



Sporopollenin as a dilution agent in artificial diets for solitary bees

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Abstract – Nutritional studies often require precise control of nutrients via dilution of artificial diets with indigestible material, but such studies in bees are limited. Common diluents like cellulose typically result in total mortality of bee larvae, making quantitative studies difficult. We investigated potential alternative dietary dilution agents, sporopollenin (pollen exines) and agar. We reared *Osmia bicornis* larvae on pollen diluted with these substances, alongside undiluted controls. Sporopollenin neither prevented nor improved survival, suggesting it is a suitable diluent. Agar appeared marginally to increase survival and its suitability requires further research. Both substances reduced cocoon weight, and sporopollenin also prolonged development, suggesting processing costs. Determining the physiological mechanisms driving these responses requires further work. Our findings should facilitate studies involving nutritional manipulations for solitary bees.

Artificial diet / Dilution agent / Solitary bee / Nutritional ecology / Geometric framework

1. INTRODUCTION

Artificial diets are integral to studies of animal nutritional ecology, because they allow hypothesis testing using controlled manipulation of constituent nutrients (Roulston and Cane 2002). For example, particularly insightful in nutritional ecology has been the Geometric Framework for Nutrition (GF) (Raubenheimer and Simpson 1993). In this technique, arrays of artificial diets are created containing different ratios and densities of

macronutrients (e.g. Lee et al. 2008), making control of nutrient density via dietary dilution especially relevant. Dilution of diets with inert, indigestible material is therefore a key component in the design of these and other nutritional studies using artificial diets, as it allows control of overall nutrient density independent of the ratios of nutrients in the diet (House 1965; Gordon 1968). A suitable dilution agent should be indigestible and neither toxic nor nutritious. Which substance is appropriate depends on a species' nutritional ecology and physiology. While water is usually used to dilute artificial diets for liquid feeders (Abisgold et al. 1994; Lee et al. 2008; Hawley et al. 2014), cellulose is a more common dietary diluent in studies of feeding where solid artificial diets are required (Bignell 1978; Slansky and Wheeler 1991; Wheeler and Slansky 1991) and is frequently used in studies conducted under the GF (Raubenheimer and Simpson 1993; Le Gall and Behmer 2014; Solon-Biet et al. 2014).

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Understanding bee nutrition is particularly important at present (Wood et al. 2016; Goulson and Nicholls 2016; Filipiak 2019). Bees, managed and wild, are globally important for their role in pollination, which is central to natural and agricultural ecosystems (Breeze et al. 2011). Unfortunately, though, bees are declining—partly due to poor nutrition (Naug 2009). Despite this, we have a poor understanding of how bees deal with variable quality food in patchy modern landscapes (Filipiak et al. 2017; Filipiak 2019). We know particularly little about larval nutrition, the stage where all growth occurs in bees. Addressing this knowledge gap could have implications for predicting pollination services and designing mitigation/conservation measures. Unfortunately, most model bee species are highly social, which is problematic for studying larval nutrition: in the most social species, foragers contribute nutrition to a collective pool that is used to feed larvae, obscuring individual feeding relationships (Schmickl and Karsai 2016). Any such studies must therefore either use intensive *in vitro* feeding of individual larvae (e.g. Helm et al. 2017) or else focus on colony-level outcomes (Brodschneider and Crailsheim 2010; Roger et al. 2017). In contrast, solitary bees are excellent model species for manipulative studies of larval nutrition, as mothers provision young with foraged pollen individually inside sealed cells, meaning we can easily link a foraging adult to its individual offspring (Killewald et al. 2019). By manipulating or replacing the pollen ball, we can study larval health directly (Levin and Haydak 1957). Despite this opportunity for experimental research, solitary bee nutrition is relatively poorly studied (Roulston and Cane 2002; Filipiak 2019). Artificial diet protocols have been developed for some economically important solitary bees (Nelson et al. 1972; Fichter et al. 1981) but with limited success in terms of larval survival. In a recent study of pollen nutrition, researchers resorted to harvesting natural provisions from bee cells in the field to provide a suitable base for minimal manipulation, adding only protein-rich royal jelly (Fischman et al. 2017).

To construct an array of artificial diets with different nutrient densities for solitary bees, as

would be required for a study conducted under the GF, cellulose would initially seem a natural candidate for a dilution agent. Just as with other consumers of solid food, bees are not known to digest cellulose (Martin 1983). However, previous anecdotal observations indicate that powdered cellulose may have toxic effects for bees (Ruedenauer et al. 2016) perhaps because of dehydration, or the possibility of formation of plugs in the larval midgut. Other indigestible diluents, e.g. glass powder, have been reported to produce similarly uniform mortality (Konzmann and Lunau 2014; see Ruedenauer et al. 2016). Consistent with this, in pilot trials, we found that substituting the original pollen balls with replacements consisting of 70% honeybee pollen and 30% powdered cellulose resulted in total mortality of *Osmia bicornis* larvae within a few days (12/12 larvae, A. Austin, pers. obs.).

In this study, we address this problem by investigating the suitability of two alternative diluents for use in solid artificial pollen diets for *O. bicornis* larvae: bacteriological agar and sporopollenin. Agar is a non-toxic jelly-like mixture of the polysaccharides agarose and agaropectin, indigestible for many animals and commonly used as a matrix in which to embed other nutrients (e.g. Burns et al. 2012). We are unaware of studies specifically investigating the excreta of agar-fed insects to check the extent of modification during digestion. However, agar is nutritionally inert for many insects. One percent agar has been used as a starvation medium for *Drosophila* (Shell et al. 2018) and was insufficient for development of *Agria* flies (House 1969) although it appeared to promote growth in *Tribolium* beetles (Sial et al. 2017). Sporopollenin is an ecologically relevant material for solitary bee larvae, constituting the exine of pollen, their exclusive food source. It is chemically extremely resistant to degradation and is recognized as one of nature's most stable molecules (Qu and Meredith 2018). While many pollen-feeding insects are able to extract pollen nutrients by piercing or rupturing the exine, only a tiny number are known actually to digest it (Roulston and Cane 2000). Accordingly, while bees extract and digest pollen cytoplasm

extremely efficiently (Wightman and Rogers 1978; Schmidt and Buchmann 1985), they do not digest sporopollenin. Rather, it is excreted intact both by adults (Suárez-Cervera et al. 1994; Roulston and Cane 2000) and larvae (Peng and Dobson 1997). All four *Osmia* spp. studied by Suárez-Cervera et al. (1994) excreted intact the exines of all studied species of pollen. Here, we investigate the diluent potential of both whole pollen exines and exines crushed to increase density of indigestible material; we are not aware of any studies examining the nutritional effects of sporopollenin in crushed or powdered form.

We reared *O. bicornis* larvae on 5 diets varying in dilution and quantity. The diets we substituted for natural pollen balls were (A) an equivalent weight of pure honeybee pollen, (B) a reduced weight of honeybee pollen, and three diets consisting of a reduced weight of honeybee pollen supplemented with dilution agents: (C) agar, (D) whole pollen exines (composed of sporopollenin) and (E) crushed pollen exines (see Table 1 for details). If agar and/or sporopollenin are suitable diluents (i.e. indigestible and neither toxic nor nutritious), the following predictions should be true:

- P1 Diets diluted with sporopollenin and/or agar should, owing to processing costs, have marginally but not drastically lower survival or fitness (e.g. lower cocoon mass, longer development time) than those receiving the same amount of undiluted nutrients.
- P2 Diets diluted with sporopollenin and/or agar should not have *higher* survival or fitness than those receiving the same amount of undiluted nutrients. If this occurred, it could mean some component of the dilution agent was being metabolized, or facilitating digestion/utilization of nutrients from other sources.
- P3 Diets diluted with crushed sporopollenin may have marginally higher mortality/lower fitness than those diluted with whole sporopollenin (owing to the larger amount of material [=processing costs] packed into the space of the pollen ball).

2. METHODS

Osmia bicornis is a univoltine, cavity nesting solitary bee providing commercially relevant ecosystem services (Jauker et al. 2012; Schulze et al. 2012). Diapausing *O. bicornis* cocoons (Mauerbienen®) were released in June 2019 inside experimental nests in a south-facing location on the University of Hull campus, and adults allowed to emerge and breed. The nests consisted of styrofoam (Styrodur®) blocks with a 9 × 9 × c.150 mm groove cut out of the surface and covered with a transparent acrylic slide for observation, modified from Strohm et al. (2002) (Fig. S1a), and were housed within custom-built outdoor wooden boxes (Fig. S1b). Completed nests (distinguished by a mud plug) were brought into the laboratory. When larvae were old enough to be handled, generally 2–3 days after hatching, they were each weighed and then placed into a single-cell nest (similar to experimental nests but shorter, housing just 1 larval cell) following the removal of any egg fragments. Within this nest each larva was randomly allocated to a treatment group and provided with one of the five experimental diets (details and sample sizes in Table 1). All larvae were placed into an environmental chamber (Sanyo® MLR-351H) at 19.5°C and 75% humidity.

Diets consisted of varying mixtures of pollen and, in some cases, a diluent. The pollen used in all of the diets was honey bee pollen obtained in bulk (buywholefoodsonline.co.uk®) and homogenized to ensure consistency across pollen balls; honey bee pollen is sufficient for normal development in *O. bicornis* (Austin and Gilbert and M. Filipiak, pers. comm.). The alternative of using conspecific *Osmia* pollen balls as a substrate was not possible because they could not be obtained in sufficient quantities for experimental requirements and, moreover, are impractical for bulk use in artificial diets because they would severely limit the quantity of diets researchers could feasibly produce.

Our “full” pollen ball treatment (diet A) weighed 0.35g, close to the largest field pollen ball mass recorded in Budde and Lunau (2007). The reduced pollen ball treatment (diet B) was identical in composition but 70% of the mass,

Table I. Experimental diets used in this study. For details, see text

Group	n	Description	Diluent	Pollen volume (ml) (%)	Diluent volume (ml) (%)	Total diet weight (mg)
A	14	Full pollen ball (100%)	–	0.87 (100)	–	0.35
B	15	Reduced pollen ball (70%)	–	0.61 (100)	–	0.245
C	13	Reduced pollen ball	Agar 3%	0.61 (70)	0.26 (30)	0.307
D	14	Reduced pollen ball	Sporopollenin whole	0.61 (70)	0.26 (30)	0.280
E	14	Reduced pollen ball	Sporopollenin crushed	0.61 (70)	0.26 (30)	0.280

Note: To ensure accuracy, pollen balls were made up in batches of 15 (13 and 9.1 ml for full and reduced pollen balls, respectively, and 3.9 ml of diluent in the diluted treatments)

i.e. 0.245 g. In the diluted diets, the diluents used were (diet C) 3% bacteriological agar (Agar No. 1 [LP0011], Thermo Fisher Scientific ®), made by adding 3 g of agar powder to 100 ml of purified water; (diet D) whole, empty pollen exines (Sporomex®) (see [supplementary methods for details of preparation](#)); and (diet E) pollen exines as in (2) but crushed in a ball mill. Experimental diet make-up is given in Table I. We calculated pollen/diluent ratios based on volume rather than weight due to the large difference in density between the pollen and sporopollenin. In formulating our diets, we made the assumption that the “nutrient” concentration equated to the amount of pollen; that is, that the pollen ball consisted of only digestible nutrients. All diets were eaten readily by all larvae.

We assessed the proportion of larvae surviving the experiment, up to a maximum of 70 days, and the time to death for non-surviving larvae, as well as the latency to spin cocoons during that period. Nests were checked every weekday for signs of spinning or death and the date of each was recorded. In addition to weighing larvae initially, we assessed final cocoon weight by weighing cocoons 21 days after the date larvae started spinning.

2.1. Statistical analyses

All analyses were performed in R version 3.5.1 (R Core Team 2018). We compared survival of larvae (as a binary Y/N variable) among

treatments using generalized linear models with binomial errors and time to death using survival regression, censored at 70 days for surviving larvae, using the *survival* package (Therneau 2015). We used one-way ANOVAs to compare cocoon weights and latency to spin cocoons (note that we did not use survival analysis for latency because only 1 surviving larva failed to spin a cocoon, so in practice the data were not censored). In all analyses, we used planned orthogonal contrasts, the formally correct and preferable approach when there are specific *a priori* predictions (see Introduction) (Day and Quinn 1989). Planned contrasts perform only those targeted orthogonal comparisons among treatment groups of interest that correspond to biological predictions. Contrasts and their corresponding predictions are given in Table II.

3. RESULTS

3.1. Survival

There were only marginal differences among treatment groups in the probability of surviving to pupation (GLM with binomial errors, $\chi^2_4=9.085$, $p = 0.059$). Survival to pupation was very high on both full pollen balls (group A) and on agar-diluted reduced pollen balls (group C), while the undiluted reduced pollen balls and the two sporopollenin-diluted diets (groups B, D and E, respectively) all resulted in marginally lower survival (Fig. 1).

Table II. Planned contrasts among diets used in the analyses in this study, and how they relate to the predictions set out in the introduction

Prediction (see Introduction)	Contrast	Groups compared	Specific question
Improved survival and fitness on full diets compared to reduced diets	1	A vs. pooled B-E	Are full diets different from reduced diets?
P1 and P2. Survival or fitness on diets diluted with sporopollenin or agar should (P1) be <i>similar or slightly lower</i> than on the same amount of undiluted nutrients and (P2) should <i>not be higher</i>	2	B vs. pooled C-E	Are diluted diets different from diets containing the same amount of undiluted nutrients?
	3	C vs. pooled D-E	Are diets diluted with agar different from those diluted with sporopollenin?
P3. Diets diluted with crushed sporopollenin may have marginally higher mortality/lower fitness than those diluted with whole sporopollenin	4	D vs E	Are whole pollen exines different from crushed pollen exines?

Diets as in Table I.

Similarly, survival times were only marginally different among treatment groups (survival regression, $\chi^2_4=9.20$, $p=0.056$). Among larvae that died before pupation, survival ranged from 1 to 54 d. Larvae on full diets and agar-diluted reduced diets nearly all survived to pupation, while larvae fed undiluted reduced diets and sporopollenin-diluted reduced diets again survived marginally less long (Fig. 2).

3.2. Cocoon weight

Larvae in different diet treatments did not differ in weight at the outset of the experiment

(ANOVA, $F_{4, 65} = 1.541$, $p = 0.201$), weighing a mean \pm SE of 0.033 ± 0.002 g (range 0.014–0.118 g). However, diet treatment affected eventual cocoon weight 21 d after spinning (ANOVA, $F_{4,40}=4.37$, $p=0.005$, Fig. 3). Cocoon weight ranged from 0.038 to 0.211 g. Larvae raised on a full pollen ball were heavier than larvae raised on the reduced pollen ball treatments pooled together (Contrast 1, $t = -3.50$, $p = 0.001$). Larvae raised on undiluted reduced diets were heavier than those raised on the diluted diets, irrespective of diluent (Contrast 2, $t = -3.09$, $p = 0.003$). Contrasts among the diluted diets were not significant, indicating that larvae developing on these diets did not

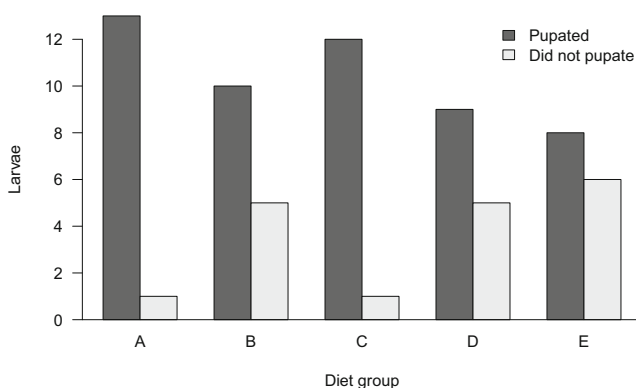


Figure 1. Number of larvae surviving to pupation in each treatment. Treatment groups as in Table I: **A** full diet, **B** reduced diet, **C** reduced diet diluted with agar, **D** reduced diet diluted with whole exines and **E** reduced diet diluted with crushed exines

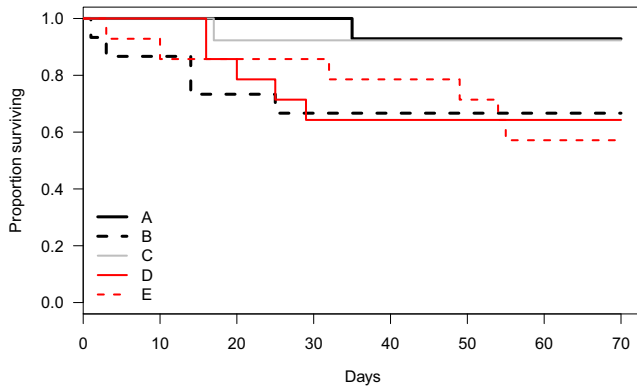


Figure 2. Survival curves for larvae in each treatment. Treatments in Table I: **A** full diet, **B** reduced diet, **C** reduced diet diluted with agar, **D** reduced diet diluted with whole exines and **E** reduced diet diluted with crushed exines

differ in weight (Contrast 3, $t = -0.75$, $p = 0.452$; Contrast 4, $t = -0.96$, $p = 0.342$).

3.3. Latency to spin cocoon

Diet treatment also affected the latency time before spinning a cocoon (i.e. pupating; ANOVA, $F_{4,48} = 3.81$, $p = 0.009$, Fig. 4). Latency time ranged from 13 to 49 days. Larvae in diluted treatment groups waited longer before spinning a cocoon than those in the undiluted reduced pollen ball treatment (Contrast 2, $t = 2.82$, $p = 0.006$), and

among diluted diets, larvae raised on sporopollenin-diluted diets waited longer before spinning cocoons than those on agar-diluted diets (Contrast 3, $t = 2.40$, $p = 0.02$). Other contrasts were not significant (Contrast 1, $t = 0.97$, $p = 0.34$; Contrast 4, $t = 1.26$, $p = 0.21$).

4. DISCUSSION

Given that sporopollenin is a normal constituent of bees' diets which they tend to excrete in relatively unmodified form (Suárez-Cervera et al.

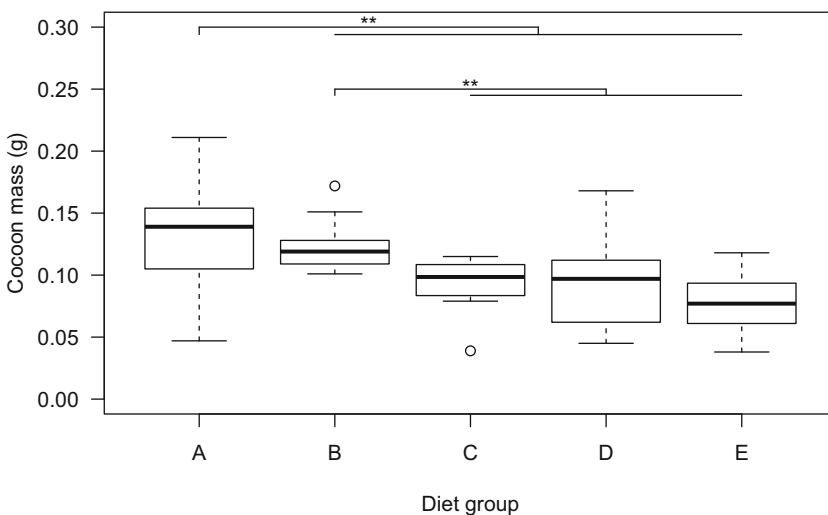


Figure 3. Cocoon weight (median \pm IQR [boxes], 95%CI [whiskers]) of larvae in each treatment. Treatments as in Table I: **A** full diet, **B** reduced diet, **C** reduced diet diluted with agar, **D** reduced diet diluted with whole exines and **E** reduced diet diluted with crushed exines. Significance bars indicate contrasts in Table II (other contrasts non-significant) (Key: $\rightarrow p < 0.05$, $** p < 0.01$, $*** p < 0.001$).

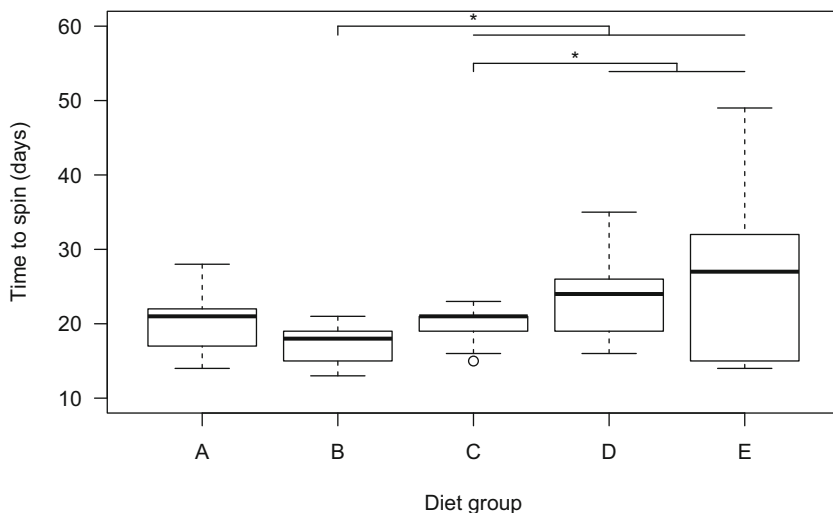


Figure 4. Latency to spin cocoon (median \pm IQR [boxes], 95%CI [whiskers]) for larvae in each treatment. Treatments as in Table I: **A** full diet, **B** reduced diet, **C** reduced diet diluted with agar, **D** reduced diet diluted with whole exines and **E** reduced diet diluted with crushed exines. Significance bars indicate contrasts in Table II (other contrasts non-significant) (Key: $\rightarrow p < 0.05$, $** p < 0.01$, $*** p < 0.001$)

1994; Roulston and Cane 2000; Peng and Dobson 1997), it is perhaps unsurprising that we should have found that sporopollenin was neither toxic nor nutritious to *Osmia* larvae. Instead, the patterns of growth and/or mortality we observed were similar to those seen in many other species consuming diets diluted with biologically inert, indigestible matter. Our study did not detect a change in mortality compared with undiluted diets, reflecting previous studies in many taxa (Raubenheimer and Simpson 1993; Lee et al. 2004; Solon-Biet et al. 2014), but individuals consuming diluted diets matured at smaller sizes (Fig. 3) and, in the case of sporopollenin, took longer to develop (Fig. 4) than those consuming undiluted food. Examples of this syndrome include fall armyworms (Wheeler and Slansky 1991), velvetbean caterpillars (Slansky and Wheeler 1991), African cotton leafworms (Lee et al. 2004) and diamondback moth caterpillars (Warbrick-Smith et al. 2009).

Our findings therefore suggest that sporopollenin (whole or crushed) is a suitable dilution agent in artificial diets for bee larvae. This should facilitate studies that require control of nutrient density within artificial diets for bee larvae and in particular studies conducted under the GF.

Among our samples, diets diluted with crushed exines did not have significantly higher mortality, lower cocoon mass or latency to spin cocoons than those diluted with whole exines. Any difference in processing costs as a result of crushing the exines, if existent, may therefore have been too small for our study to detect. Nevertheless we should point out that we do not yet know whether bees can digest crushed pollen exines, whereas whole exines tend to be excreted intact (Suárez-Cervera et al. 1994). Any potential digestion of sporopollenin may have possible future fitness consequences unmeasured by this study. Until these are quantified, or digestion of crushed exines is ruled out, we recommend researchers use whole exines in future studies.

The suitability of agar as a dilution agent we regard as less clear and requires further study. While agar was clearly not toxic to the larvae, marginal trends in our data leave open the possibility that it may have increased survival. In this study, bees on agar-diluted reduced diets appeared to survive just as well as those on a full diet, so it may be that they enjoy survival advantages over those on undiluted reduced pollen balls, even if these advantages were barely detectable (Figs. 1 and 2). Agar can provide carbon sources for

various bacterial and fungal growth (Payton et al. 1976); moreover, substances previously thought of as indigestible diluents have been shown to be digested and used by some subjects (e.g. fructan, Barbehenn et al. 2004). Note that if we retrospectively group diet C with the full diet (diet A), then the statistical contrast in mortality between (A + C) and the other diets with reduced nutrition (B + D + E) was statistically significant at the 0.01 level (GLM with binomial errors as described above).

We believe, however, that it is unlikely that larvae were digesting and using agar. While we are not aware of studies of the excretion of agar by insects, agar alone did not sustain development in *Agria* flies (House 1969), and pupal mass was reduced in the tephritid *Dacus oleae* when (Tsitsipis 1977) above a minimum threshold that prevented drowning in the liquid diet (Tsitsipis 1977). Agar can affect digestibility of other substances such as protein, typically negatively (e.g. Harmuth-Hoene and Schwerdtfeger 1979). Nevertheless, addition of agar to a normal diet increased population growth rate in *Tribolium* beetles (Sial et al. 2017), so we cannot rule out similar effects in bees. Conceivably, impurities in the agar may have provided a resource that enhanced survival (Dadd 2003 and references therein). Finally, the agar may have provided an additional water source for developing bee larvae over the other reduced diets, which may have prolonged survival without affecting growth—consistent with the marginal trend we observed.

The observed pattern of smaller cocoon weights when feeding on a diluted diet (and prolonged latency to pupate, in the case of sporopollenin) might potentially be accounted for by two non-exclusive mechanisms (Martin and Van't Hof 1988). First, animals eating dilute diets typically display a compensatory feeding response, i.e. they eat more to compensate for dilution (e.g. Timmins et al. 1988). Yet, despite this compensatory feeding response, the animal may nevertheless ingest fewer nutrients on diluted diets, reducing pupal mass and prolonging development—typically because the dilution agent imposes volumetric constraints upon feeding (Lee et al. 2004). In this study, we did not assess compensatory feeding; our study subjects were provided with a fixed amount of food,

reflecting natural situations in which larvae are fed a pollen ball whose mass is determined by the parent. However, we note that we did observe compensatory feeding in a companion study that also used sporopollenin as a dilution agent, but where we constantly replenished the food (Austin and Gilbert 2020).

Second, the animal may incur specific physiological costs of ingesting and/or digesting excesses of the dilution agent, known as processing costs (Martin and Van't Hof 1988). Some species are able to accommodate a remarkable degree of dietary dilution via compensatory feeding without suffering processing costs (Raubenheimer and Simpson 1993). However, others are less tolerant of dilution of their normal diet and exhibit reduced pupal mass and extended development time as a result (Slansky and Wheeler 1991), particularly extreme specialists (Warbrick-Smith et al. 2009). The nature and extent of processing costs is highly species-dependent, illustrated by several studies of caterpillar feeding. In tobacco hornworms (*Manduca sexta*), diets diluted with cellulose took longer to eat and reduced the efficiency of nutrient absorption, caused in part by speeding the passage of food through the gut (Timmins et al. 1988). Similar reductions in efficiency of nutrient absorption were seen in African cotton leafworms (*Spodoptera littoralis*) consuming cellulose-diluted diets (Lee et al. 2004). However, in velvetbean caterpillars (*Anticarsia gemmatilis*), cellulose did not affect the efficiency of digestion or absorption of nutrients, while dilution with water actually increased it (Slansky and Wheeler 1991). In fall armyworms, the efficiency of nutrient absorption increased on diets diluted with both water (Slansky and Wheeler 1989) and cellulose (Wheeler and Slansky 1991). However, both fall armyworms (Wheeler and Slansky 1991) and velvetbean caterpillars (Slansky and Wheeler 1989) suffered a reduction in conversion efficiency of food into biomass when their diets were diluted, whereas in southern armyworm caterpillars (*S. eridania*), cellulose did not appear to affect conversion efficiency (Peterson et al. 1988). Especially in light of such wide variation in the nature of processing costs among species, it will require further research to determine whether either or both of these two physiological

mechanisms contribute to the patterns of cocoon mass and development we observed in *O. bicornis* in response to dietary dilution.

It might be thought surprising that cellulose should prove lethal to developing bees. In addition to sporopollenin, cellulose is also an important constituent of the pollen wall, as it forms the majority of the intine (Stanley and Linskens 1974) and therefore also forms a routine part of the diet of larval bees. Alongside sporopollenin, pollen-derived cellulose is also commonly excreted relatively unmodified by *Osmia* larvae, although in pollen species where the intine is thin, the intine may “disappear” in micrographs of faeces compared to those of undigested pollen (Suárez-Cervera et al. 1994, p. 203). In addition, intine-derived cellulose was apparently digested by the specialist *Chelostoma* feeding on pollen of its host *Ranunculus* (Peng and Dobson 1997). Naturally occurring pollen, including intine-derived cellulose, is clearly not harmful to bees. Why powdered cellulose might be uniformly lethal to *O. bicornis* and other bees is therefore a matter for further research. One possibility is that powdered cellulose is relatively hygroscopic compared with the intact form. Slansky & Wheeler (1991, p. 109) note that they would “expect” the two forms to behave substantially differently; Ruedenauer et al. (2016) speculate that powdered cellulose may cause dehydration or form plugs in the larval alimentary tract.

This study has focused on broad individual-level outcomes such as mortality and cocoon weight; we did not, for example, quantify nutrients ingested or excreted. A useful aim for future research would now be a more quantitative, mechanistic assessment of how larvae respond to increasing dilution of pollen with indigestible sporopollenin, including a full feeding budget of ingestion, digestion, nutrient absorption and conversion efficiency at different levels of dilution. Additionally, further work should include examination of larval faeces to assess degradation of the dilution agent. We note that larvae may also be able to employ additional post-ingestive mechanisms for regulating nutrient intake when faced with a diluted diet, such as selective storage or excretion (Telang et al. 2002; Jonas and Joern 2013).

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AUTHOR CONTRIBUTIONS

JDJG, AA and TB conceived the study. All authors designed the experiment. FT and SW collected all data. JDJG conducted the analyses and wrote the manuscript.

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General summary

Studies of nutrition in wild animals often require precise control of nutrients by diluting artificial diets with indigestible material. Although understanding the nutrition of bee larvae is extremely important, studies of bees using artificial diets are limited. Common dilution agents such as cellulose typically kill bee larvae outright, making studies involving dilution agents difficult. In this study, we address this problem by investigating the suitability of alternative dilution agents for use in artificial pollen diets for the larvae of *Osmia bicornis*, a commercially important solitary bee. The agents we looked at were sporopollenin (a relatively inert substance that forms the outer capsule of pollen, the bees' normal diet) and agar, a non-toxic jelly-like substance often used as a matrix on which to culture bacteria which is indigestible to most animals. We reared field-collected larvae on 5 diets: full pollen ball, reduced pollen ball, reduced pollen ball diluted with agar, reduced pollen ball

diluted with whole pollen capsules and reduced pollen ball diluted with crushed pollen capsules. Pollen capsules (whole or crushed) were neither toxic nor nutritious to *Osmia* larvae. Instead, larvae grew and survived fairly similarly to other species consuming diets diluted with inert, indigestible matter. Compared with undiluted diets, survival was slightly reduced, cocoons were slightly smaller and development was slightly delayed. This suggests sporopollenin is not toxic but imposes “processing costs”, i.e. it somehow makes the whole diet harder to digest. Agar may be less good as a dilution agent, as larvae appeared to survive marginally better on agar-diluted diets than on undiluted reduced diets. We could not determine in this study exactly which physiological mechanisms led to the patterns of survival and growth that we saw. Nevertheless, our findings suggest that sporopollenin is a suitable dietary dilution agent for *Osmia* larvae, and should lay the foundation for more studies involving artificial diets for bee larvae.

La sporopollénine comme agent de dilution dans les régimes alimentaires artificiels pour les abeilles solitaires.

alimentation artificielle / agent de dilution / abeille solitaire / écologie nutritionnelle.

Sporopollenin als ein Verdünnungsmittel in künstliche Diäten für Solitärbiene Künstliche.

Diät/ Verdünnungsmittel / Solitärbiene / Ernährungsökologie.

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