

A COMPARISON OF FORAGING, DEFENSIVE, SELF-
GROOMING AND DRONE BEHAVIOUR BETWEEN NATIVE AND
NON-NATIVE SUBSPECIES OF EUROPEAN HONEY BEE (*APIS
MELLIFERA*) IN SOUTHWESTERN SWEDEN

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Abstract

Twenty-four subspecies of European honey bee, *Apis mellifera*, have evolved to different environmental conditions within Europe. However, studies comparing the behaviour of different *A.mellifera* subspecies are few, and knowledge of how behavioural adaptations differ between native and non-native subspecies remains limited. This research project addresses this gap in knowledge by comparing foraging, defensive, self-grooming and drone behaviours, which were video-recorded simultaneously at the hive entrances of four *A.mellifera* subspecies in one locality in Southwestern Sweden. The endangered Nordic brown bee (*A.m mellifera*) was the model native subspecies in this study, in addition to three commonly-imported non-native subspecies; *A.m ligustica*; *A.m carnica* and Buckfast (hybrid). It was predicted that the four subspecies would show behavioural differences due to high genetic variation between subspecies and adaptation to different environmental conditions.

The results suggest that the four subspecies differed significantly in their overall behaviour. In particular, guarding behaviour and male drone activity showed significant differences between subspecies, with *A.m mellifera* recording the greatest number of observations of both. These key findings align with the expected outcome of behavioural adaptations driven by natural selection acting upon the subspecies in different environments. The results also revealed that foraging was relatively low in *A.m mellifera*, but pollen-foraging and self-grooming behaviour showed significant similarities between the subspecies. The findings provide important insight into the behavioural adaptations of *A.mellifera* subspecies, with potential evidence of colony-level trade-offs between foraging and guarding behaviour. This could potentially guide hive management, in order to reduce widespread colony collapse and improve conservation efforts of *A.mellifera* subspecies.

Layman Summary

Twenty-four subspecies of European honey bee have evolved to different environmental conditions within Europe. Honey bees are important pollinators of flowers and crops. However, bee populations have collapsed in recent years due to habitat loss and climate change, which impacts the availability of flowers and causes an invasion of harmful parasitic mites. The Nordic brown bee (also called the British Black Bee, Native Irish Honey Bee or simply Dark Bee), was the first subspecies to colonise Northern Europe. The Nordic bee has important adaptations to its environment, including good flight strength in cold and windy conditions, and a significant drive to collect pollen. However, this subspecies has a negative reputation in the beekeeping community due to its relative aggression. Since the 20th century, beekeepers in Northern Europe have favoured imported, non-native honey bees like the Italian bee, the Carniolan bee and the Buckfast bee.

Evidence suggests that honey bees are more likely to survive and provide important pollination services when kept in their native environment, but research comparing the behaviour of native and non-native subspecies remains limited. This research project aimed to investigate the behavioural adaptations of the Nordic bee in its native environment of Southwestern Sweden, compared to three non-native subspecies. Understanding how behaviour differs between subspecies could help us develop new ways of managing and conserving the brown bee, and also helps us predict how climate change might continue to impact each subspecies.

The results of this study reveal behavioural differences between honey bee subspecies. A key finding is that the Nordic bee recorded much greater male drone bee activity. Male drones leave the hive to mate with queen bees, so this suggests that this native subspecies is attempting to

mate more often than non-native subspecies. The Nordic bee also showed the greatest amount of guarding activity, with more 'guard bees' waiting outside the hive to check if landing bees belong to their own colony, or if they are robber bees trying to steal honey. This supports evidence that the Nordic bee is comparatively aggressive. Interestingly, the Nordic bee recorded the lowest number of foragers. This might be because less worker bees were available due to the significant number carrying out guarding behaviour throughout the day. Further study is required to confirm this finding. The four subspecies carried out pollen-foraging and self-grooming behaviours to a similar extent, which might suggest that the non-native subspecies have locally-adapted to this environment in Sweden. To conclude, this study provides an important insight into how behaviour differs between different subspecies of European honey bee. The results provide an insight into how honey bees have adapted to their native environments, and can potentially be used to guide hive management and maximise pollination services.

Research Article

1. Introduction

European honey bees (*Apis mellifera*) express many behaviours in their lifetime, transitioning from roles inside the hive, such as cleaning and nursing larvae, to external tasks, like guarding and foraging, as they age (Seeley and Morse, 1976). *A. mellifera* is the sole pollinator of many flowering plants (Breeze et al., 2011), and a key pollinator of cultivated crops (Klein et al., 2007). The total economic value of global insect pollination is estimated at €153 billion annually, and the demand for agricultural pollination is only growing (Gallai et al., 2009, Aizen and Harder, 2009). However, ambient temperatures are increasing globally, which reduces honey bee foraging and results in decreased pollination of flowering plants (Blazyte-Cereskiene et al., 2010), (Abou-Shaara et al., 2012). This has severe implications for ecosystem services, food production and floral biodiversity.

Climate change, particularly global warming, is a key driver in European honey bee decline (Reddy et al., 2012). Global warming is linked to increasing incidence of *Varroa destructor* mites, and the biological invasion of other parasites such as the small hive beetle (*Aethina tumida*) both of which have a direct and severe impact on individual bee health (van Dooremalen et al., 2012, Cornelissen et al., 2019). A further effect of global climate change is the decline in floral diversity, which impacts availability of food resources for honey bees (Goulson et al., 2015), (Kuchling et al., 2018). Recent studies also suggest that climate change could disrupt honey bee overwintering strategies, however this requires further study (Norrström et al., 2021). Human activities, specifically land-use intensification, further impact honey bees by creating fragmentation between colonies (Potts et al., 2016). All of these effects directly impact honey bee behaviour, physiology and colony survival, and contribute to significant annual colony loss of managed honey bees (Le Conte and Navajas, 2008, Neumann and Carreck, 2010, Brodschneider et al., 2018).

Twenty-four known subspecies of *A. mellifera* have evolved by allopatric separation and adaptation to different environmental conditions within Europe (Ruttner, 1988). High genetic variability exists between these subspecies (De La Rúa et al., 2009). This pattern of allopatric diversification is consistent with the theory of ecological speciation, whereby populations of a species become differentially adapted to different environments through the process of natural selection (Nosil, 2012). The Nordic brown bee subspecies (*Apis mellifera mellifera*) is endangered (De La Rúa et al., 2009). *A. m mellifera* was the first subspecies to colonise Northern Europe, adapting to the Nordic-Baltic's short summers and cold winters, and co-evolving with local flora and fauna

(Ruottinen et al., 2014). Further adaptations, such as winter hardiness, good flight strength in cold and windy conditions, and a significant drive to collect pollen, explain why *A.m mellifera* is ecologically important in the Northern European climate (Ruttner, 1988). A Europe-wide colony development study suggests that variation between subspecies is maintained due to natural selection favouring phenotypes with important local adaptations (Hatjina et al., 2014). Therefore, it can be inferred that locally-adapted subspecies have a higher survival ability, and that *A.m mellifera* has a higher fitness in the Nordic-Baltic region compared to non-native subspecies.

However, *A.m mellifera* has suffered from a negative reputation in Northern Europe due to subjectively undesirable qualities, such as higher aggression and higher swarming tendency relative to imported subspecies (Ruttner, 1988). This is despite that these negative qualities are often associated with poor management (Ruottinen et al., 2014). As a result, beekeepers favoured southern European subspecies (particularly *A.m ligustica*) or synthetic hybrid subspecies (Buckfast) with the progression of the 20th century. This favouritism has led to significant habitat loss and fragmentation in *A.m mellifera* (Jensen et al., 2005). Therefore, the genetic diversity required to maintain healthy populations of *A.m mellifera* has substantially declined (De La Rúa et al., 2009).

A.m mellifera is particularly susceptible to colony loss, due to low genetic diversity and low levels of gene flow between *A.m mellifera* colonies (Ruottinen et al., 2014). The Nordic Genetic Resource Centre conducted literature studies, seminars and fieldwork across Europe from February 2011 – December 2012 to determine the conservation status of *A.m. mellifera*. Populations of special conservation interest were highlighted in Ireland, Scotland, Norway, Denmark and Sweden (Ruottinen et al., 2014). However, maintaining the current genetic purity status of *A.m mellifera* in these areas relies on isolated mating stations, where purebred virgin queens are mated with drone males to prevent hybridisation from other subspecies (Böttcher, 1947). Current management in Europe does not prevent gene flow between different subspecies (Soland-Reckeweg et al., 2009). The NordGen project suggested that the best possibilities for *in situ* conservation lie in the Nordic countries, particularly Sweden and Norway, where permanent populations of *A.m mellifera* exist and where hive density is less than 0.5 hives/km² (Ruottinen et al., 2014).

Studies suggest that *A.mellifera* subspecies have different behavioural adaptations (Costa et al., 2012, Büchler et al., 2014, Uzunov et al., 2014). Colonies of *A. mellifera* are genetically distinct due to individual genotype-environment interactions, and colonies kept close to their genotypic origin have greater overwintering survival and larger population size (Büchler et al., 2014, Hatjina et al., 2014). Additionally, colonies kept in environmental conditions similar to their genotypic origin have been found to collect more pollen compared to non-native colonies (Taha and Al-Kahtani, 2019). However, studies comparing behaviour between different *A.mellifera* subspecies are few, and our understanding of how specific behavioural adaptations differ between native and non-native subspecies remains limited. Understanding behavioural differences between subspecies has an important implication, as it could potentially help us predict how each subspecies might react to climate change, as well as providing greater insight into the foraging activity, defensiveness and hygiene of each subspecies.

My research project addresses this knowledge gap by comparing the simultaneous behaviour of four *A.mellifera* sub-species (Nordic *Apis mellifera mellifera*; Italian *A.m. ligustica*; Carniolan *A.m carnica*; hybrid *A.m 'Buckfast'*), in one locality, at different times of the day. Data was collected in 2021 during a field study at Nordens Ark, Sweden. A novel filming method was developed for this project, to assist the direct comparison of multiple behaviours between subspecies. This study is the first to investigate multiple behaviours at the hive entrances of different *A.mellifera* subspecies simultaneously. The initiative was developed at Nordens Ark as part of the cross-border

INTERREG project “Supporting Nordic Brown Bees – A Unique Resource For Our Ecosystem”. The Nordic bee (*A.m mellifera*) is the model native subspecies in this study. It was predicted that *A.m mellifera* would differ significantly in its behaviour compared to the three non-native subspecies due to adaptation to the region of study. The main aim of this research project is to highlight behavioural differences between different subspecies of *A. mellifera*.

Hypothesis:

H0: Behaviour does not significantly differ between four *Apis mellifera* subspecies

H1: Given the different origins of four *Apis mellifera* subspecies, behaviour significantly differs between these subspecies

2. Methods

2.1 Apiary design

The apiary used in this field study was established in Summer 2019 at Nordens Ark, Southwestern Sweden (Lat/Long 58.442481°N, 11.437202°E, 25m asl). The local landscape is comprised of meadows, coniferous and deciduous forest, with a slight oceanic climate. Sixteen beehives were placed in two lines of 8 beehives each. Beehives were installed in pairs, with one hive entrance of the pair orientated to the southwest and the other to the southeast, at an angle of approximately 90°. The sixteen beehives housed four colonies of four different subspecies of *A.mellifera*; Nordic *A.m mellifera* (model subspecies); Italian *A.m. ligustica*; Carniolan *A.m carnica*; and hybrid ‘Buckfast’. The experimental design of the Nordens Ark apiary is fully detailed in the first publication from the INTERREG bee project (Norrström et al., 2021).

2.2 Recording video footage

From 28th July 2021 – 9th September 2021, 1-hour of video footage was filmed simultaneously at the hive entrances of four different *A.mellifera* subspecies in the Nordens Ark apiary (Section 2.1) every day for 45 days total. To begin each filming session, four cameras (‘Ricoh WG-60 Model R02090’) were attached to four tripods (‘Gorilla Pod’). Each tripod was then attached onto a wooden post distanced 200mm from the hive entrances, and the cameras were positioned so that the hive entrance was entirely visible on screen. All four cameras were started within 15 seconds so that filming was close as close to simultaneous as possible. Recordings were stopped after 60 minutes.

A different hive (colony) from each subspecies was filmed each day on rotation. Recording period (morning/afternoon/evening) and specific hour of recording was randomly selected each day, for a total of 15 days of each recording period. 1-hour recording times were randomised within recording periods as follows; Morning 0700-1100; Afternoon 1200-1600; Evening 1700-2200. In total, 180 1-hour films were recorded (4x1 hour x 45 days = 45 hours per subspecies).

2.3 Reviewing video footage

The 1-hour films were reviewed to quantify behaviours observed on and around the hive entrance, and address the project’s aim of highlighting differences in behaviour between subspecies. To count entrances and exits of worker bees, and entrances and exits of drone bees, a 2-minute period was randomly selected using an online number randomiser from 1-30 (e.g number 4 = 4th 2-

minute period = 6min-8min of video). The 2-minute period was the same for each date of films (ie. for each four hives filmed simultaneously). An exit was counted when a bee exited via the hive gate. An entrance was counted when a bee flew directly into the hive gate, or flew to land on the hive gate and crawled inside. Bees which exited via the gate and immediately re-entered were not counted.

To count all other behaviours observed on and around the hive entrance, a 5-minute period was randomly selected using an online number randomiser from 1-12. (e.g number 4 = 4th 5-minute period = 15min-20min of video). The 5-minute period was the same for each date of films. All behaviours observed during the 5-minute clip were recorded. A behaviour guide was designed to include all behaviours observed during trial observations (Table 1). A behaviour was only counted when it could be clearly recognised by the description in the behaviour guide. If there was any doubt about a behaviour, (ie. assumed to be a particular behaviour but out-of-focus or partially out of frame), it was excluded. The results are therefore minimum values.

Table 1: Behaviour guide designed for this project to include all the behaviours observed during trial observations, in the two weeks before data collection, as described by literature.

Behaviour:	Described by:	Brief Description:
Allo-grooming	(Moore et al., 1995)	Bee (worker or drone) grooming another worker with legs, proboscis or mandibles
Defence	(Butler and Free, 1952)	Worker bee stinging, biting, grasping, pushing or dragging a robber bee or invading hornet
Fanning (air circulation)	(Lindauer, 1954)	Worker bee stationary, fanning wings with abdomen raised upwards
Fanning (projecting Nasonov pheromone)	(Avitabile et al., 1975, Free, 1987)	Worker bee stationary, fanning wings with abdomen raised upwards, last segment of abdomen pointed downwards, pale yellow-orange Nasonov gland exposed
Foraging	(Seeley and Kolmes, 1991)	Worker bee returning to hive without pollen or resin (assumed to have been foraging for nectar/water/propolis, or returning from orientation flight) Counted as bee flying into hive entrance or flying to land at hive entrance and crawling inside
Guarding	(Butler and Free, 1952, Free, 1954)	Worker bee touching returning bees with their antennae
Pollen-foraging		Worker returning to hive with pollen in corbiculae (pollen baskets) on legs
Undertaking	(Trumbo et al., 1997)	'Undertaker' workers dragging dead bee out of the hive
Resin-collecting	(Meyer and Ulrich, 1956)	Worker returning to hive with shiny resin in corbiculae (pollen baskets) on legs
Self-grooming	(Peng et al., 1987, Boecking et al., 1993)	Bee (drone or worker) cleaning self with legs, proboscis or mandibles

2.4 Data analysis

Data analysis was conducted on the numbers of behavioural observations using IBM SPSS Statistics Version 26 (IBM, 2019). A one-way MANOVA was conducted using the 'Multivariate Test' function on SPSS between the six most-commonly observed behaviour variables (worker exits, worker entrances, foraging, guarding, pollen foraging and self-grooming) and honey bee subspecies (*A.m mellifera*, *A.m ligustica*, *A.m carnica*, Buckfast). A number of behaviours (fanning, defence, undertaking, resin-collecting, allo-grooming) were removed from analysis due to a limited number of observations. MANOVAs test for a statistically significant difference between independent groups (subspecies) in multiple dependent variables (behaviours). A p-value <0.05 suggests that the hypothesis is supported. This MANOVA therefore addresses the project's aim by testing for a significant difference in behaviour between the four subspecies.

Further analysis was conducted with univariate ANOVAs ('Tests of Between-Subjects Effects' function on SPSS) between subspecies and each behaviour, including drone entrances and exits. ANOVAs test for significant differences between independent groups (subspecies) on one dependent variable (behaviour). These ANOVAs therefore further address the project's aim of highlighting behavioural differences between *A.mellifera* subspecies. A p-value <0.05 suggests that the subspecies show significant differences in the tested behaviour.

Comparison of means tests and standard deviations were calculated using the 'Compare Means' function on SPSS. Comparison of means tests calculate the mean number of observations of a particular behaviour for each subspecies. This further addresses the project's aim by facilitating direct comparison of behaviour means between subspecies. Standard deviations also illustrate behavioural differences by revealing how dispersed each subspecies' dataset is in relation to the mean.

3. Results

3.1 Key findings: differences in drone activity and guarding behaviour

Statistical analysis suggested that behaviour differed significantly between the four *A.mellifera* subspecies. The one-way MANOVA suggested that there was a statistically significant difference between the four subspecies in multiple behaviour variables (Wilks' Lambda = 0.833, p = 0.024). P-value = <0.05, which supports the hypothesis.

The results reveal differences in drone behaviour between the subspecies. There was a statistically significant difference between subspecies in the number of male drones entering the hive (ANOVA, p = 0.023). However, the effect of subspecies on drone exits was non-significant (ANOVA, p=0.215). Drone entrances and exits were greatest in *A.m mellifera*. *A.m mellifera* recorded an average of 1.76 drone entrances ($SD=5.666$, $N=45$) and 1.71 drone exits ($SD=6.423$, $N=45$) per 2-minute clip. In comparison, the three non-native subspecies recorded very little drone activity. Buckfast recorded an average of 0.44 drone entrances ($SD=1.374$, $N=45$) and 0.87 exits ($SD=3.259$, $N=45$) per 5-minutes, whilst *A.m carnica* recorded 0.18 entrances ($M=0.18$, $SD=0.806$) and 0.60 exits ($M=0.60$, $SD=0.2.666$) per 2-minutes. *A.m ligustica* showed the least drone behaviour, with an average of 0.02 entrances ($SD=0.149$, $N=45$) and 0.02 exits ($SD=0.149$, $N=45$) per 2-minutes. The greatest drone activity occurred in the afternoon, however, small numbers of *A.m mellifera* and *A.m carnica* drones were also active in the morning and/or evening (Table 2).

Table 2: Mean values of drone bee entrances and exits in a 2-minute video clip for each subspecies during different times of the day.

Subspecies	Drone Exits (morning)	Drone Entrances (morning)	Drone Exits (afternoon)	Drone Entrances (afternoon)	Drone Exits (evening)	Drone Entrances (evening)
<i>A.m carnica</i>	0.0	0.1	1.3	0.1	0.5	0.3
Buckfast	0.0	0.0	2.6	1.3	0.0	0.0
<i>A.m mellifera</i>	0.3	0.6	4.8	4.7	0.0	0.0
<i>A.m ligustica</i>	0.0	0.0	0.1	0.1	0.0	0.0

My results also reveal differences in guarding behaviour between the four subspecies. There was a statistically significant difference between subspecies in number of guard bees observed per 5-minutes (ANOVA, $p=0.01$). *A.m. mellifera* showed the greatest guarding activity, with an average of 19.0 guard bees observed per 5-minutes ($SD=17.4$, $N=45$). *A.m carnica* recorded an average of 15.6 guard bees ($SD=14.6$, $N=45$), followed by Buckfast, with an average of 10.6 guard bees observed per 5-minutes ($SD=12.3$, $N=45$). *A.m ligustica* showed the least guarding activity, with an average of 8.20 guard bees observed per 5-minute clip ($SD=8.93$, $N=45$). The total mean number of guard bees observed per 5 minutes was 13.4 ($SD=14.2$, $N=180$). The results also revealed some differences between subspecies at different times of the day, with *A.m mellifera* and *A.m carnica* showing relatively high guarding activity in the evening (Table 3) (See Appendix II, Fig. S2).

Table 3: Table showing the mean number of guard bees seen on the hive in a 5-minute video clip in the morning, afternoon and evening.

Subspecies	Number of guard bees (morning)	Number of guard bees (afternoon)	Number of guard bees (evening)
<i>A.m carnica</i>	13	16	18
Buckfast	10	15	7
<i>A.m mellifera</i>	15	24	18
<i>A.m ligustica</i>	5	16	4

3.2 Differences in foraging behaviour

The results reveal differences between subspecies in the number of worker bees entering and exiting the hive. Worker entrances and exits were lowest in *A.m mellifera*. *A.m mellifera* recorded an average of 40.6 exits ($SD=36.19$, $N=45$) and 35.2 entrances ($SD=35.77$, $N=45$) per 2-minutes. *A.m carnica* had the highest number of worker entrances and exits, with an average of 58.4 exits ($SD=63.94$, $N=45$) and 56.5 entrances ($SD=62.61$, $N=45$) per 2-minutes. *A.m ligustica* recorded an average of 46.6 exits ($SD=52.14$, $N=45$) and 50.5 entrances ($SD=68.35$, $N=45$), followed by Buckfast, which recorded an average of 42.6 exits ($SD=52.14$, $N=45$) and 41.7 entrances ($SD=49.81$, $N=45$) per 2-minutes. The total averages were 47.1 worker exits ($SD=52.1$, $N=180$), and 46.0 entrances ($SD=55.7$, $N=180$) observed per 2-minutes. The effect of subspecies on worker entrances and worker exits was found to be non-significant (ANOVA, $p = 0.371$; $p=0.282$,

respectively). *A.m mellifera* and *A.m carnica* recorded a relatively high number of worker entrances and exits in the evening (Fig. 1), (see Appendix II, Table S5).

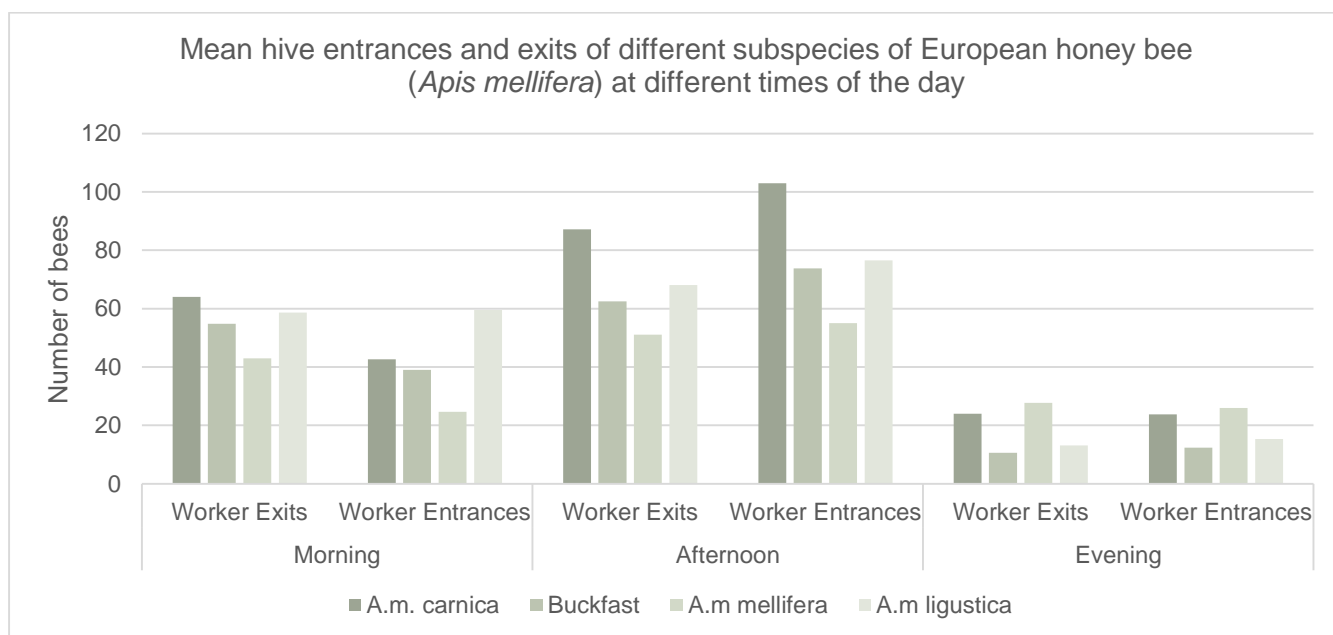


Fig. 1: Bar chart showing mean values of worker bee entrances and exits from the hive in a 2-minute video clip for each subspecies during different times of the day.

The results also reveal differences between subspecies in terms of foraging behaviour. Foraging activity was lowest in *A.m mellifera*. The average number of *A.m mellifera* bees returning from foraging was 81.9 ($SD=89.5$, $N=45$) per 5-minutes. Foraging activity was highest in *A.m carnica*, with an average of 119 forager bees ($SD=137.0$, $N=45$) observed per 5-minutes, followed by *A.m ligustica* with 106 bees ($SD=110.0$, $N=45$), then Buckfast, with 90.7 bees ($SD=119.7$, $N=45$). The total average number of returning foragers observed per 5-minute clip was 99.3 ($SD=115.2$, $N=180$). The effect of subspecies on foraging activity was found to be non-significant (ANOVA, $p=0.446$). *A.m mellifera* and *A.m carnica* recorded a relatively high number of returning foragers in the evening (see Appendix II, Table S6).

The results suggest pollen-foraging behaviour was similar between the subspecies. The four subspecies recorded similar numbers of returning pollen foragers, with the greatest pollen-foraging activity occurring in the afternoon, and the lowest in the evening (See Appendix II, Table S7). The effect of subspecies on pollen foraging behaviour was found to be non-significant (ANOVA, $p=0.988$). *A.m carnica* recorded an average of 7.98 bees returning with pollen ($SD=25.3$, $N=45$) per 5-minutes, compared to 7.84 *A.m mellifera* bees ($SD=17.4$, $N=45$), 7.60 Buckfast bees ($SD=16.6$, $N=45$) and 6.69 *A.m ligustica* bees ($SD=13.4$, $N=45$). The overall mean number of returning pollen-foragers was 7.52 per 5-minutes ($SD=18.57$, $N=180$). *A.m mellifera* and *A.m carnica* recorded a relatively high number of returning pollen-foragers in the evening (See Appendix II, Table S7).

3.3 Differences in self-grooming behaviour

The findings suggest that self-grooming behaviour was similar between the subspecies. The effect of subspecies on self-grooming was found to be non-significant (ANOVA, $p=0.914$). *A.m mellifera* recorded the highest self-grooming activity, with an average of 17.4 self-grooming bees ($SD=16.04$, $N=45$) observed per 5-minutes. This result is followed closely by *A.m ligustica* with an

average of 16.6 self-grooming bees ($SD=12.89$, $N=45$), then Buckfast, with average 16.4 bees ($SD=17.47$, $N=45$), and *A.m carnica*, with 15.2 self-grooming bees per 5-minutes ($SD=12.9$, $N=45$). The overall mean was 16.4 self-grooming bees observed per 5-minutes ($SD=14.9$, $N=180$). However, the results reveal some differences in self-grooming behaviour at different times of the day (See Appendix II, Table S8, Fig. S3).

4. Discussion

4.1 Key Findings

As predicted, the key finding of this research project is that the four studied *A.mellifera* subspecies showed differences in their behaviour. The most significant differences between subspecies were observed in drone behaviour and guarding behaviour, with the native *A.m mellifera* subspecies recording the greatest number of observations of both. Interestingly, the results also revealed that foraging activity and hive entrances and exits of worker bees were lowest in *A.m mellifera*. However, pollen-foraging and self-grooming behaviour showed similarities between the subspecies. It is not apparent from this dataset alone if the behaviour of the non-native subspecies was close to or significantly different from their “natural” behaviour (ie. behaviour exhibited in the region to which they have adapted), and therefore this requires further study. The findings of this project fill a previous gap in comparative *A.mellifera* behavioural studies, and partially contribute to our limited understanding of specific behavioural differences between native and non-native subspecies.

4.2 Discussion of Findings

The drone behaviour results suggest that *A.m mellifera* carried out more mating flights than the three non-native subspecies (Table 2). Male drones exit the hive to gather in drone congregation areas to mate with a queen, and die shortly after mating (Koeniger et al., 2005). However, this does not imply that mating was more successful in *A.m mellifera*, as the drone entrances were likely adults that have not mated returning to the hive to feed (Gary, 1992), or juvenile drones returning from an orientation flight (Howell and Usinger, 1933). My results are consistent with literature which suggests that mature drones usually begin mating flights in the afternoon (Hellmich et al., 1991). However, my results suggest that *A.m mellifera* and *A.m carnica* began mating flights earlier than the other subspecies (Table 2). *A.m carnica* drones were also observed in the evening, which supports evidence of drone mating flights occurring later in the day as the season progresses (Taber, 1964). A possible cause for these differences between subspecies may be that drone mating flights are triggered by different climatic conditions for each subspecies. Indeed, evidence suggests that photoperiod, temperature and light intensity influence flight activity of honey bees (Kefuss and Nye, 1970). For this reason, climate change might have a severe impact on drone mating flights.

The results support the general understanding that *A.m. mellifera* is comparatively aggressive and defensive of robber bees (Ruottinen et al., 2014) (Table 3). The results also support evidence of high variability in defensive behaviour between *A.mellifera* subspecies (Uzunov et al., 2014). A possible explanation for this variability in defensive behaviour could be that foraging behaviour and guarding behaviour are mutually-exclusive tasks. The oldest workers in a honeybee colony carry out the roles of foraging and nest defense, as these behaviours require the strongest flight

capabilities (Breed et al., 1990, Suarez et al., 1996). Therefore, foraging and guarding behaviours are believed to be subject to a colony-level trade-off (Giray et al., 2000, Nouvian et al., 2015, Rivera-Marchand et al., 2008). This could explain the negative correlation between foraging and guarding in both *A.m mellifera* and *A.m ligustica*, but the relationship is less clear for Buckfast and *A.m carnica* (see Appendix II, Fig. S1, Fig. S2).

It was observed that guarding activity of all subspecies increased after the evening of 2nd September 2021, when honey was harvested from the apiary and replaced with sugar solution for overwintering (see Appendix I: Raw Data). This supports literature which suggests that defensive behaviour (aggression, guarding) in honey bees increases when colony resources are limited (Siefert et al., 2021). Because honey resources are essential for brood rearing in late winter (Seeley, 1995), it is perhaps critical that there are more guard bees present at the hive entrance to prevent robber bees from stealing the colony's limited resources. My data seems to corroborate this, but further experimental research could aim to correlate guarding and defensive behaviour with the weight of honey in the hive. This could be important for understanding role allocation trade-offs in *A.mellifera* workers, and how the species adapts to changing conditions within the hive.

Literature suggests that *A.m mellifera* has a comparatively low number of foraging worker bees compared to other *A. mellifera* subspecies, due to its high capability for brood-rearing (Ruttner, 1988). It is possible that the lower foraging activity in *A.m mellifera* could be attributed to comparatively more nurse bees than the other subspecies. A further possible explanation is that *A.m mellifera* colonies do not grow particularly large, meaning numbers of worker bees are comparatively limited (Ruottinen et al., 2014). Alternatively, the lower levels of foraging in *A.m mellifera* could be a reflection of competition from the non-native subspecies present in the same apiary. *A.m mellifera* could also be impacted by competition from native wild pollinators with the same floral niche (Rasmussen et al., 2021). However, a limitation of this explanation is that wild pollinators are often more adversely impacted by competition than managed bees (Mallinger et al., 2017). There are currently no studies comparing the behaviour of managed and wild honey bee colonies of the same subspecies, however, this could potentially reveal important behavioural distinctions which could impact selective breeding between managed and wild colonies.

My results support the general opinion that *A.m mellifera* is comparatively unproductive, and this perhaps reinforces some negative opinions of *A.m mellifera* in the Nordic beekeeping community (see Appendix II, Table S5, Table S6). However, when considering the low number of *A.m mellifera* workers observed entering the hive, the pollen foraging activity in this subspecies is still relatively high (Appendix II, Table S7). This was expected, as *A.m mellifera* is known for its significant drive for pollen collection (Ruttner, 1988). The similarities in pollen-foraging behaviour might suggest that the colonies of non-native *A.m ligustica*, *A.m carnica* and Buckfast have locally adapted to the flora of Southwestern Sweden. This could have an important implication for the success of these non-native subspecies in this region, as multiple studies have suggested that local adaptation to an environment is a key influence of survival of *A.mellifera* colonies (Costa et al., 2012, Büchler et al., 2014, Uzunov et al., 2014, Hatjina et al., 2014).

The similarities in self-grooming behaviour between the subspecies (Appendix II, Table S8) is likely due to a common defense against *Varroa destructor* mites. *Varroa* mites shifted from Eastern honey bee (*Apis cerana*) hosts to *Apis mellifera* in the last century, and are now found in colonies worldwide (Rosenkranz et al., 2010). Although the evidence is not conclusive, self-grooming behaviour in *A. mellifera* has been correlated with lower mite infestation levels in colonies, and is believed to provide some degree of *Varroa* resistance (Arechavaleta-Velasco and Guzmán-Novoa,

2001, Currie and Tahmasbi, 2008, Guzman-Novoa et al., 2012). Therefore, it is unclear why Buckfast showed differences in self-grooming behaviour at different times of the day (see Appendix II, Table S8).

An improvement to this research project would be an increase in the length of video clips used to review the behaviours, specifically those which were removed from analysis due to insufficient data, so that possible differences between subspecies could be clearly observed. However, the 2-minute clips and 5-minute clips were suitable for the behaviours discussed above. These behaviours were observed commonly, and it is therefore unlikely that increased observation time would significantly change these results.

4.3 Conclusion

This research project fulfils its aim by providing a greater scientific understanding of how foraging, defensive, self-grooming and drone behaviour differs between *A. mellifera* subspecies. The results support the hypothesis, suggesting that behaviour significantly differs between native and non-native subspecies. The key finding of the study is that guarding behaviour and drone activity were significantly greater in the native *A. m mellifera*. However, foraging behaviour and worker entrances and exits were lowest in *A. m mellifera*. This lends itself to further study of the colony-level trade-offs which might determine role allocation of workers, specifically factors which might trigger or influence the apparent trade-off between hive defence and foraging. *A. m mellifera* could be used as a model subspecies for this research, as the results for this subspecies suggest that foraging, pollen-foraging and guarding were mutually-exclusive tasks. These findings advance this field of research by providing potential evidence of a foraging-guarding trade-off, which could be used to guide hive management for the endangered *A. m mellifera* subspecies.

In conclusion, this research provides evidence that *A. mellifera* subspecies are adapted to different environmental conditions. Understanding behavioural adaptations between subspecies has an important implication in helping us predict the extent to which climate change, floral resource availability and disease might continue to impact this essential species of pollinators (Neumann and Carreck, 2010, Potts et al., 2010, Brodschneider et al., 2018).

5. Bibliography:

5.1 Literature

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5.2 Software

IBM Corp., 2019. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp. Available at: <https://hadoop.apache.org>

6. Appendices

6.1 Appendix I: Raw Data used in analysis (Section 3.1)

Table S1: Table of raw data - numbers of observations of each behaviour, collected by reviewing video clips of *A.m ligustica* recorded on different dates

Sub-species	Date	Recording Period	Worker Exits	Worker Entrances	Drone Exits	Drone Entrances	Foraging	Guarding	Pollen foragers	Self-grooming
A.m ligustica	SUN 1st August 2021	Morning	20	21	0	0	76	4	0	25
A.m ligustica	MON 2nd August 2021	Morning	12	6	0	0	15	0	0	6
A.m ligustica	TUE 3rd August 2021	Morning	67	25	0	0	74	0	47	17
A.m ligustica	MON 9th August 2021	Morning	74	30	0	0	62	1	1	25
A.m ligustica	TUE 10th August 2021	Morning	1	3	0	0	3	0	0	2
A.m ligustica	FRI 13th August 2021	Morning	240	385	0	0	251	5	0	10
A.m ligustica	SAT 21st August 2021	Morning	78	102	0	0	270	18	6	17
A.m ligustica	SAT 14th August 2021	Morning	5	3	0	0	3	0	0	4
A.m ligustica	SUN 15th August 2021	Morning	72	84	0	0	182	0	7	10
A.m ligustica	MON 23rd August 2021	Morning	8	6	0	0	8	9	0	13
A.m ligustica	WED 25th August 2021	Morning	96	103	0	0	221	14	22	25
A.m ligustica	FRI 27th August 2021	Morning	15	8	0	0	0	0	0	0
A.m ligustica	SAT 28th August 2021	Morning	42	59	0	0	223	13	11	34
A.m ligustica	MON 30th August 2021	Morning	148	58	0	0	99	8	0	22
A.m ligustica	FRI 3rd September 2021	Morning	2	0	0	0	6	0	0	2
A.m ligustica	WED 28th July 2021	Afternoon	134	143	0	0	366	15	4	10
A.m ligustica	FRI 30th July 2021	Afternoon	143	137	1	1	192	6	34	35
A.m ligustica	SAT 31st July 2021	Afternoon	23	9	0	0	33	0	0	33
A.m ligustica	WED 4th August 2021	Afternoon	63	49	0	0	87	17	37	21
A.m ligustica	SUN 8th August 2021	Afternoon	8	3	0	0	37	20	0	3
A.m ligustica	WED 11th August 2021	Afternoon	64	134	0	0	294	9	3	34
A.m ligustica	THU 12th August 2021	Afternoon	128	147	0	0	322	25	61	42
A.m ligustica	MON 16th August 2021	Afternoon	37	117	0	0	107	35	2	32
A.m ligustica	FRI 20th August 2021	Afternoon	82	108	0	0	303	17	19	25
A.m ligustica	THU 26th August 2021	Afternoon	28	17	0	0	40	7	2	50
A.m ligustica	SUN 29th August 2021	Afternoon	98	83	0	0	219	16	12	26
A.m ligustica	TUE 31st August 2021	Afternoon	118	95	0	0	273	13	3	10
A.m ligustica	SUN 5th September 2021	Afternoon	49	42	0	0	190	28	8	12
A.m ligustica	MON 6th September 2021	Afternoon	29	29	0	0	51	11	10	14
A.m ligustica	FRI 10th September 2021	Afternoon	18	35	0	0	71	22	6	12
A.m ligustica	THU 29th July 2021	Evening	23	19	0	0	32	0	0	9
A.m ligustica	THU 5th August 2021	Evening	0	0	0	0	0	0	0	0
A.m ligustica	FRI 6th August 2021	Evening	18	64	0	0	277	1	1	17
A.m ligustica	SAT 7th August 2021	Evening	0	2	0	0	10	0	0	1
A.m ligustica	TUE 17th August 2021	Evening	0	1	0	0	1	0	0	1
A.m ligustica	WED 18th August 2021	Evening	20	14	0	0	18	4	2	27
A.m ligustica	THU 19th August 2021	Evening	5	6	0	0	5	3	0	1
A.m ligustica	MON 23 rd August 2021	Evening	0	1	0	0	7	0	0	3
A.m ligustica	TUE 24th August 2021	Evening	3	6	0	0	5	6	0	2
A.m ligustica	WED 1st September 2021	Evening	21	14	0	0	55	1	2	21
A.m ligustica	THU 2nd September 2021*	Evening	13	15	0	0	110	21	0	38
A.m ligustica	SAT 4th September 2021	Evening	2	6	0	0	7	1	0	5
A.m ligustica	TUE 7th September 2021	Evening	41	29	0	0	44	6	1	19
A.m ligustica	WED 8th September 2021	Evening	44	47	0	0	77	6	0	23
A.m ligustica	THU 9th September 2021	Evening	7	6	0	0	48	7	0	9

Table S2: Table of raw data - numbers of observations of each behaviour, collected by reviewing video clips of *A.m mellifera* recorded on different dates

Sub-species	Date	Recording Period	Worker Exits	Worker Entrances	Drone Exits	Drone Entrances	Foraging	Guarding	Pollen foragers	Self-grooming
A.m mellifera	SUN 1st August 2021	Morning	21	22	0	0	55	1	1	17
A.m mellifera	MON 2nd August 2021	Morning	2	0	0	0	0	0	0	3
A.m mellifera	TUE 3rd August 2021	Morning	27	21	0	0	52	3	20	17
A.m mellifera	MON 9th August 2021	Morning	7	7	0	0	21	19	1	6
A.m mellifera	TUE 10th August 2021	Morning	4	8	0	0	21	24	0	5
A.m mellifera	FRI 13th August 2021	Morning	99	43	0	0	52	10	0	10
A.m mellifera	SAT 21st August 2021	Morning	71	63	0	0	84	42	42	18
A.m mellifera	SAT 14th August 2021	Morning	1	0	0	0	1	0	0	4
A.m mellifera	SUN 15th August 2021	Morning	107	77	5	9	285	18	2	24
A.m mellifera	MON 23rd August 2021	Morning	4	4	0	0	9	2	0	14
A.m mellifera	WED 25th August 2021	Morning	82	43	0	0	90	16	13	11
A.m mellifera	FRI 27th August 2021	Morning	3	4	0	0	5	0	0	3
A.m mellifera	SAT 28th August 2021	Morning	53	48	0	0	114	38	11	7
A.m mellifera	MON 30th August 2021	Morning	140	21	0	0	70	12	0	20
A.m mellifera	FRI 3rd September 2021	Morning	24	9	0	0	36	33	0	7
A.m mellifera	WED 28th July 2021	Afternoon	65	49	0	0	71	9	55	51
A.m mellifera	FRI 30th July 2021	Afternoon	59	81	34	14	136	15	7	54
A.m mellifera	SAT 31st July 2021	Afternoon	61	23	0	0	76	3	0	58
A.m mellifera	WED 4th August 2021	Afternoon	32	70	0	0	185	83	24	15
A.m mellifera	SUN 8th August 2021	Afternoon	4	11	0	0	66	17	3	8
A.m mellifera	WED 11th August 2021	Afternoon	53	83	27	30	189	11	3	18
A.m mellifera	THU 12th August 2021	Afternoon	71	185	4	7	494	55	93	23
A.m mellifera	MON 16th August 2021	Afternoon	49	36	0	0	101	42	2	9
A.m mellifera	FRI 20th August 2021	Afternoon	125	108	2	0	186	21	29	12
A.m mellifera	THU 26th August 2021	Afternoon	22	12	0	0	35	27	5	32
A.m mellifera	SUN 29th August 2021	Afternoon	68	24	0	0	65	14	18	14
A.m mellifera	TUE 31st August 2021	Afternoon	63	76	4	18	142	14	1	66
A.m mellifera	SUN 5th September 2021	Afternoon	25	44	0	0	114	33	5	17
A.m mellifera	MON 6th September 2021	Afternoon	14	2	0	0	0	3	0	6
A.m mellifera	FRI 10th September 2021	Afternoon	56	22	1	1	43	17	3	3
A.m mellifera	THU 29th July 2021	Evening	9	8	0	0	9	4	1	14
A.m mellifera	THU 5th August 2021	Evening	4	4	0	0	0	0	0	0
A.m mellifera	FRI 6th August 2021	Evening	90	81	0	0	163	21	3	5
A.m mellifera	SAT 7th August 2021	Evening	0	0	0	0	4	0	0	1
A.m mellifera	TUE 17th August 2021	Evening	2	1	0	0	3	0	0	2
A.m mellifera	WED 18th August 2021	Evening	49	34	0	0	46	16	0	43
A.m mellifera	THU 19th August 2021	Evening	9	15	0	0	28	9	0	18
A.m mellifera	MON 23 rd August 2021	Evening	26	20	0	0	62	49	0	20
A.m mellifera	TUE 24th August 2021	Evening	22	45	0	0	49	38	0	15
A.m mellifera	WED 1st September 2021	Evening	12	56	0	0	155	25	11	12
A.m mellifera	THU 2nd September 2021*	Evening	32	17	0	0	35	10	0	10
A.m mellifera	SAT 4th September 2021	Evening	75	42	0	0	117	35	0	25
A.m mellifera	TUE 7th September 2021	Evening	65	18	0	0	133	28	0	17
A.m mellifera	WED 8th September 2021	Evening	17	26	0	0	62	25	0	46
A.m mellifera	THU 9th September 2021	Evening	3	22	0	0	25	15	0	3

Table S3: Table of raw data - numbers of observations of each behaviour, collected by reviewing video clips of Buckfast recorded on different dates

Sub-species	Date	Recording Period	Worker Exits	Worker Entrances	Drone Exits	Drone Entrances	Foraging	Guarding	Pollen foragers	Self-grooming
Buckfast	SUN 1st August 2021	Morning	5	6	0	0	26	2	0	8
Buckfast	MON 2nd August 2021	Morning	0	2	0	0	5	0	0	4
Buckfast	TUE 3rd August 2021	Morning	101	71	0	0	42	14	89	24
Buckfast	MON 9th August 2021	Morning	97	28	0	0	83	0	0	70
Buckfast	TUE 10th August 2021	Morning	1	0	0	0	1	0	0	2
Buckfast	FRI 13th August 2021	Morning	189	38	0	0	72	0	0	72
Buckfast	SAT 21st August 2021	Morning	88	85	0	0	303	12	1	34
Buckfast	SAT 14th August 2021	Morning	0	1	0	0	0	0	0	0
Buckfast	SUN 15th August 2021	Morning	73	156	0	0	295	29	16	4
Buckfast	MON 23rd August 2021	Morning	28	27	0	0	37	17	0	12
Buckfast	WED 25th August 2021	Morning	142	99	0	0	169	52	9	47
Buckfast	FRI 27th August 2021	Morning	28	38	0	0	22	1	0	6
Buckfast	SAT 28th August 2021	Morning	44	20	0	0	71	5	16	25
Buckfast	MON 30th August 2021	Morning	22	7	0	0	17	14	0	11
Buckfast	FRI 3rd September 2021	Morning	4	7	0	0	7	9	0	14
Buckfast	WED 28th July 2021	Afternoon	202	117	8	3	224	20	0	31
Buckfast	FRI 30th July 2021	Afternoon	87	133	1	1	337	32	32	43
Buckfast	SAT 31st July 2021	Afternoon	23	14	0	0	51	0	0	19
Buckfast	WED 4th August 2021	Afternoon	27	39	0	0	50	5	29	12
Buckfast	SUN 8th August 2021	Afternoon	4	4	0	0	20	1	0	7
Buckfast	WED 11th August 2021	Afternoon	90	141	0	0	408	15	19	27
Buckfast	THU 12th August 2021	Afternoon	59	74	4	6	152	38	52	39
Buckfast	MON 16th August 2021	Afternoon	17	18	0	0	40	3	3	29
Buckfast	FRI 20th August 2021	Afternoon	103	81	4	3	217	6	26	24
Buckfast	THU 26th August 2021	Afternoon	7	11	0	0	15	1	0	5
Buckfast	SUN 29th August 2021	Afternoon	127	195	20	6	435	37	0	13
Buckfast	TUE 31st August 2021	Afternoon	99	129	0	0	361	12	13	16
Buckfast	SUN 5th September 2021	Afternoon	56	85	0	0	139	23	17	10
Buckfast	MON 6th September 2021	Afternoon	4	28	0	0	32	13	12	5
Buckfast	FRI 10th September 2021	Afternoon	33	38	2	1	77	16	7	4
Buckfast	THU 29th July 2021	Evening	10	10	0	0	32	0	0	6
Buckfast	THU 5th August 2021	Evening	1	1	0	0	0	0	0	0
Buckfast	FRI 6th August 2021	Evening	22	17	0	0	74	6	1	17
Buckfast	SAT 7th August 2021	Evening	0	3	0	0	29	8	0	5
Buckfast	TUE 17th August 2021	Evening	0	0	0	0	0	0	0	0
Buckfast	WED 18th August 2021	Evening	3	6	0	0	26	28	0	9
Buckfast	THU 19th August 2021	Evening	2	4	0	0	3	2	0	0
Buckfast	MON 23 rd August 2021	Evening	1	1	0	0	0	0	0	0
Buckfast	TUE 24th August 2021	Evening	2	1	0	0	3	0	0	0
Buckfast	WED 1st September 2021	Evening	1	2	0	0	18	4	0	3
Buckfast	THU 2nd September 2021*	Evening	50	44	0	0	47	12	0	42
Buckfast	SAT 4th September 2021	Evening	4	13	0	0	21	10	0	11
Buckfast	TUE 7th September 2021	Evening	58	59	0	0	68	11	0	16
Buckfast	WED 8th September 2021	Evening	3	22	0	0	52	20	0	11
Buckfast	THU 9th September 2021	Evening	2	2	0	0	0	0	0	2

Table S4: Table of raw data - numbers of observations of each behaviour, collected by reviewing video clips of *A.m carnica* recorded on different dates

Sub-species	Date	Recording Period	Worker Exits	Worker Entrances	Drone Exits	Drone Entrances	Foraging	Guarding	Pollen foragers	Self-grooming
A.m carnica	SUN 1st August 2021	Morning	57	64	0	0	125	4	1	28
A.m carnica	MON 2nd August 2021	Morning	13	6	0	0	18	0	0	13
A.m carnica	TUE 3rd August 2021	Morning	107	39	0	0	85	1	25	8
A.m carnica	MON 9th August 2021	Morning	95	14	0	0	37	8	0	1
A.m carnica	TUE 10th August 2021	Morning	1	8	0	0	17	13	0	19
A.m carnica	FRI 13th August 2021	Morning	154	41	0	0	53	16	1	12
A.m carnica	SAT 21st August 2021	Morning	201	143	0	0	330	40	21	27
A.m carnica	SAT 14th August 2021	Morning	2	3	0	0	7	0	0	3
A.m carnica	SUN 15th August 2021	Morning	64	111	0	1	162	7	7	32
A.m carnica	MON 23rd August 2021	Morning	7	9	0	0	27	4	0	19
A.m carnica	WED 25th August 2021	Morning	138	95	0	0	322	43	23	8
A.m carnica	FRI 27th August 2021	Morning	15	21	0	0	11	0	0	0
A.m carnica	SAT 28th August 2021	Morning	58	36	0	0	118	28	1	3
A.m carnica	MON 30th August 2021	Morning	45	40	0	0	89	20	0	11
A.m carnica	FRI 3rd September 2021	Morning	4	10	0	0	25	15	0	20
A.m carnica	WED 28th July 2021	Afternoon	205	117	16	2	180	3	0	38
A.m carnica	FRI 30th July 2021	Afternoon	106	117	0	0	211	4	42	50
A.m carnica	SAT 31st July 2021	Afternoon	12	15	0	0	37	0	0	16
A.m carnica	WED 4th August 2021	Afternoon	111	172	0	0	180	5	164	13
A.m carnica	SUN 8th August 2021	Afternoon	52	23	0	0	69	1	2	17
A.m carnica	WED 11th August 2021	Afternoon	44	140	0	0	309	7	18	22
A.m carnica	THU 12th August 2021	Afternoon	223	255	0	0	540	31	17	54
A.m carnica	MON 16th August 2021	Afternoon	21	68	0	0	127	42	3	0
A.m carnica	FRI 20th August 2021	Afternoon	112	175	0	0	217	14	3	26
A.m carnica	THU 26th August 2021	Afternoon	12	32	0	0	51	29	1	29
A.m carnica	SUN 29th August 2021	Afternoon	200	210	0	0	495	24	3	31
A.m carnica	TUE 31st August 2021	Afternoon	115	103	0	0	401	24	12	9
A.m carnica	SUN 5th September 2021	Afternoon	38	52	0	0	189	25	2	13
A.m carnica	MON 6th September 2021	Afternoon	10	12	0	0	26	7	0	4
A.m carnica	FRI 10th September 2021	Afternoon	46	54	3	0	131	17	3	9
A.m carnica	THU 29th July 2021	Evening	14	29	0	0	41	2	0	29
A.m carnica	THU 5th August 2021	Evening	0	0	0	0	0	0	0	0
A.m carnica	FRI 6th August 2021	Evening	68	121	0	0	343	34	2	7
A.m carnica	SAT 7th August 2021	Evening	1	4	0	0	15	2	0	0
A.m carnica	TUE 17th August 2021	Evening	1	3	0	0	6	9	0	3
A.m carnica	WED 18th August 2021	Evening	10	14	0	0	36	64	0	21
A.m carnica	THU 19th August 2021	Evening	0	2	0	0	10	0	0	4
A.m carnica	MON 23 rd August 2021	Evening	5	5	8	5	7	13	0	8
A.m carnica	TUE 24th August 2021	Evening	2	8	0	0	4	15	1	7
A.m carnica	WED 1st September 2021	Evening	7	17	0	0	50	19	4	2
A.m carnica	THU 2nd September 2021*	Evening	49	25	0	0	43	20	0	18
A.m carnica	SAT 4th September 2021	Evening	9	13	0	0	23	34	0	21
A.m carnica	TUE 7th September 2021	Evening	106	76	0	0	109	19	1	8
A.m carnica	WED 8th September 2021	Evening	88	36	0	0	59	27	2	15
A.m carnica	THU 9th September 2021	Evening	0	3	0	0	1	13	0	4

6.1 Appendix II: Supplementary Figures for Results (Section 5)

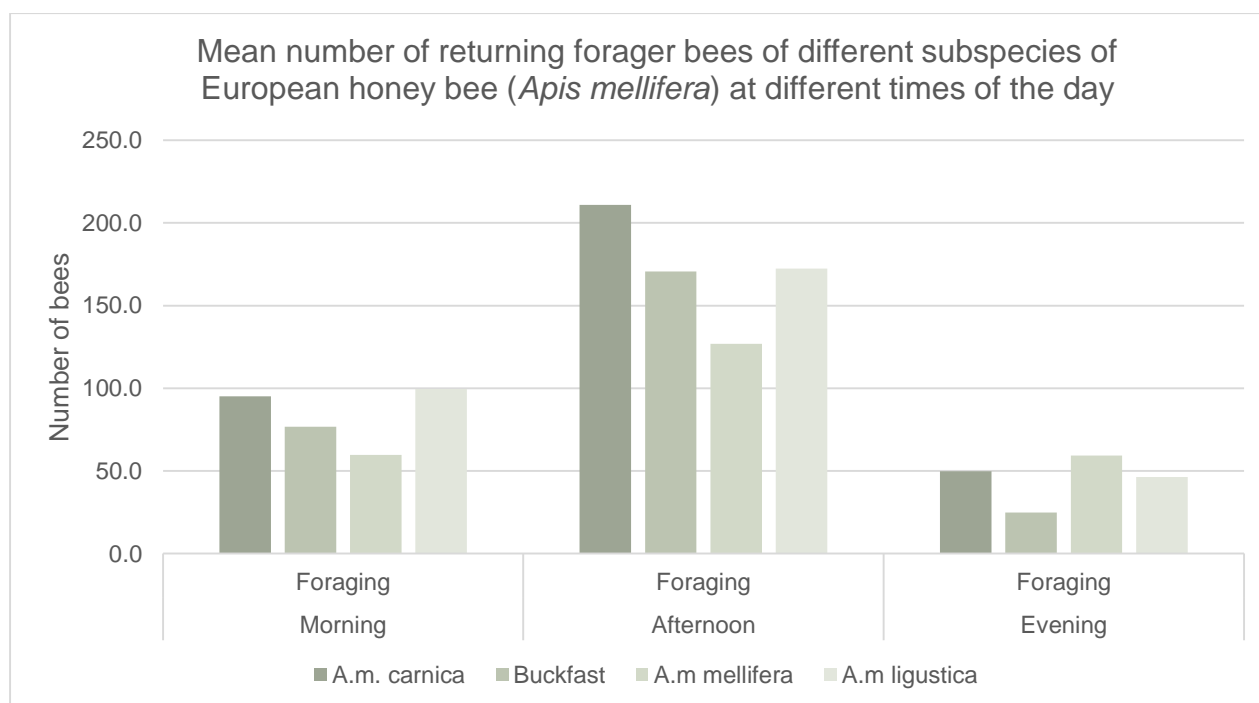
6.2.1 Foraging activity

Table S5: Mean values of worker bee entrances and exits from the hive in a 2-minute video clip for each subspecies during different times of the day.

Subspecies	Worker Exits (morning)	Worker Entrances (morning)	Worker Exits (afternoon)	Worker Entrances (afternoon)	Worker Exits (evening)	Worker Entrances (evening)
<i>A.m carnica</i>	64	43	87	103	24	24
Buckfast	55	39	63	74	11	12
<i>A.m mellifera</i>	43	25	51	55	28	26
<i>A.m ligustica</i>	59	60	68	77	13	15

Table S6: Mean values of returning foraging bees in a 5-minute video clip for each subspecies during different times of the day.

Subspecies	Number of foragers (morning)	Number of foragers (afternoon)	Number of foragers (evening)
<i>A.m carnica</i>	95	211	50
Buckfast	77	171	25
<i>A.m mellifera</i>	60	127	59
<i>A.m ligustica</i>	100	172	46



Supplementary Figure S1: Bar chart showing mean numbers of returning forager bees observed in a 5-minute video clip for each subspecies during different times of the day.

Table S7: Mean number of bees returning to the hive with pollen in a 5-minute video clip in morning, afternoon and evening.

Subspecies	Number of pollen foragers (morning)	Number of pollen foragers (afternoon)	Number of pollen foragers (evening)
<i>A.m carnica</i>	5.3	18.0	0.7
Buckfast	8.7	14.0	0.1
<i>A.m mellifera</i>	6.0	16.5	1.0
<i>A.m ligustica</i>	6.3	13.4	0.4

6.2.2 Guarding behaviour

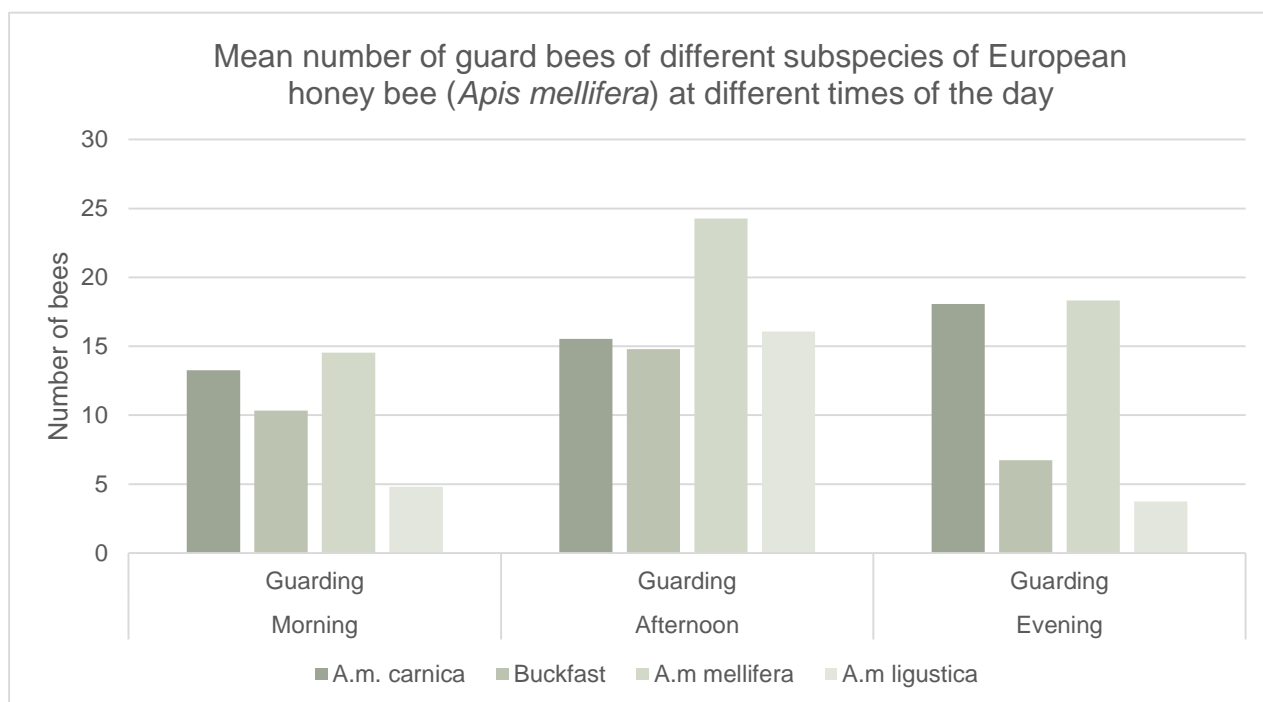


Fig. S2: Bar chart showing mean numbers of guard bees observed on the hive in a 5-minute video clip for each subspecies during different times of the day.

6.2.3 Self-grooming

Table S8: Mean number of bees exhibiting self-grooming behaviour observed in a 5-minute video clip in the morning, afternoon and evening.

Subspecies	Number of self-grooming bees (morning)	Number of self-grooming bees (afternoon)	Number of self-grooming bees (evening)
<i>A.m carnica</i>	14	22	10
Buckfast	22	19	8
<i>A.m mellifera</i>	11	26	15
<i>A.m ligustica</i>	14	24	12

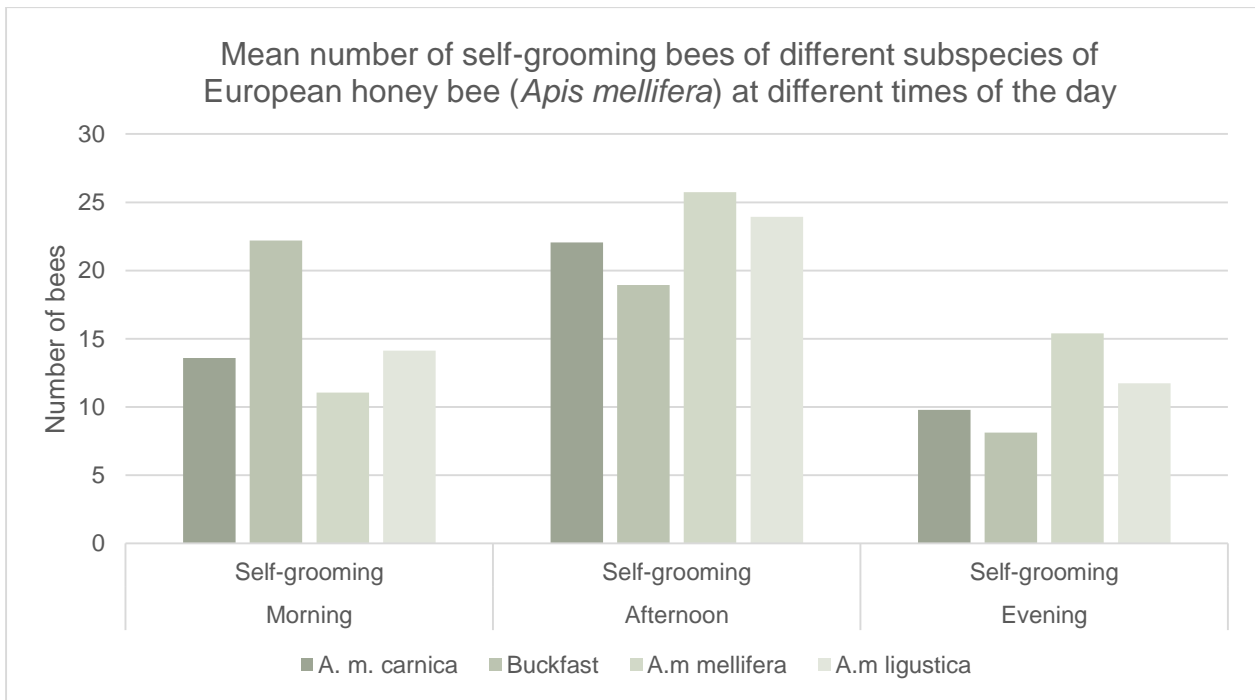


Fig. S3: Bar chart showing mean numbers of self-grooming bees observed in a 5-minute video clip for each subspecies during different times of the day.