Lower pollen nutritional quality delays nest building and egg laying in *Bombus terrestris audax* micro-colonies leading to reduced biomass gain

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Short Title: Pollen quality and nest initiation in *Bombus*

Abstract The performance of *Bombus terrestris* micro-colonies fed five diets differing in pollen species composition and level of nine essential amino acids (EAA; leucine, lysine, valine, arginine, isoleucine, phenylalanine, threonine, histidine, methionine), was assessed for 37 days by recording total biomass gain, nest building initiation, brood production (eggs, small and large larvae, pupae, drones), nectar and pollen consumption. Stronger colony performance was linked to higher amino acid levels but no consistent differences in biomass gain were recorded between mono- and poly-species diets. Poorest performance occurred in micro-colonies offered pure oilseed rape (OSR) pollen which contained the lowest EAA levels. Reduced micro-colony development (delayed nest initiation and lower brood production), was related to OSR proportion in the diet and lower EAA levels. Results are discussed in relation to selection of plant species in the design of habitats to promote bee populations.

Key Words: *Bombus terrestris audax*, nutrition, amino acid profile, pollen mixing, colony performance

1. INTRODUCTION

Bumblebees (*Bombus* spp.) are a key group of highly efficient wild pollinators, which forage on a wide variety of flowers and plants (Reynolds and Fenster 2008). Foraging behaviour of individual workers is highly selective (Harmon-Threatt et al. 2017) and it has been proposed that the nutritional content of pollen affects flower selection (Nicolson 2011). Individual foragers of *Bombus terrestris* maintain a degree of floral consistency (Goulson 2009) while differences in preferences between foragers result in the utilization of

47 a wider range of plant species at the colony level (Free 1970). The resultant poly-floral
48 larval diets have been reported to strengthen colony development (Baloglu and Gurel
49 2015).
50 The importance of pollen diversity is widely recognized and habitat management schemes
51 have focused on increasing floral diversity to enhance pollinator populations (Carvell et al.
52 2006). In the UK, environmental stewardship schemes promote a range of species mixes
53 and sowing options to enhance botanical diversity of arable landscapes (e.g. Carvell et al.
54 2007), but further work is required to optimize their impact on wild bee colonies (Albrecht
55 et al. 2007).
56 The ratio and level of the major nutritional components of pollen, including proteins, amino
57 acids, lipids (including phytosterols), carbohydrates, vitamins, carotenoids and flavonoids
58 are related to its nutritional value for bumblebees, and the degree to which pollen meets the
59 larval requirements varies between plant species (Filipiak 2019; Vanderplanck et al. 2014;
60 Somme et al. 2015). Bumblebees have been shown to favour more protein-rich pollens
61 (Leonhardt and Blüthgen 2012; Kitaoka and Nieh 2009) but amino acid composition is
62 thought to be a better determinate of pollen quality for bees than total protein content; for
63 example, *Eucalyptus* spp. pollen has a high protein content but is deficient in the essential
64 amino acid isoleucine (Nicolson 2011).
65 Assessing the role of selective foraging on colony success relies on an understanding of the
66 effect of pollen diet on bumblebee colony performance. Colony development and brood
67 production, bee physiology and immune system function have all been studied (Dance et al.
68 2017), with colony fitness being assessed using parameters such as egg production, larval
69 weight, larval ejection, adult body size, adult longevity, and the number of active foragers

(Tasei and Aupinel 2008a; Kitaoka and Nieh 2009; Kriesell et al. 2017; Vanderplanck et al. 2014). Colony responses to defined pollen diets may be investigated using laboratory based micro-colony experiments to identify response parameters that can subsequently be verified in queen-right colonies (Génissel et al. 2002; Tasei and Aupinel 2008b). Micro-colony studies suggest that mixed-species pollen diets are more favourable than mono-species diets (Génissel et al. 2002; Vanderplanck et al. 2014), and as amino acid content of pollen is thought to be a primary driver of bumblebee colony success (Moerman et al. 2017), pollen diversity may increase the potential for both essential amino acids and other essential nutritional components being included. Studies in bumblebees are limited, however, and further work comparing nutritional content of pollen diets with colony performance are required to identify specific biological mechanisms leading to colony level outcomes. This study investigates the effect of five defined pollen diets on the development of *B. terrestris audax* micro-colonies to test the hypothesis that colony performance is defined, in part, by their amino acid profiles and the diversity of pollen species included.

2. MATERIALS AND METHODS

Queenless *B. terrestris audax* micro-colonies were established using worker bees from stock colonies obtained from Agralan Ltd., Swindon, UK (originating from Biobest®, Belgium). Prior to use, colonies were fed *ad libitum* on Biobest standard pollen mix and proprietary liquid sugar solution and maintained for a 7 day acclimation period in a CE room at 27°C, 65% RH, with an 8:16 Light-Dark cycle (Elston et al. 2013).

Micro-colony arenas (modified from Elston et al. 2013) consisted of 500 ml open-topped - plastic containers (11 cm diameter × 7 cm deep), closed with muslin mesh. The base of

each arena was lined with filter paper and a small ball of cotton wool was added to encourage nest building.

Artificial nectar solution (60%, w/v Rowse Pure Honey and water) was offered *ad libitum* to micro-colonies in lidded plastic feeding tubes (length = 10cm, Φ = 1cm, with an upward-facing feeding hole (Φ = 2mm) pierced at one end) inserted at a 30° angle through a hole in the side of the colony cages. Pollen was offered using a similar tube but with a 10 x 20 mm feeding trough, inserted horizontally at 180° in each direction from the nectar tube.

Both feeding tubes were weighed, re-filled and re-weighed at 2 day intervals ensuring that a minimum of 2g of pollen and 8 ml nectar where available throughout the experiment. Three worker bees were transferred from stock colonies to each micro-colony cage at the start of the experiment, bees that remained inactive for 1 hour after transfer were replaced.

2.1. Treatments

One of five commercially sourced pollen species or pollen mixes were offered to micro-colonies:

Controls: Biobest standard pollen mix (from Biobest®, Belgium; "Standard pollen mix").

Experimental treatments:

T1 - Organic Chestnut Pollen mix (TOCA®, Spain; *Castanea sativa;* "Chestnut pollen mix"),

T2 - Pure *Camellia* pollen (Simianshan®, China; *Camellia*. Spp."; "Camellia"),

T3 - Pure oilseed rape pollen (Simianshan®; China; *Brassica napus*; "OSR"),

T4 - A 50%:50% mixture of Biobest standard pollen mix and pure OSR pollen ("Standard pollen/OSR").

All pollen treatments were homogenised using a wet and dry grinder (Andrew James Ltd., UK), and stored at -20°C until used in the experiment. Treatments were replicated 15-18 times. The experiment was run under the conditions used during acclimation and was terminated after 37 days (by which point drone production had been recorded in all treatments).

2.2. Palynological analysis

Three sample slides were prepared from each treatment for pollen composition analysis, using the method of Moore et al. (1991). Pollen identification was carried out at 400× magnification using a Microtec compound microscope (TEC Microscopes Ltd., UK). A minimum of 50 grains selected at random from each slide, were identified to at least genus (Moore et al. 1991) and percentage contribution of each genus/species to the sample was determined. Pollen grains that could not be identified were recorded as 'unknown'.

2.3. Amino acid analysis

Sub-samples of the homogenised pollen mixes from each treatment were analysed (Alta Bioscience, Birmingham) to determine amino acid content according to European Pharmacopoeia methodology (https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition); this is an ISO 17025:2005-accredited method with a limit of quantification of 5 nmol. This method reflects the total sum of amino acids (protein incorporated and free in solution), excluding tryptophan and cysteine/cystine (which are usually lost during acid hydrolysis) and creatine and creatinine (which cannot be analysed using this method). The

results were presented in two groups, essential amino acids, which must be obtained from the diet, and non-essential amino acids, which can be supplemented by the diet.

2.4. Assessments

2.4.1. Mortality

Mortality (if any) in each micro-colony was recorded at the end of each 2-day period and dead bees were removed but not replaced.

2.4.2. Nectar and pollen consumption

Consumption of nectar and pollen by each micro-colony was calculated from the difference between feeder weight at the start and end of each 2-day period and expressed as mean consumption (g) per bee (taking account of recorded mortality).

2.4.3. Nest building

Each micro-colony was observed at the end of each 2-day assessment period and the first nest building activity (either wax cell or honey pot construction) recorded.

2.4.4. Final micro-colony performance

After the 37-day experimental period, micro-colonies were euthanized by freezing at -20°C for 24-hours. Nests (including all the wax material and brood inside) were weighed and dissected, and the number of eggs, small larvae (<0.8 cm across when curled), large larvae and pupae recorded. The number of drones produced were counted and weighed. The sum

of the drone weight and nest weight (including immature bees) was recorded as 'colony biomass gain'.

2.5. Statistical analysis

Statistical analysis was conducted using R Studio 0.99.903 (R Studio Team 2015). All data was checked for normality and Log or sqrt transformations applied where necessary. Factor reduction was conducted following normal conventions, allowing for the removal of non-significant terms and interactions in order to reach the minimum adequate model for all statistical tests conducted (Crawley 2013).

2.5.1. Pollen amino acid composition

Square root transformation was applied to normalise data for total amino acid (TAA) content, total non-essential amino acids (NAA) and total essential amino acids (EAA) of each pollen treatment (g/100g) prior to application of ANOVA. Tukey post-hoc tests were used to confirm where significant differences occurred between treatments.

2.5.2. Nectar and pollen consumption

Data on consumption of nectar and pollen were subjected to square root and log transformations respectively to meet assumptions of normality. The effects of treatments on nectar and pollen consumption were analysed using repeated measures ANOVA and Tukey's post-hoc test to confirm where significant differences occurred.

2.5.3. Nest initiation

Time before nest initiation (first nest building activity) was compared between treatments using generalized linear model (GLM) with binomial error structure.

2.5.4. Worker mortality and brood production data

The number of dead workers, eggs, larvae (both early and late instars), pupae and drones present were compared between treatments using GLM with Poisson error distribution, and quasi-Poisson error distribution where data was over-dispersed.

2.5.5. Colony biomass gain

The weight gain of the colony in each treatment was analysed using repeated measures ANOVA. Turkey's post-hoc test was used to confirm where significant differences occurred.

3. RESULTS

3.1. Palynological analysis

Sweet chestnut pollen (*Castanea sativa*) represented 65.5% of the grains identified from pollen marketed as "Organic Chestnut Pollen", with the remainder dominated by *Prunus* spp., *Lotus corniculatus* and *B. napus* (Table I). These species also constituted 51.2% of the commercially sourced Standard pollen mix which included eight different genera. Camellia and OSR treatments were found to be pure.

3.2. Pollen amino acid composition

3.2.1. Total amino acid content

There was a significant difference between TAA content of treatments (F = 126.3, d.f. = 4,10, p <0.001; Figure 1a). Tukey post-hoc tests confirmed that TAA was higher in the Camellia pollen treatment than in all other treatments (p <0.001). The OSR treatment had a significantly lower TAA content than all other treatments (p <0.001), but no difference was recorded between the Chestnut pollen mix, Standard pollen mix and Standard pollen/OSR pollen mix (p >0.05).

3.2.2. Total non-essential amino acid

A significant difference between the total NAA content of treatments was identified (F = 94.95, d.f. = 4, 10, p <0.001). Camellia pollen had higher levels of NAA than all other treatments (p <0.001), and OSR lower levels than both the Chestnut pollen mix and the Standard pollen mix treatments (p <0.001).

3.2.3. Total essential amino acids

Significant differences were also recorded between the total EAA content of the pollen treatments (F = 112.7, d.f. = 4, 10, p <0.001; Figure 1b). Tukey post-hoc tests confirmed that Camellia pollen had higher levels of EAA than all other treatments (p <0.001), with OSR having lower levels than the other treatments (p <0.001). The Standard pollen mix had higher levels of EAA than the Chestnut pollen mix (p <0.05) and the Standard pollen/ OSR mix (p <0.001).

3.2.4. Individual essential amino acids

There was a statistically significant interaction between treatment and the level of individual EAAs (F = 13.77, d.f. = 32, 90, p <0.001). Tukey post-hoc tests confirmed that significant differences in levels of individual EAAs occurred between treatments (Figure 2).

Leucine: Camellia pollen contained higher levels of leucine when compared to all other treatments (p <0.001). The Chestnut pollen mix and the Standard pollen mix both had higher levels than the Standard pollen / OSR mix (p <0.05, p <0.05) and OSR (p <0.001, p <0.05), but did not vary from each other (p >0.05). Finally, the Standard pollen/OSR mix had higher levels than OSR (p < 0.001).

Lysine: Camellia pollen and the Standard pollen mix had higher levels of lysine when compared to all other treatments (p <0.001). The Chestnut pollen mix contained higher levels than the Standard pollen/OSR mix (p <0.05) and OSR (p <0.001).

Valine: Camellia pollen had higher levels of valine (p <0.001) and OSR lower levels than all other treatments (p <0.01).

Arginine: Higher levels of arginine were recorded in the Camellia pollen treatment when compared to all other treatments (p <0.001). The Chestnut pollen mix and Standard pollen mix both had higher levels than were found in OSR (p <0.001) but did not vary from each other (p >0.05).

Isoleucine: Camellia pollen had higher levels of isoleucine compared to all other treatments (p <0.001). The Chestnut pollen mix and the Standard pollen mix both had higher levels than OSR (p <0.001) but did not vary from each other (p >0.05). The Standard pollen/OSR mix also had higher levels than OSR (p <0.05).

Phenylalanine: Camellia pollen had higher levels of phenylalanine compared to all other treatments (p <0.001). The Chestnut and the Standard pollen mix had higher levels than were recorded in OSR (p <0.001, p <0.001), with the Standard pollen mix also having higher levels than then Standard pollen / OSR mix (p <0.001).

Threonine: The Standard pollen mix and Camellia pollen had higher levels of threonine compared to all other treatments (p <0.001).

Histidine: OSR contained lower levels of histidine than were recorded in Camellia, the Chestnut pollen mix or the Standard pollen mix (p <0.05).

Methionine: Camellia pollen had higher levels of methionine compared all other treatments (p <0.001), with the exception of the Standard pollen mix (p >0.05). The Standard pollen mix was found to contain higher levels than were recorded in OSR (p <0.01).

3.3. Worker mortality

Mortality of workers was low (6.3%) and GLM found no significant differences between treatments (p >0.05).

3.4. Consumption of honey solution

Repeated measures ANOVA showed that consumption per bee varied with day (F = 14.55, d.f. = 1, 943, p <0.001), but was not affected by treatment (F = 1.97, d.f. = 4, 74, p >0.05).

3.5. Pollen consumption

Repeated measures ANOVA indicated that consumption per bee varied with both treatment (F = 12.11, d.f. = 4, 69, p <0.001) and day (F = 15.55, d.f. = 1, 1085, p <0.001). No interaction between treatment and day was found (F = 2.33, d.f. = 4, 1338, p <0.05). A significantly higher weight of pollen was consumed when bees were offered the Standard pollen or Chestnut pollen mixes than when the Standard pollen/OSR, Camellia or OSR pollen were available (Table II).

3.6. Micro-colony biomass gain

Micro-colony biomass gain over the 37 days of the experiment varied significantly between treatments (F = 5.81, d.f. = 4, 74, p <0.001; Figure 3). Tukey post-hoc analyses confirmed that lower colony biomass was recorded in the pure OSR and Standard pollen/OSR mix treatments when compared to that recorded in the Chestnut pollen treatment which attained the highest biomass gain (p <0.01, p <0.01 respectively). Biomass gain in the Camelia and Standard pollen mix treatments were not significantly different and were lower (p <0.05) than in the Chestnut pollen mix, and higher (P <0.01) than in the Standard pollen mix or OSR treatments.

297 **3.7. Components of biomass gain**

298

299 ***3.7.1. Initiation of nest building***

300 The day on which nest building commenced in individual micro-colonies varied between
301 treatments (Figure 4). During the creation of the minimum adequate model no interaction
302 between day and treatment was found and so 'day' was removed from the model, although
303 overall a GLM with binomial error structure showed that the proportion of nesting micro-
304 colonies increased with time ($z = 14.95$, d.f. = 1419, $p < 0.001$).

305 More than 90% of micro-colonies in the Camellia and Chestnut pollen treatments had
306 initiated nest building by days 7 and 11 respectively, with all having done so earlier (days
307 21 and 11) than in other treatments. Most (90%) of micro-colonies in the Standard pollen
308 mix treatment had commenced nest building by day 27, with all having done so by day 31.
309 All micro-colonies offered the pure OSR treatment displayed nest building activity by day
310 33, but only 92% of micro-colonies in the Standard pollen/OSR mix treatment had initiated
311 nest building after 37 days (Figure 4).

312 The Camellia and Chestnut treatments did not differ significantly and were combined into a
313 single factor for analysis, these treatments had the earliest timing of nest initiation and thus
314 formed the intercept for analysis.

315 The Standard mix showed the second highest timing of initiation ($z = -3.19$, d.f. = 1419, p
316 < 0.01). The standard pollen/OSR mix and pure OSR pollen treatments did not differ, were
317 combined, and displayed later nest initiation than all other treatments ($z = -8.29$, d.f. = 1419,
318 $p < 0.001$).

319

3.7.2. Brood production

For the egg count, total larvae and total small larvae analyses, Standard pollen mix, Chestnut mix, Camelia and OSR were found not to differ and were combined into a single factor. During analysis of the mature brood data, Standard pollen mix, Chestnut mix and Camelia treatments where found not to differ and were also combined into a single factor.

Significantly more eggs were recorded in Standard pollen/OSR mix (in which later nest initiation had also been recorded), than in all other treatments (Figure 5a; $t = 3.64$, d.f. = 77, $p < 0.001$).

The total number of larvae (small + large larvae) found in the micro-colonies was lower in the Standard pollen/OSR mix treatment (Figure 5b; $t = -2.46$, d.f. = 77, $p < 0.05$) than in the other treatments. In addition, significantly fewer small larvae, were recorded in the Standard pollen/OSR mix treatment than in other treatments (Figure 5c; $t=-2.34$, d.f. = 77, $p < 0.05$).

Significantly fewer older brood (total number of large larvae, pupae and drones) were found in nests from both the Standard pollen/OSR mix treatment (Figure 5d; $t = -2.70$, d.f. = 76, $p < 0.01$) and the treatment fed pure OSR pollen ($t = -2.54$, d.f. = 76, $p < 0.05$), the two treatments displaying the latest nest initiation

4. DISCUSSION

339 Pollen represents the only protein source for brood of some pollinator species (Roulston
340 and Cane 2000). Larval diets containing diverse pollen species can favour bumblebee
341 colony development (Génissel et al. 2002; Vanderplanck et al. 2014), and nutrient content
342 (including amino acids) may provide a mechanistic basis for this observation (Moerman et
343 al. 2017). Kriesell et al. (2017) reported wide variation in amino acid content of pollen
344 species recovered from individual pollen loads of foraging bumblebees, but lower
345 variability in EEA content between loads, suggesting selective foraging may result in
346 improved nutritional quality of diets fed to larvae. Establishment of requirements for
347 important nutritional components such as amino acids will support selection, or directive
348 breeding, of plants used in habitats designed to promote pollinators.
349 This study investigated the impact of nutritionally diverse pollen sources on performance of
350 queenless *B. terrestris* micro-colonies; pollens utilised were devoid of morphological floral
351 traits that would impact the results (Westerkamp and Claβen-Bockhoff 2007). Nest
352 initiation and brood production was successful across treatments and all pollen sources
353 were utilised.
354 Low mortality of worker bees (6.3%) occurred across all treatments, honey solution
355 consumption did not differ between treatments, and both were similar to levels recorded in
356 other studies (Elston et al. 2013) implying that all diets offered at least the minimum
357 required nutrition for colony growth. Pollen consumption, however, varied significantly
358 with both time and treatment, possibly a response to nutrient content.
359 Micro-colonies offered the Chestnut pollen mix (primarily 4 genera; 65.5% sweet chestnut)
360 achieved the highest colony biomass gain, followed by pure Camellia pollen, and the
361 Standard pollen mix (8 genera; 27.6% OSR). The lowest biomass gains were recorded from

362 micro-colonies fed either pure OSR pollen, or the Standard pollen mix combined with OSR
363 pollen (8 genera; 63.8% OSR). Thus, although the highest growth rate was associated with
364 a diverse pollen source, no simple correlation between diverse pollen diets and biomass
365 gain was identified. Instead, evidence was obtained that when diets contained high
366 proportions of OSR pollen there was a depression of biomass gain. Thus, species
367 composition, as well as diversity was important.
368 There were significant differences in the timing of nest initiation (and associated egg
369 production) following establishment of micro-colonies. Colonies offered a diet of the
370 Chestnut pollen mix or pure *Camellia* pollen commenced nest building activities earlier
371 than those offered the Standard pollen mix. Micro-colonies offered higher proportions of
372 OSR pollen took significantly longer to initiate nest building, possibly reflecting egg
373 production in holometabolous insects being a nutrient limited process (Wheeler, 1996;
374 Hoover et al. 2006). Responsiveness of bumblebee queens initiating nests in spring is
375 important as it can promote synchrony with periods of optimal floral resource availability
376 (Geib et al. 2015); nest enlargement to accommodate eggs/larvae is undertaken by other
377 castes (Michener 2007).
378 Brood recorded at the end of the experiment reflected similar responses to diet. Micro-
379 colonies offered pure OSR produced fewer older brood. Significantly more eggs, but fewer
380 larvae and older brood, were also recorded in the Standard pollen/OSR mix treatment than
381 in other treatments, suggesting that later nest building resulted in a later egg laying/hatch in
382 these treatments. Consequently, micro-colonies offered pollen with a high proportion of
383 OSR had fewer older brood (total number of large larvae, pupae and drones) and lower

384 colony biomass at the end of the experiment than those offered diets with lower levels of
385 OSR pollen.
386 Previous micro-colony studies of the effect of nutrition on colony development rarely
387 consider potential effects on nest building activity (Génissel et al. 2002). Many have
388 terminated experiments earlier than in the current work (thus data on later colony
389 development were not collected) (Tasei and Aupinel 2008b) or encountered both oophagy
390 and larval ejection, with associated difficulties when interpreting results (Génissel et al.
391 2002). This study indicates that assessment of colony success should not rely on the
392 presence of larvae alone but in addition consider a range of other parameters. In this
393 respect, total biomass gain may be a comprehensive parameter reflecting overall brood
394 production or growth of the colony.
395 Carbohydrates, lipids, protein, sterols, vitamins, minerals, and starch have all been
396 implicated as essential nutrients for honey bees but amino acid composition is most often
397 used to assess nutritional quality (Cook et al. 2003). The suggestion that amino acid content
398 of larval pollen resources is a key factor determining bumblebee colony performance
399 (Moerman et al. 2017) may offer a partial mechanism explaining the results obtained in this
400 study.
401 Significant differences between treatments in the levels of nine of the amino acids reported
402 as essential for honeybees (DeGroot 1953) were recorded in the current study. The lowest
403 level of each was found in the pure OSR pollen with significantly higher levels in pollens
404 consumed by the highest performing colonies (Chestnut mix, Camellia, Standard mix).
405 Cook et al. (2003) reported that honeybees preferentially foraged on oilseed rape compared
406 to field bean (*Vicia faba*) pollen reflecting higher levels of valine, leucine and isoleucine.

Although bumblebee micro-colonies performed least well when fed on OSR pollen in the current study, it is notable that these three essential amino acids were present in lower quantities than in the other pollen diets investigated. In addition, when the three diets each containing different proportions of OSR pollen were offered to the bumblebee micro-colonies, those offered the diet containing the lowest proportion of OSR (thus the highest levels of the EAA) performed significantly better than those with the pure OSR pollen. This supports the suggestion that polylectic bees such as bumblebees may ameliorate the impact of nutritional deficiencies of some pollens by collecting from multiple species. Bumblebees frequently exploit flowers from several plant species in single foraging flights (Leonhardt and Blüthgen 2012; Kriesell et al. 2017), and 2-8 species have been recorded in pollen loads taken from *Bombus lucorum* and *Bombus pascuorum* (Free 1970).

Previous studies suggest that species-rich habitats offer better resources than habitats containing lower floral diversity (Dance et al. 2017; Hass et al. 2018). This is thought to result from potential nutritional limitation of mono-species pollens, whereas poly-floral pollens may be nutritionally-complimentary to each other. Such theory has been widely accepted and diversity has been a key factor when creating and promoting pollinator-friendly land use, such as in environmental stewardship schemes. This study provides further data confirming the principle, and quantifying the impact on colony success using a wider range of colony characteristics than employed in most previous work, in combination with quantification of levels of total amino acids, total and individual EAA, and total non-EEA in dietary pollen. It was concluded that, in each case, colony performance was linked (in part) to amino acid content. The contention that nutritional deficiencies in individual pollen species could be ameliorated by selected poly-floral larval diets was supported.

Future work should concentrate on analysis of key nutritional components of pollen, to support more informed selection of plant species for stewardship schemes designed to increase bumble bee abundance.

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AUTHORS CONTRIBUTION:

JR, KW conceived the ideas and collected data; JR, HT, KW, designed methodology; JR, AC, KW analysed the data; all authors contributed to drafting the manuscript.

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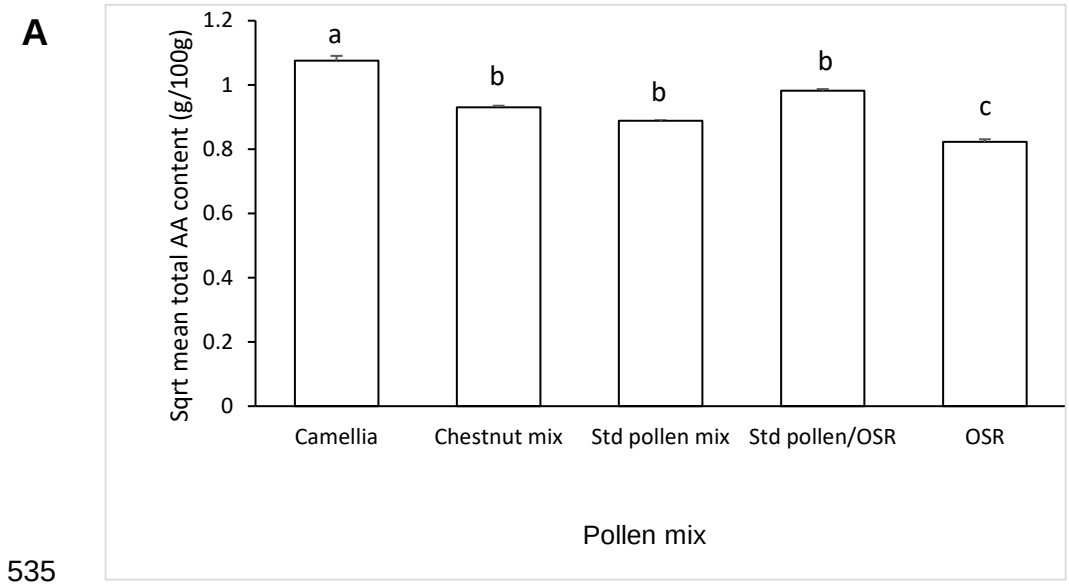
Figures



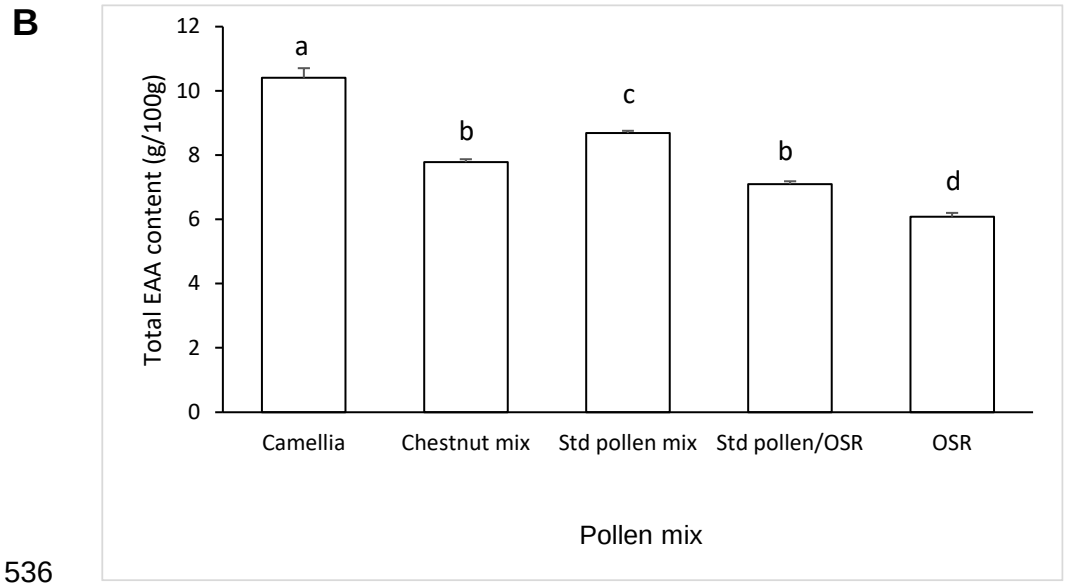


Figure 1. Amino acid content of the five pollen mixes (g/100g) - (a) Total amino acid (AA), (b) Total essential amino acid (EAA) used in treatments. Mean (± S.E.) of three samples from each treatment. Bars with the same letter are not significantly different ($p > 0.05$).

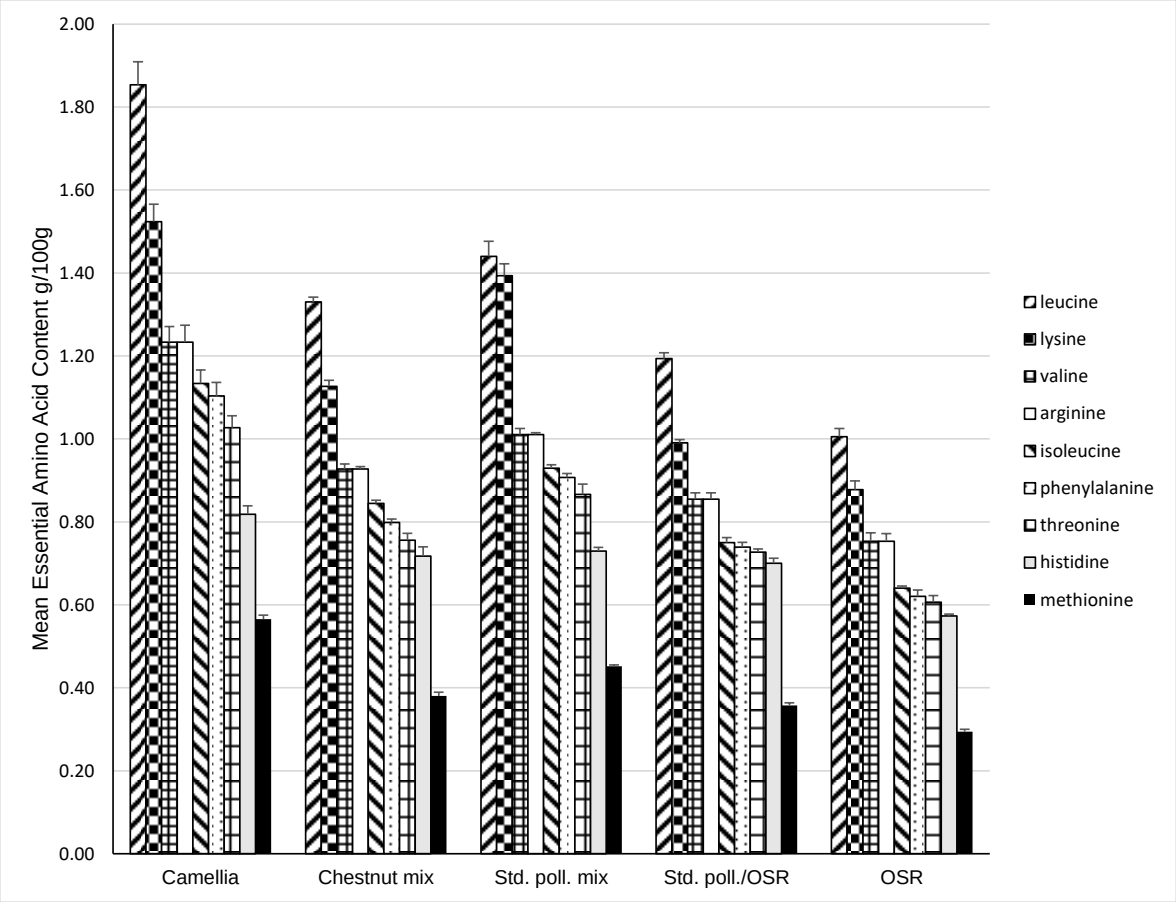
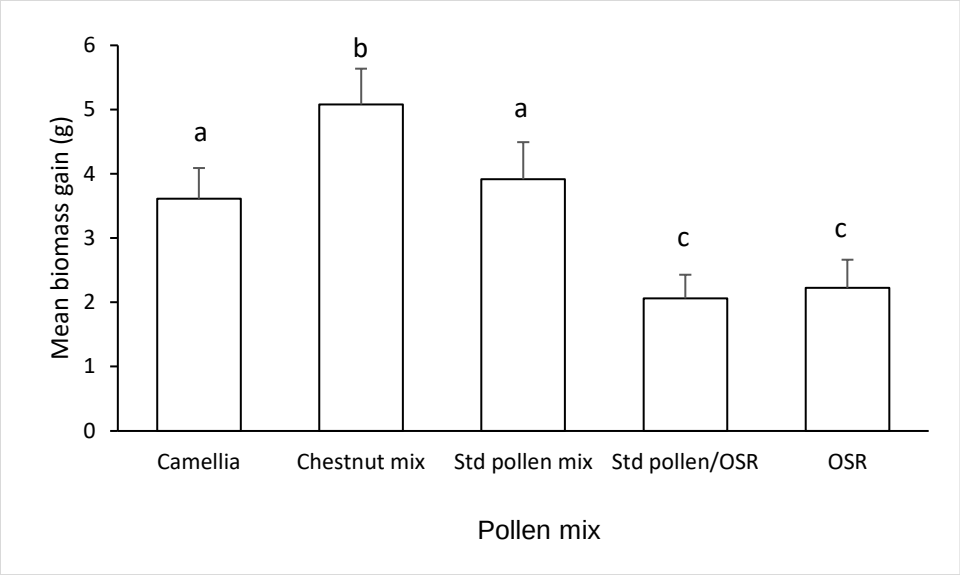


Figure 2. The levels of nine essential amino acids in the five pollen mixes used in treatments. Mean (± S.E.) of assessments (g/100g) of three samples from each treatment.



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552 **Figure 3.** Mean (± S.E.) biomass gain (g) of micro-colonies exposed to treatments offered
553 five pollen mixes, over the 37 days of the experiment. Bars with the same letter are not
554 significantly different (p >0.05).

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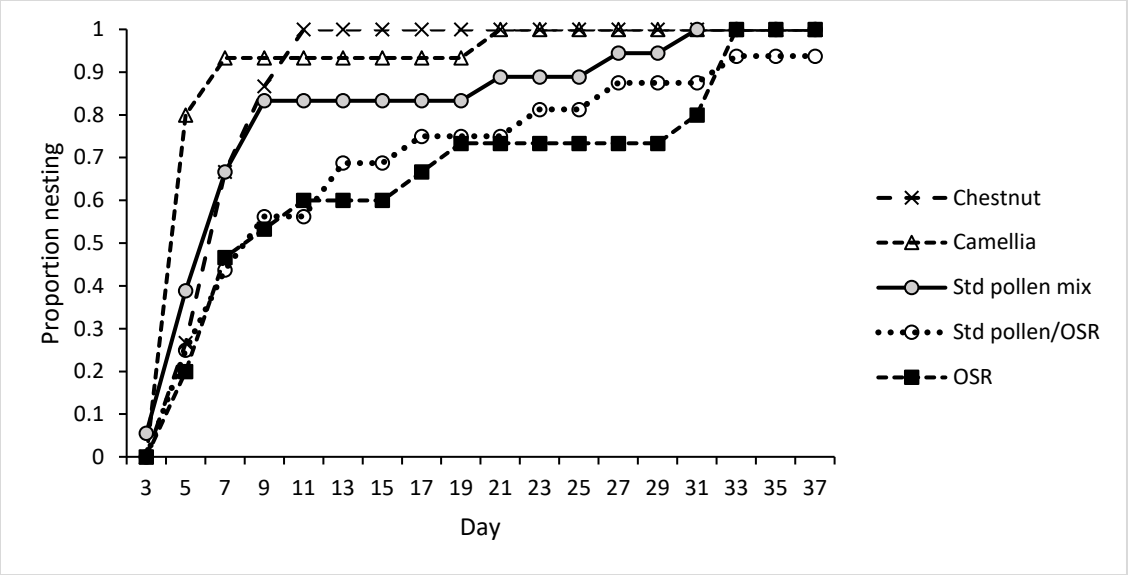

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560 **Figure 4.** Cumulative proportion of micro-colonies displaying nest building activity on
561 sequential assessment days, when offered five different pollen mixes.

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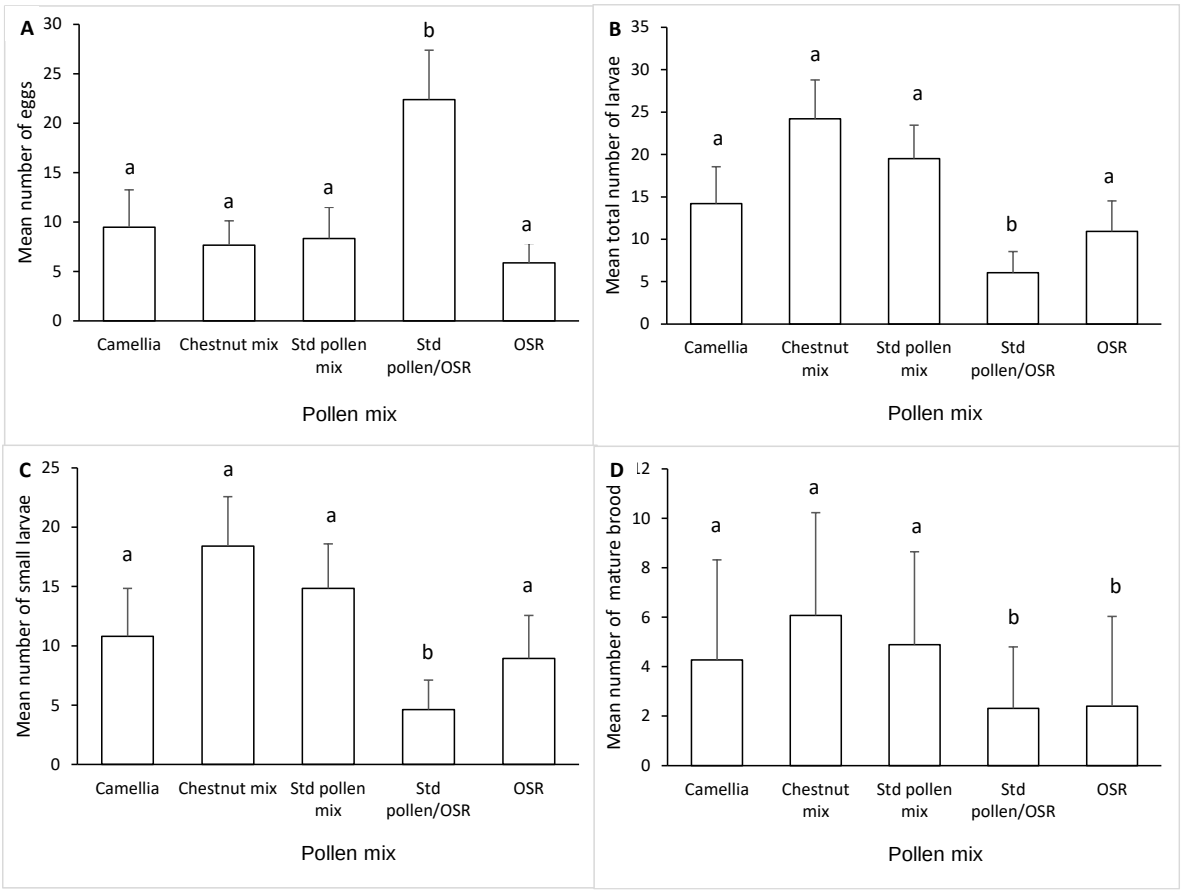

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565 **Figure 5.** Mean (± S.E.) number of brood per micro-colony recorded (day 37 of the
566 experiment) in treatments offered different commercially sourced pollen mixtures. Mean
567 number of: A = of eggs, B = total larvae (small + Large), C = small larvae, and D = older
568 "brood" (including large larvae, pupae and drones). Error bars show ±1 standard error of
569 the mean, calculated from linear models.

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Tables

Table I. Palynological analysis of the commercially sourced pollens used in the experimental treatments. The percentage of pollen grains for each species is a mean of three samples.

Commercial Name	Pollen Species	% Pollen Grains
Standard Pollen Mix	*Brassica napus*	27.6
	Salix spp.	18.8
	Taraxacum officinale	16.3
	Prunus spp.	14.2
	Ranunculus repens	8.1
	Pinus spp.	5.6
	Castanea sativa	5.2
	Lotus corniculatus	4.2
Camellia	*Camellia* spp.	100.0
Chestnut Pollen mix	*Castanea sativa*	65.5
	Prunus spp.	17.2
	Lotus corniculatus	9.5
	Brassica napus	7.0
	Unknown	0.8
Oilseed rape	*Brassica napus*	100.0

584 **Table II.** Post-hoc *t*-test analysis of weight of pollen consumed by micro-colonies offered
585 different pollen mixes.

T- test results

	Standard pollen	Chestnut	Camellia	Oilseed rape
Chestnut pollen mix	*p* >0.5	-	-	-
Camellia	***p* <0.01**	***p* <0.001**	-	-
Oilseed rape	***p* <0.001**	***p* <0.001**	*p* >0.05	-
Standard mix / OSR	***p* <0.001**	***p* <0.001**	*p* >0.05	*p* >0.05

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