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Pollination Efficiency of Native Bees and Honeybees to Apple in Norway

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Master of Science in Biology

I beg a million pardons. Abuse me to any degree but forgive me: it is all an illusion about the bees. I do so hope you have not wasted any time for my stupid blunder – I hate myself, I hate clover and I hate bees.

Charles Darwin,
Letter to John Lubbock, 1862

Abstract

Crop pollination is traditionally managed with the European honeybee (*Apis mellifera*). However, the supply of honeybees may not be sufficient to meet the increasing global pollination demands and beekeepers are met with increased challenges with honeybee health. In addition, accumulating evidence shows that traditional guidelines for crop pollination are not promoting optimal yields. Consequently, new management solutions for pollination are warranted and interest in wild bees as crop pollinators has emerged. To determine the differences between pollinator genera in single-visit pollination efficiency on apple, pollinators were allowed to forage on previously unvisited flowers, and the pollinated pistils analyzed for pollen quantity and germinated pollen tubes. Solitary bees and bumblebees had a higher pollination efficiency to apple compared with honeybees; they had higher single-visit pollen deposition effectiveness and deposited more pollen grains that formed pollen tubes. However, honeybee abundance is usually much higher compared with the native bees. Therefore, increasing the abundance of native bees with targeted management efforts has the potential to increase overall pollination services to apple. Moreover, it appears that the relative importance of functional traits in apple pollination varies among bee genera; traits that facilitate stigmatic pollen deposition of solitary bees are not key traits for corbiculate species. Therefore, it is important to gather knowledge on different species and their contributions to pollination in different cropping systems.

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Introduction

One third of global crops benefit from insect pollination (Klein et al. 2007) accounting for substantial economic value (Potts et al. 2016). Traditionally, crop pollination has been managed with a single species, the European honeybee (*Apis mellifera*). However, while agriculture is becoming more pollinator dependent (Aizen et al. 2008, Aizen et al. 2019), and the supply of honeybees may not be sufficient to meet global pollination demands (Aizen et al. 2009), beekeepers are met with honeybee health problems associated with industrial agriculture (Evans et al. 2021, Shanahan 2022). Moreover, accumulating evidence show that the custom of increasing honeybee density for better pollination is not promoting optimal crop yields, and relying solely on this practice may in fact have the opposite effect, as honeybee dominance is associated with lower pollinator diversity (Rollin et al. 2019, Weekers et al. 2022).

Several wild bee species are shown to be efficient pollinators of various crops (Danforth et al. 2019) and increased pollinator abundance and diversity is found to improve pollination and to promote better crop yields (Hoehn et al. 2008, Garibaldi et al. 2013, Brittain et al. 2013a, Mallinger et al. 2015, Blitzer et al. 2016). However, some of the main drivers of terrestrial arthropod declines are associated with crop production (Stoate et al. 2001, Geiger et al. 2010, Seibold et al. 2019), and pollinating insects are also affected (Koh et al. 2016, Powney et al. 2019). Gathering crop-specific knowledge of focal pollinator species is therefore fundamentally important for the improvement of pollination management, while overall monitoring of crop pollination is urged for securing food production (Willmer 2011, Potts et al. 2016, Garibaldi et al. 2020).

Single-visit pollen deposition effectiveness, the quantity of deposited pollen grains on a previously unvisited stigma, is frequently used for measuring pollinator performance (Willmer 2011, King et al. 2013) and indicates the degree of morphological and behavioral trait matching between pollinator and the host flower (Martins et al. 2015, Roquer-Beni et al. 2022). Indeed, functional traits such as the body-placement of pollen transporting hairs (Stavert et al. 2016), bee size and hairiness (Phillips et al. 2018), and foraging behavior (Roquer-Beni et al. 2022) have been used to predict single-visit pollen deposition effectiveness. Moreover, the degree of trait matching between a pollinator and their host plant is found to be a good predictor of plant reproductive success (Garibaldi et al. 2015).

Pollination is a key event affecting seed set and fruit quality of several fruit trees such as the apple (Garratt et al. 2014, Eeraerts et al. 2020, Belien et al. 2021). Most apple cultivars are self-incompatible and require pollen from a genetically compatible cultivar, delivered by an insect pollinator (Ferree et al. 2003). Therefore, in a cropping system with self-incompatible plants, changing trees and tree rows is an important pollinator trait facilitating cross-pollination (Willmer 2011). The interaction of female and male parentage affects pollen tube germination rate on the stigmatic surface; self-pollen has significantly lower germination rate compared with cross-compatible pollen (Speranza et al. 1988, Lord et al. 2002, Jahed et al. 2017). Nevertheless, high stigmatic pollen density is known to increase the germination rate (Zhang et al. 2010), and to significantly predict plant reproductive success in apple (Park et al. 2016).

The length of flower visits has been linked to both single-visit pollen deposition and pollination efficiency, although with contrasting effects on different crops (Thomson et al. 2001, Ivey et al. 2003, Roquer-Beni et al. 2022). In addition, visit length on a single flower partially determines pollinator visitation rate, the number of flowers visited per unit time (Inouye 1980, Harder 1983) – another key component of pollinator performance (Willmer 2011, Danforth et al. 2019). Bee size and proboscis length mediates nectar extraction efficiency and is therefore associated with the length of flower visits (Inouye 1980, Plowright et al. 1997, Peat et al. 2005, Balfour et al. 2013, Klumpers et al. 2019).

This thesis aims to determine the contributions of different pollinators on apple pollination. Data is collected from pollinator visits on virgin apple flowers to measure the differences between bee genera in single-visit pollination efficiency; the number of deposited pollen grains and the proportion of germinated pollen tubes on apple stigmas receiving a single visit. The following supporting questions will be answered: 1) What are the differences between bee genera in number of deposited pollen grains after a single visit on virgin apple flowers? 2) What are the differences between bee genera in consequent proportions of germinated pollen tubes? 3) How does visit length impact the number of deposited pollen grains? Based on previous research, I predict that visits from pollinators with distinct traits will result in different 1) stigmatic pollen loads, 2) different proportions of germinated pollen tubes, and that 3) visit length does not significantly affect pollen deposition on apple

pistils. I will then discuss the traits associated with pollen deposition, pollen tube germination and visit length.

Materials and methods

Study system

Data collection was conducted in three conventionally managed apple orchards in Norway. Two orchards in Hardanger (Lofthus: 60.321908, 6.665189 and Djonno: 60.458660, 6.750554) and one in Svelvik (59.571507, 10.393375) (Figure 1). Hardanger has a typical regional geography for western Norway composed of fjords and high mountain ridges while Svelvik has relatively low variation in topography and a more open landscape. Both areas have an annual average temperature of ca 8° C while the annual average precipitation differs from 1600 mm in Hardanger to 750 mm in Svelvik. The monthly average temperature for May in both areas is ca 11° C while the monthly average precipitation for May is 34 mm in Svelvik and 103 mm in Hardanger (Klimaservicesenter 2023).

Conventionally cultivated, mass blooming fruit and berry crops dominate the landscape in both the Svelvik and Lofthus study sites. Both sites also have a low abundance of natural habitat. The landscape surrounding the study site in Djonno has, in general, less agricultural land, and more natural habitat compared with the other study sites. Honeybees were used as a managed pollinator at each site.

Study species

Apple is a deciduous fruit tree in the genus *Malus* (Rosaceae) native to the temperate zone and is among the most important fruit crops globally (Pardo et al. 2020). Apple has perfect flowers with five stamens and a compound pistil of five carpels (hence five stigmas). (Ferree et al. 2003). The apple flowering period is approximately two weeks long during spring. As a mature fruit can potentially contain ten seeds, to produce an apple with a full complement of seeds, at least ten compatible and viable pollen grains must be transferred to receptive stigmatic surfaces (Sheffield et al. 2005). Undeveloped seeds negatively impact fruit quality which has direct consequences on economic productivity (Garratt et al. 2014, Samnegard et al. 2019).

Species of the genera *Lasioglossum*, *Andrena* and *Bombus* are often found on early flowering fruit tree crops in the temperate zone and likely to be important wild pollinators of apple (Willmer 2011). These genera have been observed in apple orchards in Hardanger already in 1956 (Løken 1956).

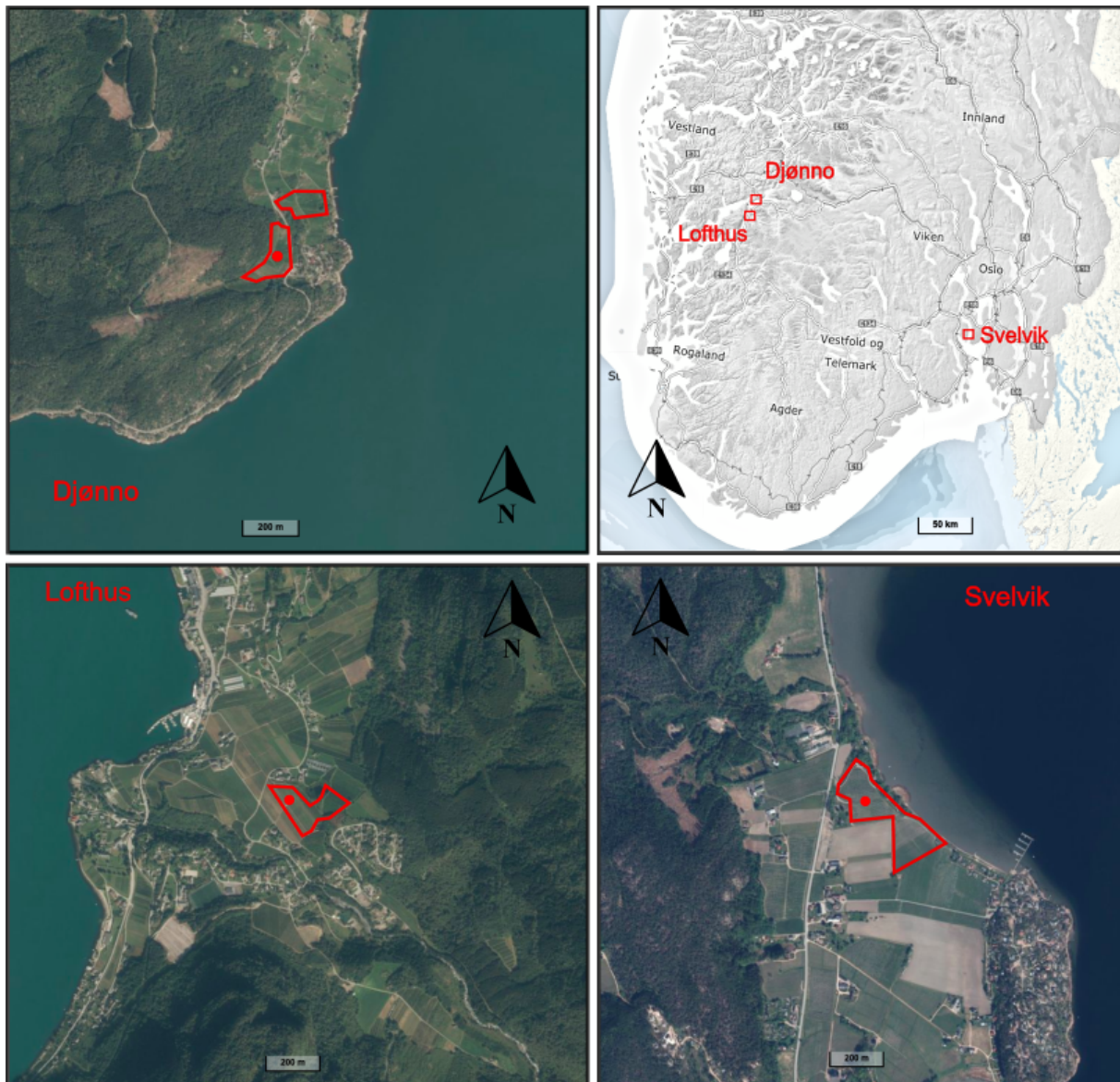


Figure 1. Location of the study areas and the experimental orchards. The red polygon indicates the areas where flower observations were conducted while the red dot indicates the coordinates for each orchard. Lofthus (60.321908, 6.665189), Djønno (60.458660, 6.750554), and Svelvik (59.571507, 10.393375).

Lasioglossum. A solitary genus in the family Halictidae with mainly ground nesting species which often form nesting aggregations. Females of the identified species has forewing length between 5-7 mm (Falk 2019) and represent the smallest species in this study. The genus has short-tongued bees with proboscis length between 1.23-2.56 mm for a selection of species (Cariveau et al. 2016).

Andrena. A large solitary genus in the family Andrenidae with mainly ground nesting species, many of which may form nesting aggregations. Females of the identified species has forewing length between 9.5-10.5 mm; similar size as the honeybee. The genus has short-tongued bees with proboscis length between 1.41-2.68 mm for a selection of species (Cariveau et al. 2016).

Bombus. A genus of social and cleptoparasitic species in the family Apidae of which the majority nests underground. The social species form colonies of up to several hundred individuals. Females of the identified species has forewing length between 13-16 mm (Falk 2019) and have proboscis length between 7-8.6 mm (Willmer 2011).

Apis. The only representative of *Apis*, family Apidae, is the European honeybee, *Apis mellifera*, by far the most commonly used managed pollinator globally. Honeybee workers have forewing length of 9-10 mm (Falk 2019), and the mean proboscis length is 4.97 mm (Cariveau et al. 2016).

Data collection

Data was collected during apple bloom in 2022. Pre-bloom, flowers of randomly selected branches of apple trees were isolated with pollinator exclusion cages (Figure 2). During full bloom, cages were removed, and the exposed virgin flowers observed until a pollinator landed on a flower. Pollinator was allowed to work on the flower undisturbed (Figure 3). At the end of visit, the pollinator and the visited flower were collected, and visit length was noted. Anthers and petals were then removed from flowers before placing the pistil in a 5 ml centrifuge tube. Samples were frozen in the evening of the day of collection and kept frozen until further analysis. In the absence of a pollinator visit, the flower was re-caged for later observation. The field experiment was part of a larger apple pollination study in Norway where several field workers gather data from single visits on apple flowers. I then further analyzed a proportion of the collected flowers for pollen deposition and pollen tube germination and generated data for this thesis. All the collected bees were identified to genus, while most (157 of 165 bees) specimens were identified to species.



Figure 2. Pollinator exclusion cages on apple flowers. Image courtesy of Helene Müller Haugan.



Figure 3. A Red mason bee (*Osmia bicornis*) foraging on an apple flower. Stigmas are in contact with the metasomal scopa.

Pistil analysis

Frozen pistils were defrosted in room temperature and fixed with 4% formaldehyde alcohol acetic acid (FAA). Prior to analysis, FAA was carefully removed with pipetting, avoiding contact with the stigmas, and pistils submerged in 5M sodium hydroxide (NaOH) for 14 hours. Pistils were then gently rinsed by pipetting with double distilled water and dark-incubated for a minimum of one hour in 0.1 % aniline blue buffered in 0.15M potassium hydroxide (KOH). Next, dyed styles were dissected from the pistils and gently compressed between a microscope slide and a cover glass with a few drops of fresh staining solution. Sets of 20 pistils were analyzed per day to avoid sample degradation towards the end of the day. Pistils were photographed in bright-field microscopy with a Leica DM2500 light microscope, and all pollen grains counted manually from the images (Figure 4). Germinated pollen tubes were counted manually under fluorescence light (Figure 5) (Yoder et al. 2009).

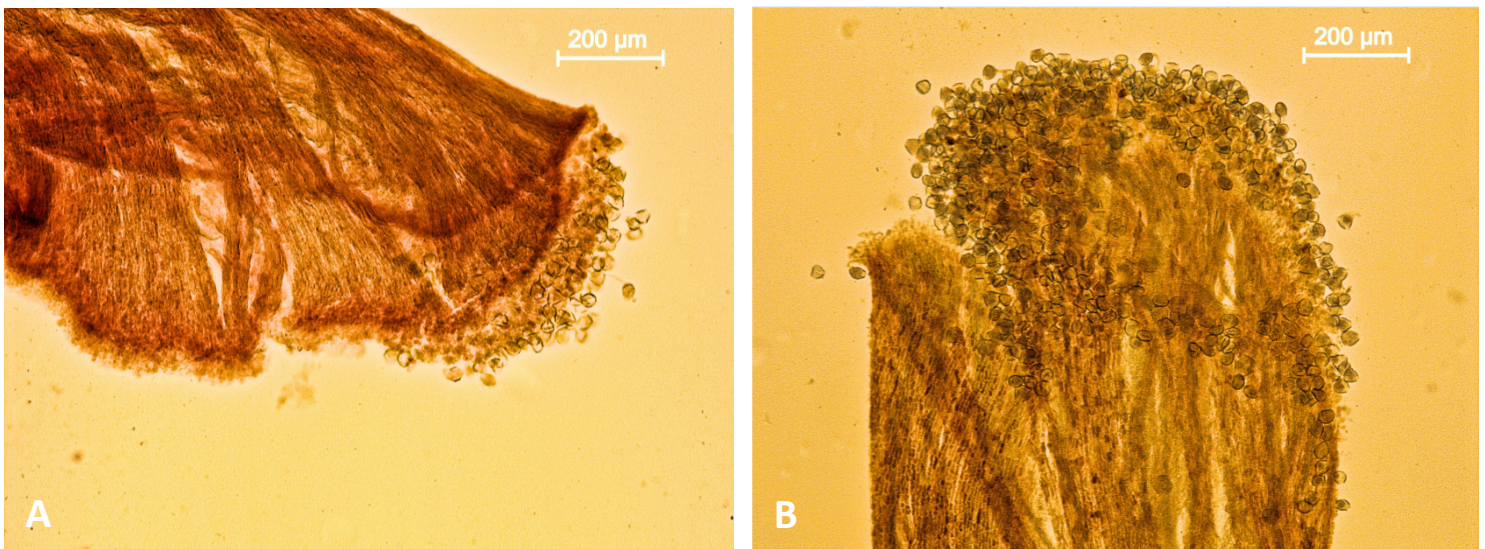


Figure 4. Apple stigmas with pollen viewed with brightfield microscopy. A) Low quantity of pollen partially covering the stigma. B) Stigma covered with high quantity of pollen.

Several pistils were tested for optimizing the protocol for softening and dyeing apple stigmas before analyzing the rest of the samples. It was clear that the time used for pistil softening is a delicate process; when pistils are excessively softened, pollen grains are released from the stigma papillae, and when softening is insufficient, the stigma and pistil tissue remains hard, and a proportion of pollen grains are not readily visible.

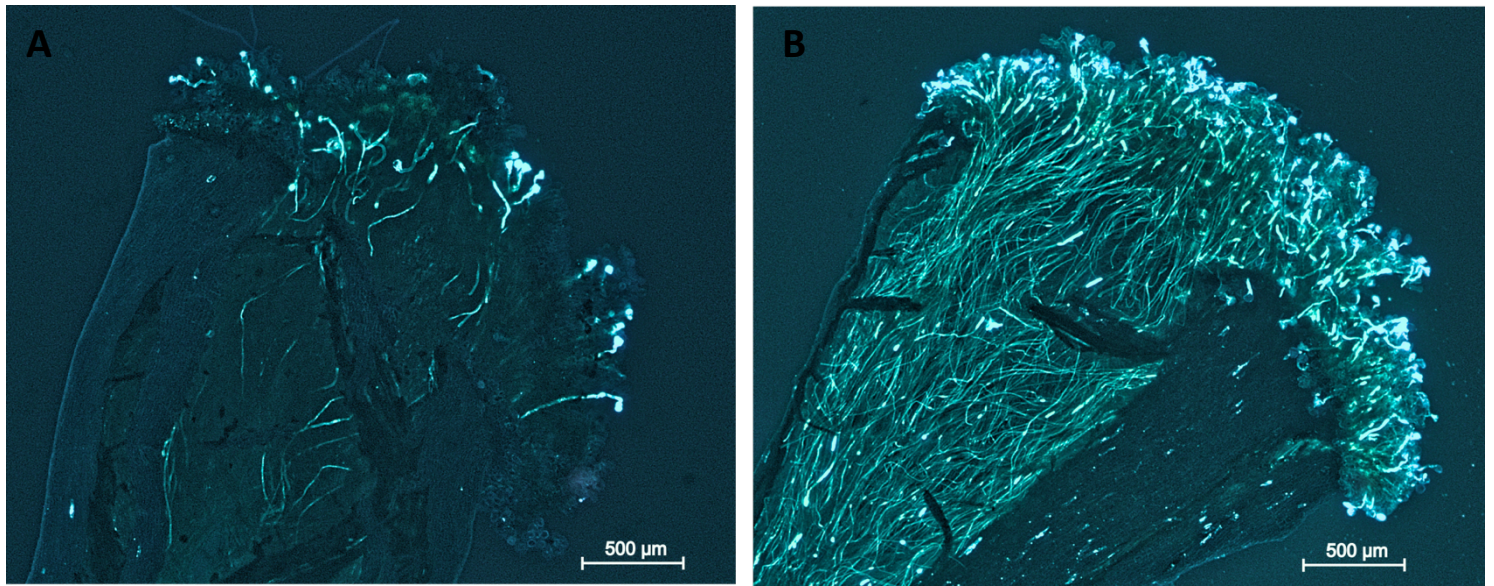


Figure 5. Top parts of apple stigmas with pollen tubes viewed with fluorescence microscopy. A) Stigma with a low density of pollen tubes. B) Stigma with a high density of pollen tubes.

Data analysis

To compare single-visit pollen deposition between pollinators, a generalized linear mixed effects model (GLMM) was fitted to predict number of deposited grains with pollinator genus and visit length. Zero-inflated negative binomial prior was assumed due to overdispersion (overdispersion test $z = 12.264$, $p < 0.001$) and the potential for a large proportion of structural zeros; possibly resulting from field workers interrupting the foraging bee or non-receptive stigmas. The interaction of date and orchard was included as a random effect. This allowed for the accounting for differences in pollen deposition due to changes in weather conditions or general pollen availability throughout the flowering season. The model was fitted using the brms package for the R statistical platform; an implementation of Bayesian modelling software that allows for more complex regression relationships than those supported in the base R functions (Bürkner 2017). Markov chain Monte Carlo (MCMC) simulation was used for posterior sampling. For both models, four chains were run, each for 15000 iterations including a burn-in period of 5000 iterations, yielding 40000 samples for each parameter. The estimated values for the parameters and their 95% credible intervals (CI) were reported under the posterior distribution. Model convergence was assessed using Rhat (Brooks 1998).

Next, a logistic regression model was fitted to test differences in pollen tube germination between bee genera. Pollen tube germination was modelled with a binomial distribution and logit link function. Pollen grains were classified as 0 (not germinated) or 1 (germinated). Finally, a generalized linear model was used to test differences in visit length between bee genera (Dobson et al. 2018). Visit length was modelled with a normal distribution and log link function. An initial analysis determined throughout the assessment of information criteria metrics (AIC for frequentist models and WAIC for Bayesian models) showed that models using taxonomic information at the genus level performed better than models aggregating solitary bees to one category (Watanabe et al. 2010, Portet 2020).

All analyses were performed in R version 4.2.2 (R Core Team 2020), and JASP version 0.17.1 (Love et al. 2019).

Results

165 flowers receiving a single pollinator visit were included in the analysis. 109 visits in Djønno, 15 in Lofthus and 41 in Svelvik. The majority of the solitary bee and bumblebee visits were from Djønno. Of the observed single visits, 29 were by *Andrena*, 67 by *Apis*, 49 by *Bombus*, and 20 visits by *Lasioglossum*. 19 visits resulted in zero deposited pollen grains.

Table 1. Mean and standard deviation of deposited pollen grains, germinated pollen tubes, and visit length on apple pistils for each genus.

	<i>Andrena</i> (n = 29)	<i>Apis</i> (n = 67)	<i>Bombus</i> (n = 49)	<i>Lasioglossum</i> (n = 20)
Deposited pollen grains				
Mean	325.3	198.9	309.5	360.3
SD	252	203.4	292.9	354.6
Germinated pollen tubes				
Mean	150.8	75.6	138.7	137.6
SD	120.3	80.2	132	136
Visit length				
Mean	34.5	10.2	8	36.9
SD	71	10.3	8	54.6

Pollen grain deposition

Pollen grain deposition ranged from 0 to 1360 per pistil (mean \pm SD: 273.5 \pm 266.9) while the stigmatic pollen deposition ranged from 0 to 370 (67.5 \pm 63.4). *Lasioglossum* showed the highest mean value of deposited pollen grains after a single visit on pistils (mean \pm SD: 360.3 \pm 354.6), followed by *Andrena* (325.3 \pm 252), *Bombus* (309.5 \pm 292.9), and *Apis* (198.9 \pm 203.4) (Table 1).

There was a marginal positive effect of visit length on stigmatic pollen grain deposition (estimate = 0, SE = 0, 95% CI = [0.00, 0.01]). Compared with *Andrena*, *Bombus* deposited most pollen grains on a single-visit on apple pistils (estimate = 0.17, SE 0.13, CI = [-0.08, 0.41]), followed by *Lasioglossum* (estimate = 0.05, SE 0.15, 95% CI = [-0.24, 0.34]), while

Apis deposited least pollen grains (estimate = -0.36, SE 0.14, 95% CI = [-0.64, 0.09]) (Table 2).

Figure 6 shows the predicted single-visit pollen deposition at mean visit length. Compared with *Apis*, the posterior probability of *Bombus* depositing more pollen grains per single visit was 0.999, followed by *Andrena* and *Lasioglossum* with a probability of 0.995. The probability for *Lasioglossum* depositing more pollen grains than *Andrena* was 0.632. Each parameter estimation converged at Rhat < 1.00 (Appendix).

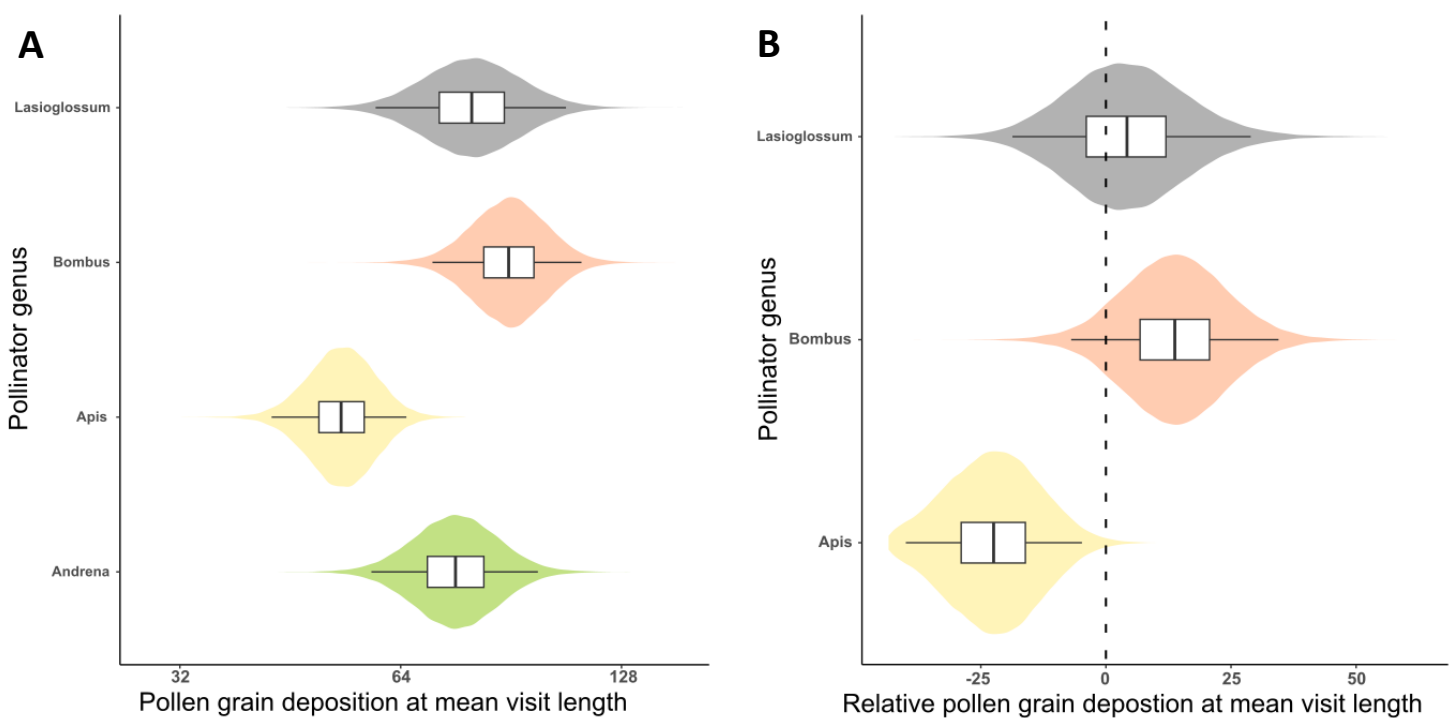


Figure 6. Predicted single-visit pollen deposition (log transformed) at mean visit length (all visits). The density curves show the full credible intervals, box-plot show the 50% credible intervals, and whiskers show the 95% credible intervals. A) Predicted pollen deposition by genus. *Bombus* deposited the highest number of pollen grains per single visit, followed by *Lasioglossum*, *Andrena*, and *Apis*. B) Predicted pollen deposition relative to *Andrena*. Effect sizes indicate the likelihood for pollen grain deposition of *Apis*, *Bombus* and *Lasioglossum* per single-visit at mean visit length relative to *Andrena* (vertical line).

Figure 7A shows the effect of visit length on single-visit pollen deposition. Across genera, the quantity of deposited pollen grains was similar at short and long visit lengths, although an overall marginal increase in deposited pollen grains can be observed with increasing visit length. The long visits of the solitary genera did not result in higher pollen deposition. There were no considerable differences in pollen deposition throughout the sampling period between orchards (Figure 7B). Two dates in Djönno and Lofthus had a marginal positive effect on pollen deposition.

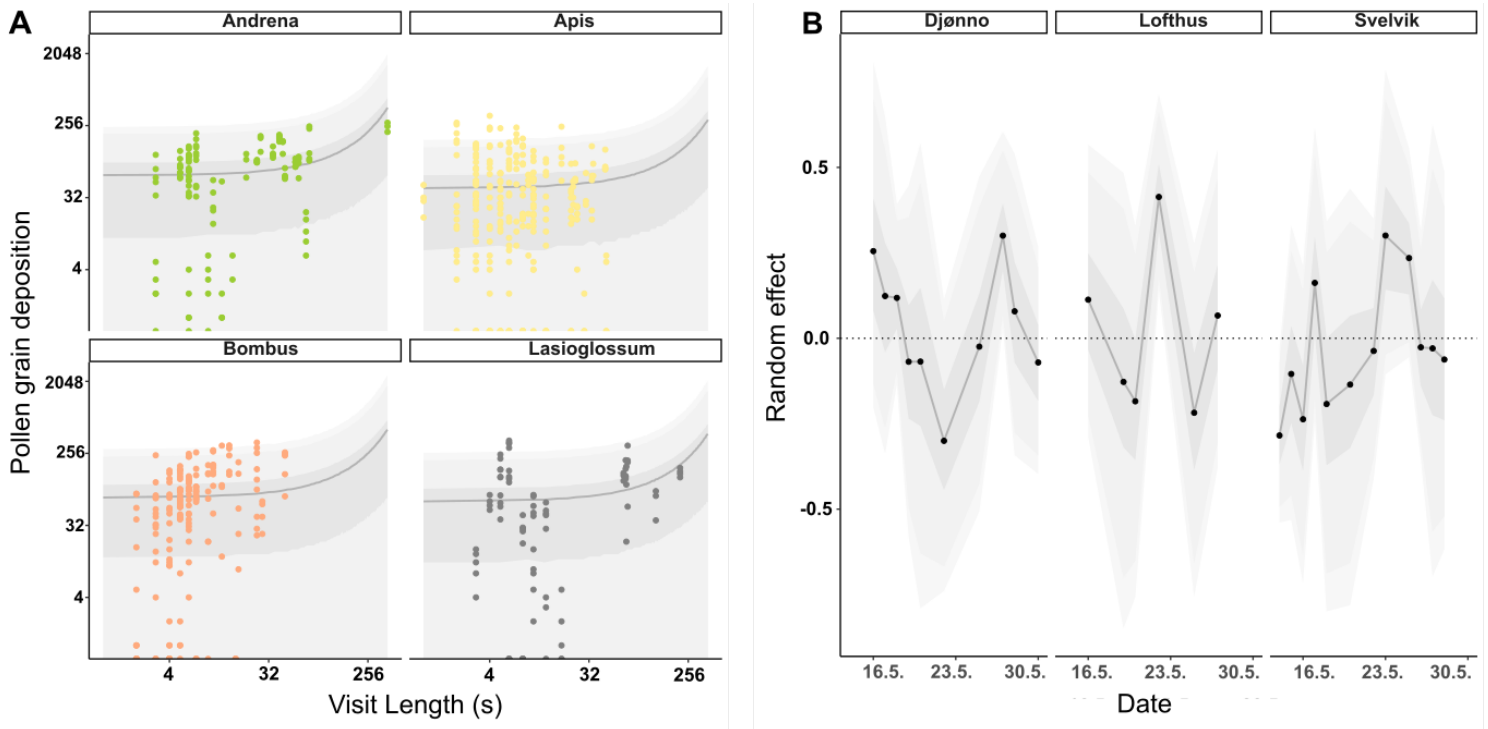


Figure 7. A) The effect of visit length (seconds) on pollen deposition for each genus. Dots represent pollinator visits. B) Random effect of date for each experimental orchard on pollen deposition. The horizontal line indicates mean single-visit pollen deposition across orchards, while the dots represent relative mean deposition rates from different sampling dates. The dark-grey areas in both figures indicate the 50% credible intervals and the light-grey areas 95% credible intervals.

Pollen tube germination

Pollen tube germination ranged from 0 to 534 per pistil (mean \pm SD: 115 ± 115.7) while the stigmatic pollen tube germination ranged from 0 to 145 (28.7 ± 27.9). *Andrena* showed the highest mean value of germinated pollen tubes after a single visit on pistils (mean \pm SD: 150.8 ± 120.3), followed by *Bombus* (138.7 ± 132), *Lasioglossum* (137.6 ± 136), and *Apis* (75.6 ± 80.2) (Table 1). For all visits, relationship between the proportion of germinated pollen tubes and deposited pollen grains was asymptotic (Figure 8).

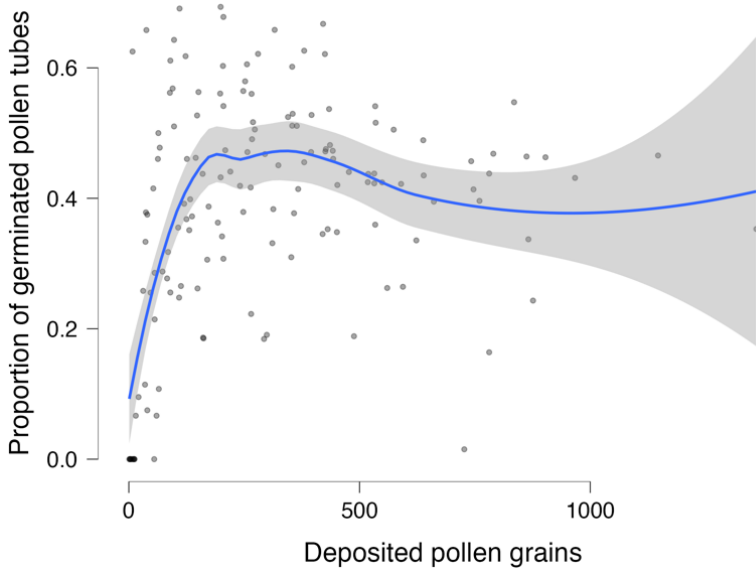


Figure 8. Relationship between number of deposited pollen grains on apple pistils and the proportion of germinated pollen tubes. The regression line represents a locally estimated scatterplot smoothing (LOESS) with 95% confidence intervals.

Results of the GLM showed significant differences between genera in the proportion of germinated pollen tubes after a single visit. Pollen grains deposited by *Andrena* on a single visit were most likely to germinate, followed by *Bombus* (estimate = -0.063, SE = 0.029, $p < 0.011$), *Lasioglossum* (estimate = -0.336, SE = 0.032, $p < 0.001$), and *Apis* (estimate = -0.034, SE = 0.03, $p < 0.001$) (Table 2).

Pollen deposited by *Lasioglossum* and *Apis* visits had significantly lower probability to result in germinated pollen tubes compared with *Andrena*. Figure 9 shows the data distribution of pollen tube germination by genus. The density peaks of *Lasioglossum*, *Andrena*, and *Bombus* are similar, even though the mean of *Lasioglossum* is significantly lower than *Andrena*. Each genus has a small peak on the low section of the density curve indicating zero germinated pollen tubes.

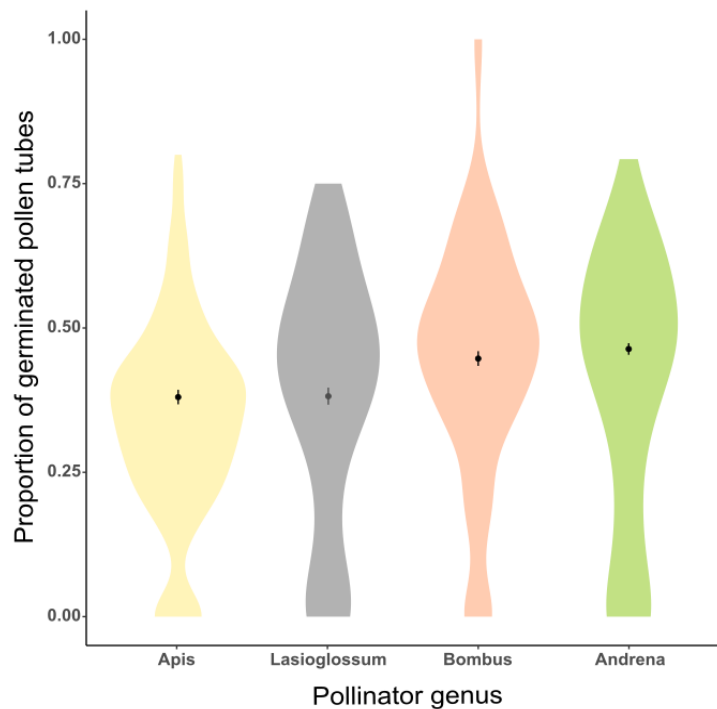


Figure 9. Violin plot showing the proportions of germinated pollen tubes by genus as a probability distribution with a box-plot element. The width of the probability curve shows data density at a particular proportion. The box-plot element indicates the means and the 95% confidence intervals of visit. Pollen grains deposited by *Andrena* were most likely to germinate, followed by *Bombus*, *Lasioglossum*, and *Apis*.

Visit length

Visit length ranged from 1 to 386 seconds (mean \pm SD: 17.1 ± 37.7). *Lasioglossum* had the longest visit length (mean \pm SD: 36.9 ± 54.6), followed by *Andrena* (34.5 ± 71), *Apis* (10.2 ± 10.3), and *Bombus* (8 ± 8) (Table 1).

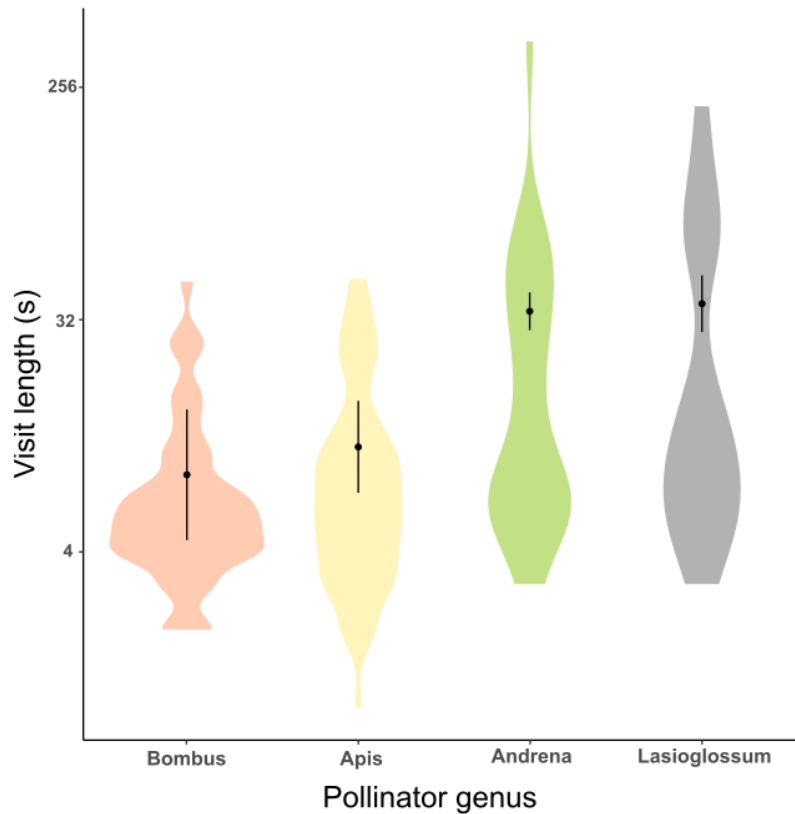


Figure 10. Violin plot showing predicted visit length in seconds (log transformed) by genus as a probability distribution with a box-plot element. The width of the probability curve shows data density at a particular visit length. The box-plot element indicates the means and the 95% confidence intervals of visit lengths. *Lasioglossum* had the longest visits, followed by *Andrena*, *Apis*, and *Bombus*.

The GLM showed significant differences in visit length between genera. Compared with *Andrena*, *Lasioglossum* had the longest mean visit length (estimate = 0.07, SE 0.13, $p = 0.601$), while *Apis* visits were shorter (estimate = -1.21, SE 0.21, $p < 0.001$), followed by *Bombus* (estimate = -1.46, SE 0.30, $p < 0.001$) (Table 2). Violin plot from the GLM shows the data distribution of visit length by genus (Figure 10). Large proportion of visit lengths were shorter than the mean for all genera, and most visits are actually more closely related than the significantly different means. Visit length for each genus has a multimodal distribution.

Table 2. Model outputs for the generated models. The generalized linear models of visit length and pollen tube germination, and the generalized linear mixed effects model for deposited pollen grains at mean visit length.

Pollen grain deposition at mean visit length (GLMM)					
	estimate	est. error	95% Credible interval		rhat
			lower bound	upper bound	
Intercept	4.25	0.14	3.97	4.52	1.00
Visit length	0	0	0	0.01	1.00
<i>Apis</i>	-0.36	0.14	-0.64	-0.09	1.00
<i>Bombus</i>	0.17	0.13	-0.08	0.41	1.00
<i>Lasioglossum</i>	0.05	0.15	-0.24	0.34	1.00

Pollen tube germination (GLM)					
	estimate	SE	95% Confidence interval		p value
			lower bound	upper bound	
Intercept	-0.15	0.02	-0.19	-0.10	< .001
<i>Apis</i>	-0.34	0.03	-0.40	-0.29	< .001
<i>Bombus</i>	-0.063	0.026	-0.114	-0.011	0.011
<i>Lasioglossum</i>	-0.336	0.032	-0.399	-0.274	< .001

Visit length (GLM)					
	estimate	SE	95% Confidence interval		p value
			lower bound	upper bound	
Intercept	3.54	0.09	3.06	3.86	< .001
<i>Apis</i>	-1.21	0.21	-3.12	-0.43	< .001
<i>Bombus</i>	-1.46	0.30		-0.51	< .001
<i>Lasioglossum</i>	0.07	0.13	-0.57	0.66	0.601

Discussion

I found differences in single-visit pollination efficiency and visit length between genera. Bumblebees and solitary bees deposited significantly more pollen per single visit than honeybees, suggesting that differences in distinct traits may mediate the size of deposited pollen loads. As predicted, *Lasioglossum* and *Andrena*, which share similar traits, deposited more closely matching pollen loads that were different from the pollen loads deposited by honeybees and bumblebees. The results of pollen tube germination partially contrasted the prediction of trait driven similarities between genera; pollen grains deposited by *Lasioglossum* had significantly lower germination probability when compared with *Andrena*. As predicted, visit length had only a marginal positive effect on single-visit pollen deposition, and most of the pollination appears to happen on impact or early during the flower visits. Hence, it is likely that factors other than flower visit length dictates the size of pollen loads. The solitary bee genera had significantly longer flower visits compared with honeybees and bumblebees, and there was an inverse relationship between bee size and visit length.

Pollen grain deposition

Structures for pollen transportation

Females of honeybees and bumblebees have corbiculae on their hind tibia which are used for pollen transport (Thorp 2000). Female bees groom extracted body-pollen, mix it with nectar, and compress the resulted moist and dense pellet on their hind tibia; corbiculate pollen is less accessible and adherent to stigmas than dry pollen attached on pollen collecting hair (Parker et al. 2015). In contrast to corbiculate species, solitary bees transport pollen on long and dense hair (scopa). Scopal pollen is not mixed with nectar, is therefore dry and more adherent to stigma, and is found on various body parts of solitary bees (Danforth et al. 2019). Consequently, solitary bees often have their pollen more widely distributed on their bodies and carry more available body pollen compared with bumblebees and honeybees (Willmer 2011, Woodcock et al. 2013). It could therefore be expected that corbiculate species deposit less pollen on stigmas than solitary bees. While honeybees in my study deposited the least pollen grains per single-visit, the solitary bees

and bumblebees had more similar pollen loads. Thus, it is likely that not only pollen transportation structures mediate pollen deposition on apple stigmas.

The majority of *Andrena* have their scopa widely distributed on the hind trochanter, femur, and tibia, in addition to propodeum, while *Lasioglossum* have scopa mainly on underside of the metasoma, in addition to the hind legs and propodeum (Danforth et al. 2019).

Despite being the smallest bee genera, *Lasioglossum* and *Andrena* deposited relatively large pollen loads demonstrating the advantage of widely distributed scopal hairs with dry pollen. *Lasioglossum* deposited more pollen grains than *Andrena*. Considering that most of pollen deposition seems to happen when pollinators land on apple flowers, metasomal scopa (on *Lasioglossum*) is likely to contact directly the stigmas and may be a highly beneficial structure for apple pollination (Figure 3).

Size and hairiness

Pollinator size is found to affect pollen extraction and deposition rates and large pollinators are usually responsible for greater quantities (Goulson et al. 2002, Willmer et al. 2014, Földesi et al. 2021). Despite both transporting corbiculate pollen, bumblebees are found depositing larger single-visit pollen loads on apple and pear stigmas compared with honeybees (Thomson et al. 2001, Zhang et al. 2015). Contrasting these results, Eeraerts et al. (2020) found the opposite to be true for sweet cherry (*Prunus avium*), and hypothesized the cause being morphological mismatch between the small flowers and large bumblebees, nevertheless demonstrating the importance of a crop-specific assessment of pollen deposition. In my study, the native bee genera (*Andrena*, *Lasioglossum* and *Bombus*) deposited largest pollen loads per single visit while representing smallest and largest body sizes. Hence, pollen deposition on apple stigmas is likely not only size-dependent, although it may be advantageous for bumblebees considering the low availability of corbiculate pollen.

Roquer-Beni et al. (2020) generated a pollinator hairiness index using the length and density of body hair and found that bumblebees are substantially hairier than honeybees and species in *Andrena* and *Lasioglossum*. Phillips et al. (2018) found species of *Bombus* and *Andrena* depositing more pollen per single visit on flowers of oilseed rape (*Brassica napus L.*) than honeybees and that increased hairiness had a positive effect on pollen deposition.

Perhaps the large size and hairiness partially contribute to the high pollen loads of bumblebees, while the combination of inconspicuous hairiness and medium size limits the pollen deposition of honeybees. An additional benefit of the greater hairiness of bumblebees is improved insulation which aids foraging in lower temperatures. As developing ovules are aborted mostly in mid- and late maturing fruits (O'Donnell et al. 1993), pollination in the beginning of stigma receptivity is likely beneficial, especially for early blooming apple cultivars.

Foraging behavior

Social bees have physical contact with each other for various reasons which may affect pollen movement. Honeybees have colony sizes up to 65000 individuals and an impressive capacity to utilize pollen and nectar resources (Seeley 1986) such as in a mass flowering crop. During the movement inside a hive, honeybees are likely to have some degree of pollen exchange between foraging individuals, referred to as in-hive pollen transfer, which may facilitate cross pollination (Free et al. 1972, Degrandi-Hoffman et al. 1986, Dag et al. 2000, Calderone et al. 2002). An obvious advantage contrasting the isolated life of solitary bees. Although in-hive pollen transfer is less studied on bumblebees, colony sizes up to several hundreds of individuals is likely to facilitate pollen exchange to some degree.

However, considering honeybees as individual pollinators presents their performance in a different light because foraging workers have different tasks. Some workers forage for nectar while others forage mainly for pollen, and the proportion of workers that forage for nectar or pollen is largely dependent on the developmental status of the colony (Camazine 1993). The distinct foraging behaviors between workers have been shown to affect pollen deposition; individual foragers frequently make less stigma contact, due to the tendency of foraging from the side rather than from top of the flower reproductive parts (Vicens et al. 2000, Woodcock et al. 2013, Eeraerts et al. 2020, Roquer-Beni et al. 2022). The two density peaks in visit length distribution of honeybees in my study (Figure 10), as well as several visits resulting in zero deposited pollen (Figure 7A), may indicate distinct foraging types.

Stern et al. (2001) found that sequentially introducing honeybee colonies, rather than all colonies simultaneously, decreased the proportion of laterally approaching side-workers

and resulted in an overall increase in cross-pollination within an experimental apple orchard. They hypothesized that after honeybees learn how to forage nectar from the host plant, they switch from top working to side working due to lower energy costs. Although, the easier nectary access may be due to a loosening of stamen filaments later in flowering or differences in flower morphology between cultivars (Schneider et al. 2002, Farkas et al. 2007).

Contrasting honeybee foraging behavior, species in *Lasioglossum*, *Andrena*, and *Bombus* have shown more frequent stigma contact and higher pollen deposition rates on various crops (Woodcock et al. 2013, Martins et al. 2015, Bernauer et al. 2022). As large body size is obviously not facilitating the large pollen loads of *Andrena* and *Lasioglossum*, frequent stigma contact combined with widely distributed, dry pollen availability is likely to be highly important for small-sized bees. Stigma contact during single visits could be an important behavioral trait to quantify when studying pollen deposition.

Pollen tube germination

Self-incompatibility

Flowering plants have different strategies to avoid self-fertilization. In self-incompatible plants, the most common mechanism is gametophytic self-incompatibility (GSI) via the RNase system, regulated by the S-genotype. Self-pollen tube growth is halted in the upper third part of the style when pistil and pollen share the same S-allele (Lord et al. 2002). However, Certal et al. (1999) found that in apple the distribution of S-proteins is quite distinctive from other Rosaceae species, as the compounds inhibiting self-pollen tube growth are evenly expressed throughout the entire stylar tissue including the stigmas. The long history of breeding has resulted in a multitude of cultivars and semi- and cross-incompatibilities have become more prevalent as more cultivars are developed (Alston 1995, Sakurai et al. 2000, Schneider et al. 2005). Self-incompatible apple cultivars have been found to self-fertilize (Williams et al. 1977) and therefore the S-allele based compatibility has been suggested as insufficient (Petropoulou et al. 1998). Therefore, using germinated pollen tubes on stigmas as an estimate of pollen compatibility may be justified, although it is not possible to rule out that a portion of the germinated pollen are self-pollen.

Due to the RNase system, a few studies assessing pollinator performance in cropping systems with self-incompatible plants have quantified pollen tubes at the base of the style, with the aim of avoiding counting self-incompatible pollen tubes. Using this method, one should consider that a maximum of 10 pollen tubes can enter the ovary, and thus a proportion of compatible pollen will also be halted in the styles (assuming there are more than 10 deposited compatible and viable pollen grains on the five stigmas). Moreover, the myriad facilitating (e.g., pollen density) and limiting (e.g., environmental) interactions affect pollen tube growth and thus mediate the number of pollen tubes reaching the ovary (Willmer 2011) – even then, pollen compatibility would be uncertain due to the possibility of fertilization by self-pollen.

Effects of stigmatic pollen density

In addition to pollen compatibility, an important driver of pollen tube kinetics is density dependent pollen tube germination and growth. High pollen quantity is found to prime stigmas and hence promote pollen tube germination and growth rate in a density dependent manner. Large pollen loads thus facilitate competition and selection of most vigorously growing gametophytes (Janse et al. 1993, Zhang et al. 2010) and results in faster fertilization and fruit maturation (Zhang et al. 2015). My results suggest that at least 200 pollen grains are needed for an optimal proportion of germinated pollen tubes on apple pistils, which could indicate a priming effect on stigmas. Although higher pollen density did not increase the proportion of germination, the total number of germinated pollen tubes did not show a saturating curve (Figure 8). The portion of visits that resulted in zero or very low proportion of germination could be due to insufficient priming of stigmas. Moreover, stigmas are known to clog from high densities of self-pollen (Bawa et al. 1981) which could partially explain the lower proportion of germinated pollen tubes from *Lasioglossum* visits. However, due to low quantity of *Lasioglossum* visits it is not possible to make robust conclusions. Figure 5B shows a style with a high density of pollen tubes and visualizes what a clogged stigma may look like.

Janse et al. (1993) experimented with varying pollen loads on apple stigmas and found that at least 40 pollen grains per stigma are needed for optimal pollen tube growth. They also found that the number of good seeds decreased with smaller pollen loads. Park et al. (2016) studied the association between stigmatic pollen deposition and seed and fruit set of apples and found that seed and fruit set increased with high pollen loads, and followed a positive,

saturating curve. Both studies found similar linear increases up to 200 deposited pollen grains per pistil. Moreover, the limiting factor for plant reproductive success after a single visit is found to be rather pollinator contacting stigma during flower visit than the deposition of compatible pollen, further indicating the importance of the quantity of deposited pollen (Vicens et al. 2000). In the context of apple pollination, perhaps germinated pollen tubes are a good and relatively low-effort estimate of plant reproductive success.

Pollinator movement in orchards

Previous research has shown the possibility of cross-pollination of self-incompatible fruit trees increasing with the mobility of pollinators; especially with the tendency to change trees and tree rows during a single foraging trip (Willmer 2011). Depending on the potential nesting sites in the surrounding landscape and the design of the orchard, visiting two cross-compatible cultivars may require relatively long flight distances. Small bees fly shorter distances than larger ones (Greenleaf et al. 2007, Zurbuchen et al. 2010). The amount of pollen solitary bees collect from host plants decreases with increased isolation between available nesting habitat and the focal host plant (Williams et al. 2007) and may affect small-sized bees' ability to forage several individual plants in a single foraging trip. This may further explain the low proportion of germinated pollen tubes after *Lasioglossum* visits. The large body size of bumblebees likely enables longer foraging trips, and hence potentially more visits to multiple individual trees. Combining multiple tree visits with the high foraging efficiency on a single flower, bumblebees are likely to contribute considerably to pollen movement, explaining the large proportion of germinated pollen tubes found in my study.

Honeybees have strong fidelity to specific plant species, often returning to the same tree when returning from the hive (Ribbands 1949, Mayer et al. 1989). They also change trees and tree rows less frequently than bumblebees (Eeraerts et al. 2020) and are more likely to forage rather along tree rows (Kron et al. 2001), which likely affects pollen movement. Fruit tree orchards are often designed with one cultivar per tree row, or alternatively, one cultivar on a large area of tree rows, which could explain the low probability of honeybee visits resulting in pollen tube germination.

Orchard design

Although the movement pattern of pollinators increases the deposition of compatible pollen, pollinators are likely to maintain their movement patterns irrespective of the placement of different cultivars and pollinizer trees, which would result in differences of compatible pollen deposition in distinctively designed orchards. Although my results showed clear differences in proportions of germinated pollen tubes between the experimental sites, it is not possible to assess orchard design due to the low number of orchard replications. Nonetheless, the results warrant further investigations. We found more solitary bee and bumblebee visits at Djønno which may indicate greater bee abundance and activity compared with Lofthus and Svelvik sites. The landscape in Djønno is likely to facilitate higher wild bee abundance due to shorter flight distance between host flowers and nesting habitat.

Commercial apple cultivars are grown as vegetative propagated trees grafted on a rootstock, and hence individuals of the same cultivar are genetically identical. Consequently, orchards are designed with cross-compatible cultivars and pollinizer trees, providing compatible pollen and facilitating cross-pollination (Ferree et al. 2003). The selection and spatial arrangement of pollinizers and cultivars can have profound impacts on both pollination and post-pollination events (Kron et al. 2001, Kron et al. 2006, Matsumoto et al. 2008, Sáez et al. 2018, Carisio et al. 2020). Kron et al. (2001) reported a significant increase in apple seed set in trees closer to pollinizer trees despite shorter distance to honeybee hives and suggested that pollen dispersion is limited by orchard design rather than availability of honeybees.

The dates where pollen deposition rates greatly differed from the mean in my data may be due to different plant physiological and environmental impacts. Different cultivars have flowering onset at different times; stigmas may not be receptive immediately after anthesis, and negatively affect pollen adhesion, resulting in low pollen deposition or low pollen tube germination on a certain date. Moreover, temperature or other varying weather condition could have affected overall pollinator activity, and thus cause a negative or positive effect on overall pollen deposition on that date (Figure 7B).

Visit length

Nectar is the primary reward for many pollinators, and its composition is likely associated with a particular pollinators' host plant preference. Apple has a typical generalist flower; an open disc-shaped corolla, accessible pollen, and nectar in low volume, which often attracts small and short tongued bees (Willmer 2011). However, apple nectar is of excellent quality – high sugar concentration with a large proportion of sucrose – and the total volume in a full blooming orchard is immense. Supporting the optimal foraging theory (Zimmerman 1983), pollinators thus seem to forage on apple to gain the high-value reward even if the morphological match may not be optimal, which could partially explain the large size-variation in bees foraging on apple flowers (Smessaert et al. 2016).

The bee proboscis (excluding the mandibles) is a highly modified structure relative to other insects and specialized for nectar foraging. Bees are traditionally divided into “short-tongued”, and “long-tongued” families based on proboscis length which is consistently, although not always, isometric to body size (Danforth et al. 2019). Bees often select flowers with a corolla depth matching the length of their proboscis to increase foraging efficiency, although species with longer proboscis have the option of visiting a larger range of flower sizes (Inouye 1980, Ranta et al. 1980). Goulson et al. (2002) showed that large bumblebees foraged nectar faster than small individuals, while pollen foraging efficiency was not size-dependent, and suggested that the length of proboscis, rather than body size, mediates nectar foraging efficiency. At high resource density, the limiting factor for increased visitation rate is flower handling time rather than locomotion costs (Willmer 2011). As nectar is often sought in each flower visitation by bees, irrespective of pollen or nectar foraging (Woodcock et al. 2013), proboscis length may be relevant for pollination of self-incompatible crops.

Harder (1983) experimented with *Bombus spp.* foraging on artificial and real flowers and found bumblebees with long tongues having shorter visit lengths compared with short-tongued bumblebees when foraging on a single plant species. He also observed increased visit length when the nectar tube was deeper than the bee's tongue and suggested that a combination of body weight and glossa length affect a bumblebees' ability to access the nectary and the time used ingesting nectar. Additionally, he found that that the relationship between tongue length and corolla depth mediates overall flower visitation rate. My results

show that despite having only marginal differences in body size, honeybees had significantly shorter visit length compared with *Andrena*, perhaps indicating an effect of proboscis length, in addition to the behavioral aspects of foraging.

For the wild bee genera, the multimodal distributions of visit lengths I found could be partially explained by the contrasting foraging efficiencies between species, caused by differences in size and/or proboscis length (Figure 10). However, due to the low number of replicates it is not possible to make robust conclusions. The inverse relationship between visit length and pollinator size implies that apple nectar may not be easily accessible for small species. Apple nectary is positioned inside the hypanthium and filaments are connate at the base (Farkas et al. 2007), smaller species may therefore have difficulties accessing the nectar. As the amount of nectar decreases during ingestion, a short proboscis is likely to further increase flower handling time and causes the substantially longer flower visits of *Lasioglossum* and *Andrena* (Harder 1983). The results indicate that much of the pollen deposition happens in the beginning of the visits. Therefore, the main effect of visit length appears to be the number of flowers pollinators are able to visit per foraging trip which partially mediates pollen movement in the orchards.

Benefits of pollinator diversity

Functional complementarity

Apple production benefits from increased pollinator diversity (Blitzer et al. 2016, Weekers et al. 2022), which is driven by habitat quality of the surrounding landscape (Garibaldi et al. 2011, Kennedy et al. 2013). When the contribution to pollination differ among species with distinct traits (Fründ et al. 2013), a diverse pollinator community is likely to have a large range of traits resulting in increased plant reproductive success. A body of evidence show positive effects of functional trait complementarity on crop yields (Hoehn et al. 2008, Blüthgen et al. 2011, Garibaldi et al. 2015, Martins et al. 2015, Zaragoza-Trello et al. 2023). My results show that the generalist floral morphology and high-sugar nectar reward of apple attracts pollinators with different functional traits which reflect their performance in apple pollination. The solitary genera, carrying dry pollen, contributed with high pollination efficiency but had long flower visits, possibly due to their short proboscis. Honeybees had lower pollination efficiency, possibly due to corbiculate pollen and their

foraging behavior, while their short visits contributed to the number of visited flowers in the orchards. Bumblebees contributed with both high pollination efficiency and short flower visits.

The effective pollination period (EPP) is determined from the synergistic interaction of stigmatic receptivity, pollen tube growth rate and ovule longevity. According to a British study, the effective pollination period of apple in the represented study areas is 2-10 days, depending on the cultivar (Williams 1966). Although there are regional variations in exact EPPs, it is obvious that active foraging pollinator community in the beginning of the bloom is important for the delivery of viable pollen to a receptive stigma – highlighted by the fact that early- and mid-maturing fruits often have fewer aborted seeds compared to late maturing fruit (O'Donnell et al. 1993). A diverse pollinator community may increase the probability of having effective species foraging in the orchard during early bloom and displays more functional complementarity, contributing to greater overall pollination (Blüthgen et al. 2011).

Orchard fruit trees, such as the apple, pear (*Pyrus*), and sweet cherry (*Prunus*) flower two weeks on average, and outside the flowering period orchards are usually poor in floral resources. Solitary bees have an average foraging period of one month. To achieve a high reproductive success, food resources should be available throughout the whole period (Danforth et al. 2019). This can be increasingly challenging if the proportion of mass-flowering crops increases in relation to surrounding vegetation (Eeraerts 2021). The implementation of cost-effective measures, such as planting floral resources to promote wild pollinator abundance may be a good method for optimizing apple pollination. Increased floral resources has shown to enhance the abundance of bumblebees (Bommarco et al. 2021) and solitary bees such as species in *Lasioglossum* and *Andrena* (Barda et al. 2023). A simple enhancement of nesting habitat has shown to increase reproductive success of ground nesting bees with consequent positive effects on crop pollination (Cunningham-Minnick et al. 2019, Nelson et al. 2023).

Challenges in honeybee management

Honeybee health associated with industrial agriculture has become a substantial challenge (Shanahan 2022). Parasitic and pathogenic infections have caused honeybee colonies to perish, and beekeepers in the US have reported annual colony losses of 30-90 % (Evans et

al. 2021) – a global phenomenon which affect also Norwegian beekeepers (Åsen et al. 2017). Climate change is estimated to magnify these effects and may lead to increased costs of management, likely to affect the price of pollination services purchased from beekeepers (Le Conte et al. 2008, Vercelli et al. 2021). Evidently, establishing new supplementing solutions for honeybee pollination is important, although honeybees are a necessary tool for improving yields of several pollination-dependent crops and are indeed found to be most effective pollinators in certain cropping systems (Pisanty et al. 2016). The main focus in managing honeybees should rather be in correct management in a crop-specific manner (Rollin et al. 2019).

Conclusions

The solitary bees and the bumblebees had a higher pollination efficiency to apple compared with honeybees. The wild bees had a higher single-visit pollen deposition effectiveness, and they deposited more pollen grains that formed pollen tubes. However, the overall contribution of honeybees is usually much higher due to their substantial relative abundance. Targeted efforts for habitat and resource management have shown to increase the abundance of native bees and has the potential to increase overall pollination services in apple orchards. Moreover, it appears that the relative importance of functional traits in apple pollination varies among bee genera; traits that facilitate stigmatic pollen deposition of solitary bees are not key traits for corbiculate species. Therefore, it is important to gather knowledge on different species and their contributions to pollination in different cropping systems.

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Appendix

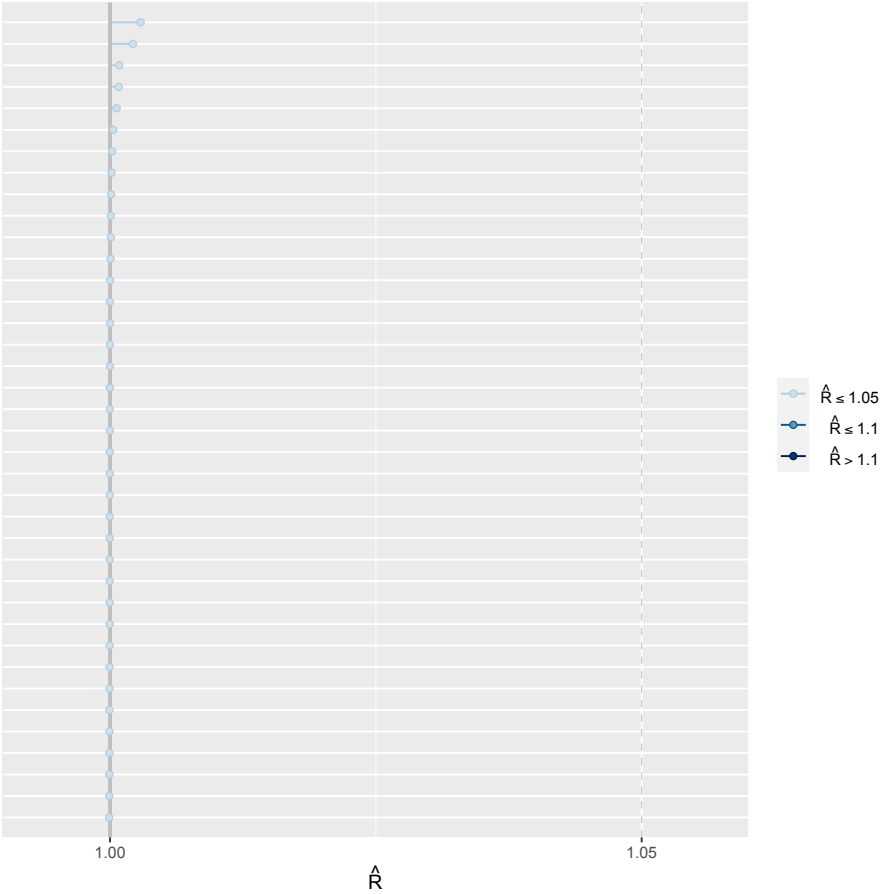


Figure 2. Parameter convergence of the MCMC posterior sampling. Each parameter estimation converged at at $R_{hat} < 1.00$.



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