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NOTES AND COMMENTS

Effect of genotype and environment on parasite and pathogen levels in one apiary - a case study

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An international collaborative experiment was run from 2009 to 2012 (Costa *et al.*, 2012) with the aim of understanding genotype-environment effects on survival and health status of honey bee colonies headed by queens of different European origins that were tested in various locations under differing environmental conditions. No chemical treatment against bee diseases was performed on any of the test colonies. The colonies were managed according to a common protocol, which included an assessment of their health status (for details, see Meixner *et al.*, 2014). One apiary located in Chalkidiki, Greece, was selected for a detailed case study analysis of parasites and pathogens, including an absolute quantification of viral titres.

The colonies were sampled regularly between autumn 2009 and autumn 2011 and analysed for *Varroa destructor* and *Nosema* spp. as described in Meixner *et al.* (2014). For virus analysis, approximately 30 worker bees from each colony were sampled in March and September of 2010, and April and October of 2011, and analysed for deformed wing virus (DWV), black queen cell virus (BQCV), acute bee paralysis virus (ABPV), Kashmir bee virus (KBV) and Israeli paralysis virus (IAPV). Extraction of RNA, qPCR and quantification of virus titers followed the methods described in Francis *et al.* (2013), with ABPV, KBV and IAPV assessed together in a single assay (AKI). Viral titres (acute paralysis complex (AKI), DWV and BQCV), *Nosema* spp. infection levels and *V. destructor* mite infestation levels during the seven sampling times were compared to the survival status of the colony at the end of the experiment. The data were analysed for patterns, trends, correlations and significant differences.

Of the 39 starting colonies, 31 could be sampled for quantitative virus analysis in the spring of 2010. They belonged to the genotypes

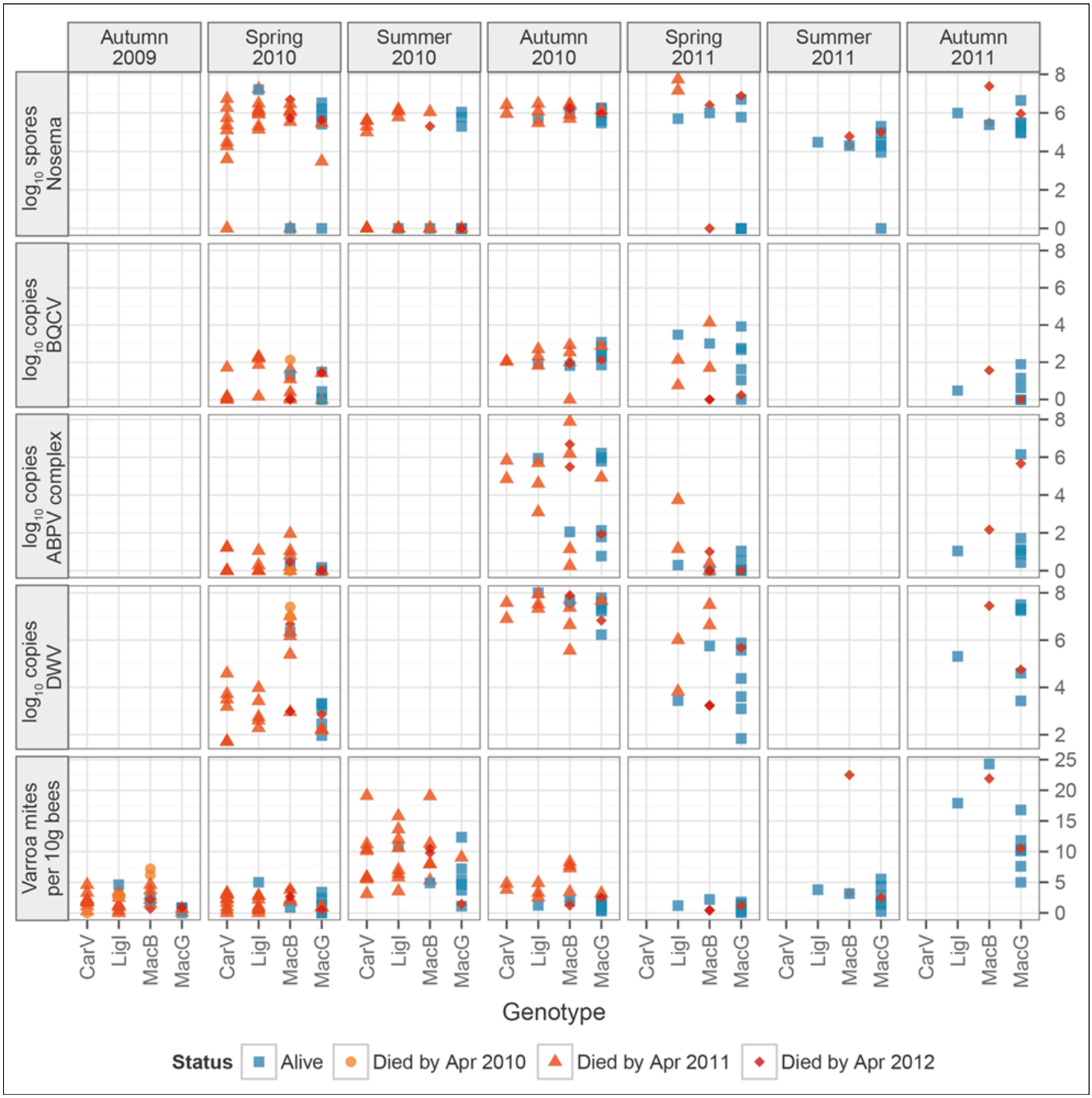

Fig. 1. Overview of results at one location (Chalkidiki, Greece). Viral titres (ABPV-KBV-IAPV (AKI), DWV and BQCV), *Nosema* spp. spore levels and *V. destructor* mite levels are shown for four genotypes (CarV, LigI, MacB and MacG (local bee)) at seven sampling periods. Survival status was determined in April every year, and colonies that remained alive after the experiment are marked 'Alive'. Viruses were sampled in spring and autumn of 2010 and 2011. *Nosema* spp. were sampled in three seasons of the years 2010 and 2011. *V. destructor* was sampled at the same times as *Nosema* spp., and also in the autumn of 2009. Note that not all colonies were sampled for all pathogens for all time periods. MacG colonies showed the lowest mite levels and DWV titres in the autumn of 2010 and 2011. The local bee (MacG) had the highest number of surviving colonies (6 of 9) at the end of the experiment.

CarV (*A. m. carnica;* n = 6), LigI (*A. m. ligustica*; n = 6), MacB (*A. m. macedonica;* n = 10) and MacG (*A. m. macedonica;* n = 9). The genotype of local origin was MacG (for details of genotypes see Francis *et al.,* 2014). At the end of the experiment, eight colonies survived, including MacG (n = 6), MacB (n = 1) and LigI (n = 1). None of the *A. m. carnica* colonies survived the second winter (2010/11).

V. destructor infestation levels generally increased over the duration of the experiment, but the local bees (MacG) showed significantly lower mite infestation rates in autumn of 2009 and 2010 compared to the three other genotypes (GLM, $p < 0.05$) (see Fig. 1.). Mite levels in October 2010 and August 2011 were negatively correlated to the survival duration of the colonies in days ($R^2 = 0.63$, $p < 0.001$).

The viral titres of the colonies and showed a strong seasonal trend (see Fig. 1.). In both years, the ABPV complex and DWV showed low titres in spring, and high titres in autumn, with a significant difference (Wilcoxon, $P < 0.05$) between them. DWV showed overall higher titres than AKI. While the titres of AKI in October 2010 were highly variable, the infection with DWV was homogeneously high. In 2010 and 2011, the number of *V. destructor* mites in autumn significantly correlated with DWV titres in the spring of the following year ($R^2 = 0.64$, $p < 0.01$). The MacG colonies consistently had the lowest mite infestation and the lowest DWV titres, while DWV titres in non-local bees were significantly higher (Wilcoxon, $W = 37$, $p < 0.05$).

Following Francis *et al.* (2013), we consider bees harbouring more than 10^7 copies of virus to represent an overt virus infection. In the case of DWV infection, we observe an increase in titres greater than 10^7 copies from 7% of the colonies in April (n = 15), to 55% of the colonies in September (n = 11) and 78% of the colonies in October (n = 23). In contrast, only one colony out of 23 had AKI titres greater than 10^7 copies in October.

Nosema spp. infection levels were higher in spring and autumn than in the summer (Fig. 1.). In spring 2010, all colonies had less than 5 million *Nosema* spp. spores per bee, except two *A. m. ligustica* colonies that harboured more than 15 million spores (significantly higher, GLM, $p < 0.05$). One of these colonies eventually died, while the other survived. A similar trend in *Nosema* spp. infection was observed in spring 2011. One LigI colony survived in spite of an extremely high *Nosema* spp. level. The BQCV infection level showed a low-high-low trend, with low titres at the beginning (spring 2010), high titres in the middle (autumn 2010 - spring 2011) and low titres towards the end (autumn 2011) of the experiment. The highest BQCV titres were observed parallel to elevated *Nosema* spp. levels in the same colonies in spring and autumn. In spring 2011, *Nosema* spp. levels were significantly correlated to BQCV titres ($R^2 = 0.68$, $p < 0.01$). In spring 2010, for example, LigI colonies had the highest *Nosema* spp. spore loads as well as the highest BQCV titres.

Although our data do not allow us to deduce a causal relationship between mortality and virus infections in this apiary, the patterns of infection differ conspicuously among introduced and local bees. A similar pattern can be observed for the infection with *Nosema* spp., with the *A. m. ligustica* origin showing the highest levels, also reflected in comparatively high BQCV titres in the same colonies. No clear patterns of survival or death of colonies in relation to *Nosema* spp. or BQCV titres emerge from these results.

Given the close proximity of the colonies in a single apiary, our findings are surprising, since a particular protection of the colonies of local origin from pathogen transfer between hives appears unlikely. Rather, our results suggest that bees of local origin are better in tune with environmental factors related to flowering patterns, climatic variation and locally prevailing apicultural methods and therefore may command more sufficient resources to fend off pathogens.

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