Developmental nutritional environment can have long-term effects on an individual’s phenotype, including health. In this study we demonstrate that two types of larval nutritional stress reduce adult honey bee resilience to viral infection. When stressed with reduced quantity of nutrition as larvae, via an acute period of starvation, honey bee worker adults had lower mass than adults that had received a normal nutritional quantity. This did not affect adult mortality. However, when inoculated with Israeli Acute Paralysis Virus, adults that had received reduced nutritional quantity as larvae had lower survival. Similarly, nutritional quality experienced as larvae significantly affected adult success at surviving viral infection. When larvae were reared in colonies that were solely fed either a high quality or low quality pollen, there was no difference in adult mortality. However, IAPV infection caused an increase in mortality of bees that had been reared with a low quality pollen. Yet, IAPV infection did not affect survival for bees reared with a high quality diet. Thus, a high quality larval diet rescued the lethal effects of adult IAPV infection.

In this study, we reproduced two nutritional stress scenarios that honey bee colonies commonly face. In Experiment 1, with acute starvation, we manipulated the pollen *quantity* that developing workers received. Both natural conditions and managerial practices can cause lapses in a colony’s pollen resources (Scofield and Mattila 2015) that result in a reduction of larval nutritional quantity. Poor weather greatly reduces pollen forager intake and results in reduced nursing behavior (Crailsheim et al. 1999). The acute starvation experienced by larvae in our experiment may reflect the reduced nursing received by workers in natural pollen dearth. However, a potentially important discrepancy between our study and natural conditions is that, by restricting nurse access to larvae, social interaction in addition to food transfer was paused. Thus, we cannot completely disentangle the effects of acute starvation from social isolation on the long-term health of workers in our study. However, in Experiment 2 we manipulated the nutritional *quality* that larvae received, presumably without perturbing their social interactions with nurses. Commercially owned honey bee colonies often face poor quality forage, including a lack of diverse forage, since they are housed at agricultural locations dominated by monoculture. This limited diversity of forage is associated with indicators of poor colony health, including hive weight and nurse bee lipid content (Dolezal et al. 2019b; St Clair et al. 2020). Additionally, commercial hives are highly susceptible to the spread of pests and pathogens because they are kept in close proximity in high density apiaries and returning foragers often enter the wrong hive (Free 1958; Seeley and Smith 2015). As much as 40% of a colony’s workers may “drift” to neighboring hives (Jay 1965). Furthermore, Geffre et al. (2020) demonstrated that guard bees are less aggressive to unfamiliar bees inoculated with IAPV than to uninfected bees. Thus, IAPV-infected bees may actually drift between colonies more frequently than healthy bees, resulting in a rapid spread of viral infection across apiaries (Geffre et al. 2020). Our results show that the dual stressors of deceased diet quality and increase viral lodes that commercial hives commonly face are compounding.

The long-term effects of larval diet restriction on adult phenotype differed between quality and quantity manipulations. Acute larval starvation produced smaller adults, with significantly less mass than unstarved bees. In contrast, there was no effect of being reared with a low quality diet on adult mass. It could be that nurse bees adjust the quantity of food they feed to brood to compensate for low quality nutrition, offsetting an effect on later adult mass and lipid stores. Even though rearing larvae on a low quality diet produced normally-sized adults, it did not produce bees with nominal immune systems. The low quality pollen used in this study (*Cistus*) has a lower concentration of trace micronutrients, including calcium and iron, than the high quality pollen (*Castanea*) (Dolezal et al. 2019a). These micronutrients may be crucial for pathogen resistance (Failla 2003).

Viral infection and diet manipulation were marked by perturbations of expression of genes associated with immune activation. In Experiment 1 (acute larval starvation), regardless of diet treatment, honey bees challenged with IAPV infection had higher expression of *dicer*, *hymenoptaecin*. Dicer is an enzyme that is part of the RNA-interference pathway: a highly conserved system that identifies and combats RNA viruses (Cerutti and Casas-Mollano 2006). Hymenoptaecin is an anitimicrobial peptide involved in honey bee immune response to infection by bacteria (Casteels et al. 1993) and viruses (Ryabov et al. 2016). In Experiment 1, *hopscotch* was upregulated in starved bees and bees treated with IAPV. Hopscotch, a JAK tyrosine kinase (Binari and Perrimon 1994), is a component of the JAK/STAT signaling pathway associated with honey bee immunity (Evans et al. 2006; Siede et al. 2012). In Experiment 2 (pollen quality limitation), regardless of diet treatment, honey bees challenged with IAPV infection had higher expression of *cactus* than control bees. The Cactus protein is a component of the Toll immunity signaling pathway (Valanne et al. 2011), which exhibits antimicrobial activity in honey bees (Richard et al. 2012). It is curious that in both the diet quantity and diet quality experiments, viral infection caused expression differences of immune genes regardless of diet treatment, and that the affected genes differed between diet experiments. This reaffirms that diet quantity and diet quality are fundamentally different forms of developmental nutrition. As expected, viral infection causes an upregulation of genes associated with the immune system. However, the type of nutritional environment experienced by individuals during development can affect how the immune system responds to infection, and subsequently, the immune system’s efficacy at successfully staving off the infection. In our study, viral infection caused higher immune gene expression differences between workers from starved and unstarved treatments than between workers from high and low quality diet treatments (among the immune genes we measured). This may be indicative that the long-term effects of larval diet quantity on adult immunity may be more pronounced than larval diet quality.

We find that reduction of both nutritional quantity and quality cause observable reductions in resilience to infection, providing evidence for the importance of developmental nutrition in producing worker bees that are patent against infection as adults. These results have important ramifications in our understanding of the interplay within the network of environmental stresses faced by pollinators… **Adam, this more your bread & butter. Do you want to finish off this paragraph about implications for bee health/beekeeping practices?**

The conditions individuals experience during early development can have long-term effects (Lindström 1999; Metcalfe and Monaghan 2001; Monaghan 2008).

Lets reframe this comparison. These experiments are different in several ways, but one is a comparison of quality and quantity. In theory, the starvation is just a quantity variant – bees get more or less nursing/food, but they are in identical colonies so there is no variation in the quality of their food.

In the chronic, we fed them all ad lib and colonies were the same size. But we did vary the quality of the diet fed to the colony. We don’t know exactly how this affected the quality or quantity of the diet fed to the larvae, but it stands to reason that they must have been similar in many ways because the mass/lipids weren’t different. So this experiment can be used to discuss what quality means and the potential for changes in quality to have important, but ultimately subtle, effects on adult phenotypes.

We might also then talk about the issue with the starvation diet being a reduction in both food and social contact/nursing overall, which may have other non-nutritional aspects we are not able to account for.

* 1. How do these scenarios correspond to conditions or events that actual honey bee colonies might experience?
     1. Chronic
        1. Migratory colonies fed one monoculture at a time
        2. “Green desert” of agriculture landscape (Dolezal et al., 2020 *PNAS*)
     2. Starvation
        1. ?? why can’t I think of a good real-world example of this?
        2. Umm.. droughts?
  2. Quality vs quantity or, why was there no difference in mass/lipids for chronic?
     1. Potential compensation by increasing diet intake, but overall low diet quality will result in lower health.
     2. Reduction in ‘quality’ not just quantity. My RSOS paper and some other papers have pointed at things like micronutrients as important factors for immunity. SO while that wouldn’t affect weight or lipids it could affect immune responses (this then links to III well)

1. Discussion of genes-of-interest expression
2. Implications for honey bee practices
3. Early life effects are long-reaching

From Scofield and Mattila

Honey bee larvae are probably routinely exposed to the short-term nutritional stress that

was experienced by our focal individuals, either seasonally or because of management practices

that limit nutrient availability. This is suggested by the overlap between the weight range for

pollen-stressed and unstressed workers in this study (mean 71–113 mg across treatments and

trials) and weights reported previously for workers at adult emergence (81–140 mg; reviewed

by [102]). Larvae undergo a 700-fold weight gain during the 5–6 days that they are nursed

[103], but a single day of bad weather reduces nursing activity by more than one half, even

when colonies have stored pollen [104]. This response to poor weather likely explains in our

study why confinement alone (without pollen stress) produced workers that were slightly

smaller than those reared in unconfined colonies (but with few behavioral effects). Over the

long term, the number of small workers in colonies increases with repeated bouts of bad weather

[54] and the heaviest workers are reared at times when pollen is readily available within a

season [68], so differences among our treatments in emergence weights likely reflect adjustments

made to brood provisioning in colonies in response to changes in both foraging opportunity

and pollen stores (but note that confinement had no effect on the behavior of

abundantly supplied control workers). Because the window during which larvae are fed is so

brief, day-to-day changes in attention from nurses have the potential to generate nutritional

stress for developing workers and the corresponding deficits in adult function that we demonstrated

here. It is worth noting that pollen-limited workers often looked similar in size to control

workers, so it would be difficult to determine by visual inspection alone that workers in

managed colonies had been subjected to such stress.

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