was performed on a ABI 3730XL. Genotypes were called in GeneMarker, each checked by eye.

We re-amplified and re-genotyped 88 samples that successfully amplified the first time to check for genotyping errors but found no evidence of dropout or other errors. Paternity was assigned to workers by assuming a single mother queen in each colony (all genotypes were consistent with a single mother), and deducing the minimum number of males necessary to explain worker genotypes. We also used the maximum likelihood method of the program Colony 2 (Jones and Wang 2010) to assign workers to patrilines. Colony 2 parameters were polyandry for females, monandry for males, allele frequency updating, no inbreeding, maternal sibship for all daughters from each colony, and a weak paternal sibship size prior of 16, chosen based on the results of a previous paternity study in this species (Foster and Ratnieks 2001). These two approaches resulted in identical paternity assignments in all cases. Within each colony, we then calculated patriline number and effective paternity, which describes the queen mating frequency and accounts for unevenness in the frequency of offspring from each patriline and the sample size of daughters analysed ($k_{e3}$; Nielsen et al. 2003). This latter measure is an effective descriptor of genetic diversity within colonies and is inversely related to intracolony relatedness. The allele frequencies at the seven microsatellite loci, estimated by Colony 2, were used to calculate the population-level non-detection error, which is the probability that two males have the same genotype and thus a male mate goes undetected (Jaffé 2014).

## Data analysis

Our design had a small number of nests, but a large number of wasps were sampled from within each nest.

### *Individual-level effects*

Correlations between individual KBV loads and immune gene expression were analysed with linear regression. To test for effects of patriline membership on individual wasp viral loads or immune gene expression, of *Dicer* or *Superkiller*, we created linear models (lm() function in R ver. 3.3.0) and used model selection calculating the corrected Akaike information criterion (AICc) and model weights with the *MuMIn* package (Barton 2016). Since patriline membership and colony membership are not independent (because patrilines are unique to colonies), it was not possible to include them both in the same model. Furthermore, a model with just patriline membership as a predictor could show a significant effect merely as a result of differences between colonies, since patrilines are unique to colonies. To overcome this problem, we compared three models for each response variable: a model with no predictors (null), a model with colony membership as the only predictor, and a model with patriline as the only predictor. If patriline membership explained most of variation in a response variable (beyond information present in colony membership), the patriline-only model would be preferred relative to the colony-only model. AICc model weights were used to determine the preferred model. Such information-theoretic model comparisons do not involve p-values to determine the importance of predictors: if predictors were not important, the null model would be preferred (Burnham et al. 2011). To complement this analysis, within nests we used unequal variances t-tests or one-way ANOVA (for colonies with two or three patrilines with at least five workers within each, respectively) to see if patriline membership affected KBV loads or gene expression.

### *Colony-level effects*

To test our hypothesis that wasp nest size (represented by total cell number) varies with viral load, immune response and effective paternity ($k_{e3}$) we used a permutational MANCOVA, with 999 permutations. We used the *adonis* function in the *vegan* package in R (Oksanen et al. 2016). We included $k_{e3}$ as a predictive variable in the model despite having only one value per nest, as it explained a considerable amount of variation among nests and enabled us to estimate the role of covariates. Viral load and the expression of *Dicer* and *Superkiller* were used as covariates because they varied in value among wasps within each nest. We used a blocked design based on levels of queen production, with three levels each containing two nests: no queens produced, a small number of queens ($<$ 1000 cells), and large queen numbers ($>$ 1000 cells) (Fig. 1A). We then used Pearson correlation to test if the factors have positive or negative correlations with colony cell number.

All boxplot and scatterplot graphs were created using the *ggplot2* package in R (Wickham 2009). Boxplots show the mean, the 25th and 75th percentiles, with whiskers extending from the hinge to the highest or lowest value that is within 1.5 × the inter-quartile range of the hinge. Outliers are automatically classified as values $>$ 1.5 fold higher than the inter-quartile range, but were not excluded from the statistical analyses.

## Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.pb2rp> (Dobelmann et al. 2017).

# Results

The six collected wasp nests varied considerably in size and queen production. For the purpose of presentation, we rank ordered nests by their size from smallest to largest based on worker cell number and named them A to F (Fig. 1A). Two nests (A and C) failed to produce queen cells, whereas nests B and D showed a medium queen cell production (up to 1000 cells), and the largest nests E and F were highly reproductive (over 1000 cells) (Supplementary material Appendix 1

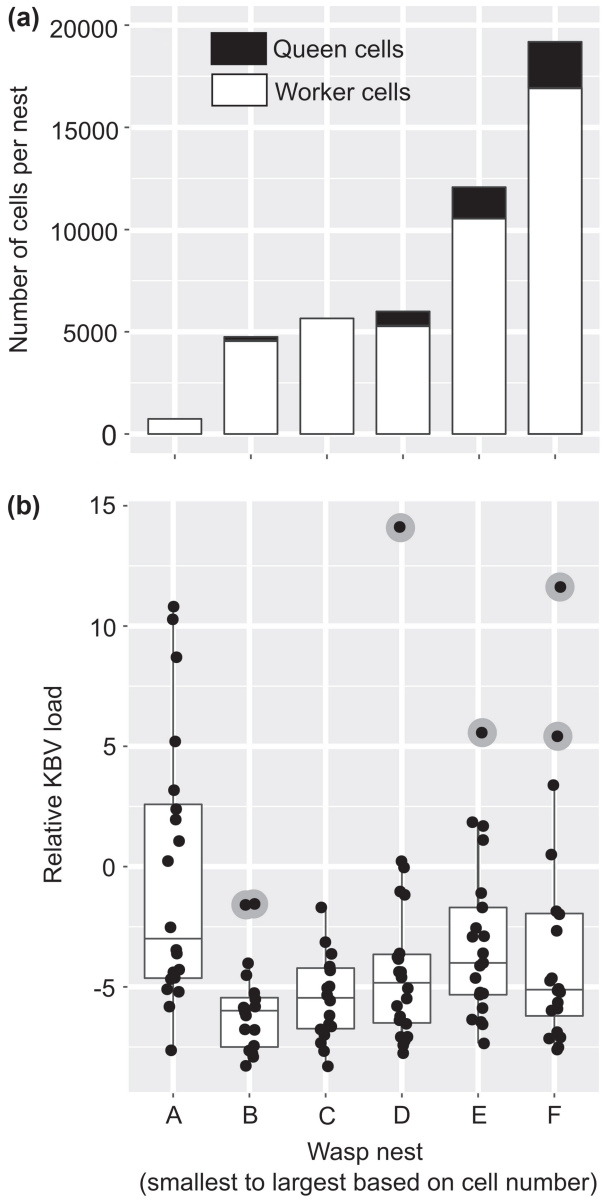


Figure 1. (a) The size of the six common wasp nests used in the study, shown in the number of cells for both workers and queens. There was a 19-fold difference in the range of nest size, with two nests producing no new queens at all. (b) Relative viral loads between nests. Data shown are the amounts of RNA from the KBV primer relative to the reference genes *Pros54* and *eIF3-S8* (Supplementary material Appendix 1 Table A1). No wasp sampled was devoid of KBV. Outliers are identified by the grey circles.

Table A2). Nest A was the smallest nest containing 740 worker cells and no queen cells. The largest nest (nest F) had the highest number of queen cells (2246).

We determined KBV prevalence and viral loads within and among nests. All wasps were found to be infected with this virus, but viral loads differed substantially among individuals within nests (Fig. 1B). Every nest contained workers with relatively low infection levels. Highly infected workers were found in nest A, D and F. However, in nest D only a single worker with a high viral load was identified as an outlier. Nest A showed the most variable viral loads among wasps, with an overall highest mean KBV load. KBV replication, indicating parasitism by the virus, was detected in individual wasp samples from every nest.

The variation in immune gene expression across nests was much smaller than the variation in viral loads. The RNAi pathway gene *Dicer* (Fig. 2A) showed the highest mean expression levels in nest A and lowest levels were found in nest C and F. The second immune gene *Superkiller* (Fig. 2B) showed lower expression values compared to *Dicer* with less variation across nests, although variation within nests remained high. We observed statistically significant positive relationships between the expression of *Dicer* and *Superkiller* with KBV infection levels (Pearson $r = 0.206$, $p = 0.023$ and $r = 0.182$, $p = 0.046$, respectively; Fig. 2C and 2D). The effect, however, was not strong, with less than 4% of the variation in immune gene expression explained by KBV loads. Amplified sequences did not vary between nests, which is consistent with a single strain being present, and closely matched KBV found in *Apis mellifera* (Fig. 3). Phylogenetic analysis of immune genes, *Dicer* and *Superkiller,* positioned amplified sequences of *Vespula vulgaris* with other hymenopteran species (Fig. 3).

Next we examined the variation and effects of polyandry on wasp colonies. All workers in a nest had microsatellite genotypes consistent with a single mother, suggesting that no foreign queens or workers had been recruited. Two to three patrilines were observed in each colony. The estimate of non-detection error was 0.00093, indicating that the probability of two males possessing indistinguishable genotypes is extremely negligible, and thus that our loci had sufficient genetic diversity to accurately determine paternity. No two inferred father genotypes from different colonies were genetically identical, indicating that each colony contained unique fathers. The (harmonic) mean effective paternity ($k_{e3}$), which takes into account the relative contribution of each male to the progeny, was 1.87 across the six colonies. Among colonies, the effective paternity varied from 1.21 in nest B to 3.14 in nest F (Supplementary material Appendix 1 Table A2).

For individuals, we examined the effect of patriline membership on gene expression and KBV loads. We found no evidence that patriline membership affected any of these response variables. To see if patriline membership explained variation in KBV load, *Dicer* or *Superkiller* expression better than colony membership alone, we compared the AICc values of three linear models (null, colony, patriline). The best supported model for KBV and *Dicer* contained 'colony' as a predictor (Table 1), indicating that adding information about patriline membership does not explain more of the variation in individual KBV load. For *Superkiller* the best supported model is the 'null' model (Table 1) showing that patriline and colony membership do not affect the expression. Extremely low model weights for models with patriline membership as a predictor (Table 1) mean that we have high confidence that patriline is not informative for these response variables. To see if there were patriline effects within just some of the colonies, we compared the two or three predominant patrilines within each colony. Patrilines did not differ in KBV load, *Dicer* expression or *Superkiller* expression (all $p > 0.05$, Supplementary material Appendix 1 Table A3). Although the differences were not significant,

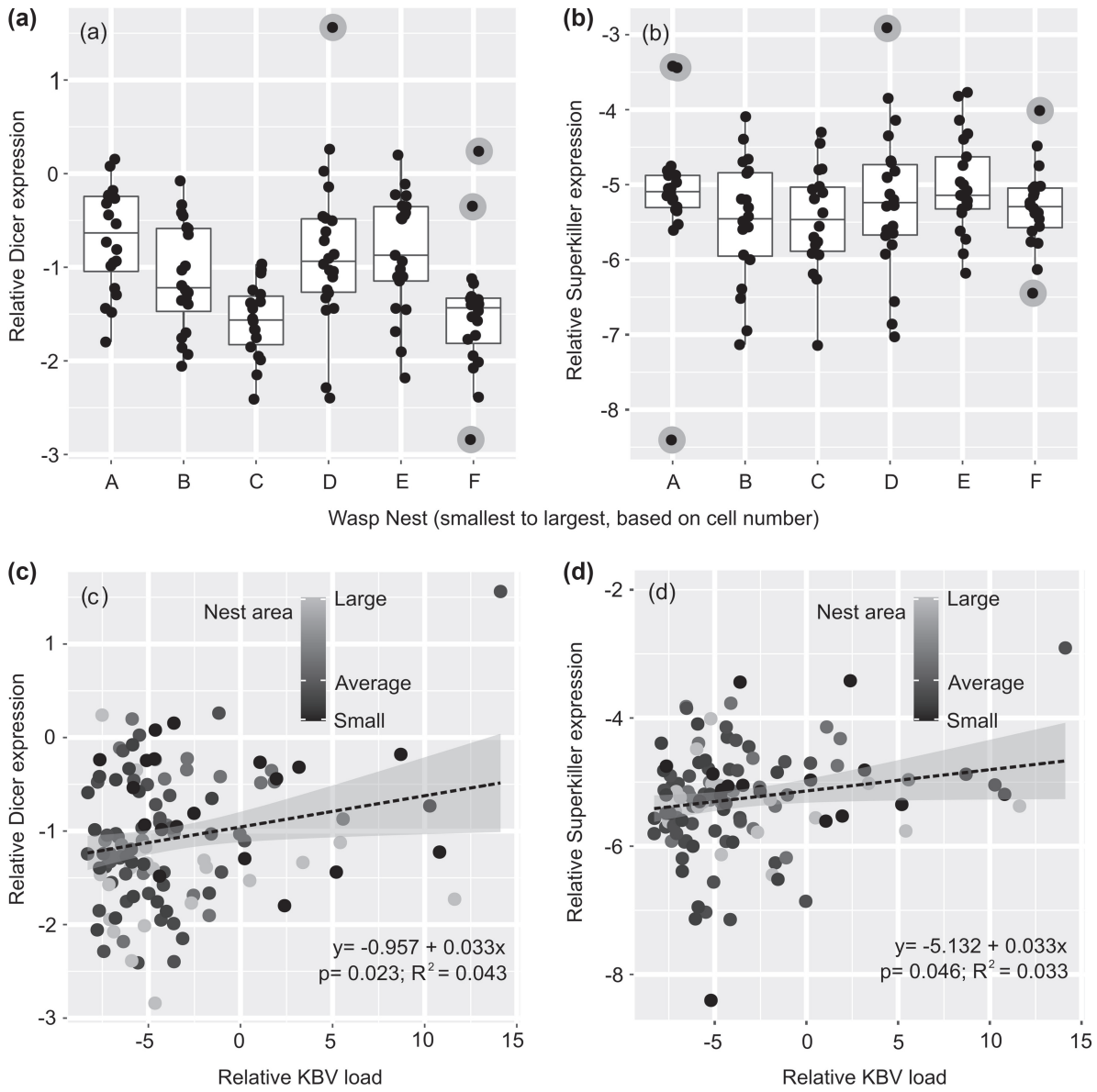


Figure 2. (a, b) The expression of the immune genes *Dicer* and *Superkiller* in wasps relative to the reference genes *Pros54* and *eIF3-S8*. Outliers are identified by the grey circles. (c, d) The relationship between the expression of the immune genes and KBV load, per wasp. Dashed lines show linear regression with standard error in grey. Wasps are coded in grey scale as being from small or larger nests.

an example of the variation in immune genes and KBV infection rates between patrilines is given in Fig. 4, for nest F, showing that KBV susceptibility could be higher in one patriline.

To assess the factors that influence colony fitness in *V. vulgaris,* we used a permutational MANCOVA with $k_{e3}$, *Dicer* and *Superkiller* expression, and KBV load as predictors for the number of cells in a nest. Our results show that nest size was significantly affected by effective paternity, viral load, and *Dicer*, whereas *Superkiller* expression had no significant effect on the nest size (Table 2). $k_{e3}$ had a strong, positive correlation with cell number (Pearson $r = 0.868$, $p < 0.001$), while *Dicer* negatively affect cell number (Person $r = –0.234$, $p = 0.011$). KBV load, although significantly affecting nest size in the permutational MANCOVA, and *Superkiller* expression showed weak, non-significant correlations with cell number (Pearson $r = –0.031$, $p = 0.7332$ and $r = 0.028$, $p = 0.763$, respectively). While *Dicer* and KBV load significantly affect cell number, there is considerable variation in colony fitness that is not explained by these variables (Fig. 1, 2A).

## Discussion

The goal of this study was to observe effects of viral infections, immune response and polyandry on fitness traits in *Vespula vulgaris.* The insights into the wasp antiviral immune response and assessment of fitness influencing factors can improve management of invasive wasp species. Colonies sampled from a single location showed enormous variation in size with larger colonies producing large numbers of new queens, substantially outperforming medium and small sized nests.

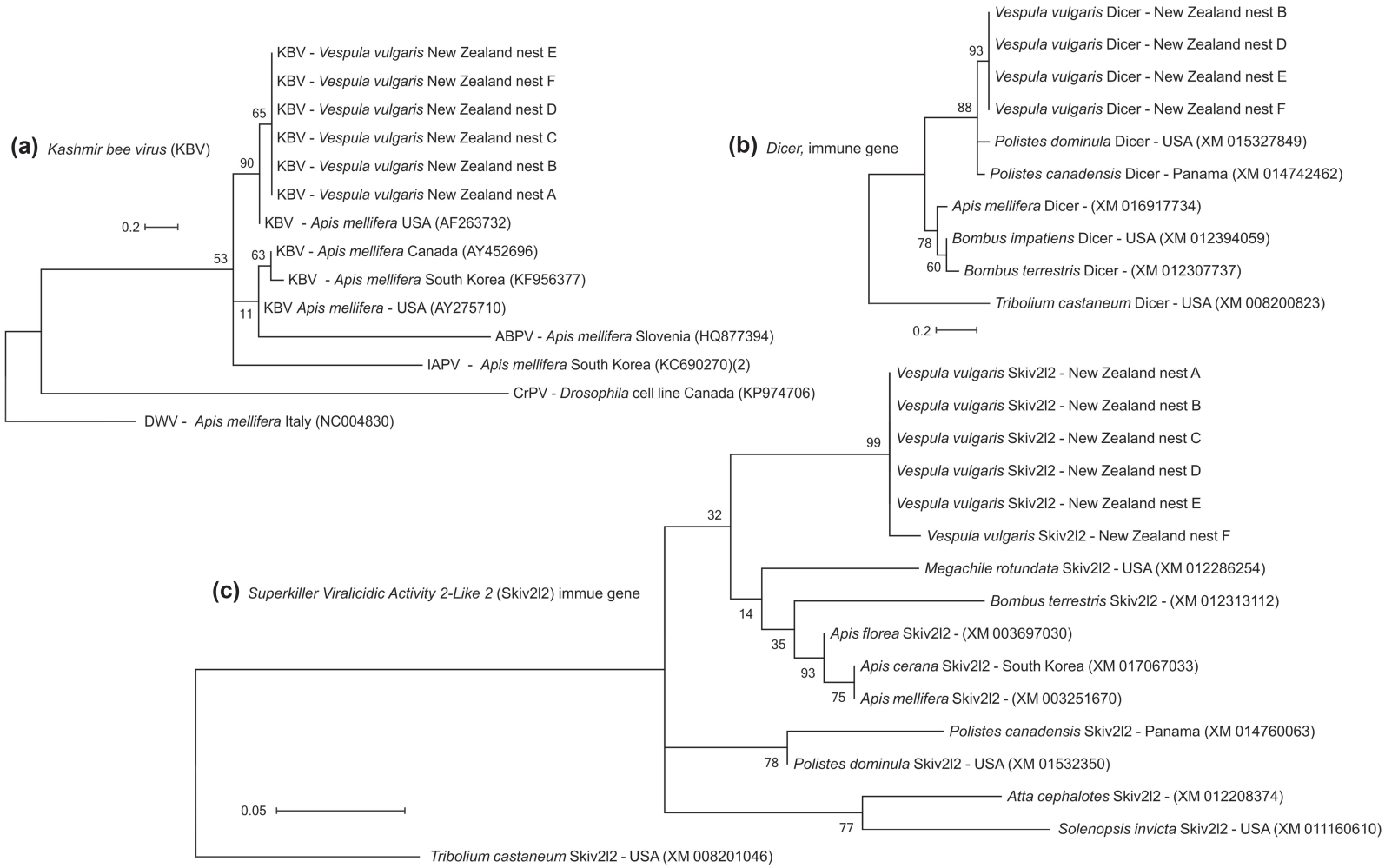


Figure 3. (a) Maximum composite likelihood tree for *Kashmir Bee Virus* (24bp of amplified sequence) and other Dicistroviridae (*Acute Bee Paralysis Virus* (ABPV), *Israeli Acute Paralysis Virus* (IAPV), *Cricket Paralysis Virus* (CrPV), *Deformed Wing Virus* (DWV) is the outgroup. (b, c) Maximum composite likelihood tree for immune genes *Dicer* (24bp) and *Superkiller* (96bp) in *Vespula vulgaris* and other hymenopterans. Trees are based on 500 bootstraps of Kimura 2-parameter model (*Superkiller*) or Jukes-Cantor model (*Dicer* and KBV). MEGA7 was used to conduct trees and scales show substitutions per site.

Table 1. Results of model selection on KBV load, *Superkiller* and *Dicer* expression. Models are ranked by ΔAICc; preferred model in bold.

| Response | model | ΔAICc | weight |
|---|---|---|---|
| KBV | **colony** | **0** | **0.98** |
| | patriline | 8.7 | 0.01 |
| | 1 | 11.8 | 0 |
| *Superkiller* | **1** | **0** | **0.92** |
| | colony | 4.97 | 0.08 |
| | patriline | 24.33 | 0 |
| *Dicer* | **colony** | **0** | **0.99** |
| | patriline | 10.33 | 0.01 |
| | 1 | 19.85 | 0 |

Table 2. Nonparametric permutational MANCOVA with parameters describing total cell number in a nest. Showing sum of squares (SS), F-statistic (F) and p-value (p) for effective paternity ($k_{e3}$), the *Kashmir Bee Virus* load (KBV) and expression of the immune genes *Dicer* and Superkiller.

| Source | SS | F | p |
|---|---|---|---|
| $k_{e3}$ | 5.793 | 72.782 | 0.001 |
| KBV | 1.169 | 14.680 | 0.002 |
| *Dicer* | 0.280 | 3.517 | 0.037 |
| *Superkiller* | 0.007 | 0.085 | 0.860 |
| Residual | 9.233 | – | – |
| Total | 16.481 | – | – |

*Kashmir Bee Virus* was observed to be highly prevalent and was detected in every individual wasp that we sampled. KBV can have substantial effects on social insect populations and significantly affected cell number in the studied colonies. Along with other viruses, KBV has been repeatedly associated with colony collapse in honey bees (Schwarz et al. 2015). Although effects of KBV on honey bees are well-studied, effects on other insects are poorly understood. Bumble bee colonies infected orally with KBV showed reduced drone production (Meeus et al. 2014). While the original host of KBV is still unknown (de Miranda et al. 2010), the high prevalence shown here in wasps provides further evidence that the effects of KBV are not limited to honey bees. High viral loads observed in some individuals and confirmation of viral replication in the common wasp indicate that KBV is parasitic in wasps. Colony A, which hosted the highest KBV levels, failed to reproduce new queens. It is possible that KBV occurs in coinfection with other parasites or even other viruses. Individual honey bees were shown to be infected with up to three more viruses additionally to KBV (Chen et al. 2004). However, it remains to be tested if KBV becomes pathogenic as a secondary infection when other viruses are present or weakens the hosts defence mechanisms and allows other pathogens to infect wasp colonies, causing immune response and colony decline. Controlled infection studies could provide insight about virulence in wasps.

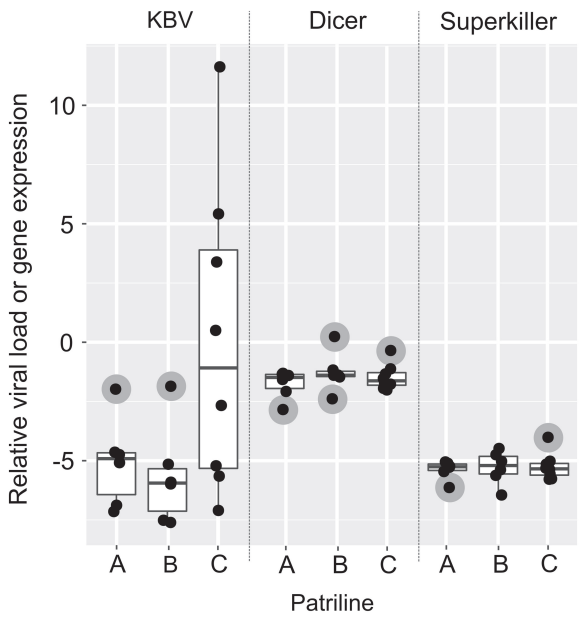


Figure 4. An example of within-nest variation, according to patriline, for KBV loads and the immune genes *Dicer* and *Superkiller*. This data is from Nest 'F', but similarly no significant difference in KBV, *Dicer* or *Superkiller* expression was observed for any nest. Outliers are identified by the grey circles.

The timing of our sampling likely played a large role in the high observed viral abundances. We collected wasp colonies in autumn, near the end of the colony life cycle. Some insect viruses, like *Black queen cell virus*, have been found to accumulate in bees over the summer and peak in abundance in autumn, although other viruses show different patterns (Runckel et al. 2011). Sampling colonies at the end of the season does not allow us to determine when a colony becomes infected and for how long it has been parasitized, but is more likely to reveal effects of virus load on fitness. It seems likely that colonies infected early in the season might show slow growth and delayed offspring production compared to colonies infected later. Future studies with multiple samples taken across the season could reveal whether the timing of infection explains mature colony viral load. Alternatively, intrinsic resistance, or coinfection with other pathogens, may explain colony-level variation in virus load and colony response to infection. Regardless of the mechanism by which KBV infection influences colonies, our work agrees with previous studies that indicate KBV can be prevalent in wasps (Lester et al. 2015), and tentatively suggests that it may have colony-level consequences. Although the effect on colony productivity was statistically significant, the correlation between viral load and colony size was weak and insignificant. Our sample was quite small (six colonies), which could have affected the permutational MANCOVA, and a larger sample may make this effect more pronounced. Furthermore, the fact that KBV levels predict viral immune response at the individual level suggests that wasps likely expend resources to combat KBV infections, making an effect of KBV likely.

The correlation we observed between KBV and *Dicer* expression corresponds with previous work showing increased *Dicer-2* expression upon Dicistroviridae infections in bumble bees (Niu et al. 2016). The expression effects we detected were small, but sampled workers were randomly selected, not age-controlled. The presence of highly infected group members can lead to changes in physiological immune response by social immunization (Masri and Cremer 2014), which would weaken the individual-level association between

viral load and immune response. Of the factors we studied, our analysis indicated that *Dicer* expression was one of the main predictors of colony fitness, and indicated that *Dicer*-mediated immune response via RNAi is important in viral defence in wasps. Immune defence is costly and requires resources (Hawley and Altizer 2011) which could result in reduced colony productivity and reproduction.

Colony-level properties such as genetic diversity are predicted to mediate the degree to which colonies are affected by pathogens (Cremer et al. 2007). Pathogens such as KBV may not be equally adapted to all host genotypes present within the wasp nests, this phenomenon may be observed if some patrilines had consistently different infection rates (Sherman et al. 1988, Schmid-Hempel 1994, Crozier and Fjerdingstad 2001, van Baalen and Beekman 2006). Support for this hypothesis comes from experiments with leaf-cutter ants, where resistance to a fungal parasite varied between patrilines (Hughes and Boomsma 2004). In contrast, we found no evidence that patrilines differ in their susceptibility or immune gene response to KBV infection. However, a strong association between genetic diversity and colony reproduction across our six study colonies reinforces the benefits of polyandry for wasp colony fitness (Goodisman et al. 2007, Johnson et al. 2009). It is noteworthy that the average effective paternity that we observed in this invaded range of *V. vulgaris* (average effective paternity 1.87) corresponds with estimates from the native range (average effective paternity 1.9; Foster and Ratnieks 2001).

Taken together, this means that although polyandry and viral immune gene expression are both likely to influence colony fitness in *V. vulgaris*, the benefits of polyandry do not accrue by conferring colony-level resistance specifically to KBV or by variation in RNAi immune response. We examined the evidence that patrilines in a genetically diverse colony affect KBV susceptibility and immune gene expression but no effects of patriline membership could be detected. These results indicate that there is no genetic variation in KBV susceptibility or immunocompetence in this invasive *V. vulgaris* population. Such variation might occur in different populations of these wasps within the invaded or native range. Whether polyandry benefits result from effects of other, yet unmeasured pathogens, or whether they come from effects unrelated to disease (Crozier and Fjerdingstad 2001) remain to be determined.

*Acknowledgements* – We thank E. C. Brenton-Rule and J. Haywood for help with data analysis, J. Kurtz for supervision and comments on an early version of the manuscript.
*Funding* – This research supports the New Zealand National Science Challenge on Novel Wasp Control Strategies under the Biological Heritage programme. It was supported by a Royal Society of New Zealand (RSNZ) Marsden Grant, and an RSNZ James Cook Fellowship, to PJL.

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Supplementary material (available online as Appendix oik-04117 at <www.oikosjournal.org/appendix/appendix oik-04117>). Appendix 1.