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Resident viruses, but not honeybee-associated viruses, impair solitary bee fitness in the field

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Viruses can impact individual host fitness and host population dynamics, especially following host shifts. Thus, the decline of wild solitary bee populations over the last few decades may be linked to viruses or other pathogens. However, evidence for the impact of viruses—transmitted from other genera or resident in solitary bees—on their fitness remains scarce. Here, by assessing solitary bee (*Osmia cornuta*) foraging, offspring sex ratio, survival and body mass across seven locations in northern Switzerland, we show that resident viruses—but not honeybee-associated viruses—can impact fitness proxies in the field. Loads of *Osmia*-resident viruses (Ganda bee virus—GABV; Scaldis River bee virus—SRBV) and honeybee-associated viruses (black queen cell virus—BQCV; deformed wing virus B—DWV-B) were quantified in foraging females. Prevalence and loads of GABV and SRBV were higher than BQCV and DWV-B. Females with high SRBV or GABV loads had reduced offspring survival or lower male offspring body mass, respectively. Honeybee-associated viruses had no impact on *O. cornuta* fitness proxies. We demonstrate that viruses can affect solitary bee fitness negatively, but the degree of impact varies with viral species and provenance. Further research is needed to unravel the dynamics of multi-host pathogens in pollinator communities.

1. Background

Multiple, interacting stressors are driving observed wild bee declines [1–3] and are jeopardizing the important pollination services they provide [4,5]. Among these stressors, pathogens are suspected to affect the fitness and population dynamics of solitary bee pollinators [6,7]. Pathogen transmission from managed bee colonies to wild pollinator species or virus sharing between genera appear to be widespread [6–8]. However, the effects of transmission and potential emergence of novel pathogens in solitary wild pollinator species remain poorly understood [9]. Particularly, the relative impacts of pathogens—resident in a host species or transmitted from a phylogenetically more distant host—on host fitness remain unknown and

should be quantified to deepen our understanding of pathogen risks to wild pollinator species.

Viruses and other microorganisms may contribute to a healthy multi-host community, for example, by decreasing the abundance of dominant pollinators and thus reducing competition, as observed in parasitoid leaf-miner communities [10], and pointed out by Brown [11]. However, virus infections often have fitness-related costs for the individual host [12,13], particularly by affecting their fertility [14]. These costs likely emerge due to a reproduction–immunity trade-off [15], where energetic resources allocated to activate an immune system lead to a reduction of resources invested in reproduction (or vice versa). Such a trade-off has been observed in a variety of female insects, including Hymenoptera [15]. However, offspring could also be directly negatively affected by pathogens [16], which would also decrease the fitness of the parents.

A wide range of viruses challenge the immune system of wild pollinators and managed honeybees (*Apis mellifera*) [8,16–19]. Deformed wing virus (DWV) and black queen cell virus (BQCV) are among the most prevalent and widespread viruses in honeybees across Europe [20,21] and infections can result for example in larval death or decreased life span of adult honeybees [16,20]. There is increasing evidence for transmission of these honeybee-associated viruses from honeybees to wild pollinators [7,17,22,23], likely through shared use of floral resources [24,25]. Findings of replicating viruses in solitary bees [17,26,27], and high viral titres even in spiders [28], suggest a wider host range than the honeybee alone. In addition to the honeybee-associated viruses, there are viruses that are likely resident in wild pollinator species, such as Ganda bee virus (GABV) and Scaldis River bee virus (SRBV), so far only detected in the solitary bee *Osmia cornuta* [18,19]. It is, however, unknown whether these 'resident' or the honeybee-associated viruses have negative consequences on the fitness of *O. cornuta* (or other species). Honeybee-associated viruses have often been found in experiments to negatively affect survival of bumblebees, which are taxonomically closely related to honeybees [7,29] (but see [30]). As an example for a potential reproduction–immunity trade-off, bumblebee colonies experimentally treated with honeybee-associated viruses produced fewer drones [31]. Whereas, survival of two solitary bee species was unaffected by a mixture of common viruses harmful to honeybees [32], and DWV did not replicate in the solitary bee *Osmia bicornis* [33] although replicating DWV was found in the abdomen of *O. cornuta* [26]. Hitherto the effects of viruses on pollinator health and fitness have mostly been quantified in laboratory studies [34] (but see [9,35]), revealing a need for a more realistic assessment of risks under field conditions. So far, these laboratory studies and field studies with bumblebees have yielded inconsistent results, e.g. on mortality [9] or colony size [35], and knowledge on sublethal effects of viruses on fitness components of solitary bee pollinators under natural conditions is still limited [36].

The majority of bee species are solitary [37]. To fight infection, solitary species depend solely on their individual immune response, whereas social species have various mechanisms of social immunity (e.g. allogrooming, corpse removal; reviewed by [38,39]). Furthermore, females of solitary bee species individually provide pollen to their own offspring, in contrast to bumblebees and other social bees, in which poor performance of individual workers can be compensated by others in the colony. The reproductive success of solitary bee females is thus directly dependent on the amount and quality of the pollen-nectar provided as food for developing offspring [40,41]. Therefore, lower investment in offspring provisioning due to energetic costs of a pathogen infection could directly translate into lower reproductive success of solitary bees (i.e. reproduction–immunity trade-off [15]), particularly because solitary species lack the potential buffering 'superorganism resilience' of eusocial bees that build large colonies [42]. Virus infections may thus have significant consequences for the reproductive performance and fitness of solitary bees. In addition, a pathogen may be transmitted vertically from infected female to eggs/larvae or horizontally to offspring through contaminated pollen or nectar provided to brood, as occurs in social honeybees [43,44] and solitary carpenter bees [45]. Whether viruses are present in larvae, pupae or adults of other solitary bee species before emergence remains largely unexplored [46].

Here, we investigated the impacts of variation in natural viral loads on the reproductive success of females of the solitary bee *O. cornuta* (Latreille) across seven agricultural sites. *Osmia cornuta* is a relatively common cavity-nesting species native to wide parts of Europe, where it is an important pollinator of fruit crops such as apple, cherry and almond [47–49]. We established experimental populations of nesting *O. cornuta* females in apple orchards to expose them to the natural pathogen community to measure the resident *Osmia*-associated viruses (GABV; SRBV) [18,19] and the honeybee (*A. mellifera*)-associated viruses (BQCV; DWV-B). Using video analysis with machine learning of individually tagged nesting females [50], we tracked their nesting activity, foraging performance and reproductive success. We related variation in these fitness components to loads of *Osmia*-associated (resident) and honeybee-associated viral loads in nesting females to address the following questions: (i) Do high loads of resident compared to honeybee-associated viruses in nesting females of *O. cornuta* have consequences on their foraging performance and reproductive success in terms of offspring survival, sex ratio and body mass? (ii) Are these viruses acquired by nesting female bees during foraging in the environment after emergence or are they already present in bees before emergence?

2. Methods

(a) Study design

We selected eight study sites (apple orchards) in agricultural landscapes characterized by a mosaic of crops (including arable crops and fruit orchards), grasslands and semi-natural habitat (SNH) remnants, such as forest patches, in northeastern Switzerland (cantons of Zürich, Thurgau and St Gallen; electronic supplementary material, figure S1; details about landscape composition in electronic supplementary material, table S1). Apple orchards were selected because this crop has been shown to be a highly suitable floral resource for *O. cornuta* in the study region and foraging females have high fidelity for apple pollen when nesting in apple orchards (>80% of collected pollen stemming from apple [51–54]). This set-up aimed to ensure:

(i) local availability of ample floral resources to minimize variability in foraging distances that affect foraging and offspring provisioning performance [51,55]; (ii) minimize significant variation in pollen composition supplied to offspring that could affect offspring performance [55,56]; and (iii) the presence of honeybees introduced for honey production/pollination services and thus the potential for honeybee-associated virus transmission. Apple orchards were separated from each other by at least 3 km to ensure independence of samples according to the foraging range of *O. cornuta* [57]. At a non-shaded, southeast-facing border of each selected orchard, we installed two trap nests (bee hotels) at 1.2 m above ground level, protected against rain with a wooden roof (electronic supplementary material, figure S2A). Trap nests consisted of wooden MDF (medium-density fibreboard) plates with drilled semicircular nesting cavities of 7, 8 and 10 mm in diameter (104, 120 and 18 cavities, respectively, per orchard site, summing up to n = 242 cavities per site). Each MDF layer was covered with a transparent plastic foil, facilitating tracking of nesting progress of *O. cornuta* and offspring production and performance (see below).

At the onset of apple bloom in spring 2022 (8 April 2022), 340 commercially sourced cocoons of *O. cornuta* (Wildbiene+Partner AG, Switzerland) were released at each site from small open boxes that were fixed below the roofs covering the trap nests (female to male ratio approximately 1 : 1.5, 47, cocoons with >12 mm length and >7 mm width were classified as females [52]). Eight days after the initial release, we confirmed that the female bees had started nesting, well synchronized with apple bloom.

To assess the density of honeybees at the trap nest locations, we captured flower-visiting honeybees along a 400 m transect within 100 m around the trap nest (four times 100 m transect in four directions from the nests, 20 min searching time in total).

(b) Foraging performance and offspring production of nesting females

To track individual *O. cornuta* female nesting activity, foraging performance and different aspects of reproductive success, we individually tagged females, videoed their foraging activity at their individual nests and used machine learning-based software for automated analysis of the videos ('Bee Tracker' [50]). We first marked 15 nesting females per site (except for one site where only seven females could be marked) by fixing unique tags used to mark honeybee queens ('Opalithplättchen', Imkereibedarf Wespi, Switzerland) with glue that is also used for marking honeybee queens (product 'Ersatzleim'; Imkereibedarf Wespi) on the thoraces of the bees (electronic supplementary material, figure S2B,C). The tagging was performed over two nights, 10–11 days after the initial release of the cocooned bees at orchard sites. Since the nights at this time of the year were still very cold (around 5°C), bees were naturally almost unable to move and therefore easy to handle for tagging. Filming started 1–2 days later to avoid any potential confounding effects on bees arising from handling/tagging. At each site, two video cameras (Canon LEGRIA HF G50 4K Camcorder) were placed at a distance of 1 m in front of each of the two trap nests (electronic supplementary material, figure S2A) to continuously record (2–3.5 h d^{-1}, between 11.00 and 16.00) the nesting and foraging activity of female bees on 4 sunny days (within an 8-day window) during peak nesting activity. We recorded the videos within this rather short 8-day window to reduce the chance of the tag becoming unfixed or of the bee dying. The machine learning 'Bee Tracker' software was used to analyse the videos [50] and to identify and track individually tagged females as a prerequisite to assess nesting activity, measures of female reproductive success and foraging trip duration. A precise time stamp of each individual entering or leaving its specific nest and identifying it according to its vertical and horizontal position in the trap nest allowed the software to identify the nest of each individual bee at high accuracy (96% of correctly identified bees, nests and events such as leaving or entering [50]). Outliers comprising trips <56 s or >36 min (representing the lowest and highest 3% of the data) were trimmed from the dataset to exclude reading errors and other types of activity other than foraging for brood cell provisions (i.e. short mud collection trips or long resting periods outside nests [50,58,59]). We could recover between 27% and 40% of the tagged bees in the videos (i.e. 4–6 out of 15 tagged bees or 2 out of 7 tagged bees at one site, respectively; mean ± s.e.: 32 ± 4.6%).

After video recording at all sites was completed (12 days after the confirmed start of nesting), we collected all tagged females at night when the females were resting in their nests. The bees were freeze-killed by placing in dry ice directly in the field before storage at −80°C in the laboratory. A single site was excluded from further analyses because all female nesting bees had disappeared shortly before the collection date due to unknown reasons and despite previously observed nesting activity. Approximately one month after the start of nesting, we covered all trap nests with a fine mesh (polyamide, mesh size 195 µm) to prevent interference from natural enemies and transferred them to a sheltered outdoor overwintering site (Agroscope Reckenholz, Zürich, Switzerland) to let the offspring complete metamorphosis and overwinter until hatching in early spring of the subsequent year (2023).

(c) Survival, sex ratio and body mass of offspring

At the end of January 2023, trap nests were transferred to a cold room (2–3°C) to control and synchronize the time of hatching. Each offspring (cocoon) was transferred into a separately labelled 2 ml Eppendorf® tube with a small hole to allow air exchange. On 6 March 2023, cocoons were placed at room temperature, and we recorded whether or not offspring successfully hatched (survival) and determined their sex morphologically using Westrich [60]. If an offspring had not hatched after 17 days at room temperature, it was considered not viable. After hatching, offspring were transferred to −80°C. The body mass of frozen hatched offspring was measured (Mettler AE163, 0.02 mg precision) after thawing. In total, 173 offspring (cocoons) were produced by tagged females: 45 females and 128 males offspring from n = 31 nests (see electronic supplementary material, table S1, for n per site).

(d) Virus quantification

All tagged females that could be re-captured (n = 48, 65.6% of all originally tagged females) were screened individually for four viruses. These viruses have been mainly detected in *O. cornuta* and *O. bicornis* (resident GABV and SRBV), hereafter *Osmia*-viruses for simplicity [18,19], and viruses originally detected in *A. mellifera* (DWV-B and BQCV), hereafter honeybee viruses. Additionally, eight females from cocoons that were not released to the field but stemming from the same batch of cocoons (i.e. generation F0) as the released bees were frozen in their cocoons, then extracted under sterile conditions and screened for the same four viruses to check for infection in the source stock.

The body mass of all females (tagged and frozen cocoons from the same generation) was measured (without the tag) prior to RNA extraction and transferred into 2 ml Eppendorf® tubes. PBS buffer (1 µl/0.5 mg tissue) and a 5 mm glass bead were added to each tube and individuals were shredded using a Retsch® (Haan, Germany) MM 300 mixer mill for 1 min at a frequency of 25 s^{-1} [61]. RNA extraction was performed using a NucleoSpin® RNA II kit (Macherey-Nagel, Oensingen, Switzerland) with 50 µl (25 mg tissue) of the homogenate, following the manufacturer's recommendations. We eluted RNA in 60 µl elution buffer and stored it at −80°C [61].

For RNA reverse transcription, we used an M-MLV RT Kit (Promega, Dübendorf, Switzerland) and followed the manufacturer's recommendations. First, 2.5 µg of template RNA was incubated in a Thermocycler (Biometra, Analytik Jena, Jena, Germany) for 5 min at 70°C together with 0.75 µl of a random hexamer oligonucleotide (100 µM) and water. To synthesize cDNA, 5 µl of 5× buffer, 1.125 µl dNTPs (10 mM) and 1 µl reverse transcriptase (M-MLV) were added and incubated at 37°C for 60 min.

To quantify the number of virus genome equivalents (GE) (i.e. viral copies), we used quantitative PCR (RT-qPCR) in a CFX96TM Real-Time PCR Detection System (BioRad©, CA, USA) (conditions: 3 min for 95°C, 40 cycles of 3 s at 95°C and 30 s at 57°C). qPCR reactions consisted of 6 µl of 2× reaction buffer (SensiFAST™ SYBR® No-ROX Kit, Meridian Bioscience, London, UK), 0.24 µl forward and reverse primer, 2.52 µl water and 3 µl of 10-fold diluted cDNA (see electronic supplementary material, table S3, for primer sequences). To verify the PCR product specificity, we analysed the melting curve of the strand dissociation by reading the fluorescence at 0.5°C increments per second from 55 to 95°C and considered the sample as positive if the curve was smooth in shape and with the melting temperature (T_m) of the respective viral PCR product. All samples were run in duplicate, and each qPCR plate contained positive (PCR product of a positive sample) and negative (ddH_2O) control wells. The negative controls consisted of 'blanks' as control of the RNA extraction and 'NTC' (non-template control) as control of the qPCR steps. Purified PCR products of known concentration (from 10^{-2} to 10^{-9} ng) were freshly prepared and run on qPCR plates as standard curves. GE absolute quantification was calculated based on those standard curves. The regression equations were used to calculate the absolute amounts of viral genome copies per PCR reaction, i.e. the starting quantity (SQ). PCR efficiencies (E) were calculated as follows: $E = 10(-1/\text{slope})$ (DWV-B: E = 1.90, slope = −3.58, y-intercept = 36.07, R^2 = 0.99, copies/qPCR dynamic range of standard curve: 7.17–7.17 × 10^7; BQCV: E = 1.97, slope = −3.39, y-intercept = 35.22, R^2 = 0.99, copies/qPCR range: 3.37–3.37 × 10^7; GABV: E = 1.95, slope = −3.44, y-intercept = 35.55, R^2 = 0.98, copies/qPCR range: 6.43–6.43 × 10^7; and SRBV: E = 1.99, slope = −3.34, y-intercept = 36.30, R^2 = 0.99, copies/qPCR range: 3.96–3.96 × 10^7). Results were expressed as viral GE per microgam of total RNA, calculated by considering dilutions from RNA extraction through cDNA synthesis and qPCR, as follows: viral load = SQ × 10 × 25 × [RNA conc.]/2.5. pPCR amplification of the 28S rRNA gene from the suborder Apocrita [62] was used as reference gene to assess the quality of the RNA extraction, cDNA synthesis and qPCR. The quantification threshold was set in the Bio-Rad CFX MAESTRO v. 1.0 software as auto calculated for all runs per target, assuring it always set in the exponential phase of the amplification curve [63]. A qPCR quantification cycle (Cq) threshold of Cq < 35 was set as cut-off value to define a positive sample [64].

(e) Statistical analyses

Due to low numbers of DWV-B copies in *O. cornuta* females (table 1, figure 1), we focused on the impact of GABV, SRBV and BQCV on the reproductive performance of *O. cornuta* nesting females. We used the raw numbers of GABV, SRBV and BQCV copies per microgram of RNA as explanatory variables in statistical models. We did not detect a correlation among the loads of the different viruses per bee (electronic supplementary material, figure S3).

To investigate the effect of viral loads of GABV, SRBV and BQCV in nesting *O. cornuta* females on foraging performance and different aspects of their reproductive success as response variables, we used (generalized) linear mixed-effects models (G)LMMs with site ID as a random factor. LMMs were fitted for mean female (mother) foraging trip duration and body mass of female and male offspring as response variables and GLMMs with binomial error distribution for offspring survival (yes or no) and sex ratio (i.e. proportion of female offspring) as response variables. In each model, we included GABV, SRBV and BQCV load of *O. cornuta* mothers as explanatory variables (z-standardized prior to model fitting). Body mass of the mothers was included as an explanatory covariate in the models to control for the strong effect that body mass can have on reproductive success of *Osmia* females [41,65]. Cover of SNH (extensively managed meadows and pastures, flower strips, hedgerows, forest) in a 500 m radius buffer around each focal orchard site was also included as explanatory variable in the models to account for possible confounding influence of the surrounding landscape on the investigated variables (although minimized by our study design, see above). For each of these five full models, we performed stepwise backwards model selection [66] until only near-significant variables were left in the model ($p < 0.1$).

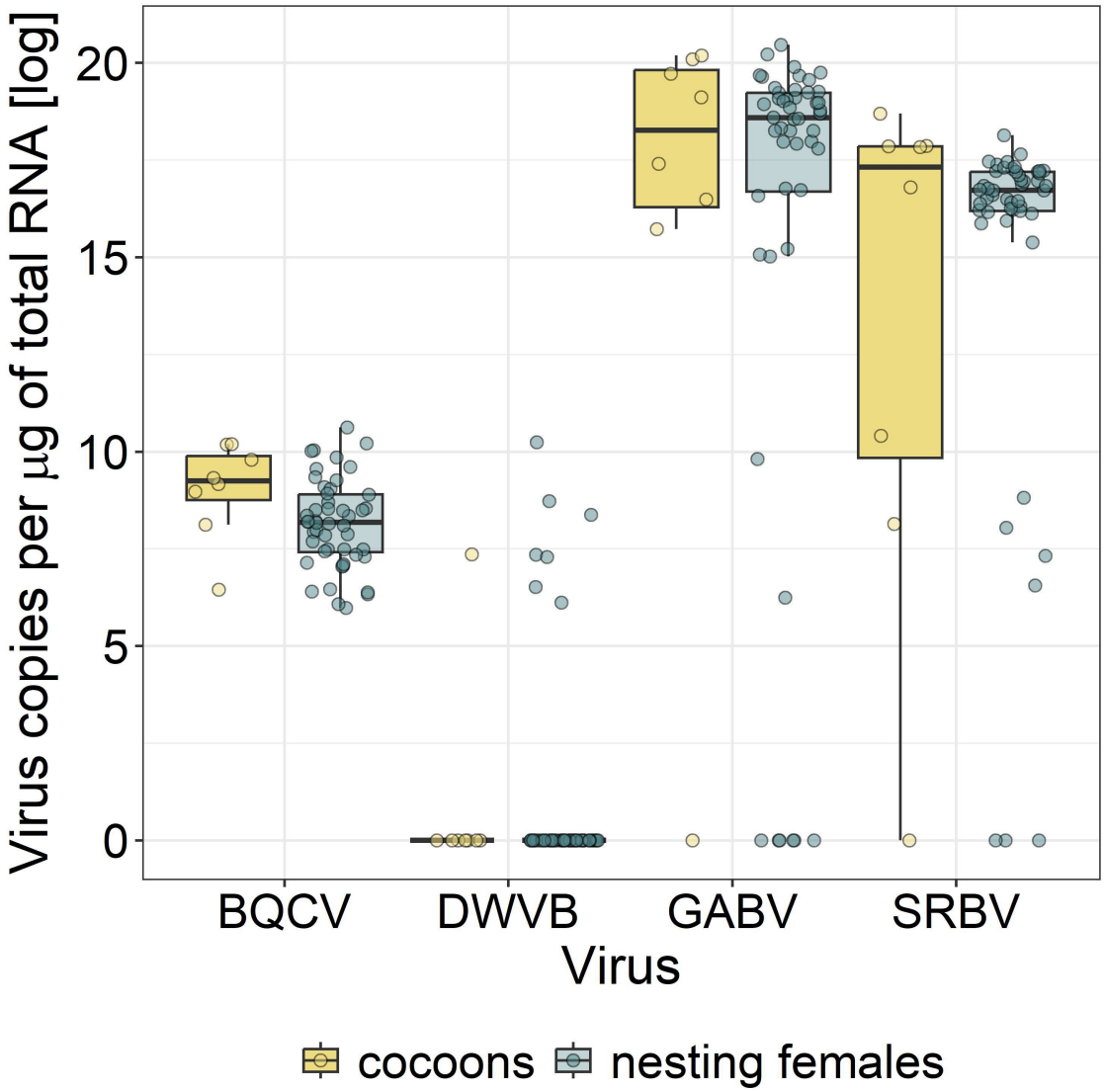

Figure 1. Boxplot of the number of GE (virus copies) per µg of total RNA per cocoon (same generation as nesting females, yellow) or per nesting female *Osmia cornuta* (blue) (log-transformed) for each of the four analysed viruses: the honeybee-associated viruses BQCV and DWV-B; and the resident *Osmia*-viruses GABV and SRBV. Circles show raw data points. BQCV, black queen cell virus; DWV-B, deformed wing virus B; GABV, Ganda bee virus; SRBV, Scaldis River bee virus.

Table 1. Median viral loads (number of GE per µg of total RNA per extract) and the range (min. and max.), as well as the number and percentage of nesting females ($n = 48$) and female cocoons ($n = 8$, same generation as nesting females) screened positively for the following viruses: BQCV, black queen cell virus; DWV-B, deformed wing virus B; GABV, Ganda bee virus; SRBV, Scaldis River bee virus.

virus	sample type	no. positive	median GE per µg of total RNA	range GE per µg of total RNA
BQCV	nesting females	48 (100%)	3.58×10^3	3.95×10^2–4.11×10^4
	cocoons	8 (100%)	1.04×10^4	6.29×10^2–2.66×10^4
DWV-B	nesting females	7 (14.6%)	0	0–2.80×10^4
	cocoons	1 (12.5%)	0	0–1.56×10^3
GABV	nesting females	42 (87.5%)	1.18×10^8	0–7.69×10^8
	cocoons	7 (87.5%)	1.19×10^8	0–5.85×10^8
SRBV	nesting females	45 (93.8%)	1.83×10^7	0–7.55×10^7
	cocoons	7 (87.5%)	3.77×10^7	0–1.31×10^8

To explore whether the local density of honeybees had an effect on loads of the honeybee virus BQCV in *Osmia* females (potentially through spillover), we related BQCV loads (log) to the mean number of honeybees counted from the four transects at a site (LMM with site ID as random factor) in an additional model.

All statistical analyses were performed in the software R v. 4.2.1 [67] and the R package *lme4* [68] was used for model fitting. All explanatory variables were scaled prior to model fitting, and there was no strong collinearity among explanatory variables in all models (VIF < 1.7 [9,69]). Inspection of residual plots confirmed no violation of model assumptions (i.e. residuals were normally distributed with homogeneity of variance) and an overdispersion test showed no overdispersion in GLMMs (R

package DHARMa [70]). Additionally, the data were inspected for normality with the Shapiro–Wilk test; where the data were not normally distributed ($p < 0.05$), we report the median and interquartile range instead of the mean and standard deviation in the descriptive statistics.

3. Results

The resident *Osmia*-viruses GABV and SRBV were highly prevalent in *O. cornuta* nesting females across all orchards as well as in (control) cocoons of the same generation not exposed to field communities, with up to 10^8 virus genome copies per microgram of total RNA (table 1, figure 1). Of the honeybee-associated viruses, DWV-B was only prevalent in 15% of all nesting females and in one out of eight (13%) screened cocoons, with a low virus load of maximally 10^4 genome copies per microgram of total RNA. BQCV was detected in all nesting females and cocoons, but also with a lower load than the *Osmia*-viruses (maximally 10^4 genome copies per microgram of total RNA) (table 1, figure 1). Viral loads of resident viruses were significantly higher than viral loads of honeybee-associated viruses (Wilcoxon signed rank test, $v = 3$, $p < 0.001$). Mean number of honeybees at a site did not affect BQCV loads in *Osmia* females (LMM: d.f. = 3, $t = -0.84$, $p = 0.465$).

(a) Foraging trip duration, offspring survival and sex ratio

The mean duration (± s.d.) of a foraging trip by a nesting female was 9.4 ± 4.7 min (range: 56 s–35 min; see electronic supplementary material, table S4, for details on foraging trip duration), but was not related to GABV, SRBV or BQCV load, body mass of the mother or SNH cover (i.e. the best model after backwards selection was the null model). Survival of offspring per nest ranged from 0% to 100% (median: 100%, interquartile range: 86.6–100%). Offspring survival was negatively related to the SRBV load of mothers (table 2, figure 2). On average, the offspring sex ratio was male biased, with a median proportion of female offspring of 20% (range: 0–100%, interquartile range: 10.6–42.9%) per nest. The proportion of female offspring was not related to GABV, SRBV or BQCV load of mothers (table 2), but was positively related to the body mass of mothers (table 2; electronic supplementary material, figure S4A).

(b) Body mass of female and male offspring

The mean body mass (± s.d.) of female offspring was 126.7 ± 24.1 mg (range: 81.8–175.5 mg) and the mean body mass of male offspring was 72.7 ± 14.1 mg (range: 34.2–95.0 mg). The body mass of male offspring was negatively related to GABV load of mothers (table 2, figure 2B). The body mass of female offspring was tentatively positively related to GABV load (non-significant trend, $p = 0.073$, table 2) and positively related to body mass of mothers (table 2; electronic supplementary material, figure S4B). Together, the resident viruses SRBV and GABV in mothers were negatively related to some of our measured components of reproductive success (offspring survival and body mass), while honeybee-associated viruses did not show any significant effect on the measured fitness components of *O. cornuta* females.

4. Discussion

Here, we show for the first time that the reproductive success of a solitary bee species, *O. cornuta*, is negatively affected when females carry the resident SRBV or GABV. SRBV loads in nesting females had a negative effect on offspring survival, while GABV loads in nesting females had a negative effect on body mass of male offspring. In contrast, the honeybee-associated BQCV did not show any negative effects on our measured components of reproductive success (and DWV-B loads were so low as to be almost absent).

Resident viruses of *O. cornuta* were prevalent and present with higher loads in overwintering (hibernating) and nesting *O. cornuta* females compared to the honeybee-associated viruses. Thus, the resident viruses GABV and SRBV appear to be widespread in *O. cornuta* in our study region, as well as across four sites in Belgium [18,19]. However, compared to the known negative consequences of honeybee-associated viruses on bumblebees in laboratory studies [7,30,71], fitness-related consequences of honeybee-associated or resident viruses in solitary bees are largely unknown. Our results demonstrate that loads of resident SRBV and GABV in nesting females modulated certain components of *O. cornuta* fitness: higher SRBV loads were associated with reduced offspring survival, while higher GABV loads were associated with lower body mass of male offspring. In contrast, honeybee-associated BQCV loads in nesting females did not influence any of the examined *O. cornuta* fitness components despite BQCV being ubiquitous—albeit at low levels—in our analysed females. Viral loads (GE per bee) in honeybees collected in the same region in Switzerland were found to be from 2.9×10^2 up to 3.8×10^{11} (BQCV) and from 1.7×10^1 up to 4×10^9 (DWV-B) [24], which is more than 10 000 times higher than the maximum levels we found in *O. cornuta* (4.23×10^4 BQCV and 3.17×10^3 DWV-B GE per bee). Honeybees classified as being clinically affected by the virus showed loads of 2.14×10^7 BQCV copies and 2.34×10^6 DWV-B copies per bee [72]. These and our own results indicate that the *O. cornuta* females in our field experiment were likely unaffected by the honeybee-associated viruses BQCV and DWV-B. This is in line with findings from a laboratory study showing that solitary bee (*Megachile rotundata* and *Colletes inaequalis*) survival was not impacted by a mixture of sacbrood virus, Israeli acute paralysis virus, DWV and BQCV, while the mixture was harmful to honeybees [32]. Furthermore, field experimental studies found no impact of honeybee-associated viruses either taken up during free foraging or experimental feeding on colony growth or worker survival of *B. terrestris* [9,35]. Also, foraging trip duration

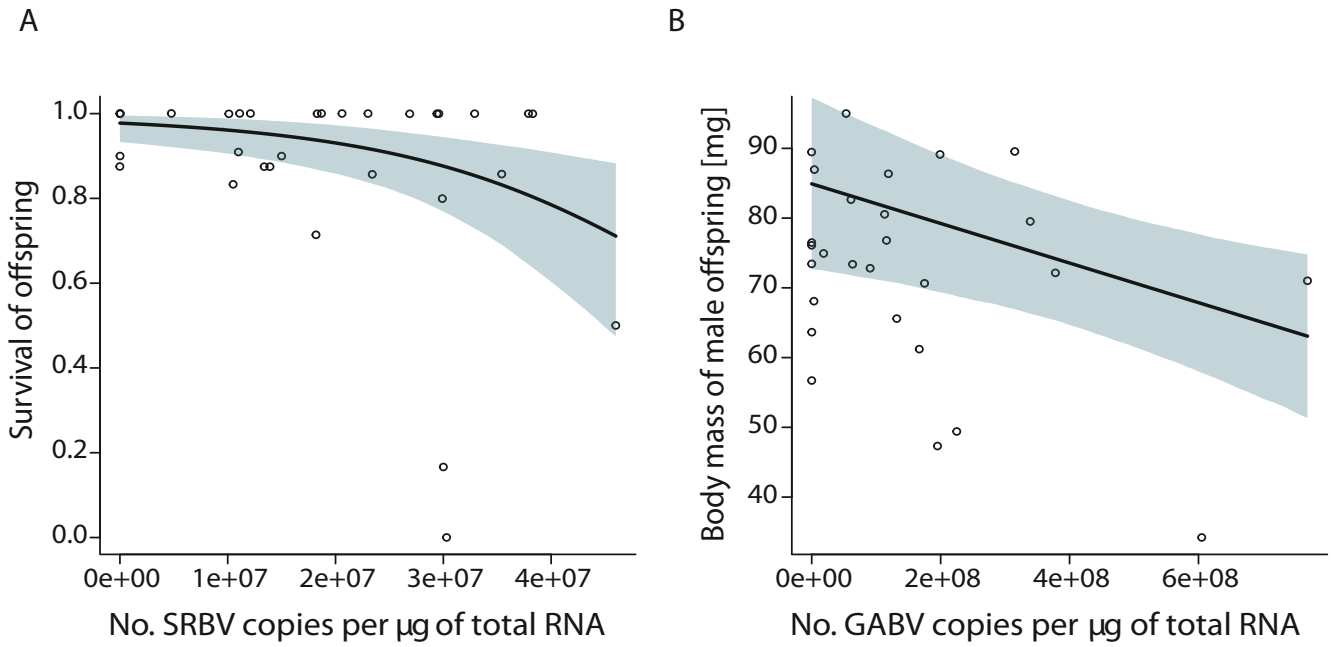

Figure 2. Relationships between the number of SRBV GE (virus copies per µg of total RNA) of nesting female *Osmia cornuta* and (A) offspring survival and (B) between GABV GE (virus copies per µg of total RNA) per nesting female and body mass of male offspring. Model predictions with 95% Bayesian credible intervals (shaded areas) are shown. Covariates in the model that were not plotted were fixed at their mean value. Circles show raw data points. SRBV, Scaldis River bee virus; GABV, Ganda bee virus.

Table 2. Results after backwards selection of models analysing the effect of mother body mass, percentage of SNH, the resident *Osmia*-viruses GABV and SRBV loads and the honeybee-associated BQCV load (number of virus copies as GE per µg of total RNA) of nesting *Osmia cornuta* females on their reproductive success (i.e. offspring survival, sex ratio and body mass). The table shows the results from likelihood ratio tests (LRT) or *F*-tests (from generalized linear mixed effects or linear mixed effects model analyses, respectively), degrees of freedom (d.f.) and *p*-values, as well as marginal and conditional *R*-squared (R^2m, R^2c).

response			LRT	*p*-value	R^2m	R^2c
sex ratio of offspring	body mass mothers (mg)		11.01	0.0009	0.34	0.49
survival of offspring	SRBV load		11.69	0.0006	0.39	0.74
		d.f.	***F*-value**	***p*-value**	**R^2m**	**R^2c**
body mass female offspring	body mass mothers (mg)	20	6.34	0.0204	0.26	0.33
	GABV load	20	3.58	0.0731		
body mass male offspring	GABV load	21	7.59	0.0117	0.16	0.54

was not affected by any of our studied variables. Thus, it appears as if honeybee-associated viruses such as BQCV, that are potentially transmitted from managed honeybees, do not cause significant harm in the field at least to adult *O. cornuta* (this study) or *B. terrestris* [9,35]. A possible reason for this might be phylogenetic distance between host species, i.e. the susceptibility of potential new hosts can vary enormously, with close relatives of the natural host typically being the most susceptible [73]. A recent study found that only a few viruses were able to replicate in multiple bee host species and host phylogenetic distance predicted virome composition [74]; it is therefore likely that BQCV may not replicate well in *O. cornuta*, similar to DWV in *O. bicornis* [33]. In contrast to these findings of limited impact of viruses, trypanosomatid and microsporidian gut pathogens (*Crithidia mellificae*, *Nosema ceranae*) known from honeybees are able to infect *Osmia* species and decrease their survival [75–77] and even affect larval development [78]. As these pathogens are mainly transmitted via the faecal–oral route, they may be like viruses easily transmitted during visits on shared flowers [79,80]. As these gut pathogens do not rely on the RNA replication machinery of the host, they may be more easily shared across species.

Altogether, our study demonstrates effects of natural infections of resident *Osmia*-viruses on solitary bee reproductive success in field conditions, but a corresponding lack of impacts of viruses likely transmitted from a more distantly related host, namely, the honeybee.

Our findings are in agreement with predictions of the reproduction–immunity trade-off hypothesis [15], although we cannot exclude other mechanisms. In solitary *Osmia* bees, the amount of food ingested as a larva largely determines cocoon mass and adult body mass [40,65,81] and likely also survival (i.e. hatching probability) of offspring. Thus, females with high viral loads might have invested less into nest provisioning due to increased energetic costs of a virus infection, leading to the observed negative relationships between SRBV loads in mothers and offspring survival or between GABV and male offspring body mass. Similar effects of viruses on reproduction were also observed in the common wasp *Vespula vulgaris*, where high loads of Kashmir bee virus were associated with smaller nest size [82]. However, *O. cornuta* females with high viral loads apparently did not shift offspring sex ratio towards less costly males to save resources, as sex ratio was only influenced by body mass

of mothers and not by their viral infection status in our study. Rather, it appears as if the reduction in a mother's investment into reproduction is relatively lower (compared to uninfected mothers) later in the season, when males are produced (thus the observed lower body mass of male offspring with higher GABV loads in mothers).

Instead of, or as well as, impacting the mother's physiology and foraging performance, and thus the amount of food provisioned per brood cell, viruses could have been transmitted either to the offspring from an infected mother (via egg to larvae) or via provisions of viral-contaminated pollen or nectar [83], and thereby affected offspring performance directly. The relatively similar viral loads of GABV and SRBV in nesting females and cocoons (of the same generation as the nesting females) indeed suggest that viruses can also be taken up by developing larvae before emergence. This may reveal an additional pathway (vertical transmission or contaminated food) by which virus infections can affect the reproductive success of females via offspring size or infection status. Developing larvae might therefore be additionally or alternatively directly exposed to viruses. Viruses in developing larvae could possibly directly negatively affect their survival, as reported for *A. mellifera* (reviewed by [16]). Through this alternative pathway, mothers with a high viral load or collecting contaminated pollen might potentially have a higher probability of infecting their offspring, and thereby reduce offspring size or survival. We are not aware of any studies analysing these transmission pathways in solitary bees, which calls for further research on this topic.

Our findings are likely not confounded by landscape effects, since reproductive success as well as foraging trip duration of *O. cornuta* was unaffected by the proportion of SNH in the landscapes around the nesting sites in our study set-up. Although the amount of SNH is usually expected to affect bee reproductive success due to its effects on foraging [55,58], the ample floral resources in the flowering orchards where the nests were located likely masked such effects, as intended by our study design. Nevertheless, our results should be interpreted with caution due to the relatively low number of sites and the relatively few samples with very high virus loads. Furthermore, we do not know whether, beyond the experimental nests monitored with the 'Bee Tracker' software, nesting females had built other nests and, consequently, we do not know their lifetime reproductive output. Furthermore, we did not assess the amount of pollen collected per foraging trip, and therefore foraging efficiency of the bees could not be estimated. Both of these aspects could also be affected by the surrounding landscape or the health (i.e. viral load) of a nesting female.

In conclusion, high loads of viruses in nesting females of *O. cornuta* under field conditions have the potential to negatively affect their reproductive success. In our study, these negative effects were found for females carrying high loads of the resident SRBV and GABV, which were frequently found in *Osmia* bees, but we did not find evidence of any negative effects of the honeybee-associated BQCV, while DWV was almost entirely absent. It remains to be further explored whether and to what extent negative effects of these viruses on reproductive success at the level of individual females can translate into negative impacts on solitary bee health at the community and population levels. We emphasize the need for further field studies with larger sample sizes (bees, bee species and field sites) to investigate individual fitness consequences and population-level impacts of different viruses and other pathogens on solitary bees and other wild pollinators, and how such effects may affect the provisioning of pollination services and ecosystem functioning.

Ethics. No permit is required to work with the solitary bee *Osmia cornuta* in Switzerland.

Data accessibility. Data and code supporting the results of this study can be found online on the Dryad Digital Repository and Zenodo [84].

Electronic supplementary material is available online [85].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. C.M.: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; O.Y.: investigation, methodology, writing—review and editing; A.S.: writing—review and editing; P.N.: funding acquisition, resources, writing—review and editing; A.J.V.: conceptualization, funding acquisition, project administration, writing—review and editing; O.S.: conceptualization, funding acquisition, writing—review and editing; R.J.P.: conceptualization, funding acquisition, writing—review and editing; H.S.: conceptualization, funding acquisition, writing—review and editing; L.P.: supervision, writing—review and editing; M.A.: conceptualization, formal analysis, funding acquisition, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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