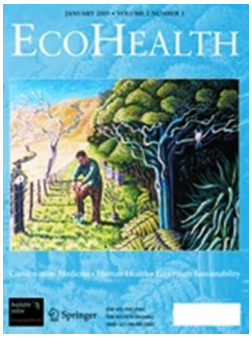

Bringing back a healthy buzz? Invertebrate parasites and reintroductions: a case study in bumblebees

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Running head: Parasites and bumblebee reintroductions

Abstract: Reintroductions can play a key role in the conservation of endangered species. Parasites may impact reintroductions, both positively and negatively, but few case studies of how to manage parasites during reintroductions exist. Bumblebees are in decline at regional and global scales, and reintroductions can be used to re-establish extinct local populations. Here we report on how the risks associated with parasites are being managed in an ongoing reintroduction of the short-haired bumblebee, *Bombus subterraneus*, to the UK. Disease risk analysis was conducted and disease risk management plans constructed to design a capture-quarantine-release system that minimized the impacts on both the bumblebees and on their natural parasites. Given that bumblebee parasites are (i) generalists, (ii) geographically ubiquitous, and (iii) show evidence of local adaptation, the disease risk management plan was designed to limit the co-introduction of parasites from the source population in Sweden to the destination site in the UK. Results suggest that this process at best eliminated, or at least severely curtailed the co-introduction of parasites, and ongoing updates of the plan enabled minimization of impacts on natural host-parasite dynamics in the Swedish source population. This study suggests that methods designed for reintroductions of vertebrate species can be successfully applied to invertebrates. Future reintroductions of invertebrates where the parasite fauna is less well-known should take advantage of next-generation barcoding and multiple survey years prior to the start of reintroductions, to develop comprehensive disease risk management plans.

Word count: 3625

Introduction and purpose:

Species reintroductions are widely regarded as an important conservation technique and have been utilised in the UK and worldwide with increasing frequency in recent years. They enable the reinforcement of declining populations and re-establishment of locally extinct populations (IUCN SSC, 2013). Given their importance for individual species, and the likelihood that their use will increase in the future, the IUCN/Species Survival Commission (SSC) Reintroduction Specialist Group (http://www.iucnsscrsg.org) recently published updated guidelines for reintroductions and other conservation translocations (IUCN SSC, 2013). Amongst other issues, these guidelines highlight the importance and complexity of considering parasites and diseases in reintroduction processes.

Parasites (defined as viruses, bacteria, fungi, protozoa, helminths, and ectoparasites) represent arguably the most common mode of life (Windsor 1998). Parasites can control host population dynamics (Hudson et al., 1998), add complexity and stability to food webs (Dobson and Hudson, 1986), and contribute considerable biomass to ecosystems (Johnson et al., 2010). The loss of parasites can disrupt host immune regulation (Dargent et al., 2013), and presumably alter host population dynamics and food-web function. In contrast, the accidental gain or spillover of parasites can lead to epidemics and host extinctions (Woolhouse et al., 2005). In particular, emerging infectious diseases (EIDs) have been argued to be one of the major threats to biodiversity (Daszak, 2000) and human health (Woolhouse et al., 2005). It is clear from these examples that parasites can have both positive and negative impacts on individuals, populations and ecosystems. Consequently, it is important to consider parasites explicitly within reintroductions, if we want reintroductions to succeed and, at the

same time, have minimal negative impacts to the areas animals and plants are being taken from and introduced to.

Initially, reintroduction programs in birds and mammals inadvertently resulted in parasite elimination as a consequence of broad-spectrum use of therapeutic agents on the host, leading to extinction of species-specific parasites (e.g., Gompper and Williams, 1998). Partially as a result of such losses, the recent IUCN recommendations are much more nuanced (IUCN SSC, 2013). There have been numerous calls to avoid such species co-extinctions (Koh et al., 2004; Jørgensen, 2015), and to manage both host and parasite during translocations (Gompper and Williams, 1998; Pizzi, 2009; Jørgensen, 2015). However, such an approach has to be balanced with the potential risks from disease associated with co-introducing parasites. Consequently, it is of value to examine and report on how parasites have been integrated into species reintroduction programs. This is particularly true for reintroductions of invertebrates, for at least three reasons: 1) invertebrates are under greater threat of extinction than other taxonomic groups (Thomas et al., 2004), 2) in terms of reintroductions, they are the least-studied group (Moehrenschlager A, pers. comm.), and 3) past and current IUCN guidelines have been largely constructed by vertebrate biologists with vertebrates in mind.

Declines in populations of bumblebees have recently become a cause for global conservation concern, both because of their intrinsic biodiversity value and the ecosystem services that they provide (Vanbergen et al., 2013). Many bumblebee species are in decline, both regionally (Williams, 1986; Nieto et al., 2014) and globally (Goulson et al., 2008; Williams and Osborne, 2009). The main driver of these declines is habitat loss through agriculture (Williams, 1986; Benton, 2006; Carvell et al., 2006; Fitzpatrick et al., 2007; Goulson et al., 2008; Xie et al., 2008; Williams et al., 2009; Nieto et al., 2014), but, more recently, emergent

diseases have been demonstrated to be serious potential threats (Thorp and Shephard, 2005; Colla et al., 2006; Brown, 2011; Cameron et al., 2011; Meeus et al., 2011; Fürst et al., 2014; Schmid-Hempel et al., 2014; McMahon et al., 2015).

Bombus subterraneus, a widespread palearctic species (Nieto et al., 2014), is in decline across parts of its range and was last seen in the UK in 1988, being declared locally extinct by the IUCN in 2000 (Gammans and Allen, 2014). A reintroduction program for this bumblebee has been running since 2009, with the intention being both to re-establish a sustainable population of this bee in the United Kingdom and to act as a flagship for bumblebee conservation in the UK more generally. Here we a) briefly describe the process used to produce a disease risk analysis and disease management plan for this reintroduction, and b) demonstrate how these documents were used to inform and manage the risks from disease and parasites during this reintroduction program.

Methods:

Disease Risk Analysis

A first disease risk analysis (DRA) based around the translocation of *B. subterraneus* queens from Sweden to the UK was conducted in 2011 (Vaughan-Higgins et al., 2012a). Here, we briefly describe how the analysis was conducted (for detailed descriptions see the published DRA; Vaughan-Higgins et al., 2012a). The DRA process followed the guidelines of Murray et al. (2004), modified for wildlife translocations for conservation purposes by Sainsbury and Vaughan-Higgins (2012), and in addition to assessing source hazards, it also assessed transport, carrier, destination, and population hazards. Hazard identification utilised information from the literature, as well as information gained from sampling and screening Swedish *B. subterraneus* queens and bumblebee workers from the reintroduction site in 2011

for parasites (see below for details). The first draft of the DRA was conducted by authors who were experts in DRA for wild animal translocation, but naïve to the field of bumblebee parasites (RV-H, AWS). It was then edited initially by an expert in bumblebee diseases (MJFB), prior to an iterative process that included discussions with invertebrate ecologists (RV-H, AWS, MJFB, GM, NG) and Natural England (for the release licence into the UK). The DRA report was submitted to the steering group responsible for overseeing the reintroduction program (Vaughan-Higgins et al., 2012a). The DRA was updated annually on the basis of results from the previous year's reintroduction (see Results).

Hazard identification: screening Swedish B. subterraneus *queens from the source population, and native bees from the putative reintroduction site for parasites.*

In order to inform the DRA for the first year of reintroduction, permission was gained in Sweden at the national level from Dr Björn Cederberg of Artdatabanken and Jord Bruksverket (for the export licence), and at the regional level from Per Levenskog of the Skane Lansstyrelsen to collect 59 *B. subterraneus* queens from the putative collection site for destructive screening. Queens were collected by NG between 16-19 May 2011 in Skane in southern Sweden (Figure 1). Collection was conducted across a large area to maximise the chance of collecting queens from multiple nests. After collection, and before departure to the UK, the bees were inspected by a Swedish licensed government vet and honeybee inspector and issued a health certificate. The bees were then transported live to Royal Holloway University of London where they were screened, after euthanasia, for macro- and micro-parasites (for viral screening, see below) using standard dissection and microscopy techniques (Rutrecht and Brown, 2008) by MJFB. Samples were then sent, along with 22 samples from the putative reintroduction site (5 workers of *B. hortorum*, 5 males of *B. lapidarius*, 2 workers of *B. pratorum*, and 10 workers of the *B. terrestris* group), for viral

screening by PCR at FERA, UK for the following viruses: Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV), Israeli Acute Bee Paralysis Virus (IAPV), Kashmir Bee Virus (KBV), Sacbrood Virus (SBV). Total nucleic acid (TNA) was extracted from single bumblebees using an adapted method from Simon-Delso et al. (2014). Briefly, single bumblebees were ground in 3 ml GITC Lysis buffer and incubated for 30 min at 65C. Following centrifugation at 6189g for 5 min, samples were loaded onto the Kingfisher Flex system and processed as described by Simon-Delso et al. (2014). Reactions were set up as described in Martin et al. (2012), which provides reagent details and reagent conditions, as well as primer details for DWV, KBV, IAPV, and ABPV. Primers and conditions for BQCV and SBV followed Chantawannakul et al. (2006). In addition to virus testing, TNA quality was assessed using a generic 18s ribosomal RNA gene.

Disease Risk Management

In 2011 / 2012, a detailed disease risk management plan (DRM) was constructed, based on the initial DRA (Vaughan-Higgins et al., 2012b). Again, here we describe briefly the process used to construct the DRM, but refer interested readers to the full document (Vaughan-Higgins et al., 2012b). The DRM was designed to minimise the risks from disease associated with the reintroduction of *B. subterraneus* queens from Sweden to the UK. The DRM built on an earlier DRM for a proposed, but not realised, reintroduction of *B. subterraneus* from a naturalised New Zealand population. Subsequently, the New Zealand populations had been found to be deeply inbred (Lye et al., 2011), and a Swedish source population was designated to replace the New Zealand population after genetic analyses showed them to be both outbred and closely related to extinct UK populations (Lye et al., 2011). The construction and writing of the DRM followed the same iterative process as described above for the DRA, and again

the DRM was annually updated based on results from the previous year's reintroduction (see Results).

Methods for minimising risk of disease from hazards (parasites)

Here we describe how queens were collected and kept in quarantine prior to release, as per the DRM (Vaughan-Higgins et al., 2012b). Briefly, on collection in Sweden queens were kept in separate vials and, except during feeding, chilled to minimize stress and energy expenditure. After queen collection was completed, queens were transported directly to a dedicated quarantine room at Royal Holloway University of London. For the first year of reintroduction the quarantine room was maintained at a temperature between 20-24°C and 50% humidity (in following years, room temperature was reduced to minimize queen activity and subsequent wing-wear during quarantine). Queens were screened for physical abnormality (none were found across the 4 years of the project; see Vaughan-Higgins et al., 2012b for further detail) and signs of disease on arrival. Each queen was then placed in a separate Perspex enclosure with *ad libitum* sugar water and pollen for nutrition (Figure 2). Queens were checked daily and serviced with dedicated tools to maintain barrier quarantine. If dead, they were immediately frozen and later dissected (see above) to screen for parasites. On either day two or three, and again on day 14 of quarantine, faecal samples were collected from each queen, and screened under x 400 for the microparasites *Apicystis bombi*, *Crithidia bombi*, and *Nosema bombi*, and larvae of the parasitic nematode *Sphaerularia bombi*. Infected animals were sacrificed and removed from quarantine (see Results). On the final day of quarantine queens were screened for physical abnormalities before being transported to the reintroduction site. Again, any queens with physical abnormalities were sacrificed and frozen for examination. Post-quarantine and dissection, all dead queens were sent to FERA, UK, for

viral screening (ABPV, BQCV, DWV, IAPV, KBV, SBV). Data are reported as prevalences, with 95% binomial confidence limits (CL).

Results:

The initial DRA in 2011 identified a total of 28 hazards, comprising 15 parasite species: these included 12 source hazards, 14 destination hazards, one carrier hazard and one transport hazard (Table 1). All the source and destination hazards were included on the basis of possible strain differences between species of parasite present in both Sweden and the UK (Vaughan-Higgins et al., 2012a).

Fifty-seven of 59 Swedish queens collected were available for parasite screening by dissection (two died during transport and were too autolysed to screen), while all 59 were submitted for viral screening, along with the 22 bees from the reintroduction site. Three Swedish queens were infected by the trypanosome gut parasite *C. bombi*, and four by the castrating parasitic nematode *S. bombi*. No queens were positive for viruses, but one worker of *B. hortorum* from the reintroduction site was positive for Acute Bee Paralysis Virus (ABPV).

The exclusion of Swedish parasites was deemed necessary for four reasons: (i) bumblebee parasites are broad generalists (Schmid-Hempel, 2008), (ii) the presence of highly pathogenic parasites in the Swedish queens that were assessed by the DRA as either medium or high risk (*C. bombi*, *S. bombi*), (iii) evidence that allopatric infections can have a higher disease impact than sympatric infections (Imhoof and Schmid-Hempel, 1998), and (iv) that both *C. bombi* and *S. bombi* parasites were known to be present in the UK (Jones and Brown, 2014), and thus exclusion from the reintroduction would not lead to their loss in UK ecosystems.

Consequently, the duration of quarantine (15 days) was designed to maximise the probability of detecting *S. bombi* infection (day 14 faecal screen), whilst early (~day 2-3) screening (and sacrifice of positives) aimed to reduce the potential for cross-infection by micro-parasites during the quarantine process.

Swedish queens were brought into quarantine for screening prior to reintroduction in 2012-2015. In 2012, 89 queens entered quarantine. Ten were infected with *C. bombi* and one with *N. bombi*. In addition, 14 died due to parasitism by the parasitoid Braconid wasp *Syntretus* sp. (Table 2). No viruses were detected in the 39 queens submitted for screening (all those which died during quarantine, including those sacrificed due to the presence of parasites) (Table 3). In 2013, 100 bees entered quarantine, and of these four were infected by *A. bombi*, 21 by *C. bombi*, two by *N. bombi*, two by *S. bombi*, and 15 by *Syntretus* sp. (Table 2). Of 50 queens sent for viral screening, only one was positive, for Black Queen Cell Virus (BQCV) (Table 3). In 2014, 100 bees entered quarantine, and of these 10 had *C. bombi*, two had *N. bombi*, and 27 were infected by *Syntretus* sp. (Table 2). As in 2012, of the 53 queens sent for viral screening, none gave a positive result (Table 3). Finally, in 2015, 67 queens entered quarantine. Of these, three were infected with *A. bombi*, 24 with *C. bombi*, and six with *Syntretus* sp. (Table 2). Again, none of the queens sent for viral screening gave a positive result (Table 3).

Results from the 2012-2015 quarantine screening, plus changes in UK national policy for bumblebee releases (Natural England, 2014), led to the following changes to the quarantine protocol. The discovery of BQCV in 2013 led to an addition to the DRA, but no modification to the DRM. In 2013, additional molecular screening of faeces for micro- and macro-parasites was introduced, in accordance with policy for the import of commercial bumblebees

(Natural England, 2014). In 2014, queens were pre-screened for micro-parasites using faecal samples in Sweden. This change was made in response to the large number of queens sacrificed in 2012 and 2013 in quarantine due to *C. bombi* infection (see above). From 2014, queens found positive for *C. bombi* on pre-screening were re-released in Sweden, where they would have a chance to contribute to the source population, as this action might be less disruptive to the native host-parasite assemblage. In 2014 two of the queens collected in Sweden were identified as infected with *C bombi* by this pre-screening, and in 2015 nine captured queens had *C. bombi*; these bees were released back to their collection sites.

Discussion:

In this study, we show that DRA and DRM designed for wildlife populations, and the resultant collection, transport and quarantine procedures, can be successfully applied to an invertebrate reintroduction to significantly reduce the risk of introducing potentially novel parasites at the same time as the target organism.

The role of parasites within reintroductions is a contentious issue, with arguments for both the explicit removal of parasites from reintroduction populations and the deliberate retention of these parasites (Gompper and Williams, 1998; Pizzi, 2009; IUCN SSC, 2013; Jørgensen, 2015). Given the diversity of host-parasite interactions, and the range of biology being dealt with in reintroductions, it is exceedingly unlikely that there is a one-size-fits-all solution to this issue. *Bombus subterraneus* shares its parasites and pathogens with congeners (Schmid-Hempel, 1998), and with respect to its viruses, a broader range of insects (Fürst et al., 2014; McMahon et al., 2015). Given that the reintroduction was to take place over a geographical barrier (the Baltic and North Seas, and the English Channel), and that previous studies in a gut trypanosome parasite of bumblebees had demonstrated higher virulence in allopatric

infections (Imhoof and Schmid-Hempel, 1998), the DRM took the stance that parasites should be eliminated, as far as possible, from the reintroduction population. The aim was to reduce parasite impact on UK native populations of bumblebees and managed honeybees in the destination environment, with the concomitant assumption that re-introduced bees would rapidly pick up the local parasite assemblage (Jones and Brown, 2014). The DRM was devised on the basis of the known biology of parasites that were found during pre-screening of the source population, as well as the broader group of bumblebee parasites and pathogens predicted to be present in Swedish *B subterraneus*. Results from the DRM clearly demonstrated the success of this approach, with five parasites (*Apicystis bombi*, *C. bombi*, *N. bombi*, *S. bombi*, *Syntretus* sp.) and one virus (BQCV) being prevented from entering the reintroduction site. However, given that viral screening can only be undertaken on bees post mortem, it remains unclear whether viruses from Sweden (potentially novel strains to UK bees) were introduced with queens. Based on the upper 95% confidence limits (CL) calculated after viral screening, we would expect that fewer than five queens released in 2012 carried viruses, with fewer than four in 2013 and approximately three in 2014 (the lower 95% CL would predict no viral co-introduction). Given the absence of viruses in the Swedish queens captured in 2011 for initial parasite-screening, it was concluded that the disease risk from introducing novel viral strains was sufficiently low such that future reintroduction of queens was a reasonable management decision. Our results largely confirm this decision.

Bumblebees are, perhaps, unusual amongst invertebrates in the degree to which their parasite fauna is known (Schmid-Hempel, 1998). While devising disease risk management for species where the parasite fauna is less well-known may pose a different problem, our results show that such a DRM plan should not be based on a single-year's screening of the source population. Our pre-screening of the source population failed to discover three parasites and

one virus. It might have been possible to have detected these if a larger sample of Swedish bumblebees had been tested before reintroduction, but this was impossible due to the need to minimize any impact on the source population. Such constraints are likely to be normal for most reintroduction projects. Given the dynamics in host-parasite populations, and the dramatic changes in prevalence these can cause (Schmid-Hempel, 2011), our pre-screening results are not surprising, but may not be immediately obvious to conservation biologists who have not previously worked on parasites or pathogens. It is also possible that parasites are left undiscovered due to screening errors (false negatives). In addition, the timing of collection of queens for pre-screening was based on best knowledge at the time. It resulted in collection at a later point in the post-hibernation emergence period than subsequent collections for reintroduction, and included collection of queens carrying pollen (indicative of colony-founding behaviour), which may have led to a biased assessment of the parasite fauna (e.g. queens infected by the parasitoid *Syntretus* are highly unlikely to start founding a colony and *C. bombi* is known to reduce colony founding success; Brown et al., 2003; Rutrecht and Brown, 2008). Of the missed parasites, one was the highly virulent microsporidian *Nosema bombi* (Rutrecht and Brown, 2009), whilst the other, the braconid wasp *Syntretus* sp., wiped out between 10 and 27% of the potential reintroduction population each year between 2012 and 2015. Introduction of either of these to the reintroduction site has the potential for significant impacts on native bees, and thus designing disease risk management on the basis of the complete DRA, rather than pre-screening alone, was essential to maximise the effectiveness of this process. This result argues both for (i) extensive multi-year screening of potential source and destination populations for parasites across a broad range of potential hosts, (combining DNA-barcoding with next-generation sequencing can give powerful insights into parasites and pathogens (Cox-Foster et al., 2007)), particularly when parasite

faunas are little known, and (ii) the use of the precautionary principle in quarantine and DRM design.

In this reintroduction project, quarantine screening was explicitly integrated into informing the living nature of the DRA and DRM. Discovery of novel parasites (e.g. BQCV) and observations of high, albeit variable prevalence, in micro-parasites led directly to revisions of both the DRA and DRM. In the case of the DRM, the introduction of pre-transport screening (in 2014) resulted in the re-release in the native environment of *C. bombi*-infected queens that would have otherwise been lethally excluded during quarantine in 2014 and 2015. All such losses unnecessarily impact on the source population. In addition, the removal of infected queens from the source population could disrupt host-parasite population dynamics, and so the retention (that is, re-release) of these queens might have minimized such disruptions. Interestingly, levels of this parasite were particularly high in 2015, perhaps due to the poor weather (2015 was the coldest May in 150 years; Goran Holmstrom, pers. comm.) limiting forage and resulting in hotspots for parasite transmission (e.g. Ruiz-Gonzalez et al., 2012). Introducing pre-transport screening minimized the impact of these dynamics on the success of the reintroduction process.

Conclusion:

In conclusion, the application of both DRA and DRM to the *B. subterraneus* reintroduction project demonstrates that IUCN guidelines to incorporate parasites, both their threat and promise, into reintroductions can be successful for invertebrate species. Our results suggest that extensive pre-screening of potential source populations, taking advantage of modern molecular techniques, is necessary for designing adequate disease risk management procedures in species where there is a paucity of information on parasite fauna. In addition,

natural variability in host-parasite population dynamics needs to be explicitly integrated into disease risk management designs.

All applicable institutional and national guidelines for the care and use of animals were followed.

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Table 1. Infectious agents identified as hazards in the risk analysis for the reintroduction of the short-haired bumblebee, *B. subterraneus* (adapted from Vaughan-Higgins et al., 2012a)

Name of Hazard	Non-native to UK	Found in Sweden	Strain differences considered?	Type of hazard	Risk category
Acute Bee Paralysis Virus (ABPV)	No	Yes	Potentially	Source Destination	Low Medium
Apicystis bombi (Neogregarine)	No	Yes	Potentially	Source Destination	Low Medium
Beauveria bassiana (Fungus)	No	Yes	Potentially	Source Destination	Low Low
Crithidia bombi (Trypanosome)	No	Yes	Potentially	Source Destination	Medium High
Deformed Wing Virus (DWV)	No	Yes	Potentially	Source Destination Carrier	Medium Medium Medium

Locustacarus buchneri (Tracheal mite)	No	Yes	Potentially	Source Destination	Low Low
Nosema bombi (Microsporidian)	No	Yes	Potentially	Source Destination	Low Low
Metarhizium anisopliae (Fungus)	No	Yes	Potentially	Source Destination	Low Low
Paecilomyces farinosus (Fungus)	No	Yes	Potentially	Source Destination	Low Low
Verticillium lecanii (Fungus)	No	Yes	Potentially	Source Destination	Low Low
Sphaerularia bombi (Nematode)	No	Yes	Potentially	Source Destination	Medium Medium
Kashmir Bee Virus (KBV)	Yes (in *Apis mellifera*)	No	Potentially	Destination	Low
Melittobia acasta	No	Yes	Potentially	Destination	Medium

(Chalcidoid wasp)					
Aspergillus candidus (Fungus)	No	Yes	-	Transport	Low
Paenibacillus larvae (Bacterium)	No	Yes	Potentially	Source Destination	Low Low

Table 2. Parasite prevalence (with 95% confidence intervals) for *B. subterraneus* queens collected in Sweden for reintroduction to the UK and tested in quarantine.

	2012 (N = 89)	2013 (N = 100)	2014 (N = 100)	2015 (N = 67)
Apicystis bombi	0 (0-0.041)	0.04 (0.011-0.100)	0 (0-0.036)	0.04 (0.001-0.125)
Crithidia bombi	0.11 (0.005-0.197)	0.21 (0.135-0.303)	0.10 (0.049-0.176)	0.36 (0.245-0.485)
Nosema bombi	0.01 (0.003-0.061)	0.02 (0.002-0.070)	0.02 (0.002-0.070)	0 (0-0.054)
Sphaerularia bombi	0 (0-0.041)	0.02 (0.002-0.070)	0 (0-0.036)	0 (0-0.054)
Syntretus sp.	0.16 (0.089-0.250)	0.15 (0.087-0.235)	0.27 (0.186-0.368)	0.09 (0.034-0.185)

Table 3. Viral prevalence (with 95% confidence intervals) for *B. subterraneus* queens that died or were sacrificed during quarantine in the UK.

	2012 (N = 39)	2013 (N = 50)	2014 (N = 53)	2015 (N = 42)
Acute Bee Paralysis Virus (ABPV)	0 (0-0.090)	0 (0-0.071)	0 (0-0.067)	0 (0-0.084)
Black Queen Cell Virus (BQCV)	0 (0-0.090)	0.02 (0.001-0.107)	0 (0-0.067)	0 (0-0.084)
Deformed Wing Virus (DWV)	0 (0-0.090)	0 (0-0.071)	0 (0-0.067)	0 (0-0.084)
Israeli Acute Bee Paralysis Virus (IAPV)	0 (0-0.090)	0 (0-0.071)	0 (0-0.067)	0 (0-0.084)
Kashmir Bee Virus (KBV)	0 (0-0.090)	0 (0-0.071)	0 (0-0.067)	0 (0-0.084)
Sacbrood Virus (SBV)	0 (0-0.090)	0 (0-0.071)	0 (0-0.067)	0 (0-0.084)

List of figures:

Figure 1. A map of southern Sweden including both the sites of collection of *B. subterraneus* queens for parasite pre-screening (see text), shown by white and black dots, and the two transects that were subsequently developed for capturing queens for reintroduction to Dungeness (Kent), shown by grey lines.

Figure 2. *B. subterraneus* queens in quarantine.

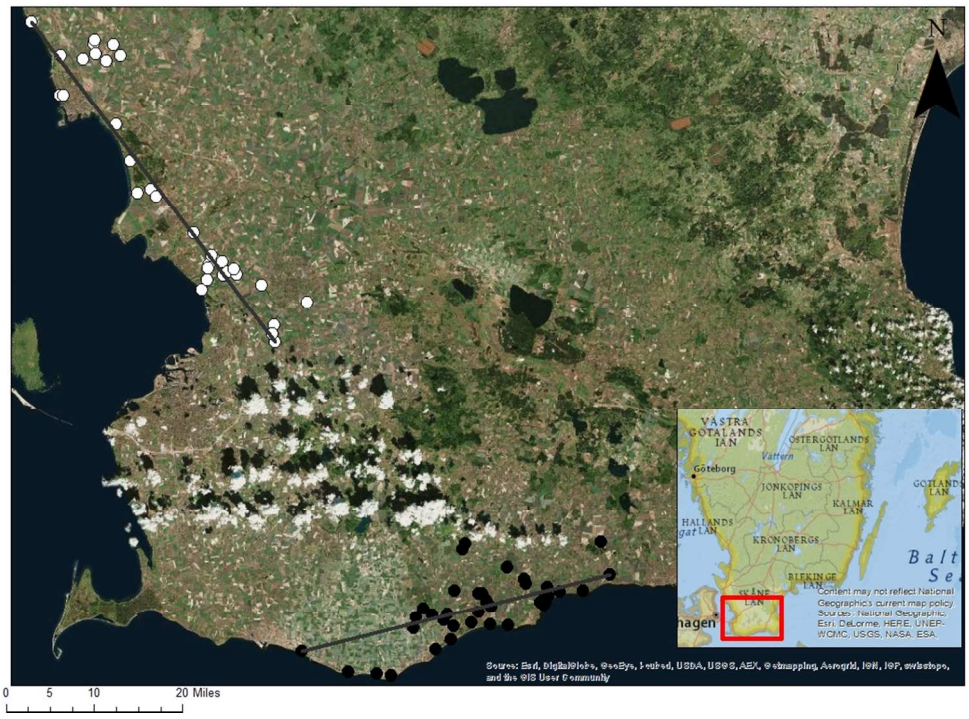

Figure 1. A map of southern Sweden including both the sites of collection of B. subterraneus queens for parasite pre-screening (see text), shown by white and black dots, and the two transects that were subsequently developed for capturing queens for reintroduction to Dungeness (Kent), shown by grey lines.
296x210mm (96 x 96 DPI)

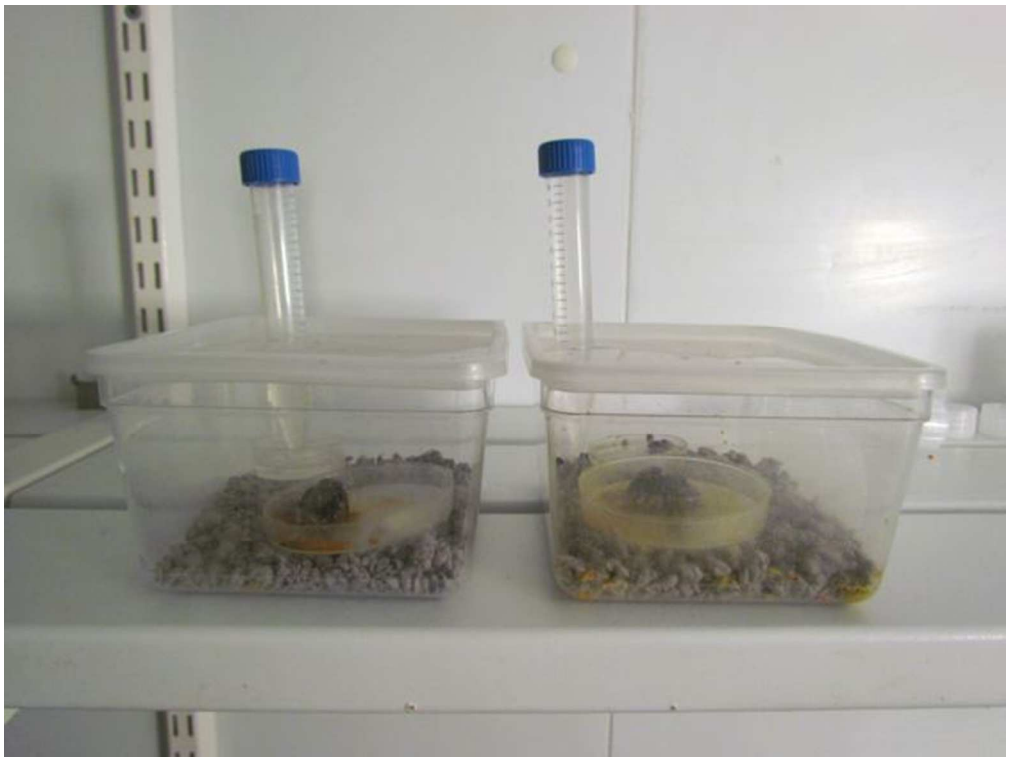

Figure 2. B. subterraneus queens in quarantine
90x67mm (180 x 180 DPI)