A pest and disease survey of the isolated Norfolk Island honey bee (Apis mellifera) population

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Norfolk Island is one of Australia’s most remote communities, located about 1,400 km east of the mainland. This report is the first documented survey of pests and diseases affecting the honey bee (Apis mellifera L.) population on Norfolk Island. The only invertebrate pest detected during the survey was the lesser wax moth (Achroia grisella), and only one honey bee virus was identified (Lake Sinai virus 1). The microsporidian parasite Nosema ceranae was also detected in the majority of adult bee samples, but Nosema apis was not present in any of the samples analyzed. Given that honey bee imports to Norfolk Island ceased in 1992, we discuss possible scenarios for N. ceranae introduction to the island. Lineage analysis also determined that Norfolk Island’s honey bees are for the most part from Eastern Europe (probably A. m. ligustica and A. m. carnica) with a small percentage from Western Europe (probably A. m. mellifera). This survey has identified a remote and isolated honey bee population that is relatively free from major pests and diseases that affect honey bees around the world. This knowledge will help inform trade policy and management strategies for maintaining the unique health status of honey bees on Norfolk Island.

Introduction

Norfolk Island is an Australian territory situated in the Coral Sea and is one of the smaller and more isolated islands in the Pacific. It is located about 1,400 km east of the Australian mainland, and the French territory of New Caledonia is more than 700 km to the north (Holloway, 1977; Figure 1). European honey bees (Apis mellifera L.) are not native to Norfolk Island, but were probably introduced between 1822 and 1855. These dates coincide with when honey bees were imported to Sydney, Australia, from England in 1822 (Weatherhead, 1866) and 1855, when Norfolk Island was closed as a penal settlement, before joining the Commonwealth of Australia in 1901. Early introductions to Australia were likely to have been from England with introductions from Italy (A. m. ligustica) and other subspecies from a broad distribution coming later (Chapman et al., 2016; Chapman, Lim, & Oldroyd, 2008; Hopkins, 1886; Koulianos & Crozier, 1997; Oxley & Oldroyd, 2009; Ruttnert, 1976). Limited honey bee introductions have occurred from Australia to Norfolk Island, with records suggesting that no introductions have occurred since 1992 (Neil Tavener, pers. comm.).

There are now about 130 managed hives of A. mellifera on Norfolk Island, and a large population of feral colonies. Most hives are managed by three beekeepers, with a small number of hobby beekeepers also managing 1–2 hives each. This small industry provides valuable honey and honey bee products to

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island residents, as well as crucial pollination services for a variety of crops. While beekeeping has historically been popular among some Norfolk residents, numbers of hives and beekeepers have remained relatively stable since the early 1990s.

Norfolk’s isolation and a lack of reported honey bee issues suggested that this population may be free of many pests and diseases. Our aim was to investigate managed and feral *A. mellifera* colonies to provide a baseline of information on honey bee pests and diseases.

![Map of Norfolk Island showing detections of lesser wax moth (*Achroia grisella*), *Nosema ceranae*, and Lake Sinai virus 1 in *Apis mellifera* colonies. Estimated Nosema spore levels are shown in the inset pie chart (n = 26).](image-url)

(B) Lineage proportion of ancestry from Eastern Europe (C; White), Western Europe (M; Grey), and Africa (A; Black) in six individuals from Norfolk Island.
present on Norfolk Island. We inspected colonies for the presence of parasitic mites, hive pests, and brood diseases and analyzed bee samples for the presence of Nosema spp., fungi, and viruses. Considering the remoteness of the honey bee population and the restricted pool from which imports of honey bee stock have occurred, we also conducted preliminary lineage testing to determine the genetic makeup of the honey bee population, according to the three major ancestral lineages, Western European [M], Eastern European [C], and African [A] (Chapman et al., 2015).

This report is the first documented honey bee health survey conducted for Norfolk Island. This can assist local government and biosecurity agencies to better understand Norfolk Island’s honey bee population and its pest and disease status, and to implement appropriate biosecurity strategies to ensure this population is protected into the future. The continual worldwide importation of honey bee stock has resulted in the establishment of non-native populations (Potts et al., 2010; Vanbergen et al., 2013) and declining pollinator populations (Mcdougall, 1973; Figure 1). The island population is currently around 1,400 residents, but has reached close to 2,000 in the past.

The majority of the island is an undulating plateau around 100 m above sea level, almost completely surrounded by cliffs and with much of the inland area consisting of small-scale agriculture and mixed farming areas as well as residential zones with gardens (Holloway, 1977). Norfolk Island National Park covers around 30% of the island and contains remnants of the native forests that originally covered the island, including stands of subtropical rainforest dominated by Norfolk Island pine (Araucaria heterophylla) (Coyne, 2011). Norfolk Island National Park also includes two additional smaller islands to the south, Nepean Island (0.1 square km) and Phillip Island (2.6 square km), which were not included in the survey.

Norfolk Island has a maritime subtropical climate tempered by the surrounding sea, and is best characterized as mild (Holloway, 1977). Diurnal and annual temperature ranges are very small: average maximum temperatures range from 18 to 19 °C in winter to 23–25 °C in summer and average minimum temperatures range from 13 to 15 °C in winter and 18–20 °C in summer (BoM, 2015). Mean annual rainfall is 1312 mm, with rainfall greatest during the winter months of May to August (BoM, 2015).

### Materials and methods

#### Description of study area

Norfolk Island is roughly 34 square kilometers in area, and is an eroded remnant of a basaltic volcano that forms part of the Norfolk Ridge, linking New Zealand and New Caledonia (Holloway, 1977; Jones & Mc Dougall, 1973; Figure 1). The island population is currently around 1,400 residents, but has reached close to 2,000 in the past.

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#### General colony health inspections and visual inspection for pests and diseases

Over two separate one-week periods, a total of 61 European honey bee colonies in 27 apiaries, and 2 feral nests were visually inspected for signs and symptoms of a range of pests and diseases. These inspections occurred in early to mid-Spring 2013 (23–27 September 2013 and 7–11 October 2013). All A. mellifera colonies inspected were managed by the three main beekeepers on Norfolk. These colonies are generally kept at permanent apiaries, ranging from 1 to 10 hives per apiary. The feral nests were located after speaking with local residents.

In all managed and feral A. mellifera colonies, observations were made on colony health by looking for dead or dying adult bees and examining multiple brood combs, when available, for pest and disease symptoms such as scattered brood and perforated cappings. We searched for clinical symptoms of brood diseases including chalkbrood (Ascosphaera apis), American foulbrood (AFB; Paenibacillus larvae), European foulbrood (EFB; Melissococcus plutonius), as well as viruses that cause clinical symptoms such as sacbrood virus (Iflavirus) and black queen cell virus (Cripavirus).

We also visually examined colonies for the presence of varroa mites (Varroa destructor and V. jacobsoni), tropilaelaps mites (Tropilaelaps clareae and T. mercedesae), braula fly (B. coeca), and small hive beetle (Aethina tumida). Healthy colonies and stored combs (that is, empty supers above brood boxes) were also examined for the presence and symptoms of greater wax moth (Galleria mellonella) and lesser wax moth (Acholeplasia griseola) and associated symptoms such as webbing, tunneling, and frass (Ellis, Graham, & Mortensen, 2013). Suspect brood samples were further examined for the presence of exotic mites through uncapping and removal of brood. Colonies with a low population and patchy brood were subjected to a powdered sugar test as according to Dietemann et al. (2013).

#### Sample collection

Samples of dead and/or dying adult bees, larvae, and pupae showing brood disease symptoms were collected in sterile 20-ml screw-top containers. Additional samples of at least 60 adult bees were collected from peripheral combs in the brood area or from honey supers to maximize the detection of Nosema (Fries et al., 2013). These adult bee samples were also tested for viruses, tracheal mites (Acarapis woodi), and amoeba disease (Malpigia-moeba mellifica). Adult bees were collected by scooping bees with a plastic cup directly from the frames or by shaking the frames into a large plastic bag. Samples were collected during September and October 2013. Six additional adult honey bee samples were collected during October 2014, and were tested for the presence of Nosema spp. and viruses. Each sample came from a single
Honey if collected from a single hive apiary, or a combined sample if multiple colonies were located in the same apiary. All samples were frozen after collection on Norfolk Island and transported frozen to mainland Australia where they were kept at −20°C until processing. All samples were transported under an Australian Government Department of Agriculture permit to import quarantine material (IP13013160).

**Honey sampling for the detection of American foulbrood (AFB) and European foulbrood (EFB)**

Eight honey samples were collected to detect *P. larvae* and *M. plutonius*. On Norfolk, honey from multiple hives is extracted at the one time, using the only extracting machine available on the island. This provides an effective avenue of collecting bulked composite honey samples that are representative of the majority of hives on the island. Honey samples were collected in February 2010, February 2012, December 2012, April 2013, September 2013, October 2013, December 2013, and April 2014. Bulked honey samples were transported in sterile 200-ml honey-proof containers to the Elizabeth MacArthur Agricultural Institute of the New South Wales Department of Primary Industries and examined for the presence of *P. larvae* according to Hornitzky (2010) and for the presence of *M. plutonius* following Hornitzky and Smith (1998).

**Examination for external mites and braula fly**

Twenty five managed colonies were randomly selected across Norfolk Island throughout September and October 2013 for the detection of the external parasitic varroa mites (*V. destructor* and *V. jacobsoni*), tropilaelaps mites (*T. clareae* and *T. mercedesae*), and for the commensalist braula fly (*B. coeca*). The method of acaricide treatment and sticky board analysis used on Norfolk followed Australia’s National Bee Pest Surveillance Program guidelines (NBPS, 2013). After two days, the sticky boards were removed, labeled, placed in a plastic bag, and brought back to the Norfolk quarantine laboratory for analysis. The sticky boards were examined under E277-LED – New Standard Maggylamp with LED and the presence of *Varroa sp.*, *Tropilaelaps sp.*, and *B. coeca* was recorded. The acaricide strips were disposed of as per the label recommendations.

**Examination for small hive beetle**

Twenty managed colonies were randomly selected across Norfolk Island throughout September and October 2013 for small hive beetle (*A. tumida*; SHB), examination using Apithor™ harborages. The Apithor harborages provides a fast, cheap, and easy quantitative diagnosis for determining whether SHB is present and provides a very efficient (96%) mortality rate of small hive beetle (Levot, 2008). After 14 days, the harborages were removed from the hives and placed into individual labeled sealable plastic bags and brought back to the laboratory. Using latex gloves, the harborages were opened and examined for the presence of small hive beetle.

**Microscopic detection of non-viral honey bee pathogens**

Adult bee samples from across Norfolk Island were examined microscopically for the presence of microsporidian parasites (*Nosema apis* and *N. ceranae*), amoeba disease (*M. mellificae*) as well as the internal Acari parasite, tracheal mite (*A. woodi*). For detection of *N. apis*, *N. ceranae*, and *M. mellificae*, 26 samples from randomly selected hives were collected and examined. Ten of these samples were collected in September 2013 and another 10 samples were collected in October 2013, while the remaining 7 samples were collected in October 2014.

To screen for *Nosema* sp. and *M. mellificae*, two preparations of 30 adult bees (60 bees in total) were homogenized by mortar and pestle in 4 ml of distilled water for each of the 27 samples. For each homogenized subsample, 2 × 5-μl aliquots were examined on a microscope slide at 400x magnification under a light microscope (Leica Microsystems). If *Nosema* spores were detected, average spore levels across the four aliquots per sample were subjectively assessed as low (<100 spores), medium (<1,000 spores), or high (>1,000 spores). The presence of *M. mellificae* cysts, which are similar in size to *Nosema* spores at 6–7 μm in diameter but completely spherical instead of oval, was also recorded (World Organisation for Animal Health, 2013).

Positive *Nosema* samples were further tested by PCR for specific identification. For each sample, DNA was extracted from a 500-μl aliquot using the High Pure PCR Template Preparation Kit (Roche Diagnostic) following the manufacturer’s tissue extraction protocol. PCR reactions were carried out in a total volume of 10 μl containing 1 × PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.4 μM forward primer, 0.4 μM reverse primer, 1 U Taq DNA polymerase (New England Biolabs), and 1-μl DNA template. PCR cycling conditions and primer sequences for *N. apis* and *N. ceranae* are shown in Table 1.

For detection of *A. woodi*, 20 samples from randomly selected hives were collected and examined. The sampling method and time of sampling are crucial for the detection of tracheal mites (Sammataro, De Guzman, George, Ochoa, & Otis, 2013). For this reason, adult bee samples were collected in early spring on two occasions, once in September 2013 and once in October 2013. Tracheal mites were screened by dissecting the tracheae from 50 adult bees from each sample and were examined under a microscope for the presence of mites and signs of mite damage.
Table 1. Diagnostic primers and PCR cycling conditions used in the Norfolk Island survey.

| Pathogen Primers | PCR cycling conditions | References |
|-------------------|------------------------|------------|
| Nosema ceranae F: CGGCCAGATGTGATAGAAAATATTTA | 94 °C (15 s), 60 °C (30 s), 72 °C (45 s) | (Martin-Hernández et al., 2007) |
| R: CCCGGATCCTACCATGAAAATACCGG | | |
| Nosema apis F: GGGGGGATGTTTACGTAACGTA | 94 °C (15 s), 60 °C (30 s), 72 °C (45 s) | (Martin-Hernández et al., 2007) |
| R: GGGGGGCTTAAAAATGTGAAAAACACTATG | | |
| Sacbrood virus F: GCTAGGATGACGTTTGG | 94 °C (30 s), 58 °C (30 s), 72 °C (40 s) | (Chen et al., 2004) |
| R: TCATCATCCATCCCTCGGA | | |
| Black queen cell virus F: TGTTCACTCCTACCACTTACACAC | 94 °C (30 s), 56 °C (30 s), 72 °C (40 s) | (Benjeddou, Leat, Allsopp, & Davison, 2001) |
| R: GCCAAGAAGGCGATTAAACCAC | | |
| Israeli acute paralysis virus F: AGACACCAATACGGACGAA | 94 °C (30 s), 58 °C (30 s), 72 °C (40 s) | (Runckel et al., 2011) |
| R: AGATTGGTGCACAGTGC | | |
| Kashmir bee virus F: GATGAACTCTGCAATGTTGA | 94 °C (30 s), 58 °C (30 s), 72 °C (40 s) | (Runckel et al., 2011) |
| R: GTGGGTTGCTATGAGTC | | |
| Chronic bee paralysis virus F: AGTTGGTCTAGTTAACAGGAGGAGG | 94 °C (30 s), 56 °C (30 s), 72 °C (40 s) | (Ribiere, Faucon, & Pepin, 2000) |
| R: TCTAATCTTACCGAAAACGGGAG | | |
| Acute bee paralysis virus F: TGAGAACCACCTGTAATGCTG | 94 °C (30 s), 56 °C (30 s), 72 °C (40 s) | (Tontecheva et al., 2004) |
| R: ACCAGAGGTGACTGTGTTG | | |
| Slow bee paralysis virus F: GATTTCGCAATCGATTGTTG | 94 °C (30 s), 56 °C (30 s), 72 °C (40 s) | (De Miranda et al., 2010) |
| R: ACCAGTTAGTACCTCTGTAATCTCG | | |
| Deformed wing virus F: CTCTGGATCTTTCGCGACT | 94 °C (30 s), 56 °C (30 s), 72 °C (40 s) | (Singh et al., 2010) |
| R: TGCAAGAGTACTGTCACACDC | | |
| Lake Sinai virus 1 F: TTATCTGATCCGCGCAGCTCT | 94 °C (30 s), 54 °C (30 s), 72 °C (40 s) | (Runckel et al., 2011) |
| R:ATTCATGGCCCTGGAAGTGCAC | | |
| Lake Sinai virus 2 F: CGGCCGCTCTAGCTGGTGTTG | 94 °C (30 s), 54 °C (30 s), 72 °C (40 s) | (Runckel et al., 2011) |
| R: TGGCAAGCTGTGACGAATCCCT | | |

**Analysis for honey bee viruses**

A total of 15 adult bee samples and 36 individual brood samples was provided for screening of acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), Israeli acute paralysis virus (IAPV), sacbrood virus (SBV), black queen cell virus (BQCV), deformed wing virus (DWV), slow bee paralysis virus (SBPV), chronic bee paralysis virus (CBPV), and Lake Sinai viruses 1 and 2 (LSV1 and LSV2).

RNA was extracted from 500 μl of adult bee homogenate (bees homogenized by mortar and pestle in 0.01 M potassium phosphate buffer) and from individual brood samples using 800 μl of Trizol reagent (Invitrogen) following the manufacturer’s instructions. RNA was then converted to complementary DNA (cDNA) using the SuperScript III first-strand synthesis system (Invitrogen–chloroform isoamyl alcohol and SNP genotyping was performed with the Sequenom MassARRAY MALDI-TOF system (Sequenom, CA, USA). Ancestry was assigned to individuals from the Norfolk Island population based on the reference populations (Chapman et al., 2015) using STRUCTURE (v2.3.4 Pritchard, Stephens, & Donnelly, 2000) with three ancestral lineages assumed and a burn-in phase of 50,000 iterations followed by 100,000 iterations, with admixture assumed and uncorrelated allele frequencies.

**Results**

**Colony inspections and examination for hive pests and brood diseases**

Visual examination of the 61 managed colonies and 3 feral colonies revealed relatively healthy colonies, with no signs or symptoms of brood diseases or honey bee pests. The only pest recorded was the lesser wax moth (A. grisella) which was detected in five colonies in three apiaries, all of which were in the central-northern area of the island (Figure 1). Twenty five sticky boards from a total of 12 apiaries were examined for the presence of external parasitic mites (Varroa sp. and Tropilaelaps sp.) and external pests such as braula fly (B. coeca). All sticky boards were negative for the presence of varroa mites, tropilaelaps mites, and braula fly. Inspection of 20 Apithor traps from a total of 11 apiaries revealed no small hive beetle (A. tumida).
Microscopic detection of non-viral honey bee pathogens

Nosema spores were detected in all 26 samples, but no cysts of *M. mellificae* were observed. Spore levels were estimated to be low in nine samples, medium in 11 samples, and high in six samples (Figure 1). All samples were found PCR positive for *N. ceranae*, but *N. apis* was not detected (Figure 1). Sequencing of all positive samples confirmed 100% similarity with *N. ceranae*. The reliability of the *N. apis* protocol was also confirmed by positive amplification of *N. apis* from Western Australian bee samples.

Analysis for honey bee viruses

Lake Sinai virus 1 (LSV1) was the only virus detected from honey bees on Norfolk Island. LSV1 was detected in 15 of the adult bee samples collected from 10 apiaries, but not in any of the 36 brood samples tested (Figure 1, Supplementary Table). None of the other viruses tested for were detected, despite several of these being common in mainland Australia.

All LSV1-positive samples were sequenced (GenBank accession numbers: KT380002–KT380005) and compared with reference genomes. A phylogenetic analysis of LSV1 sequences from Norfolk Island suggests there are two genetic groups, with approximately 3% difference between them. Both Norfolk Island LSV1 groups were distinct (approximately 15%) from LSV1 isolates collected from Cairns (Queensland, Australia) *Apis mellifera* and *Apis cerana* in 2012 (Roberts & Anderson, 2013). Norfolk Island sequences were also more distantly related to US isolates, showing 20–30% difference (Figure 2).

Lineage testing

On average, the Norfolk Island samples were mainly of Eastern European heritage (average 80.7%, range
Honey sampling for American foulbrood and European foulbrood

Eight honey samples collected from 2010–2014 extraction dates were negative for both *P. larvae* and *M. plutonius*.

Discussion

The Norfolk Island Quarantine Survey demonstrated that the island’s honey bee population is free from many of the damaging honey bee pests and diseases that affect honey bee colonies around the world. The only pests and diseases detected on Norfolk Island were the lesser wax moth (*A. grisella*), Lake Sinai virus 1, and *N. ceranae*. The survey has confirmed that Norfolk Island is free from parasitic mites, bacterial and fungal brood diseases, and several key hive pests, gut parasites, and viruses.

The detection of *N. ceranae* on Norfolk Island is particularly relevant for Australia, as the first detection of *N. ceranae* in Australia occurred in 2007 (Giersch, Berg, Galea, & Hornitzky, 2009). This emerging pathogen was first detected in 1994 on Asian honey bees (*A. cerana*) from China (Fries, Feng, Silva, Slemenda, & Pieniazek, 1996). In 2005, *A. mellifera* colonies in Taiwan were found to be infected with *N. ceranae* (Huang, Jiang, Chen, & Wang, 2005). *Nosema ceranae* has since been detected worldwide on *A. mellifera*, including in Europe (Chauzat et al., 2007; Fries, Martin, Meana, García-Palencia, & Higes, 2006; Higes, Martin, & Meana, 2006; Paxton, Klee, Korpela, & Fries, 2007), North America (Chen, Evans, Smith, & Pettis, 2008; Williams, Shafer, Rogers, Shutler, & Stewart, 2008), and South America (Invernizzi et al., 2009). Further testing of historical bee samples has demonstrated that *N. ceranae* has been present for longer than originally thought, with bee samples from the US in 1996 (Chen et al., 2008) and in France from 2002 (Chauzat et al., 2007) testing positive to *N. ceranae*. Similarly, our results for Norfolk Island suggest a possibly longer presence of *N. ceranae* in Australia, taking into account the last recorded imports from Australian apiaries in Queensland and New South Wales in 1992.

There are three possible avenues for the arrival of *N. ceranae* on Norfolk Island. Firstly, honey bee stock may have been introduced to Norfolk Island illegally since 1992. Considering the island's small population, small size of the honey bee industry, little knowledge of queen breeding among local beekeepers, and no clear commercial incentive, it is unlikely that any illegal imports have occurred.

Secondly, *N. ceranae* may have arrived via the honey pathway. Although honey can contain *N. ceranae* spores (Giersch et al., 2009) and is a plausible avenue of reinfection, the viability of spores in honey is still unclear (Higes, Martín-Hernández, & Meana, 2010). It is also unlikely that *N. ceranae* entered via this pathway given the limited quantities of honey imported for retail purposes to Norfolk Island (around 1 ton per year). Importantly, other honey bee diseases that are readily transmitted through honey, such as *P. larvae* and *M. plutonius*, were not detected on Norfolk Island as part of this survey.

Lastly, honey bee stock imported to Norfolk Island before 1992 may have been infected with *N. ceranae*. The rapid, long-distance dispersal of *N. ceranae* throughout worldwide honey bee populations is believed to have been due to the transport of infected honey bee stock (Klee et al., 2007). The trade in queen bees and worker bees is considered a source of infective *N. ceranae* spores, and this pathway may explain the presence of *N. ceranae* in Norfolk Island *A. mellifera* colonies, implying that *N. ceranae* was present in Australian honey bee stocks well before their official detection in 2007.

The absence of *N. apis* also raises another interesting scenario. *Apis mellifera* has been transported around the world for beekeeping purposes, and *N. apis* has been associated with it throughout its global range (Klee et al., 2007). Considering the global distribution of *N. apis* and the well-established population of *N. apis* in Australian honey bee stock (Giersch et al., 2009), it is possible that *N. apis* once infected the Norfolk Island honey bee population but has since disappeared. The climate of Norfolk Island may be unfavorable to *N. apis*; the notion that *N. ceranae* can establish more readily in warm or subtropical climates at the expense of *N. apis* has been supported in other studies in Spain and Italy (Fenoy, Rueda, Higes, Martín-Hernández, & Del Águila, 2009; Klee et al., 2007; Martín-Hernández et al., 2009). On the Australian mainland, *N. ceranae* has high prevalence in warmer tropical areas and *N. apis* remains more common in the cooler regions of southern Australia (Giersch et al., 2009).

The detection of only LSV1 and no other common honey bee viruses on Norfolk Island was also unexpected. Several viruses, including sacbrood virus and black queen cell virus, were common in Australia prior to 1992 (Dall, 1985; Hornitzky, 1987) and are likely to have been introduced to Norfolk prior to cessation of honey bee imports to the island in 1992. Despite sampling in two consecutive spring seasons, when viruses are typically more prevalent, these viruses were not detected. Further testing at different times of the year and with more sensitive techniques (for example, quantitative PCR, next-generation sequencing) may reveal additional viruses (De Miranda et al., 2013). In addition, no viruses were detected in the suspected diseased brood. These samples were collected on the basis of being uncapped and not showing signs of other non-viral...
brood diseases such as chalkbrood. The unhealthy brood may therefore be due to cell disruption by wax moth larvae or nutritional or temperature stress.

Analysis of the LSV1 isolates revealed a surprising level of diversity for such a small island. This virus has also been found to have considerable heterogeneity around the world and even within single bees (Ravoet, De Smit, Wenseleers, & De Graaf, 2015). The two well-supported genetic groups were distinct from Australian and US isolates but may reflect regional differences in the origins of Norfolk Island’s bees. Examination of more Australian isolates may provide insights into these possible origins.

European foulbrood (*M. plutonius*) was not detected in Norfolk Island during this survey, but further testing should be conducted to provide more conclusive evidence of whether Norfolk Island is free from this bacterial disease. *Melissococcus plutonius* can be isolated from honey by cultivation; however, the bacterial culture method seems to be very insensitive, detecting less than 0.2% of the bacterial cells (Hornitzky & Smith, 1998; Forsgren, Budge, Charrière, & Hornitzky, 2013). To confirm the presence or absence of *M. plutonius* on Norfolk Island, a hemi-nested PCR could be conducted to detect *M. plutonius* in honey bees and their products as according to McKee, Djordjevic, Goodman, and Hornitzky (2003). No laboratories in Australia are currently using this method.

Considering uncertainty over the dates of the arrival of honey bee stock on Norfolk Island, it was important to understand the genetic makeup of the honey bee population. Our genetic data are consistent with multiple introductions of the importation of previously hybridized bees from the mainland (Chapman et al., 2015). There have been a number of introductions of bees into Australia from the African, Eastern European, Western European, and Middle Eastern lineages, but introductions from Africa and the Middle East were limited (Goodacre, 1935; Weatherhead, 1986; Gulliford, 2005). The honey bees of Norfolk Island are predominantly of the Eastern European lineage (75.6–84.6%), with a substantial contribution from Western Europe (14.3–22.1%) and a minor contribution from Africa (0.4–3.0%). This is in contrast to the rest of Australia, which varies significantly in the proportion of ancestry from each of these lineages (Chapman et al., 2015).

It may be that the small population on Norfolk Island has led to homology in the degree of hybridization or that local conditions have selected for hybrid individuals of this genetic background. This has been demonstrated elsewhere in Australia; in the cool Tasmanian highlands, the honey bees are predominantly of the subspecies *A. m. mellifera*, which evolved in colder climes, while elsewhere in Tasmania, other subspecies are predominant (Chapman et al., 2016; Oldroyd, Cornuet, Rowe, Rinderer, & Crozier, 1995). Given the closed population of Norfolk Island, future studies should include estimates of genetic diversity, though the absence of patchy broodcomb suggests inbreeding is not yet a problem (Zayed, 2009). Closed population breeding programs can adequately maintain genetic diversity in honey bee populations (Chapman et al., 2008), and such a program may need to be instigated to ensure the continued health of the honey bee population on Norfolk in the absence of further importations.

It is critical that Norfolk Island’s freedom from many honey bee pests and diseases is preserved into the future. The Australian and Norfolk Island Government should consider the recommendations of Mutinelli (2011), who outlines the main honey bee diseases and the products that could act as vectors and preventive measures that could prevent their spread, including inspections, testing, certification, and sourcing honey bee products from disease-free areas or even a ban on importation (Mutinelli, 2011). Efforts should also be made by the appropriate agencies to include Norfolk Island in Australia’s National Bee Pest Surveillance Program, which involves undertaking routine, continuing surveillance for exotic and established honey bee pests and diseases. A combination of these measures will strengthen Norfolk Island’s biosecurity system and ensure that this unique honey bee population is protected into the future.

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