Mendoza, 16 de septiembre de 2022

Estimada Arq. Soledad Barros

Coordinadora Unidad Evaluaciones Ambientales

Secretaría de Ambiente y Ordenamiento Territorial-Mendoza. Ref.: EX-2022-01605275-GDEMZA-SAYOT

Pedido de inclusión al expediente de referencia

En nuestra Manifestación General de Impacto Ambiental hemos argumentado varias de las razones por las cuales; la utilización de colmenas *de Bombus pauloensis (ex B. atratus*) en invernaderos mallados de la provincia de Mendoza; no provocarían un daño ambiental dada su imposibilidad de multiplicarse en el ambiente.

Una de las razones presentadas fue que el polen de tomate es un polen pobre en proteínas que provoca un crecimiento limitado de la colmena. Este crecimiento limitado también conlleva a una pobre producción de reinas nuevas. Al no haber producción de reinas nuevas, los riesgos de establecimiento en la región son muy reducidos. La producción de reinas nuevas en colmenas que pecorean sobre tomate en invernadero fue medida en condiciones de laboratorio. Pero, dada que esta información se usó para mejorar el sistema productivo de colmenas, no fue publicada en ninguna revista científica.

Una nueva publicación, que adjuntamos, de Wynants y colaboradores (2022) sostiene este argumento: las colmenas de *Bombus terrestris* alimentadas con polen monofloral de bajo contenido proteico no pueden producir reinas nuevas. Esta publicación reafirma lo expresado en la MGIA y reafirma que no habría producción de reinas nuevas en colmenas alimentadas con el polen del cultivo de tomate.

En la medida que las colmenas sean usadas sobre tomate en invernaderos, la producción de nuevas reinas se vería muy limitada y su hipotético establecimiento en la región sería altamente improbable.

Este argumento es plenamente coincidente con la falta de detección de *B. pauloensis* en los monitoreos llevados a cabo en la provincia de Mendoza durante los años 2020 (Maggi y colaboradores) y 2022 (Silvina Vélez)

Sin más saludamos a ud muy atte

Lic Laura Valles Ing. Agr. Carlos Silvestre

Brometan SRL

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ORIGINAL ARTICLE

Ecological Entomology

Re-evaluation of a method used to study nutritional effects on bumble bees

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Abstract

1. Bee declines are an increasingly recognised problem globally. Nutritional stress due to agricultural intensification is one of the drivers of bee declines. Therefore, understanding the nutritional requirements of bees is crucial to mitigate the effects of food scarcity on bee populations.
2. Laboratory studies evaluating nutritional effects on bumble bees often use microcolonies as a model system for effects on queenright colonies. Microcolonies consist of workers, whereby one worker will lay male-destined unfertilized eggs. Consequently, microcolonies exclusively produce males, while queenright colonies produce (female) workers and reproductives (males and queens).
3. A comparison between *Bombus terrestris* (L.) microcolonies and queenright colonies was made by feeding three diets of varying nutritional quality.
4. The experimental diets affected most fitness parameters of microcolonies differently than those of queenright colonies. Low-protein, largely monofloral, pollen affected queenright colony fitness by reducing colony size and increasing larval mortality, while these fitness parameters were not affected in microcolonies. High-protein polyfloral pollen reduced larval mortality in queenright colonies but did not affect larval mortality in microcolonies. For both colony types, a similar result was obtained when assessing offspring body mass, which was reduced by the low-quality diet and increased by the high-quality diet.
5. In conclusion, our results demonstrate that microcolonies are inaccurate model systems and their use can lead to erroneous conclusions in terms of effects of nutritional stress or pollen quality on bumble bees. Our results further highlight the importance of high-quality (here high-protein polyfloral) pollen for sustaining colony development of bumble bees.

KEYWORDS
bumble bees, fitness, microcolonies, pollen quality

INTRODUCTION

Global pollinator declines pose a major threat to wild plant diversity and human food security (Gegear et al., 2021; IPBES, 2019; Klein et al., 2007). Bee declines are of particular concern because bees are a major group of pollinators globally (Potts et al., 2010; Winfree et al., 2011). Drivers of bee declines include habitat loss and fragmentation, increased use of pesticides, declines in food resources and nest availability, climate change, and an increased prevalence of bee pathogens (Goulson et al., 2015; Sgolastra et al., 2020; Vanbergen &

Initiative, 2013). Agricultural landscapes are increasingly dominated by monoculture crops, which causes nutritional stress for pollinators due to a reduced availability and diversity of pollen sources resulting in an imbalanced diet (Baude et al., 2016; Kammerer et al., 2021; Kleijn & Raemakers, 2008; Scheper et al., 2014). Both food deprivation and poor-quality food can lead to nutritional stress, which can render bumble bees more susceptible to other stressors, such as diseases (Roger et al., 2017) and pesticides (Linguadoca et al., 2021). Food deprivation can also be caused by exposure to other stressors, such as pesticides that decrease foraging success (Muth & Leonard, 2019; Siviter et al., 2021). These factors, and the complex interactions between them, contribute to population declines because a reduced access to high-quality pollen negatively affects colony health and reproductive success (e.g., Kämper et al., 2016; Leza et al., 2018).

As various types of nutritional stress impact bee fitness in different ways, accurate methods to evaluate their effects are needed (Cabrera et al., 2016; Klinger et al., 2019). Many studies evaluating the impact of environmental factors, such as diet and pesticide exposure, on bumble bee fitness, use microcolonies. A microcolony consists of workers only, in which typically one worker will take on the role of becoming a 'pseudoqueen' by laying unfertilized eggs (Regali & Rasmont, 1995). Bumble bees, like other Hymenoptera, have a haplodiploid sex determination, whereby haploid males develop from unfertilized eggs, a process known as arrhenotokous parthenogenesis, while diploid females develop from fertilised eggs (Grimaldi et al., 2005). Bumble bee workers are not able to mate and can only lay unfertilized eggs that will develop into males (Wilson, 1971). As a result, all offspring produced in microcolonies are male. There are a number of advantages to using microcolonies over queenright colonies, such as the ease to create and maintain them, the possibility for larger sample sizes, and easier standardisation among microcolonies (Cabrera et al., 2016; Klinger et al., 2019). These advantages have led to the widespread use of microcolonies in bumble bee research.

A general assumption of results obtained using microcolonies is that they are representative—at least in terms of the factors under study—for queenright colonies (Dance et al., 2017; Klinger et al., 2019; Tasei & Aupinel, 2008a). However, the extrapolation of conclusions drawn on the basis of laboratory-reared microcolonies, to wild, foraging, queenright colonies has been debated (Mommaerts et al., 2010; Van Oystaeyen et al., 2021) and only few studies have directly compared results obtained using both types of colonies under identical conditions. One exception is a study on dietary effects by Tasei and Aupinel (2008a), in which multiple diets differing in quality were tested in bumble bee microcolonies and queenright colonies. The authors concluded that—based on the evaluated fitness parameters—the quality ranking of diets was identical for both colony types. However, a direct comparison of results obtained with both colony types was not possible because different parameters were evaluated for both colony types. Out of all evaluated parameters in the study, mean larval weight was the best diet quality predictor in microcolonies, whereas body size of new queens and pollen consumption were the best predictors in queenright colonies. A recent study assessed pesticide side-effects on both colony types under identical conditions using similar fitness parameters (i.e., reproductive output) and concluded that microcolony results conflicted with results obtained using queenright colonies in the laboratory and freely-foraging queenright colonies (Van Oystaeyen et al., 2021). This begs the question to what extent microcolonies can be relied on in bumble bee nutrition research.

Here, we compare *Bombus terrestris* microcolonies to queenright colonies under identical laboratory conditions by exposing them to three diets of differing quality. Similar fitness parameters are measured in both colony types: larval mortality, total number of offspring produced, and body mass of offspring.

MATERIALS AND METHODS

Bumble bee rearing and experimental design

Sixty queenright *B. terrestris* colonies and 30 *B. terrestris* microcolonies were reared at Biobest Group NV (Westerlo, Belgium), with each colony type reared on three different pollen diets ($N_{queenright} = 20$, $N_{microcol} = 10$). All colonies were kept at 28°C and 60% relative humidity and continuous darkness (except during feeding and monitoring). Queens used for colony foundation were hibernated for 15 weeks and then placed into a plastic nest box (16 cm × 16 cm × 10.5 cm) where they immediately received the diet treatment. One week after awakening from hibernation, a single callow bumble bee worker was added to queens in the nest boxes to stimulate egg laying by the queen. Each microcolony consisted of 10 callow workers placed together in a plastic nest box (16 cm × 16 cm × 10.5 cm) where they immediately received the diet treatment. Both colony types were placed in the same climate room and colony types were alternated so that both treatments were spread homogeneously over the available space. All colonies were fed ad libitum pollen diet and 50° Brix sugar water (Biogluc®, Belgosuc, Belgium).

All queens/microcolonies were assigned randomly to one of the three pollen quality treatments. A quality rank was assigned to the pollen treatments in a pre-trial carried out by the commercial breeding company Biobest Group NV, whereby queenright colonies were fed with different pollen diets and the number of produced workers was evaluated after 9 weeks (unpublished results). Subsequently, based on the number of produced workers, a quality ranking was assigned to different pollen by comparison to a standard. For this study, we chose three pollen diets, the standard pollen diet used in the commercial rearing, the top-ranking pollen, and the lowest-ranking pollen available at the time. The pollen treatments consisted of: (1) polyfloral pollen consisting of a blend of different pollen used for the commercial breeding of bumble bees with 18% (wet weight, w wt) protein content (further referred to as 'control diet'), (2) a high-quality polyfloral pollen mix with 22% (w wt) protein content (further referred to as 'high-quality pollen diet'), and (3) a low-quality monofloral pollen diet with 14% (w wt) protein content, consisting mainly of *Cistus* pollen (main type of pollen determined by microscopic analysis at ×400

magnification); further referred to as 'low-quality pollen diet'. Crude protein content of the pollen was analysed by the Kjeldahl method (SGS, Belgium). The level of monoflorality was determined based on the different pollen pellet colours combined with microscopic analysis. Pollen was considered as monofloral when they consisted of the same pollen type (plant species) for more than 60% of the sample. For polyfloral pollen, the exact plant composition was unknown. All pollen types were freshly frozen honey bee-collected and gamma-irradiated pollen obtained from Biobest Group NV.

After 9 weeks of development, colonies were frozen at −21°C and the number of eggs, larvae, pupae, queen larvae, queen pupae, adult males, adult workers, as well as larvae that had died during the experiment (i.e. larvae deposited in the colony's waste pile), were counted. The total colony size was defined as all produced offspring in all different developmental stages, that is the sum of all eggs, larvae, pupae, and adult offspring (both males and workers in case of queenright colonies). Predicted number of adults was defined as the summed number of pupae and number of adult offspring (both males and workers). An average wet weight of workers and males was obtained per colony by weighing all individuals of the same sex together and dividing this weight by the total number of individuals of that sex. For further statistical analysis, we used the average male weight for microcolonies and average worker weight for queenright colonies. Only a few queenright colonies produced males by the time that the experiment was stopped and thus their weight was not used in any further analyses (Data S1).

Statistical analyses

All statistical analyses were performed in R version 4.0.0 (R Core Team, 2019). For all statistical analyses in this study, a significance level of $\alpha = 0.05$ was used. Model selection, including the decision of excluding/including interaction effects between fixed factors, was based on the Akaike information criterion (AIC). Model validation, that is assessment of the model distribution, outliers and dispersion, was analysed using package *DHARMa* (Hartig, 2020). All generalised linear models (GLM) were performed using the package *nlme* (Bates et al., 2015) and post hoc comparisons were made using the *contrast* function in package *emmeans* (Searle et al., 1980), with a Tukey *p*-value adjustment.

A GLM with a negative binomial distribution was used to analyse total colony size (i.e., a sum of eggs, larvae, pupae, and adults) in the function of colony type (queenright colony or microcolony) and pollen diet (fixed factors), as well as the interaction effect between both fixed factors. The number of predicted adults was analysed in terms of colony type and pollen diet using a GLM with Gaussian distribution including a significant interaction term. This variable was calculated as the sum of the number of pupae and adults and reflects the future number of adults. The rationale for this variable is that larval mortality occurred frequently, while pupal mortality was never observed. The number of pupae thus more accurately reflects future adult numbers. Adult body mass, that is, worker body mass in queenright colonies and male body mass in microcolonies, was analysed using a GLM with gaussian distribution and the interaction term was not retained in the model. A GLM with quasibinomial distribution was used to analyse the percentage of dead larvae (calculated as the number of dead larvae divided by the sum of live and dead larvae, pupae, and adults) in function of colony type and pollen diet, including the interaction effect between both fixed factors.

RESULTS

There was a significant interaction between colony type and pollen diet for the total colony size, demonstrating that the effect of diet on the total colony size is different between the two colony types ($\chi^2 = 11.4$, $p = 0.003$). Colony size of microcolonies was neither affected by high-quality (HQ) pollen nor by low-quality (LQ) pollen compared to the control diet ($z = -0.05$, $p = 1$; $z = 0.26$, $p = 0.96$, respectively). By contrast, in queenright colonies, total colony size was significantly reduced by LQ pollen compared to the control diet ($z = -4.23$, $p < 0.0001$), while HQ pollen did not have a significant influence on total colony size compared to the control ($z = 0.73$, $p = 0.71$). The total colony size of queenright colonies was larger than that of microcolonies for all pollen diets but this difference between colony types was the smallest for LQ pollen (control: $z = -5.73$, $p < 0.0001$; HQ: $z = -6.38$, $p < 0.0001$; LQ: $z = -1.99$, $p = 0.047$) (Figure 1a).

A similar pattern is observed for the number of predicted adults (pupae + adults), with a significant interaction between colony type and pollen diet ($\chi^2 = 11.5$, $p < 0.0001$). In microcolonies, the predicted number of adults is not affected by HQ or LQ diet compared to the control (HQ: $z = 0.8$, $P = 0.67$; LQ: $z = 0.63$, $p = 0.78$). By contrast, in queenright colonies, the LQ diet reduced the number of predicted adults ($z = -5.44$, $p < 0.0001$) and the HQ diet increased the number of predicted adults ($z = 2.63$, $p = 0.02$) (Figure 1b).

Also for the percentage of dead larvae, there was a significant interaction between colony type and pollen diet ($\chi^2 = 40.78$, $p < 0.001$). In queenright colonies, larval mortality increased in the LQ treatment and was reduced in the HQ treatment compared to the control ($z = 4.68$, $p < 0.0001$; $z = -2.25$, $p = 0.048$, respectively). For microcolonies, no significant differences were detected compared to the control (HQ: $z = -0.8$, $p = 0.67$, LQ: $z = -1.14$, $p = 0.45$). For the control and HQ diet, larval mortality was significantly higher in microcolonies than in queenright colonies (control: $z = 4.47$, $p < 0.0001$; HQ: $z = 3.7$, $p = 0.0002$) but there was no difference in larval mortality between both colony types when fed the LQ diet ($z = -0.97$, $p = 0.33$) (Figure 1c).

In contrast to previous parameters, offspring body mass was not affected differently by pollen diet when comparing both colony types, that is, there was no significant interaction between colony type and pollen diet, while both pollen diet ($F_{2,79} = 41.95$, $p < 0.0001$) and colony type ($F_{1,79} = 83.91$, $p < 0.0001$) independently influenced this parameter. For both colony types, LQ pollen reduced adult body mass compared to the control ($t = -6.22$, $p < 0.0001$), while HQ pollen

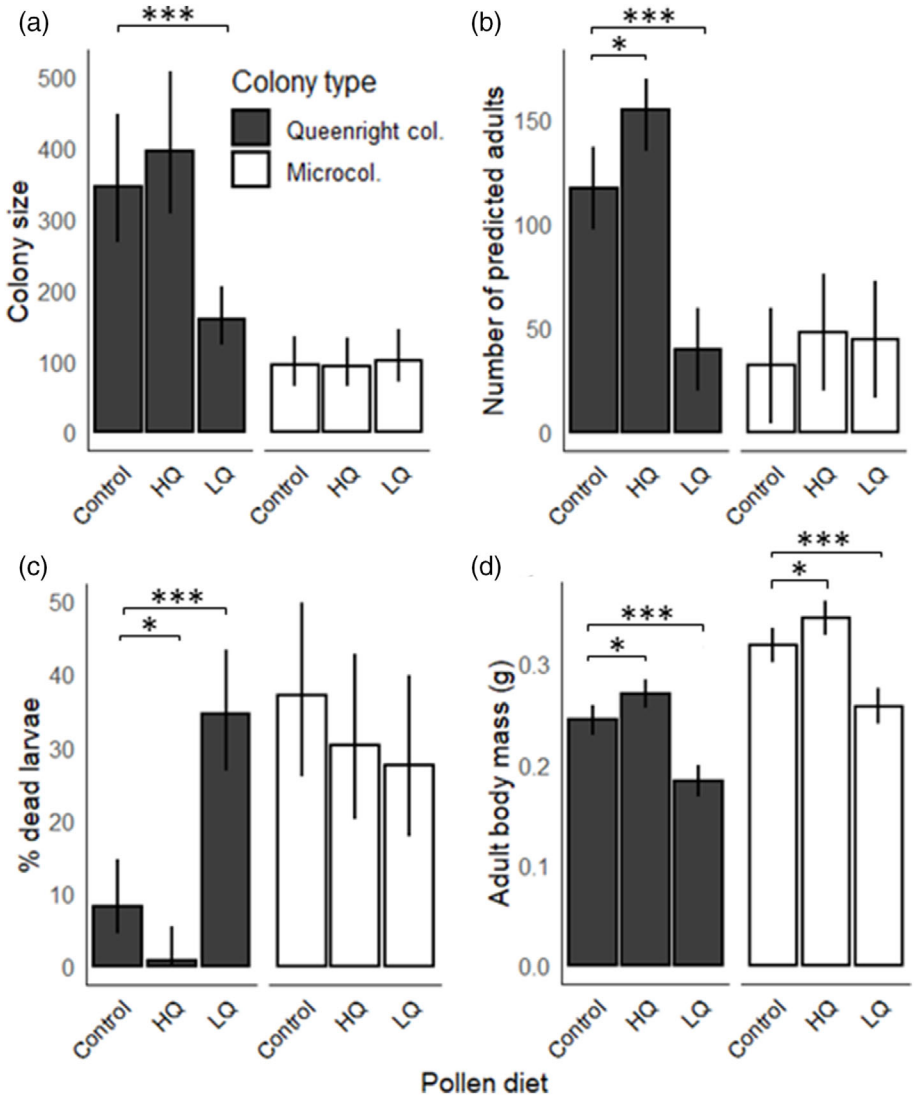

FIGURE 1 Different fitness parameters evaluated in queenright colonies (grey) and microcolonies (white) in response to three diets of varying quality ("Control" = Control pollen, "HQ" = high-quality pollen, "LQ" = low-quality pollen) and composition: (a) the total colony size, that is the total number of offspring (eggs, larvae, pupae, and adults), (b) the number of predicted adults (pupae and adults), (c) the percentage of dead larvae, and (d) the adult body mass (drones for microcolonies, and workers for queenright colonies). The marginal means are shown with bars indicating the 95% confidence interval. Asterisks represent the level of significance (*$p < 0.05$; ***$p < 0.001$).

increased body mass ($t = 2.78$, $p = 0.014$) (Figure 1d). Males produced in microcolonies were on average heavier than workers produced in queenright colonies ($t = 6.43$, $p < 0.0001$), with a mean body mass of 307 mg (±34 SD) for males and 236 mg (±57 SD) for workers.

DISCUSSION

In bumble bee laboratory research, microcolonies are often used to evaluate pesticide and nutritional stress on fitness parameters, resting on the assumption that results can—at least to some extent—be extrapolated to queenright colonies (Klinger et al., 2019; Tasei & Aupinel, 2008a). However, a recent study showed that this assumption does not hold for pesticide side effects (Van Oystaeyen et al., 2021). In this study, we show that microcolonies are also an inadequate model to study the effects of nutritional stress or pollen quality on bumble bees. Our results highlight that microcolony studies risk significantly underestimating the effects of anthropogenically induced stress on wild bumble bee populations because negative effects of low-quality pollen could not be detected by using microcolonies for most colony development parameters, such as colony size, number of produced offspring, and larval mortality.

A direct comparison between microcolonies and queenright colonies in terms of colony fitness—defined in terms of reproductive output, that is number of offspring produced—revealed contrasting results. While in queenright colonies a low-protein monofloral pollen diet, consisting mainly of *Cistus* pollen, reduced both total colony size (all developmental stages and adults together) and number of

predicted adults (pupae and adults together), there were no effects detected on these parameters in microcolonies. The results obtained here using queenright colonies are in line with previous research on queenright bumble bee colonies that demonstrate a negative impact on colony development by a low-protein diet (Vaudo et al., 2016) and monofloral pollen (e.g., Baloglu & Gurel, 2015; Hass et al., 2019), and *Cistus* pollen in particular (Baloglu & Gurel, 2015). Our results further support findings that bumble bees require different floral resources to improve colony fitness (Wintermantel et al., 2022).

The finding that microcolonies and queenright colonies respond differently to the tested diets could point to different nutritional requirements between the sexes during larval development. To date, knowledge on nutritional requirements for bumble bee development is limited and differences in nutrition between the sexes in social bees are virtually unknown. In solitary bees, there is scant literature on the nutritional differences between the sexes during larval development. For instance, females of the halictid bee *Megalopta genalis* provide nutritionally different food to male and female larvae, causing females to exhibit more variation in body weight compared to males, as a result of a more variable nutrition (Kapheim et al., 2011). Another example is the solitary mason bee *Osmia bicornis*, for which female larvae were shown to have higher demands for phosphorus, copper, and zinc compared to male larvae, and were consequently provided with pollen richer in these elements by their mothers (Filipiak, 2019).

Another potential explanation for the conflicting results between microcolonies and queenright colonies could be differences in reproductive physiology between both female castes founding these different colony types, that is workers in microcolonies versus queens in queenright colonies. Bumble bee workers are incapable of mating and thus can only lay unfertilised eggs that develop into males (Bourke, 1988). By contrast, a successfully mated queen will store sperm in her spermatheca and will control the release of sperm cells to fertilise an egg while it is passing through the oviduct (Baer, 2015; Plowright & Plowright, 1990). Inside the spermathecae, sperm is nourished by spermathecal gland secretions that ensure viability (Pascini & Martins, 2017). We suggest that nutritional effects (e.g., low protein content) on stored sperm in the spermatheca could occur when proteins produced by the spermathecal glands, needed to ensure sperm viability (Baer et al., 2009; Gonzalez et al., 2018), are affected by poor nutrition. These effects are irrelevant in workers producing males, as no sperm is needed to produce the haploid males. Thus, if poor nutrition affects sperm quality (either directly and/or via reduced quality of spermathecal gland secretions), negative effects on the number or quality of offspring would only be observed in queenright colonies. In addition, stored sperm cells themselves may be affected by the poor nutritional intake of the queen. In many animal species, including humans, nutrition is a known factor influencing sperm quality and quantity (e.g., Salas-Huetos et al., 2019), which has also been demonstrated for male insects (e.g., Bunning et al., 2015). However, it remains to be investigated whether the quality of sperm cells stored in the spermatheca could also be directly affected by the nutritional status of the queen.

Dietary effects on larval mortality were inconsistent when comparing both colony types. In queenright colonies, the low-quality monofloral diet increased larval mortality and the high-quality polyfloral diet reduced larval mortality compared to the control pollen diet, while in microcolonies diet did not have any measurable effect on larval mortality. These results provide a mechanistic explanation for the observed effects on colony size and predicted number of adults in queenright colonies, as a higher larval ejection rate will result in a lower number of offspring. Previous studies have demonstrated that nutritional stress due to poor pollen quality causes larval mortality in bees (Bortolotti et al., 2020; Nicholls et al., 2021). Larvae need to feed on pollen to obtain nutrients required to complete their development, among which proteins, vitamins, and sterols are thought to be the most important (DeGroot, 1953; Katsumata et al., 1967; Togasawa et al., 1967; Vanderplanck et al., 2014). Consequently, nutritional insufficiencies in the diet can lead to an increased larval mortality or a smaller adult body size (Brodschneider & Crailsheim, 2010). As such, this study confirms the importance of the availability of high-quality pollen resources (such as the HQ diet in this study) for larval development and hence colony development, whereby protein content and high source plant diversity can determine quality (Wintermantel et al., 2022). A further noteworthy finding is that larval mortality, overall, was considerably higher in microcolonies (37.1% on average for the control treatment) than in queenright colonies (8.2% on average for the control treatment), while receiving the same pollen, sugar water, and while exposed to identical conditions. Similar observations were made in the study of Van Oystaeyen et al. (2021), where higher larval ejection rates were found in microcolonies than in queenright colonies. Larval mortality in microcolonies has also been reported in other studies, ranging on average between 4.5% and 100%, depending on pollen treatment (Génissel et al., 2002; Roger et al., 2017; Tasei & Aupinel, 2008b). Proximate explanations for the inherently higher larval mortality in microcolonies compared to queenright colonies remain to be investigated. We propose that this can be related to differences in larval nutritional requirement, as described above, or to differences in susceptibility to stressors between sexes (male larvae in microcolonies vs. worker larvae in queenright colonies), the latter of which has indeed been demonstrated for stressors in honey bees (McAfee et al., 2022). Alternatively, we propose that a higher level of conflict among workers in queenless (micro)colonies (Sibbald & Plowright, 2013) could reduce brood care (feeding and incubation) compared to colonies with a dominant queen, which can ultimately lead to an increased larval mortality in microcolonies. Further, brood care can be influenced by the number of workers available to partake in brood care, which is limited to 10 in microcolonies, while queenright colonies contained over 50 workers on average by the end of the experiment.

Offspring body mass was the single fitness parameter measured in this study that showed consistent changes according to diet treatment for both colony types. For both microcolonies and queenright colonies, body mass was reduced when colonies were fed with a low-quality diet and increased when fed with a high-quality diet. Previous studies have argued that offspring body mass or size is an adequate

parameter to evaluate the health impact of poor-quality diets in bumble bee microcolonies (Roger et al., 2017; Tasei & Aupinel, 2008b). We argue that offspring body mass or size is still an inferior fitness parameter compared to direct reproductive output for three reasons. First, we demonstrate that dietary effects were more pronounced on reproductive output parameters, such as total colony size or number of predicted offspring, than on worker body mass in queenright colonies. For example, the total colony size of queenright colonies fed with the high-quality diet was 2.5 times higher than that of colonies fed with the low-quality diet, while worker body mass was 1.7 times higher when colonies were fed with high-quality pollen versus low-quality pollen. Second, there are stricter biological constraints to bumble bee body size than to colony size, for example due to high energetic costs associated with large body sizes (Pyke, 1978). When comparing body mass and reproductive output parameters, another limitation of body mass is that it cannot be zero. A critical body mass for metamorphosis or a minimal nutrient intake is likely to exist, as described for other holometabolic insects (De Moed et al., 1999). In *B. terrestris*, adult workers have never been observed to weigh below 100 mg (personal observations). These biological constraints may obscure dietary effects. Third, worker body mass or body size is dependent on the number of workers present in the colony (or colony age), as well as on whether brood is tended mostly by the mother queen or mostly by the workers, independent of external factors such as diet (Shpigler et al., 2013).

We further argue that laboratory studies addressing the effects of environmental stressors, such as poor nutrition or pesticide exposure, should include the evaluation of colony output of new sexuals produced in queenright colonies, that is, queens and males, and their subsequent reproductive success (López-Uribe et al., 2020; Straub et al., 2015; Van Oystaeyen et al., 2021). Not only is the number of produced sexuals an important fitness parameter for the mother colony, but gyne production in wild bumble bees also has profound consequences at the population level. The ability to assess gyne production is an additional incentive for using queenright colonies over microcolonies when assessing colony fitness.

In conclusion, our results demonstrate that microcolonies are inadequate model systems to study dietary effects on bumble bee colony fitness, impeding direct extrapolations of results to queenright colonies or to natural bumble bee populations. Evaluation of the reproductive output of queenright bumble bee colonies in the laboratory provides a more robust and more reliable picture of dietary colony-level effects.

AUTHOR CONTRIBUTIONS

Enya Wynants: data analysis; visualisation; writing. **Felix Wäckers**: supervision; writing; **Annette Van Oystaeyen**: conceptualization; methodology; experimental analysis; data analysis; supervision; writing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Supplementary dataset S1. The dataset generated in this study on which the statistical analyses were based.

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