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Research article

Varroa destructor (Mesostigmata: Varroidae) Parasitism and Climate Differentially Influence the Prevalence, Levels, and Overt Infections of Deformed Wing Virus in Honey Bees (Hymenoptera: Apidae)

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Abstract

The prevalence and loads of deformed wing virus (DWV) between honey bee (*Apis mellifera* L.) colonies from a tropical and a temperate environment were compared. The interaction between these environments and the mite *Varroa destructor* in relation to DWV prevalence, levels, and overt infections, was also analyzed. *V. destructor* rates were determined, and samples of mites, adult bees, brood parasitized with varroa mites and brood not infested by mites were analyzed. DWV was detected in 100% of the mites and its prevalence and loads in honey bees were significantly higher in colonies from the temperate climate than in colonies from the tropical climate. Significant interactions were found between climate and type of sample, with the highest levels of DWV found in varroa-parasitized brood from temperate climate colonies. Additionally, overt infections were observed only in the temperate climate. *Varroa* parasitism and DWV loads in bees from colonies with overt infections were significantly higher than in bees from colonies with covert infections. These results suggest that interactions between climate, *V. destructor*, and possibly other factors, may play a significant role in the prevalence and levels of DWV in honey bee colonies, as well as in the development of overt infections. Several hypotheses are discussed to explain these results.

Key words: deformed wing virus, *Varroa destructor*, climate, *Apis mellifera*

The unprecedented loss of honey bee (Hymenoptera: Apidae) colonies experienced in many countries during the last decade has frequently been linked to the parasitic mite *Varroa destructor* (Mesostigmata: Varroidae) (vanEngelsdorp et al. 2008, Guzman-Novoa et al. 2010, Le Conte et al. 2010, Dainat et al. 2012a,b, Dainat and Neumann 2013) as well as to bee viruses carried and transmitted by this mite (Kevan et al. 2006, Cox-Foster et al. 2007, Berthoud et al. 2010, Carreck et al. 2010, Genersch and Aubert 2010, Dainat et al. 2012a). Several viruses are vectored by *V. destructor* (Tentcheva et al. 2004, Carreck et al. 2010, Santillan-Galicia et al. 2010), but the role this mite plays in both their transmission and multiplication is still not well understood. Furthermore, little is known as to how abiotic factors such as climate affect the efficiency of varroa as a vector and promoter of virus multiplication in honey bees.

Deformed wing virus (DWV; Iflaviridae) is one of the most commonly found viruses in honey bee colonies around the world and is transmitted by *V. destructor* (de Miranda and Genersch 2010). Studies have demonstrated that the newly acquired virulence of several viruses, particularly DWV, has been observed in relation to *V. destructor* acting as a vector and activator of virus multiplication (Genersch and Aubert 2010). For example, it is well established that varroa mites increase both the incidence and titers of DWV in honey bees (de Miranda and Genersch 2010). However, comparative studies in different climates aimed at investigating how varroa mites and climate interact to affect virus infections in honey bee colonies have not been conducted.

DWV infections can be vertically transmitted by queens and drones or horizontally transmitted by nurse bees (Yue and Genersch 2005, Chen et al. 2006, Yue et al. 2007, de Miranda and Fries 2008, de Miranda and Genersch 2010). In the absence of varroa mites, these transmission routes result in covert infections (infections without clinical symptoms) that are not considered serious (de Miranda and Genersch 2010). In the presence of *V. destructor*, however, infection levels tend to be higher and more virulent, resulting in many cases in overt outbreaks characterized by noticeable clinical

symptoms such as wing deformities, reduction in body size, and cuticle discoloration in affected insects (Ball and Bailey 1997, Bowen-Walker et al. 1999, de Miranda and Genersch 2010, Mockel et al. 2011). Bees with overt DWV infections usually die soon after emergence (Ball and Bailey 1997, Dainat et al. 2012a).

The varroa mite may favor the ability of DWV to establish an infection and replicate in honey bees by not only vectoring and transmitting the virus but also by suppressing immune responses of bees when feeding on their hemolymph (Shen et al. 2005, Yang and Cox-Foster 2005) and by promoting virus replication within itself and in infested bees. Yue and Genersch (2005) demonstrated that DWV multiplies and accumulates in the mites. They suggested that virus replication within the mite ensures large numbers of viral particles that are inoculated when varroa parasitizes honey bees, thus ensuring transmission and multiplication efficiency of the virus.

Variation in susceptibility to *V. destructor* parasitism and reproduction has been documented in different genotypes and subspecies of honey bees (Page and Guzman-Novoa 1997). For example, several studies conducted in Brazil and Mexico have demonstrated that Africanized honey bees are more resistant to varroa infestations than their European counterparts (Moretto et al. 1991, Guzman-Novoa et al. 1996, 1999, 2012a, Medina-Flores et al. 2014). However, almost no information exists on whether this resistance is also reflected in their susceptibility to virus infections transmitted by *V. destructor* (Hamiduzzaman et al. 2015).

In the past several years, it has become more evident that high infestation rates of *V. destructor* in association with high DWV loads are factors linked to overt infections, bee mortality and colony losses. However, the effect that abiotic factors such as climate working alone or in combination with biotic factors such as varroa mites have on the mechanisms leading from covert to overt infections in bees is not well understood. Therefore, this study was aimed at testing the hypothesis that the interaction between climate and *V. destructor* parasitism influences the prevalence, relative levels and symptoms of DWV infections in honey bee colonies from temperate and tropical environments.

Materials and Methods

Study Areas

Surveys of varroa mite infestations and DWV presence and loads in honey bee colonies were carried out in two climatic regions of Mexico, one tropical in Nayarit state (20°55′N, 104°24′W) and one temperate in the Federal District (19°03′N, 98°57′W). *V. destructor* has been present in Mexico since at least 1992 (Chihu et al. 1992), whereas several honey bee viruses, including DWV, have only been recently identified in the country (Guzman-Novoa et al. 2012b, 2013) although they probably have been present for a long time. The relevant nectar flow seasons and climatic characteristics of each region where the colonies were sampled are as follows. The tropical region is located 800 m above sea level, has a mean annual precipitation of 1,200 mm, and its average annual temperature is 23°C (Intituto Nacional de Estadistica, Geografía e Informática [INEGI] 2015). During late spring (May), when the survey took place, the mean daily temperature is 27°C. Two main nectar flows occur in this region, one during spring and another in the fall. The temperate region is located 2,400 m above sea level, has a mean annual precipitation of 700 mm, and its average annual temperature is 15°C (INEGI 2015). During late spring (May), when the survey took place, the average temperature is 17°C. Similar to the tropical region, two main nectar flows occur in this region, one during spring and one during fall.

Sample Collection

Samples of varroa mites, brood, and adult worker bees were collected from 80 commercial honey bee colonies in 6 municipalities (39 colonies were sampled in the temperate region and 41 in the tropical region). The studied colonies had not been treated against varroa mites or requeened for at least one year, and were sampled during late spring, 2013 (May), after the honey harvest. At this time of the year, colonies are populated but queens start to decrease their egg-laying rate relative to early spring (similar to early fall in colder climates). Additionally, late spring is when varroa infestation rates are at their highest levels in colonies from both environments (Medina-Flores et al. 2014).

To determine varroa infestation rates in adult bees, ~300 workers were collected in a glass jar containing 70% ethanol from brood-nest frames in each hive. To determine virus presence and loads in adult bees, five samples per colony were collected. Each sample consisted of five workers that were captured with forceps from brood nest combs and transferred into a sterile 2 ml microcentrifuge tube. To each microcentrifuge tube, 1200 µl of RNAlater solution (Life Technologies Inc., Burlington, Canada) was added. The samples were then lightly crushed with sterile forceps for each tube to ensure the RNAlater solution would penetrate the tissues. The samples were kept frozen (−20°C) until RNA extraction. To determine varroa infestation rates as well as virus presence and levels in worker brood, three 10×10 cm sections of comb containing pupae were collected from each hive and temporarily preserved by refrigeration (4°C). Brood for virus analyses were immediately transferred to microcentrifuge tubes and treated as described for adult bees. Brood to be used to determine *V. destructor* infestation levels were kept refrigerated until analyzed. In addition to the above, at least 10 mites per colony were collected as per Arechavaleta-Velasco and Guzman-Novoa (2001) to detect if DWV was present in varroa mites as well as to determine the haplotype of the mites. Mites were placed in microcentrifuge tubes with RNAlater solution as previously described.

At the same time that samples were collected, two independent observers inspected each hive by looking at bees on all combs in search for individuals showing apparent clinical symptoms associated with overt infections by DWV, such as curled or deformed wings and small bodies. If detected, a note was recorded for each colony.

RNA Extraction, cDNA Synthesis, and PCR Reactions

Total RNA was extracted from at least three samples per colony after homogenizing the five bees (brood or adults) or five mites per sample (Chen et al. 2000). All items that were used for macerating bees or mites, or for extracting RNA were thoroughly washed and autoclaved prior to these procedures to prevent contamination. The amount of extracted RNA was determined with a spectrophotometer (Nanovue GE Healthcare, Cambridge, UK) using a CF of 40. For cDNA synthesis, 2 µg of total RNA was reverse transcribed using Oligo $(dT)_{18}$ (0.5 µg/µl) and M-MuLV RT (200 U/µl) with the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, Burlington, CA), following the instructions of the manufacturer.

Multiplex simultaneous reactions were done combining one set of DWV-specific primers with one set of primers for a bee constitutive control gene. Primers for the constitutive honey bee gene were for the ribosomal protein RpS5 gene (Thompson et al. 2007). Primers for DWV were those used by Guzman-Novoa et al. (2012) in previous research. All primers were obtained from Laboratory

Services at the University of Guelph (Guelph, Ontario). All PCR reactions were done with a Mastercycler (Eppendorf, Mississauga, ON, Canada). Each 15 µl of reaction contained 1.5 µl of 10x PCR buffer (New England BioLabs, Pickering, ON, Canada), 0.5 µl 10 mM of dNTPs (Bio Basic Inc., Markham, ON, Canada), 1 µl each of 10 µM forward and reverse primers for RpS5 and 10 µM forward and reverse primers for DWV, 0.2 µl 5 U/µl of *Taq* polymerase (New England BioLabs), 1 µl of the cDNA sample, and 7.8 µl of dd H_2O. The PCR conditions were 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 60 s at 58°C and 60 s at 72°C, and a final extension step at 72°C for 10 min.

Separation and Quantification of PCR Products

PCR products were separated on 1% TAE agarose gels and stained with ethidium bromide. A 100 bp DNA ladder (Bio Basic Inc.) was included in each gel. Images of the gels were captured using a digital camera with a Benchtop UV Transilluminator (BioDoc-It Imaging System, Upland, CA).

The intensity of the amplified bands was quantified in pixels using the Scion Image (Scion Corporation, Frederick, MD, USA) (Dean et al. 2002). Semiquantification was determined from the ratio of intensity between the band of the target virus and the band of the honey bee gene, *RpS5*, to obtain the relative quantification units (RQUs) of viral RNA. The intensity of the bands of the *RpS5* gene was constant at all time points. To determine whether quantification at 35 amplification cycles was affected by signal saturation of the band intensities, which could affect the accuracy of relative quantifications, randomly selected samples with high, medium and low RQUs of DWV were also quantified in the same manner with 25 and 30 amplification cycles.

Determination of Varroa Infestation Rates

V. destructor infestation levels in adult bees were determined as per De Jong et al. (1982). Briefly, the jars containing the bees were agitated for 30 min on a mechanical shaker (Eberbach, Ann Arbor, MI, USA), and the mites that were dislodged from the bees' bodies were strained. The number of mites per 100 bees was calculated for each sample and an average infestation rate was calculated for each environment. *V. destructor* infestation rates were also determined in the brood of each colony by inspecting 200 cells containing pupae under a stereoscopic microscope. The percentage of parasitized cells was calculated as above.

Mite Haplotype Determinations

V. destructor haplotype determinations were performed to find out if varroa mites in the samples from the two climates were the same or different, which could be associated to differences in mite pathogenicity (Solignac et al. 2005). For determining haplotypes, a random collection of 20 varroa mites from each location were individually assessed for mt DNA type, and all were of the Korean haplotype (Solignac et al. 2005).

DWV Identity

To confirm the identity of DWV, PCR products were sequenced (Laboratory services, University of Guelph) and the sequences blasted against GenBank accesions (accession No. NC_004830). In all cases, identity was >97%. We also conducted an alignment of the partial sequence of the putative RNA helicase amplified from DWV samples from Mexico's tropical and temperate climates with 10 other putative RNA helicase sequences of DWV samples from Poland, Austria, Hungary, Slovenia, Germany, Nepal, Sri Lanka, United Arab Emirates and Canada (Genbank accession nos. DQ224291.2, DQ224281.2, DQ224298.2, DQ224300.2, DQ224294.2, DQ224305.2, DQ224306.2, DQ224308.2, DQ224310.2, and DQ224311.2, respectively). These samples were used by Berényi et al. (2007) to indicate genetic variation associated with a recent global distribution of DWV. The sequences were aligned with MUSCLE sequence alignment software (EMBL- EBI, UK) (http://www.ebi.ac.uk/Tools/msa/muscle/).

Morphometric Analyses of Adult Bees

In addition to the above, the morphological type of bees (Africanized or European), as well as their forewing length, were determined by subjecting 30 workers per colony to the Fast Africanized Identification System (FABIS; Sylvester and Rinderer 1987). These assessments were done to find out if bees from the two different environments varied in morphological traits associated to African and European ancestry, which could potentially explain results from this study, since it has been found that Africanized bees are relatively more resistant than European bees to *V. destructor* parasitism (Guzman-Novoa et al. 1996, 1999, 2012a, Medina-Flores et al. 2014).

Statistical Analyses

Chi square tests were used to compare proportions of DWV positive and negative samples, as well as proportions of samples of bees with Africanized and European morphotypes between the colonies of the tropical and temperate environments. To test for differences in relative amounts of DWV RNA between bees of colonies from the two climates, RQU data were log-transformed and subjected to analyses of variance (ANOVA). Data on *V. destructor* infestation rates were arcsine-square root transformed to correct for non-normal distribution and then factorial ANOVAs were performed on the data to test for effects of climate on *V. destructor* infestation levels as well as for effects of climate, mite parasitism and for interactions between mite parasitism and climate on DWV loads. When significant differences were detected, means were separated with multiple comparison Scheffe tests. Pearson correlation analysis was used to detect significant relationships between data on varroa infestation rates and DWV levels in each environment. Student *t* tests were used to compare two treatments for different variables including DWV loads and *V. destructor* infestation rates between colonies with overt and covert DWV infections, as well as to compare the wing length of bees from the two environments. All statistical analyses were performed with the R Statistical Program (R Development Core Team, Auckland, New Zealand).

Results

DWV Prevalence in Mites and in Bees

RT-PCR analysis of mites confirmed the presence of DWV in all samples from both environments. In adult bees as well as in varroa-infested brood and brood not infested by varroa, DWV was significantly more prevalent in the temperate environment than in the tropical environment (Table 1). When comparisons were made within the same environment, DWV prevalence was significantly higher in adult bees than in varroa-infested brood in the tropical environment, but not in the temperate environment. However, DWV was significantly more prevalent in adults compared with noninfested brood in both environments. No differences in prevalence were detected between infested and noninfested brood in either of the two environments (Table 1).

Table 1. Percentage of honey bee colonies located in a temperate and in a tropical environment in which DWV was found at detectable levels in samples of adult bees, brood infested by *V. destructor* mites and brood not infested with mites ($n = 80$)

Type of sample	Temperate	Tropical	χ^2
Adult bees	87.2	65.8	5.0*
Varroa-infested brood	69.2	34.1	9.8**
Noninfested brood	51.3	21.9	7.4*
χ^2 adult bees versus varroa-infested brood	3.7^{ns}	8.2**	
χ^2 adult bees versus noninfested brood	11.8***	16.0***	
χ^2 varroa-infested versus noninfested brood	2.6^{ns}	1.5^{ns}	

χ^2 tests were used for comparisons. ns, not significant, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

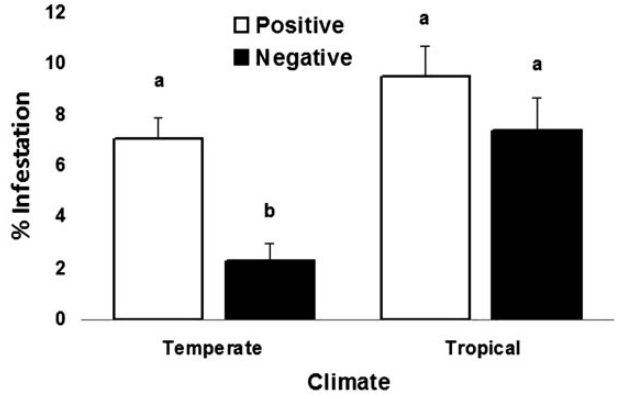

Fig. 1. Percent *V. destructor* infestation (±SE) in adult worker honey bees from colonies where DWV was detected (positive) or was not found (negative) in temperate and tropical climates ($n = 80$). Different letters indicate significant differences of means based on analyses of variance and Scheffe tests performed on arcsine-square root transformed data. Nontransformed values are presented.

V. destructor Parasitism and DWV Prevalence in Bees

Mean varroa infestation levels in adult bees and brood did not differ between colonies from the two environments. For adult bees, mite infestation levels were 6.5 ± 0.6 and $8.3 \pm 0.6\%$ in the temperate and tropical environments, respectively, whereas for brood, infestation rates in these environments were 13.9 ± 1.4 and $13.8 \pm 0.9\%$, respectively ($F = 1.0$; df = 1, 158; $P > 0.05$). In the tropical environment, no differences were found when mite infestation levels of DWV positive colonies were compared with those of colonies where the virus was not detected. Conversely, in the temperate environment, colonies where DWV was detected had significantly higher mite infestation rates than DWV negative colonies ($F = 8.3$; df = 1, 76; $P < 0.01$) (Fig. 1). In brood, mite infestation levels did not differ between DWV positive and DWV negative colonies in either environment ($F = 0.5$; df = 1, 76; $P > 0.05$) (Fig. 2).

V. destructor Parasitism and DWV Levels in Bees

The accuracy and reliability of the viral quantification method used was confirmed because the RQUs of DWV from random samples obtained with 25 and 30 amplification cycles were not significantly different to those obtained with 35 cycles ($F = 0.30$; $P = 0.75$). In the colonies where DWV was detected, relative viral loads were affected by type of environment ($F = 4.2$; df = 1, 125; $P < 0.05$), type of sample ($F = 4.9$; df = 2, 125; $P < 0.01$) and by the interaction between environment and type of sample ($F = 3.2$; df = 2, 125; $P < 0.05$). When all types of samples were used in the analysis, DWV relative levels were higher in the temperate climate than in the tropical climate. Furthermore, brood infested by *V. destructor* had significantly higher DWV loads than noninfested brood and adult bees in the temperate environment, but not in the tropical environment (Fig. 3).

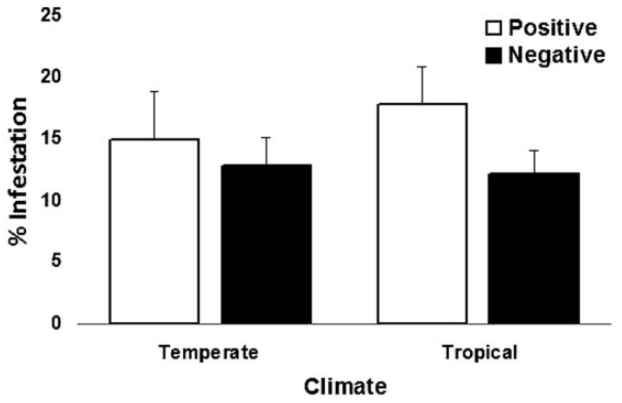

Fig. 2. Percent *V. destructor* infestation (±SE) in honey bee brood from colonies where DWV was detected (positive) or was not found (negative) in temperate and tropical climates ($n = 80$).

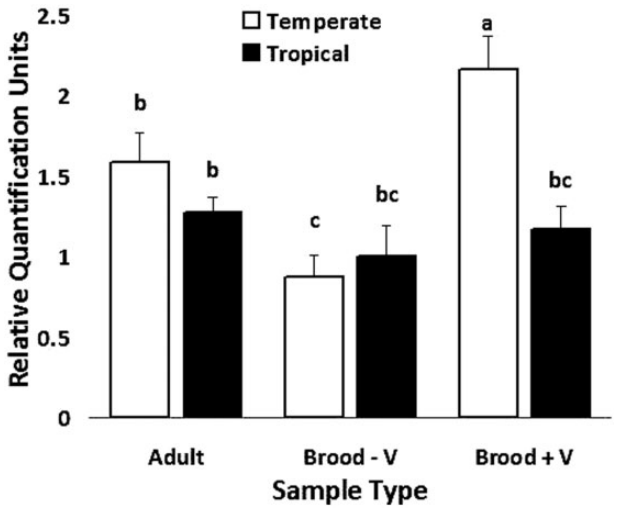

Fig. 3. Relative RT-PCR quantification units of DWV by coamplification with *Apis mellifera* RpS5 in honey bee adults, brood not parasitized by *V. destructor* (Brood − V) and brood parasitized by *V. destructor* (Brood + V) in temperate and tropical climates ($n = 80$). Different letters indicate significant differences of means based on analyses of variance and Scheffe tests performed on log-transformed data. Nontransformed values are presented.

In addition to the above, $df = 2, 15$; varroa infestation rates of adult bees were significantly correlated with relative amounts of DWV in the temperate environment ($r = 0.42$; $n = 34$; $P < 0.01$), but not in the tropical environment ($P > 0.05$). No significant correlations were found between DWV loads and mite infestation rates in brood in either environment ($P > 0.05$).

Overt DWV infections were evident in 25% of the colonies studied in the temperate climate, with adult bees showing small bodies and deformed wings. In contrast, no clinical symptoms associated with DWV infections were observed in any of the colonies from the tropical environment. Moreover, adult bees, but not brood, from the colonies showing overt infections in the temperate climate had significantly higher DWV loads than bees from the colonies

with covert infections in the same climate ($t = 2.50$; $P < 0.05$ and $t = 0.55$; $P > 0.05$; $n = 34$, for adult bees and brood, respectively) (Supp Fig. 1 [online only]). Additionally, the mite infestation rate of adult bees from colonies with overt infections was significantly higher than that of bees from colonies with covert infections ($t = 2.0$; $n = 34$; $P < 0.05$). However, no differences in mite infestation rates of the brood were found between colonies in the temperate climate with and without overt infections ($t = 0.40$; $n = 34$; $P > 0.05$) (Supp Fig. 2 [online only]).

DWV Variation

DWV isolates from both regions in Mexico did not appear to be atypical. A comparison of their RNA helicase sequence showed that there was >98% nt identity. The polymorphic sites between those samples were also polymorphic in other RNA helicase sequences of DWV from several countries (Berényi et al. 2007).

Morphotype and Fore-Wing Length of Bees

More than 75% of the bee samples from both regions were classified as European based on the morphological traits measured and no significant differences were detected for the proportion of colonies that were classified as European or as Africanized by the FABIS ($\chi^2 = 0.93$; $n = 80$; $P = 0.33$), as well for the bees' fore-wing length between the two regions ($t = 0.57$; $n = 80$; $P > 0.05$).

Discussion

DWV was detected in 100% of the mites analyzed, confirming the strong association between these parasites and the potential role of *V. destructor* as vector of DWV. Other studies have also found a high prevalence of DWV in varroa mites (Bowen-Walker et al. 1999, Nordstrom et al. 1999, Tentcheva et al. 2004, Yue and Genersch 2005, Guzman-Novoa et al. 2012b). Moreover, the results of this study confirm those of previous reports linking varroa mites with increased prevalence and levels of DWV in temperate climates (Tentcheva et al. 2006, de Miranda and Genersch 2010, Di Prisco et al. 2011, Desai et al. 2015), but in addition, this study is the first to analyze and compare DWV infections in colonies from tropical and temperate regions.

The results of this study consistently showed higher prevalence, levels and overt infections of DWV in colonies from the temperate environment than in colonies from the tropical environment despite the fact that no differences in mean varroa infestation rates were found between colonies from the temperate and the tropical environments for either adult bees or for brood. Several factors and scenarios could explain these results, including climatic effects, an interaction between climate and *V. destructor* parasitism, differences in *V. destructor* genotypes and infestation rates, differences in DWV strains, and differences in bee genotypes between the two environments.

The first two hypotheses regarding climatic effects and interactions between climate and *V. destructor* parasitism are supported by our results, particularly in temperate regions. It could be that DWV transmission and replication is favored in honey bee colonies located in temperate climates compared with tropical climates. In support of the hypothesis that colder, temperate climates, may favor the transmission, and replication of DWV in honey bees, laboratory experiments (Di Prisco et al. 2011) showed that honey bee pupae subjected to the stress of a lower temperature than that considered normal in the brood nest of a colony (30°C) had higher titers of DWV than pupae kept at 33°C. Di Prisco et al. (2011) found that cold-stress weakened the host immune responses, which may have increased the susceptibility of the bees to DWV infection. In fact, it has been demonstrated that DWV titers in winter correlate with decreased expression of immune-related genes in honey bees, but not in summer (Steinman et al. 2015). In another study, it was found that as many as 19 immune genes were downregulated in bees with increased loads of DWV that died during winter (Nazzi et al. 2012). Colder environments thus, could induce reduced immune functions, which could increase honey bee's susceptibility to DWV. It is also possible that DWV transmission and multiplication is more common in drier environments, in temperate climates, than in more humid, tropical climates. For example, the transmission and multiplication of some viruses are favored by dry conditions (Lowen et al. 2007). Humidity could perhaps affect the transmission and replication of DWV as well, although it is not clear how climatic conditions may affect the virus since it is found inside the bees in a thermoregulated environment. It may be that brood nest temperature and humidity in honey bee colonies differ between temperate and tropical regions, but to the best of our knowledge there is no comparative data published on these parameters. Therefore, further research on this matter is warranted.

Our results also suggest an interaction between climate and *V. destructor* parasitism resulting in higher prevalence and loads of DWV in honey bee colonies from a temperate climate in comparison to colonies from a tropical climate. It may be that under temperate environmental conditions, *V. destructor* becomes more efficient as a vector and activator of DWV replication. Consistent with our results, Möckel et al. (2011) had previously demonstrated that DWV is the causative agent of the deformed-wing syndrome in adult bees if DWV is transmitted to pupae by *V. destructor*. However, not all infected pupae develop the syndrome, apparently because virus replication in the mite up to a certain threshold is a prerequisite for overt DWV infections to develop (Yue and Genersch 2005, Gisder et al. 2009). Therefore, factors that favor the replication of DWV in the mites would lead to overt infections, and climate could be one of these factors. It is well known that varroa infestations in colder climates cause considerably more damage to honey bee colonies than infestations in warmer climates, which further supports our hypothesis (De Jong et al. 1984, De Jong 1997).

It has been reported that colonies with low *V. destructor* infestation rates show lower prevalence and titers of several viruses, including DWV (Emsen et al. 2015). However, our data do not support an epidemiological explanation for the lower incidence and loads of DWV in honey bee colonies from the tropical environment relative to those from the temperate environment, because there were no differences in mite infestation rates between colonies from the two climates studied. It could also be argued that the results of this study may have occurred due to possible differences in virulence of *V. destructor* populations in the two environments, but the *V. destructor* haplotype was the same in colonies from both environments.

Our results do not support the hypothesis that the tropical and temperate strains of DWV in this study were different and thus varied in virulence, because the DWV sequences from the two environments were highly similar to each other as well as to other DWV sequences from Canada, Europe and Asia. Thus, there was no evidence that the DWV from either region in this study was atypical of the variation in DWV found worldwide. Reinforcing this argument, Martin et al. (2012) demonstrated that the recent arrival of *V. destructor* into the Hawaiian islands increased the prevalence and titers of DWV and massively reduced DWV diversity, leading to the predominance of a single DWV strain. If *V. destructor* selects for the same variant in tropical and temperate environments, then, climatic

factors could explain our results, at least partially. Conversely, if *V. destructor* selects different variants of DWV in different regions, it may be that in temperate climates, it selects variants with a competitive advantage, e.g., variants that can replicate in the mite (Gisder et al. 2009). Under this scenario, these strains would prevail in the temperate climate which may result in virulence differences compared with strains selected in other regions and, hence, in differences of prevalence and virus loads. Since we only sequenced a small portion of the DWV genome, future studies are required to sequence the complete viral genome of variants collected from regions that vary in DWV prevalence and loads. Additionally, different DWV strains should be tested for virulence under different climatic conditions. These studies may help explain the different prevalence, viral levels and symptoms observed in DWV-infected bees in different environments.

Genotypic hypotheses might also explain at least part of the differences found in DWV prevalence and loads between bees of the two climates. Africanized bees have been found to be relatively resistant to varroa infestation and population growth in Mexico (Guzman-Novoa et al. 1996, 1999, 2012a, Medina-Flores et al. 2014), and this resistance could also be linked to virus resistance (Hamiduzzaman et al. 2015). However, in this study, no significant differences in wing length or in the proportion of Africanized and European morphological types were found between bees of colonies located in the tropical environment, and those of colonies from the temperate environment.

A complex interaction of several factors in tropical climates could also explain some of these results. For example, no or lower incidence of DWV in colonies from tropical regions populated with honey bees of African ancestry than that reported from bees of European countries has been noticed (Teixeira et al. 2008, Kajobe et al. 2010, Strauss et al. 2013, Adjlane et al. 2015), again suggesting a possible effect of climate and type of bee, or the interaction of these with other factors.

In summary, we show here consistent differences in DWV prevalence, levels and symptoms in bees from temperate and tropical environments, as well as an association between DWV and *V. destructor* parasitism that differs in the two environments. Our data do not allow us to explain why these interactions result in higher prevalence and loads of DWV in bees from the temperate environment than in bees from the tropical environment. Therefore, we discuss different hypotheses that could potentially explain these results. These hypotheses however, remain to be tested in further, more controlled studies.

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Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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