



Hypergravity Impact on Fertility of *Apis mellifera carnica* Queens – Case Study

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Abstract

The launch is considered the most stressful rocket flight stage due to the hypergravity occurrences. The possibility of using honey bees (*Apis mellifera*) as the extraterrestrial pollinator depends on their ability to reproduce correctly after experiencing hypergravity. The described study aims to verify the impact of a launching rocket's acceleration on honey bee queen's egg-laying behavior. Four artificially inseminated *A. mellifera carnica* queens were placed in the Human Training Centrifuge and given to the acceleration pattern of the launching *Soyuz* rocket. Next, the data on the number of food stores, eggs, larvae, and worker and drone pupae were collected from the test and control hives using the modified *Liebefeld* method. The pilot study results imply that accelerated queen's egg-laying behavior may change twofold: limiting or maximizing the number of laid eggs, with the control queen egg-laying rate remaining stable for all samples. The number of drone pupae is greater for the test sample colonies, with its earlier appearance in the hive. No impact on overwintering success was observed. Authors indicate limitations of the results and a need to continue the study to verify the occurrence of anomalies potentially related to the examined factor.

Keywords *Apis mellifera* · Fertility · Reproduction · Hypergravity · Rocket launch

Introduction

Pollinators are an essential part of the Earth's food production system. According to the Food and Agriculture Organisation of the United Nations, up to 75% of food crops depend to some extent on their work (Food and Agriculture Organization of the United Nations 2018; Gallai et al. 2009; Ollerton et al. 2011). While honey bees (*Apis mellifera*) are associated mainly with honey production, increasingly more regions rely on their pollination services (Aizen et al. 2009; Bommarco et al. 2012; Calderone 2012). The clearest examples are the great almond plantations in California,

requiring approximately 60% of commercially managed honey bees in the U.S. to be transported to the area during flowering season (Traynor 2017). Although honey bees are not the only insect species used in crop pollination (Peterson and Artz 2022; Thorp 2023), their long history with humankind makes them the first choice for extraterrestrial pollination-based food production.

From the beginning of modern beekeeping, scientists studied and described honey bee biology and behavior extensively. Due to their phenotypical and behavioral plasticity, honey bees became a model organism in the broad area of science (HuoQing and FuLiang 2009). The same plasticity makes *A. mellifera* an ideal candidate for pollinating extraterrestrial crops.

Several bee species have already been sent into space during various missions. In the early 1980s, two experiments on honey bees (*A. mellifera*) were conducted by NASA, of which one gives a significant track in the studied context. In the 1980s, a study was done on the survival, behavior, and comb construction in microgravity (Vandenberg et al. 1985). A dedicated module, the B.E.M. (Bee Enclosure Module), enabling the observation of honey bees, was placed on board the Space Shuttle Discovery. Maximum acceleration

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during the launch was around 3 Gs, and the control sample in the Johnson Space Centre was centrifuged accordingly to simulate launch and ascent conditions (Human Research Program Education Outreach Team 2011). The queen on board the Space Shuttle laid approximately 35 eggs, which failed to hatch after transferring to a standard hive after returning to Earth. After the experiment, 120 specimens appeared to die in the test sample and 350 in the control one (Vandenberg et al. 1985). Such results might suggest a negative impact of hypergravity on honey bees' survivability. Another study has also revealed that the semen of honey bees recovered from the satellite shows a decrease in its viability and the lifespan of the queen inseminated with its use (Jun et al. 2009; Pei et al. 2019).

Two other experiments with carpenter bees and honey bees have been recorded in history. The first aimed to verify different insect species (moths, houseflies, and honey bee drones) adaptation to microgravity conditions. Movement descriptions were, respectively, flying, walking on the surface, and tumbling. However, there is no additional data on the number of alive specimens before and after the space expedition. Although the other experiment was focused on carpenter bees and their ability to construct nests in microgravity, it was never finished due to the fatal crash of the space shuttle. Nonetheless, the above projects suggest that when fed properly, pollinators could function properly under microgravity conditions during long-term space travel. However, to verify this, more studies are needed.

The data focusing on *A. mellifera* in the context of space travel is scarce, and despite a thorough literature query, no more information was found. None of the research involved the queen's fecundity or fertility. This aspect is essential as the use of bees as pollinators on another celestial body can only be considered if the queen is able to reproduce and produce viable offspring.

Extraterrestrial food production, once needed, will have to face many challenges, such as space radiation, lunar or martian dust presence on-site, or water availability. One of the issues to be addressed will be the pollination of the established crops. Although not crucial for all plants, insect-pollinated fruits have been reported to be of improved quality and storage capacity (Wietzke et al. 2018). The two aspects, food quality and storability, could be essential for extraterrestrial crops. However, the possibility of using natural pollinators may be a matter of their transportation rather than the wish of the greenhouse designer. The selected pollinator species will have to survive space travel, hence the most violent of its part – a rocket launch. Moreover, survivability will not be enough, and animals will have to maintain their biological functions intact, allowing them to provide the necessary services.

Honey bees (*A. mellifera*) are one of the most well-known and the best-studied pollinator species, widely used for crop pollination. With the development of modern beekeeping (Langstroth 2012), their biology has become well-known. In addition, the ability to overwinter might be a significant advantage for the species while considering pollinating extraterrestrial crops. As travel to another celestial body may take even a couple of months, transportation of overwintered colonies at lowered temperatures may increase the chances of their survival and reduce the negative effects of microgravity conditions. Additionally, fruits pollinated by honey bees (*A. mellifera*) compared to other greenhouse pollinators, such as bumble bees (*Bombus*) show no significant differences in nutritional quality (Chen et al. 2011). Although bumble bees are the species most widely used for pollinating greenhouse crops, some studies show that both honey bees and bumble bees adapt well to solar greenhouse conditions and show comparable efficiency regarding viable pollen collection (Zhang et al. 2019). As neither bumble bees nor honey bees manage in polycarbonate greenhouses, access to ultraviolet radiation probably will be required (Blacquiere et al. 2006). In the conditions on Mars or the Moon, the agricultural facilities that will need to be developed will be unable to rely exclusively on the access of natural light, requiring supplementary lamps to guarantee sufficient photosynthesis conditions. Therefore, it is reasonable to assume that an additional ultraviolet light source will enhance pollinators' ability to cope with greenhouse conditions. Some studies suggest that using the honey bee in the greenhouse requires cyclic exposure to outdoor conditions due to declines in breeding behavior (Sabara and Winston 2003). However, the occurrence of this phenomenon would need to be verified when an artificial UV source is used in the greenhouse.

Last but not least, using honey bees would involve periodic beekeeping. This could prove to be a critical advantage for this species in the perspective of long-term missions, as some studies report a positive impact of beekeeping on human mental wellbeing (Alton and Ratnieks 2022). This aspect should be addressed in the case of missions requiring good crew performance during long periods of isolation.

This study aims to explore the relationship between exposing honey bee (*A. mellifera carnica*) queen to the acceleration characteristic for the launch of a crewed space rocket and its ability to lay eggs capable of proper development. Basing on the limited literature on the space conditions impact on honey bees (Vandenberg et al. 1985), preliminary experiments on the survivability of honey bee workers of small sounding rocket flight (Stasiowska 2020), and the decrease in honey bee semen viability after space travel (Jun et al. 2009; Pei et al. 2019), we hypothesized that *A. mellifera* queen after experiencing an acceleration

Table 1 Number of attendants in each sample per specific queen, considering the sample type and queen's individual number

Experimental sample		Control sample	
Queen's number	Number of attendants	Queen's number	Number of attendants
3V	11	1E	10
3K	14	1H	11
97	9	39	10
40	10	1D	12

of launching rocket would experience lowering of its fertility, resulting in (1) a reduction in the number of eggs laid, (2) an increase of drone egg ratio or (3) a shortening of the queen's lifespan.

Knowledge of the honey bees' performance after rocket flight may further enable cross-pollination of extraterrestrial crops when required. The species cooperated well with bumble bees, improving their mutual performance (Eeraerts et al. 2020; Howlett et al. 2019). Such a cross-pollination may improve the quality and shelf life of the fruits produced, and using more than one pollinator species would provide greater reliability in a food production system.

Materials and Methods

Honey Bees

The examined species was the Carniolan honey bee (*Apis mellifera carnica*) – Galicja line. Queens were raised and inseminated in a commercial apiary (*Pasieka Szeligów*, Wielkie Drogi, Poland) specialized in artificial insemination. To minimize the influence of genetic diversity, seven-day-old sister queens were inseminated with the semen of the brother drones of the same line but originating from different colonies. For each queen insemination, the semen of 6 drones was used. Right after the procedure, queens were

placed in queen cages with 25 worker bees, and after several hours, they were transferred to the mini mating box with a closed exit to prevent them from performing mating flights.

We established experimental and control groups. In each, four artificially inseminated queens were accompanied by a cohort of young bees (attendants) to reflect their natural environment. The exact number of attendants in each sample is presented in Table 1.

Hypergravity

Hypergravity was achieved using the Human Training Centrifuge (HTC), owned by the Military Institute of Aviation Medicine in Warsaw, Poland. HTC enables the simulation of every known acceleration pattern with values in the range of -3 to $+16Gz$. The maximal gradient of accelerations increases to $14,5$ G/s. The main arm of the device is 8 m long, and the maximum roll and pitch for angular acceleration is 8 rad/s².

During the experiment, the most frequently used rockets for crew and cargo transportation were *Soyuz*-type rockets. Therefore, the acceleration pattern characteristic for their launch was used (Fig. 1). The pattern is characterized by three gradually increasing peaks in hypergravity value occurring in the 180th second (4G), the 380th second (2,5G), and in the 645th second (3G), divided by drops of value down to 1,4G after reaching each peak. The total acceleration time was 660 s.

Environment Control Device – *BeeO!Logical* Payload

BeeO!Logical device is a rocket payload dedicated to pollinators' examination. It was designed by Stasiowska (2020) to ensure safe and stable experiment conditions, such as consistent temperature, equal air composition at the start of the experiment, and compartment volume ensuring harmless air quality (Eskov and Eskova 2015) for at least 2 h.

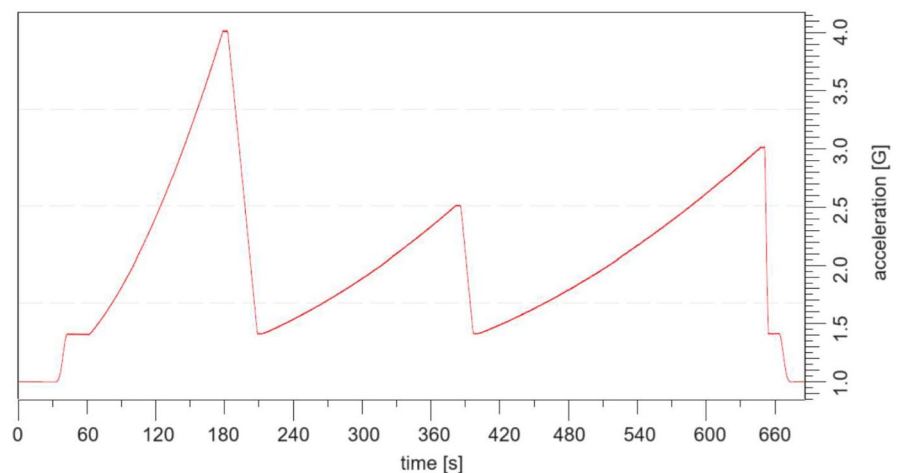
Fig. 1 Acceleration pattern of the HTC during the experiment

Fig. 2 Internal part of the *BeeO!Logical* payload with box locations and specific components marked. (Source: own materials)

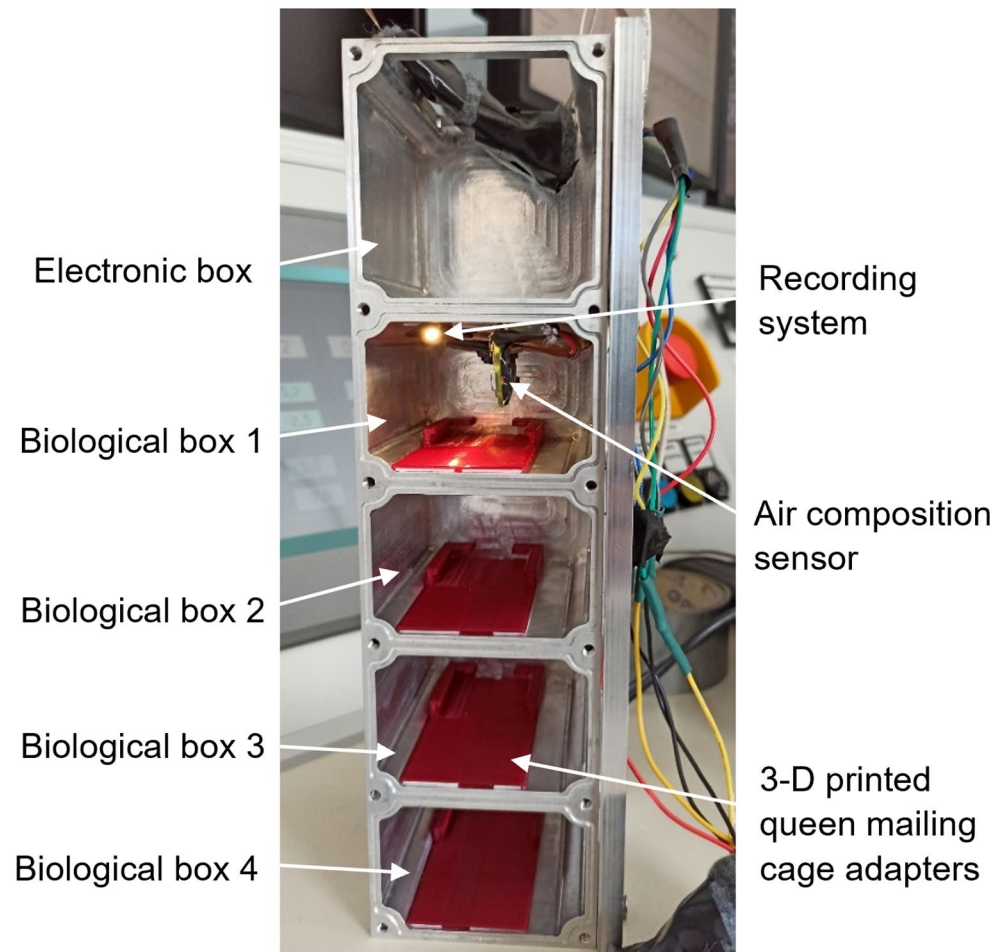


Table 2 Experiment initial procedures detailed timetable

Date	Time	Activity
30.06.2021	20:00	Queens' transportation in queen cages from Kraków to Warsaw
01.07.2021	10:37	Enclosing queens in queen cages in the <i>BeeO!Logical</i> testing devices (test and control)
01.07.2021	10:40	Placing the test sample in the HTC
01.07.2021	10:49	Centrifugation start
01.07.2021	11:00	Centrifugation end
01.07.2021	11:04	Opening testing devices and relocating queen cages with subjects to the transport box
02.07.2021	16:00	Transportation from Warsaw to <i>Pasieka Szeligów</i> for initial observation

The *BeeO!Logical* consists of an antishock case, thermal isolation, and the internal part, and when fully assembled, has a standard size of 3U (10×10×30 cm). Two identical copies of the described device exist to ensure the same internal conditions for the test and control sample. Consequently, the only external factor changing during the experiment is hypergravity.

Two groups of queens were treated using the *BeeO!Logical*, to avoid the impact of external factors on the experiment results. The total enclosed in-payload time was 27 min for the test sample and 28 min for the control.

For this particular experiment only the internal parts of the payloads were used (Fig. 2).

As the centrifuge allows the precise control of the temperature both in the payload compartment and control panel room, the additional stabilization was unnecessary.

The HTC Experiment

The experiment's detailed timetable is shown in Table 2. Briefly, bees have been transported to Warsaw for centrifuge training. Next, honey bees have been returned to the apiary for initial observation and egg-laying start.

Setting up Experimental Hives

Artificial queen replacement in the colony is a known procedure in the apiaries. To minimize the chance of queen rejections, all steps must be carefully designed to reflect the biology of the colony. Here, we followed a standard and successful procedure established in *Pasieka Szeligów*:

1. An artificially inseminated queen (AIQ) is given to the mini mating box a miniaturized version of the mating beehive used during the procedure for AIQ egg laying starts.
2. After 12–16 days, AIQ starts laying eggs.
3. AIQ is placed in the queen cage with the cohort of young nursing bees.
4. The colony that has been orphaned (queenless) for at least 9 days is chosen.
5. AIQ is given to the chosen experimental hive in the queen cage.
6. The AIQ is released from the cage by worker bees within 24–72 h and starts laying eggs.

Figure 3 presents a schematic representation of the described procedure.

Due to the fact that the queen is given to the hive that other queens primarily occupied, the frames may contain pupae, as well as food stores. Hence, there is a significant natural variation in the starting points of each colony.

After a month, five of eight queens were received in experimental beehives from the breeder and transferred to their final location (experimental apiary located at AGH University of Krakow, 50°03'60", 19°54'02"). Contents of all hives were noted, and later changes were observed with reference to day 0 (August 3, 2021), which is defined as the day of transporting the experimental hives to the final location. Two weeks later, another queen was transported, as well as the additional hive bodies filled with worker bee packages. Hive bodies were needed for the enlargement procedure, required for the successful overwintering of the colonies. The delay in queen 39 transportation was caused by its delayed acceptance in the colony. The other two queens,

97 and 1E, were not accepted in the colonies to which they were given. Therefore, it was not possible to examine them in the described analysis. As one of the rejected queens was from the test sample and the other from the control one, it was assumed that the natural factor caused a lack of acceptance, and no impact of the hypergravity was observed.

Data Acquisition Procedure

Data were acquired from August 4, 2021 (day 1) to April 29, 2022 (d. 269). Inspections were performed once to thrice weekly, resulting in 12 to 15 controls per hive in 2021. After winter, controls were conducted every other week, resulting in three inspections per hive in 2022 in total. The exact dates of inspections and the identification number of hives inspected on a specific date can be found in supplementary materials, Table S2.

Fecundity and fertility examination was performed in mini-plus beehives (experimental hives). Bee breeders and scientists primarily use this type of hive as it allows the colony to develop naturally, simultaneously limiting the required work by beekeepers. The beehives were placed in the experimental apiary in Kraków, Małopolska Region, Poland.

The external measurements of the mini-plus frame are 215 mm (± 2) per 168 mm (± 2). The internal dimensions of the frame, which the wax foundation can effectively occupy, are 150 mm (± 2) per 200 mm (± 2). The number of cells on the single side of the frame ranges from 1127 to 1176 and varies slightly with respect to the possibility of effective use of cells on the edges. Taking into account the above, the mean number of cells per side of the frame equals 1152 (rounded up), which allowed a recalculation of the area to the exact number of cells with eggs, larvae, and pupae when needed.

For the number of brood and food (both nectar and pollen) stores assessment we adapted a method developed by Liebfeld (Dainat et al. 2020; Imdorf et al. 1987). It allows rapid measurements, limiting the negative impact on colonies, and is characterized by a low variation in results (Bargen et al. 2020; Dainat et al. 2020). However, instead of

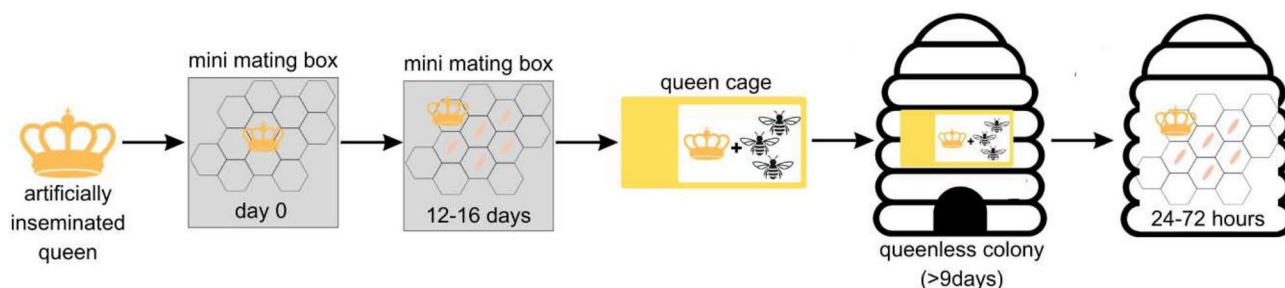


Fig. 3 Schematics of the artificial queen replacement procedure used in *Pasieka Szeligów*

decimetre square coverage, the percentage of the entire comb face was estimated while maintaining the proportion of the total to the smallest area evaluated ($10\text{dm}^2 - 0,1\text{dm}^2$ vs. $100\% - 1\%$). The variation was implemented due to the relatively small size of the mini-plus frame compared to other hive types.

Each frame was inspected separately, and data on its composition were noted. The inspection procedure started with estimating the area occupied by the capped brood due to its high accuracy. It was followed by estimating the area occupied by the food stores, eggs, and larvae. The assessment stage considered that the area must not exceed 100% in total. Due to the frame's fixed measurements, it was possible to recalculate the marked area to the exact number of cells.

We did not collect data on the number of adult bees as their abundance combines multiple factors and is not limited to queens' fertility.

Beekeeping Procedures Detailed Schedule

All colonies were subjected to typical beekeeping procedures in the experimental apiary. No honey was harvested from colonies during data acquisition. All the major observations, comments, and beekeeping events can be seen in Fig. 4.

Data Normalisation

The data collected for each successfully settled hive were prepared for graphical analysis.

During the hives' enlargement procedure on August 17 (day 14), additional frames containing food stores and brood were added to experimental hives. Hence, data on the number of eggs after the procedure was corrected. We did this to exclude eggs added to the hive along with the new hive body ("extra eggs") from the total number of eggs in the hive. By doing this, we avoided considering extra eggs from researched queens.

Thus, the number of eggs in the whole, enlarged hive noted on August 18 (d. 15) was reduced by the number of eggs from the added hive body. The August 20 (d. 17) measurement was also reduced by the number of eggs from the added hive body. After day 17, the correction was discontinued for the eggs due to the standard development time of the egg (Human et al. 2013).

Analogous correction was applied to all brood stages and food stores where necessary, e.g., where the new hive body brood or food stores were present. The last larvae correction was applied to data from day 20, and worker pupae – day 33 due to the standard development time of those brood stages (Human et al. 2013). Food stores correction was applied to

all 2021 data. No drone pupae correction was needed due to the lack of drone pupae in the added hive bodies.

Data deficiencies caused by the inspection gaps were addressed. In section, gaps were caused by intervals at which controls were performed. Each colony was controlled 1 to 3 times per week – raw data from the controls is available in Table S1 in Supplementary Materials. Data gaps between subsequent control days were filled with the number of eggs calculated proportionally to the number registered during the previous and subsequent control, considering the number of days between observations. Consequently, we have obtained daily data on the number of eggs in the hive. An analogous procedure was performed for all other parameters measured, acquiring daily data for all the parameters.

Statistical Analysis

A correlation test was performed to check the correlation between each sample. Analyses were performed using Analysis ToolPak for Excel, version 2309, and the graphs were produced using Excel Charts.

Results and Discussion

Rejected Queens

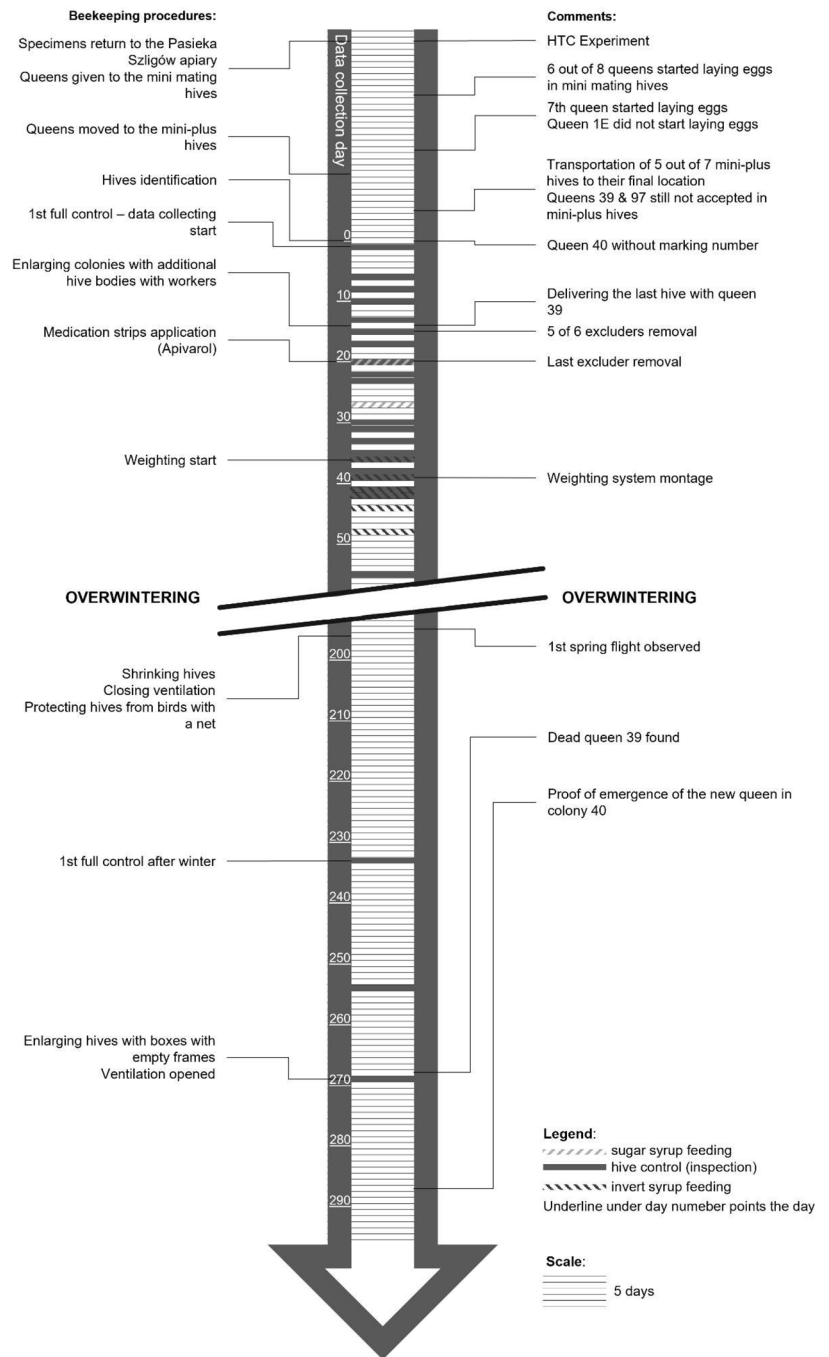
One queen in each group was excluded from the apiary data collection due to their rejection by the honey bee workers. Queen 1E was rejected in the mini mating box due to the absence of egg-laying behavior. Queen 97 was rejected after transferring to an experimental hive.

In addition, queen 39 was accepted into the colony with a significant delay (2 weeks after the start of data collection in the experimental apiary). It was decided to exclude data from this hive to avoid additional complications in the analysis.

Queen 40 had no queen marking number and differed in phenotypic traits from other AIQ, suggesting worker replacement (Tarpy et al. 2000). Therefore, it was not taken into account in the following analysis. However, this event should be kept in mind, as if the queen was replaced, it could indicate the weaker condition of queens after experiencing the hypergravity related to the rocket launch. However, this hypothesis is not supported by strong evidence and is made only on the basis of the different looks of the queen and the lack of the marking number.

The above caused only 2 out of 4 queens in each sample to be qualified for the following analysis.

Fig. 4 Detailed timeline of all beekeeping procedures and comments from observations. Underlined numbers on the arrow indicates observation day number. Feeding and inspections are marked with various patterns



Hypergravity Impact on Queen’s Egg-Laying Abilities

Fecundity, defined as the potential ability to reproduce, was assessed based on the number of eggs laid.

Basic descriptive statistical tests for the number of eggs were performed for all experimental hives. For the test, only collected data was used, excluding data gaps. The calculated parameters can be seen in Table 3.

The decision to perform the descriptive statistical analysis was made apart from the seasonality of the honey bee colony development. Due to the equal hive size and the same inspection and operation procedures used for all samples throughout the observation time, colonies should develop similarly and with comparable strength. Descriptive statistics enables the comparison of colonies in terms of the overall seasonal performance.

The sample size ranged from 15 to 18 measurements in total. The mean number of cells in the hive occupied by eggs

Table 3 Descriptive statistics of number of eggs for all hives based on data collected throughout the study

	3K	3V	1D	1H
Number of samples	18	17	15	16
Mean	1 931	1 375	1 586	1 608
Standard error	288	153	293	219
Median	1 855	1 440	1 336	1 590
Standard deviation	1 224	630	1 133	876
Sample variance	1 497 100	396 638	1 283 412	767 913
Minimum	69	346	202	115
Maximum	4 493	2 304	4 493	3 260
Sum	34 756	23 368	23 789	25 736
Confidence level (95.0%)	612	344	627	480

Table 4 Correlation strength (r_s value) between the number of eggs in subsequent hives

	3K	3V	1D	1H
3K	1	0.815132	0.552063	0.728582
3V	0.815132	1	0.363669	0.724786
1D	0.552063	0.363669	1	0.280989
1H	0.728582	0.724786	0.280989	1

throughout the measurement season varies from 1 375 to 1 931, the greatest for queen 3K and the lowest for 3V. The mean value for the control sample is comparable for both queens, settling at 1 597 (+/-11) eggs. Similarly, the greatest and lowest sample variances were calculated for the queens of the test sample. The worst and best fecundity traits seem to have queens from the test sample, and the queens of the control sample are characterised by more stable egg-laying behaviour.

The variability of the test sample in terms of the mean number of cells occupied by eggs may suggest that honey bee queens experiencing hypergravity may respond twofold: maximizing the egg production or lowering egg production, while the fecundity of queens not subjected to the hypergravity impact remains more stable throughout the whole season. This approach is supported by the historical record of research on honey bees in a space context, suggesting the negative impact of space travel on drone semen (Jun et al. 2009; Pei et al. 2019) and the increased mortality of worker bees due to acceleration (Vandenberg et al. 1985).

The correlation between all hives in terms of the number of eggs is shown in Table 4. It was calculated only for the 2021 data with consideration of the hive enlargement procedure data correction (see: Data Normalisation). Considering the lowest number of samples equal to 12 (number of samples for hive 1D minus the number of controls in 2022), the critical value should be greater than 0.5035 for $p=0.05$, to prove the correlation.

The calculated value of r_s for 1D and 1H, as well as 1D and 3V is below the critical value of 0.5035 for $p=0.05$. There is more than 5% probability that the correlation between those

is accidental. Pair 1D-3K r_s value is greater than the critical value for $p=0.05$, but lower than for $p=0.01$ (0.6713). For all other pairs the calculated r_s is greater than the critical value for both $p=0.05$ and $p=0.01$, so those correlations prove the strong relationship between these data sets.

Comparing the above to the graphs of the number of eggs laid by each queen (Fig. 5), it can be seen that the most rapid changes were observed for queen 3K. All the queens started laying more eggs after the hive enlargement procedure, probably caused by more space available for egg-laying behavior. A reduction in the number of laid eggs appeared around September 15, followed by a slight increase later, not exceeding 1500 eggs laid in the hive 3 V by the end of the season.

Changes in the number of laid eggs may be impacted by the queen's age (Simeunovic et al. 2014), food type (Fine et al. 2018), and others (Cobey 2007). Given parameters were uniform in the described experiment – all queens were the same age, inseminated in the same manner, given the same beekeeping procedures, and all the colonies were placed in the same area, having the same food source. For that reason, all the changes in egg-laying behavior are of experimental rather than environmental origin.

Hives Composition

The general composition of the hives in 2021 is shown in Fig. 6. It can be seen that all the hives were occupied in a similar way throughout the 2021 beekeeping season, reaching the maximum volume of the hive in mid-September. Notably, there was a sudden decrease in the number of cells occupied in the 3K hive after first reaching the maximum volume capacity of the hive. The drop was mainly due to a decrease in the number of stores. The reason is unknown; however, since the 3K hive was characterised generally by the best results regarding the number of eggs laid, it could have been caused by the number of adult honey bee workers present in the hive after mid-September. The other possibility is that the drop was caused by the robbery by another colony.

The hive enlargement procedure caused the sudden growth on August 18 (d. 15). To each 1-body hive, another body filled with food stores, eggs, pupae, and larvae was added to increase the chances of winter survival. This caused an increase in the total number of cells that could have been filled (from 13 824 to 27 648 cells).

An analogous comparison of the composition of the hive was done after data correction regarding the described enlargement procedure. The total number of cells occupied was reduced by the number of cells occupied in the added hive body. 'Extra cells' were subtracted, considering bees development time. In the case of food stores, if the

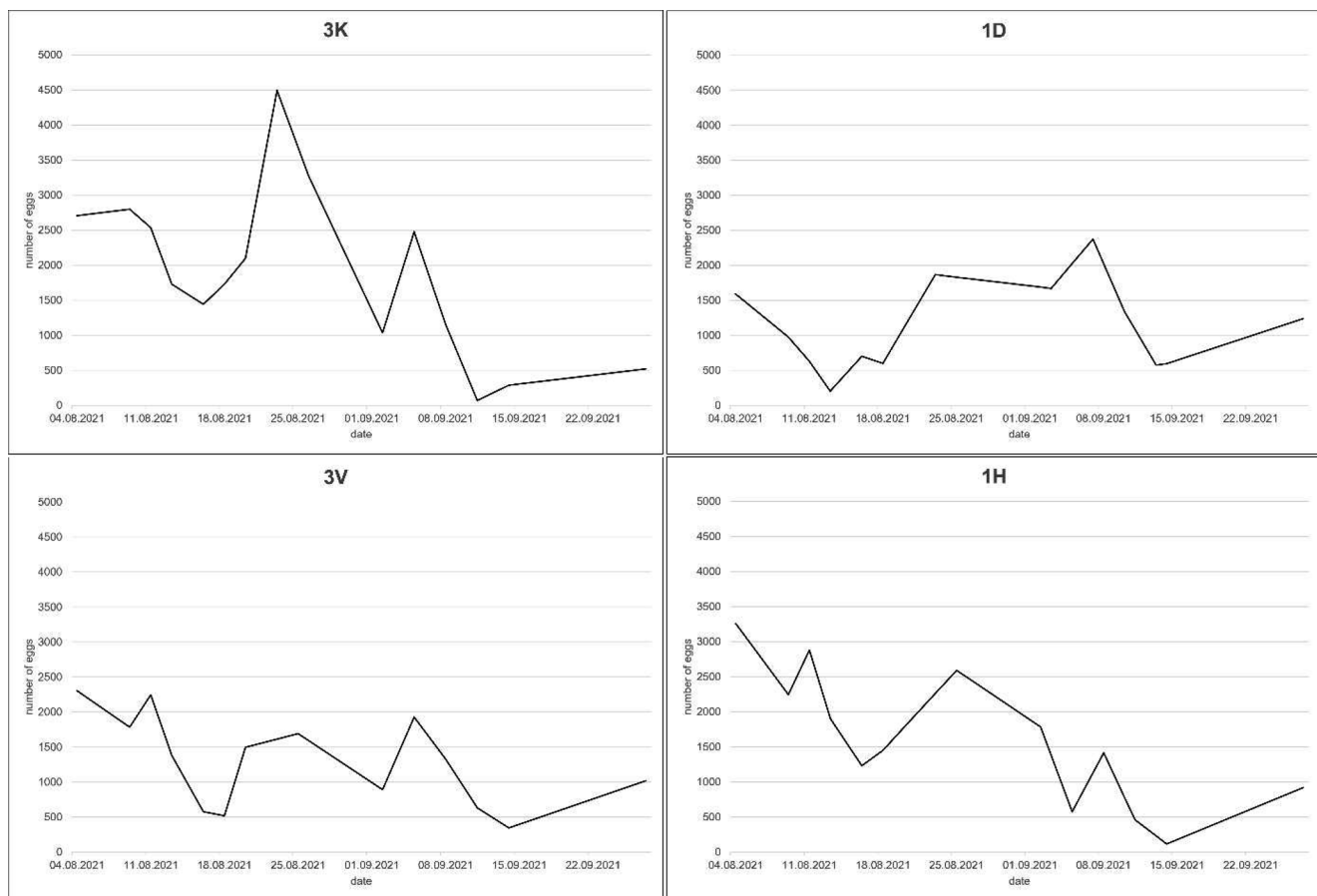


Fig. 5 Number of eggs in experimental hives throughout the 2021 season. The scale is uniform across all diagrams

number of food store cells in the new hive body was greater than in the moment of adding the body, the excess number was treated as occupied by the examined queen's offspring and included in the final statistics. Taking into account the impact of the enlargement, in terms of the amount of food stored at the end of the beekeeping season, the control sample was prepared better for overwintering than the test sample (Fig. 7).

Figure 8 compares the number of occupied cells between hives. Hive 3K reached over 11,000 cells occupied in total, while the other experimental sample hive had the lowest number of occupied cells from all the analyzed hives. The control hives were more consistent, reaching a maximum of approximately 8000 occupied cells with little or no drone pupae cells.

Development Deviations Based on the Egg-to-Larva Transition

The correctness of egg-to-larvae development was assessed by comparing the number of registered larvae with the number of eggs registered four days earlier but shifted on the time axis on the right – it was assumed that after such a time

all eggs evolved into larvae. Data did not suggest the occurrence of the starvation periods or the periods without the flow of fresh nectar, so data should not have been additionally disturbed, e.g., by brood cannibalism episodes.

The correlation for all samples was positive and greater than 0.5. 3K had the strongest correlation (0.898) between the actual and expected number of larvae. Therefore, no significant deviations in the egg to larvae development were found. Figure 9 is the visual representation of the actual and expected number of larvae in all hives.

Overwinter Survival and Spring Season Start

The readiness for overwintering was assessed based on the total mass of the hive. It indicates the amount of food stored, which is crucial for winter survival. The changes in the hive mass can be seen in Fig. 10, covering exclusively the colonies' feeding period with the invert syrup. The weight gain is similar for all hives, with no less than 0.986 correlation. The mass of the invert left on the feeder was subtracted for the last measurement.

Another issue for overwintering is pathogen infestation, with an emphasis on *Varroa destructor*. To limit its

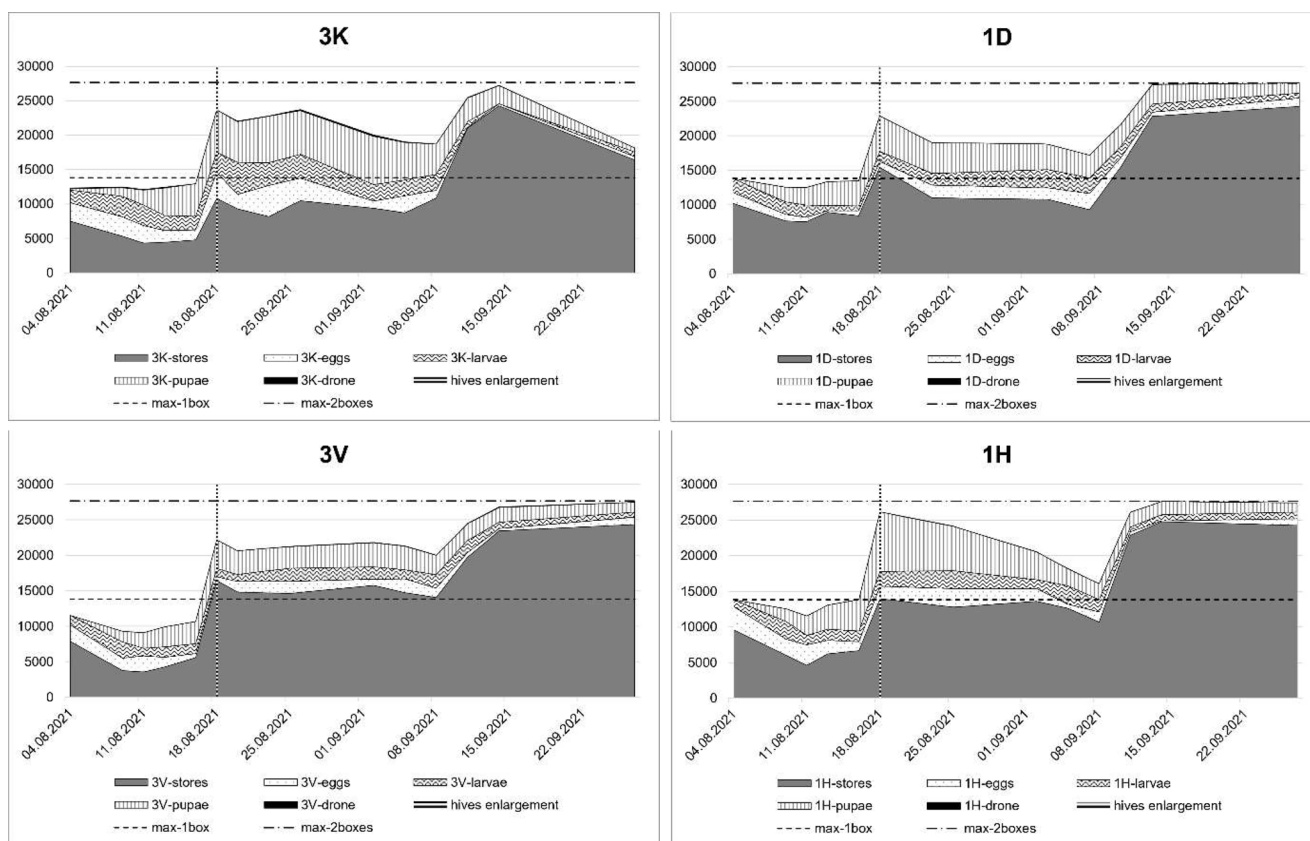


Fig. 6 Total number of cells occupied by each parameter. Similar seasonal tendencies are visible in all hives. The decrease in 3K hive volume can be seen at the end of the season; the influence of the added hive body is not considered. Horizontal dotted lines mark the maxi-

possible negative impact on overwintering success, all colonies were treated, as previously shown in Fig. 4.

All examined colonies survived the winter in good condition and correctly resumed activity in spring. The number of eggs laid at the beginning of 2022 beekeeping season can be seen in Fig. 11. In general, experimental sample colonies performed worse than the control colonies and had a greater number of drones, emphasizing hive 3K. The strongest positive correlation was calculated for hives 3K and 1D (>0.88), negative for 3V and 1H (<-0.82), while the correlation between the groups is not greater than 0.64. This may indicate a high degree of individual variability within the groups. What is more, higher drone production might be an indicator of issues with stored semen. Typically, such an issue is characteristic for older queens which run out of sperm (Boes 2010; Rangel et al. 2013). The early droning of experimental queens might suggest problems with stored semen and other physiological-related issues.

Development controls were performed until the end of April 2022. Since May, controls have been stopped due to swarming behavior in the examined colonies. Such an event causes a reduction in the number of eggs laid by the

queen, and any action performed to mitigate the swarming in the colony could directly impact the experiment results. For that reason, a decision was made to stop gathering data. Later, queen 39 was found dead, and queen 40 swarmed. All remaining colonies and experimental queens survived until the end of the 2022 season and were overwintered again.

Data Acquisition Procedure Bias

The same person took observations each time, ensuring a stable bias for all measurements, if present. As the research on *Liebefeld method* proved, the smaller size of the hive increases the precision of the estimates, and a possible variation from the real number is stable (Bargen et al. 2020; Dainat et al. 2020).

Research Limitations

The authors are aware of the limitations of the presented experiment. We discuss them in the following section. The most significant limiting factor is the size of the test and control groups, preventing us from drawing statistically

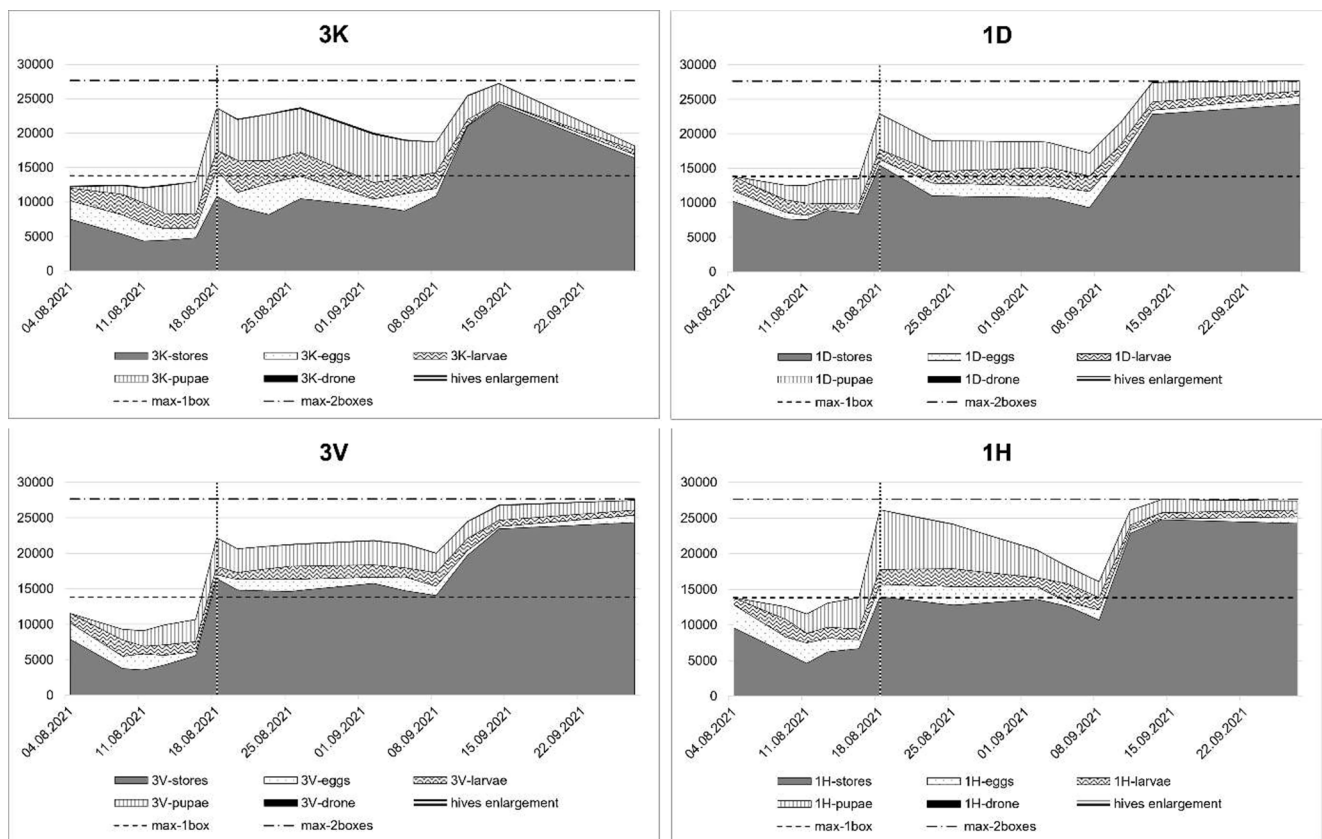


Fig. 7 Total number of cells occupied by each parameter, excluding cells occupied by brood and food stores added to the hive along with the additional hive body during the hive enlargement procedure. Horizontal dotted lines mark the maximum capacity of fully occupied hive with one and two bodies. The number of drones in a 3K hive is noticeable. The scale is identical for all graphs

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significant conclusions for the entire species. Although implementing larger sampling would provide a more significant statistical power, we strongly believe that our preliminary data are a sufficient starting point for further studies.

season, which would allow the evaluation of the fertility of the queens throughout the year.

The choice of *A. mellifera* might seem suboptimal regarding the pollination effectiveness of the species under greenhouse conditions (Li et al. 2006). However, the honey bee is a highly recognised species, and the mechanisms described on it can be extrapolated to other species with reasonably good accuracy (Maleszka 2014). Due to their well-known biology and established breeding methods, honey bees are a good starting point for studying hypergravity effects in the context of future Mars expeditions.

The fertility results and survivability of the colonies could have been biased by the hives enlargement procedure performed on August 18, 2021. Available precautions were taken to reduce bias; however, the appearance of new worker bees could have affected the functioning of the colonies. The decision on hive enlargement was made for the good of the examined subjects and for the extension of the observation time of the colonies.

Another significant limitation is the lack of spring/early summer observation. The seasonal nature of *A. mellifera* biology leads to a significant variation in development dynamics throughout the year. Young queens can be successfully inseminated (depending on the season) as late as the end of May. Considering the time from insemination through the experiment to successfully implementing queens into the colonies, the observations could not start before June. The analogous experiment should be carried out with observation throughout the whole beekeeping

The study considers only the very initial part of space travel, the rocket launch. Various acceleration patterns and G-force values should be examined for the broadening of the perspective. It is crucial due to the rapid development of space transportation systems such as those offered by SpaceX or NASA. The condition of honey bees in the context of the subsequent stages of space travel should also be of interest to the scientific community. This includes issues such as long periods of microgravity impact on the condition of the honey bee colony as well as the effectiveness of *A. mellifera* as a pollinator under lowered gravity.

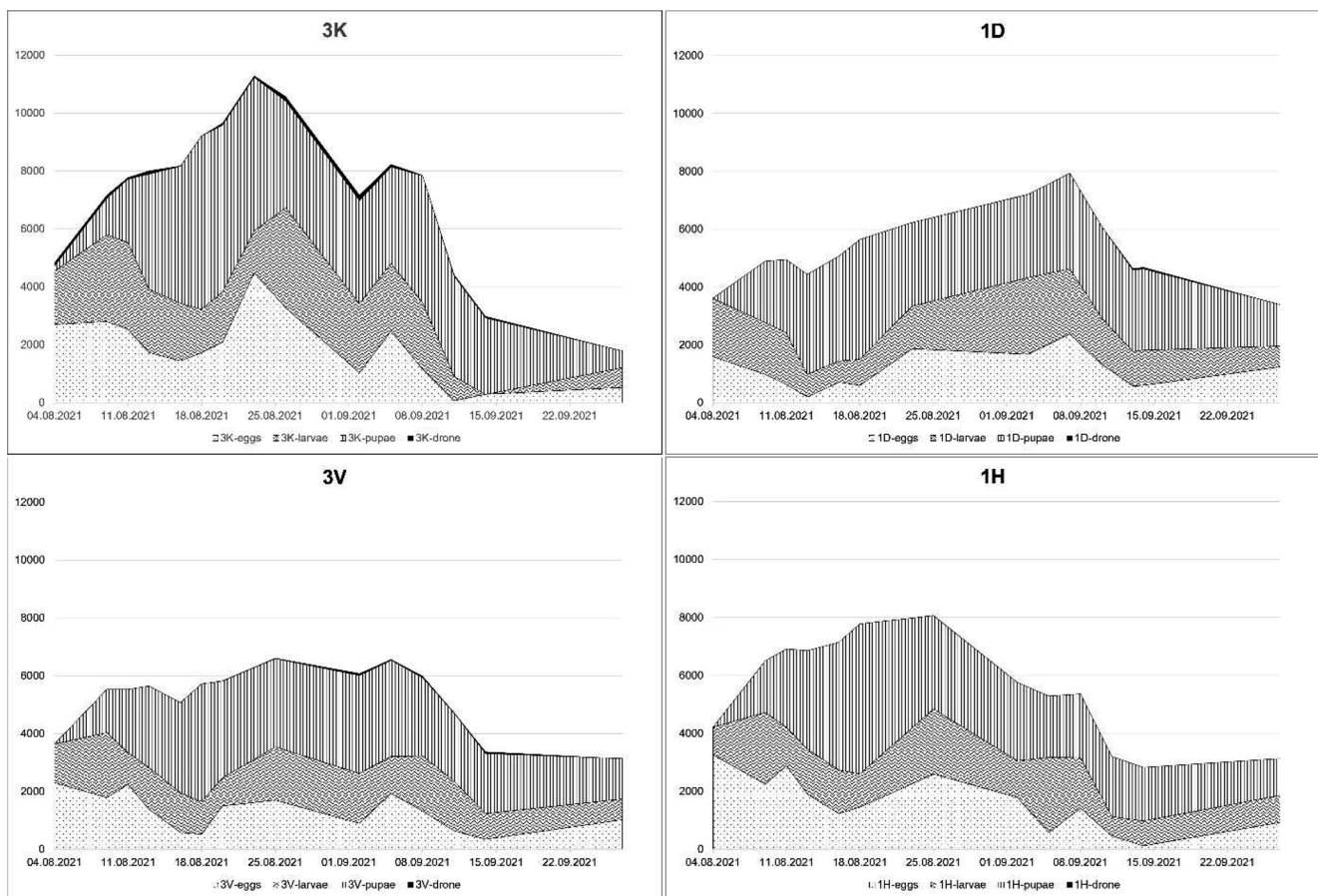


Fig. 8 Number of cells occupied by various stages of the development of bees. The maximum number of occupied cells was more than 11,000 in the 3K hive, having also the greatest part of drone pupae in the composition. The scale is uniform across all diagrams

Conclusions

The study aimed to verify the relation between giving honey bee queens to the acceleration characteristic for the crewed rocket launch and its later fertility. Although limited in sampling size, the study provided us with much-needed information about the impact of *G*-force on *A. mellifera*. Regarding the number of laid eggs, a discrepancy in response to the hypergravity was observed for test queens. No impact on overwintering success was observed. The results suggest that the topic should be further studied, and abnormalities, such as a greater number and earlier appearance of drone pupae in the test sample, should be verified in a larger group.

The extraterrestrial settlement will require the highest efficiency of all subsystems possible, including food production. Therefore, the author suggests also examining other pollinator species with an emphasis on species known as well coexisting with each other and providing different pollination techniques (Eeraerts et al. 2020), such as bumble bees (*Bombus*). This knowledge could be used as a basis for further studies on pollinators' ability to cope with space travel and extraterrestrial habitat conditions.

The use of honey bees as extraterrestrial pollinators, although not ideal, can be an advantage regarding crew wellbeing (Alton and Ratnieks 2022). For this reason, despite the many aspects that must be considered to make extraterrestrial beekeeping possible, such trials should be undertaken. Further study should consider both aspects directly concerning bees, such as greenhouse lighting, and those concerning the people working with them. Furthermore, the impact of rocket flight on the whole overwintering colony should be examined, and their ability to stay in a winter cluster in microgravity conditions should be verified. In addition, the species of plants to be cropped in honey bee-pollinated greenhouse should be composed so that the diet of humans and bees will be ongoing and well-balanced. The latter can be optimised with well-developed models of honey bee colony, such as BEEHAVE (Schmolke et al. 2020), enabling the consideration of aspects such as available nectar and pollen sources throughout the season on colony development. In further perspective, fully enclosed, naturally pollinated food production facilities, being the outcome of such research, might be used not only on Mars

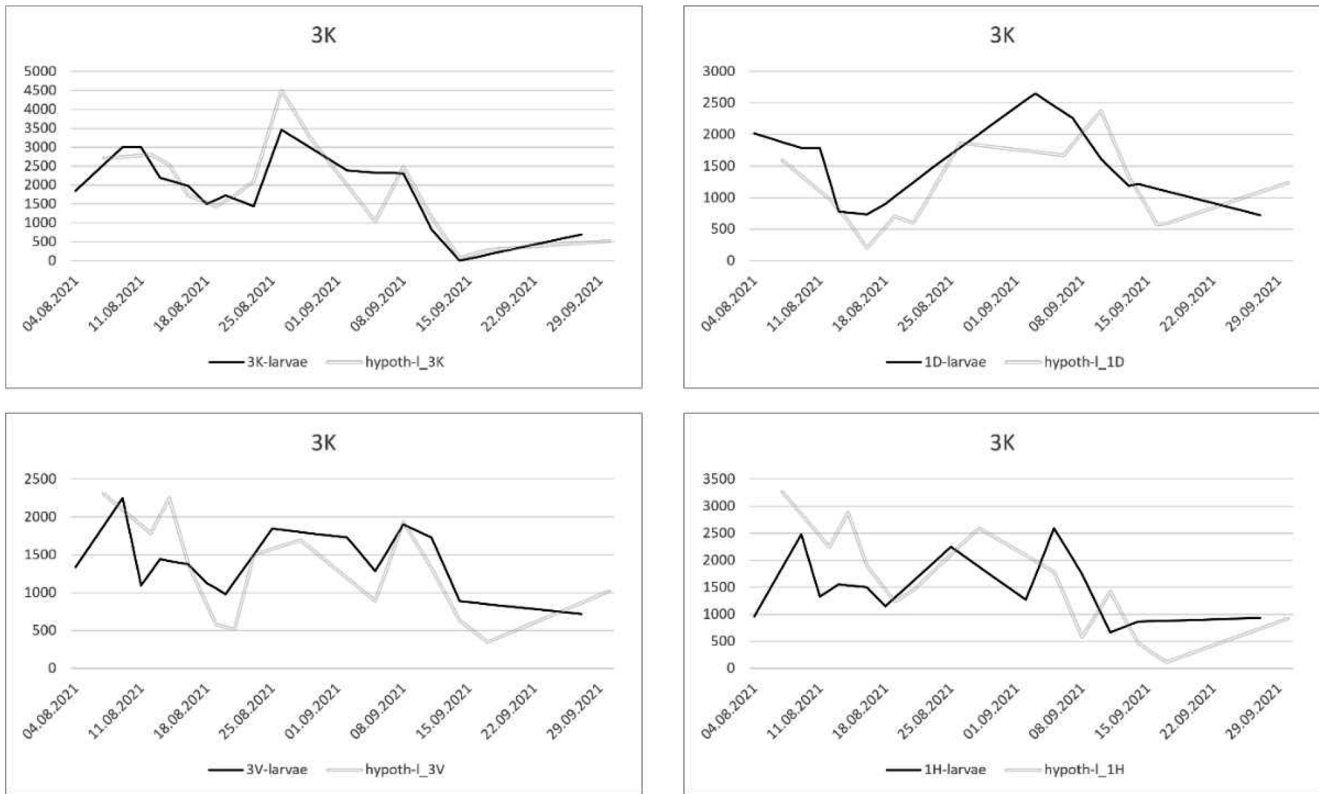


Fig. 9 The number of larvae in the hive and the expected, calculated considering the development of egg to larvae transition time. The scale is identical for all graphs

Mass of hives before the overwintering

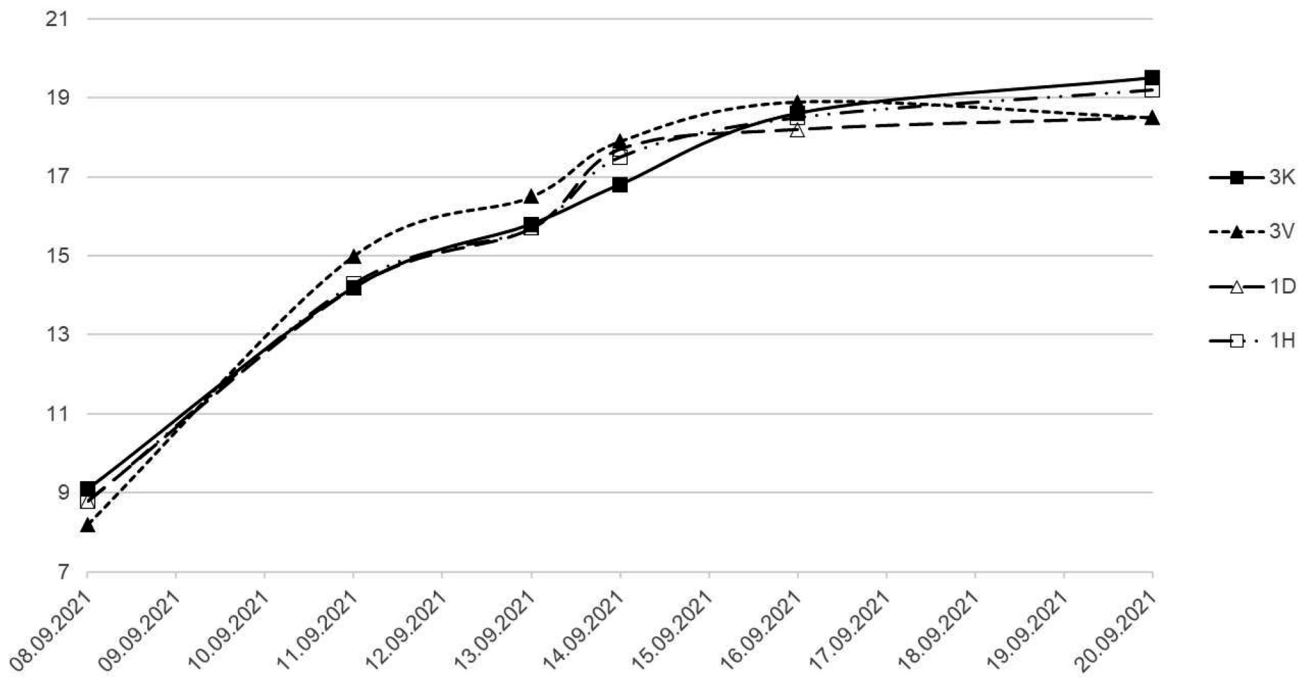
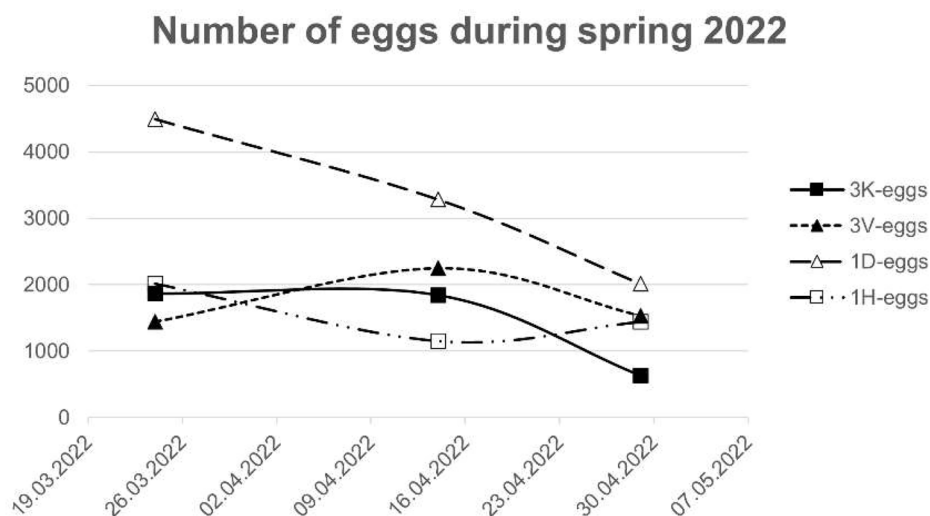


Fig. 10 Evaluation of overwinter readiness based on the hive mass: All samples reached the same mass level at the end of the beekeeping season

Fig. 11 Number of eggs laid at the beginning of beekeeping season 2022 – hive 1D had more than twice as many eggs as the rest of the hives during the first measurement. In all the hives during the last control, the number of eggs dropped below 2000



but also on Earth, in areas where fresh product availability is low, such as polar and arctic regions.

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Author Contributions D.S. designed the study, analysed the data, prepared figures and wrote and reviewed the manuscript text. M.K. participated in the conceptualisation of the experiment and writing and correcting the manuscript.

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Data Availability All raw data from colonies controls are available in Supplementary Table S1. A detailed control schedule is available in Supplementary Table S2. Data supporting the findings of this study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval According to the decision of the 2nd Local Institutional Animal Care and Use Committee in Krakow, Poland, no approval from the commission was needed for the experiment (resolution no. P3/2020).

Consent to Participate Not applicable.

Consent for Publication All the authors consent to the publication of this work.

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References

- Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.M.: How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Ann. Bot.* **103** (2009). <https://doi.org/10.1093/aob/mcp076>
- Alton, K., Ratnieks, F.: Can Beekeeping improve Mental Wellbeing during Times of Crisis? *Bee World* **99**, 40–43 (2022) <https://doi.org/10.1080/0005772X.2021.1988233>
- Bargen, H., Fauser, A., Gätschenberger, H., Gonsior, G., Knäbe, S.: Bee colony assessments with the Liebefeld method: How do individual beekeepers influence results and are photo assessments an option to reduce variability? In: *Hazards of pesticides to bees: 14th International Symposium of the ICP-PR Bee Protection Group*, October 23–25, 2019 Bern, Switzerland - Proceedings. pp. 100–105., Bern (2020)
- Blacquiere, T., van der Aa-Furnée, J., Cornelissen, B., Donders, J.N.L.C.: Behaviour of honey bees and bumble bees beneath three different greenhouse claddings. In: *Proceedings of the Netherlands Entomological Society meeting 17*. pp. 93–102. Nederlandse Entomologische Vereniging, Amsterdam (2006)
- Boes, K.E.: Honeybee colony drone production and maintenance in accordance with environmental factors: An interplay of queen and worker decisions, (2010)

- Bommarco, R., Marini, L., Vaissière, B.E.: Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia*. **169** (2012). <https://doi.org/10.1007/s00442-012-2271-6>
- Calderone, N.W.: Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992–2009. *PLoS One*. **7** (2012). <https://doi.org/10.1371/journal.pone.0037235>
- Chen, W., feng, An, J., dong, Dong, J., Ding, K., fa, Gao, S.: Flower-visiting behavior and pollination ecology of different bee species on greenhouse strawberry. *Chin. J. Ecol.* **30**, 290–296 (2011)
- Cobey, S.W.: Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. (2007)
- Dainat, B., Dietemann, V., Imdorf, A., Charrière, J.D.: A scientific note on the ‘Liebefeld Method’ to estimate honey bee colony strength: Its history, use, and translation. *Apidologie*. **51** (2020). <https://doi.org/10.1007/s13592-019-00728-2>
- Eeraerts, M., Smagghe, G., Meeus, I.: Bumble bee abundance and richness improves honey bee pollination behaviour in sweet cherry. *Basic. Appl. Ecol.* **43** (2020). <https://doi.org/10.1016/j.baec.2019.11.004>
- Eskov, E.K., Eskova, M.D.: The effect of hypoxia on the development of honeybee workers at prepupal and pupal stages. *J. Evol. Biochem. Physiol.* **51**, 216–221 (2015). <https://doi.org/10.1134/S0022093015030064>
- Fine, J.D., Shpigler, H.Y., Ray, A.M., Beach, N.J., Sankey, A.L., Cash-Ahmed, A., Huang, Z.Y., Astrauskaite, I., Chao, R., Zhao, H., Robinson, G.E.: Quantifying the effects of pollen nutrition on honey bee queen egg laying with a new laboratory system. *PLoS One*. **13** (2018). <https://doi.org/10.1371/journal.pone.0203444>
- Food and Agriculture Organization of the United Nations: Why bees matter. The importance of bees and other pollinators for food and agriculture: (2018)
- Gallai, N., Salles, J.M., Settele, J., Vaissière, B.E.: Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* **68** (2009). <https://doi.org/10.1016/j.ecolecon.2008.06.014>
- Howlett, B.G., Lankin-Vega, G.O., Jesson, L.K.: *Bombus terrestris*: A more efficient but less effective pollinator than *Apis mellifera* across surveyed white clover seed fields. *N Z. J. Crop Hort. Sci.* **47** (2019). <https://doi.org/10.1080/01140671.2018.1466341>
- Human Research Program Education Outreach Team: Space shuttle launch motion analysis: (2011)
- Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J.D., Forsgren, E., Fries, I., Hatjina, F., Hu, F.-L., Jaffé, R., Jensen, A.B., Köhler, A., Magyar, J.P., Özkýrým, A., Pirk, C.W.W., Rose, R., Strauss, U., Tanner, G., Tarpy, D.R., Van Der Steen, J.J.M., Vaudo, A., Vejsnaes, F., Wilde, J., Williams, G.R., Zheng, H.-Q.: Miscellaneous standard methods for *Apis mellifera* research. *J. Apic. Res.* **52**, 1–53 (2013). <https://doi.org/10.3896/IBRA.1.52.4.10>
- HuoQing, Z., FuLiang, H.: Honeybee: A newly emerged model organism. *Acta Entomologica Sinica*. **52**, 210–215 (2009)
- Imdorf, A., Bueahlmann, G., Gerig, L., Kilchermann, V., Wille, H.: Überprüfung Der Schätzmethode Zur Ermittlung Der Brutfläche Und Der Anzahl Arbeiterinnen in Freifliegenden Bienenvölkern. *Apidologie*. **18** (1987). <https://doi.org/10.1051/apido:19870204>
- Jun, G., Wei, S., Guiling, D., Liping, L., Zhiguang, L.: A primary report on honeybee space-flight breeding. *Acta Agriculturae Nucleatae Sinica* **23**, (2009)
- Langstroth, L.L.: A practical treatise on the hive and honey-bee. (2012)
- Li, J.-L., Peng, W.-J., Wu, J., An, J.-D., Guo, Z.-B., Tong, Y.-M., Huang, J.-X.: Strawberry pollination by *Bombus lucorum* and *Apis mellifera* in greenhouses. *Acta Entomologica Sinica* **49**, (2006)
- Maleszka, R.: The social honey bee in biomedical research: Realities and expectations, (2014)
- Ollerton, J., Winfree, R., Tarrant, S.: How many flowering plants are pollinated by animals? *Oikos*. **120** (2011). <https://doi.org/10.1111/j.1600-0706.2010.18644.x>
- Pei, W., Hu, W., Chai, Z., Zhou, G.: Current status of space radiobiological studies in China. *Life Sci. Space Res. (Amst)*. **22**, 1–7 (2019). <https://doi.org/10.1016/J.LSSR.2019.05.001>
- Peterson, S.S., Artz, D.R.: Production of solitary bees for pollination in the United States. In: *Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens* (2022)
- Rangel, J., Keller, J.J., Tarpy, D.R.: The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth. *Insectes Soc.* **60**, 65–73 (2013). <https://doi.org/10.1007/s00040-012-0267-1>
- Sabara, H.A., Winston, M.L.: Managing Honey bees (Hymenoptera: Apidae) for greenhouse tomato pollination. *J. Econ. Entomol.* **96**, 547–554 (2003). <https://doi.org/10.1093/JEE/96.3.547>
- Schmolke, A., Abi-Akar, F., Roy, C., Galic, N., Hinarejos, S.: Simulating Honey Bee large-scale colony feeding studies using the BEEHAVE Model—Part I: Model Validation. *Environ. Toxicol. Chem.* **39**, 2269–2285 (2020). <https://doi.org/10.1002/ETC.4839>
- Simeunovic, P., Stevanovic, J., Cirkovic, D., Radojicic, S., Lakic, N., Stanisic, L., Stanimirovic, Z.: Nosema ceranae and queen age influence the reproduction and productivity of the honey bee colony. *J. Apic. Res.* **53** (2014). <https://doi.org/10.3896/IBRA.1.53.5.09>
- Stasiowska, D.: Can we be with Bee on Mars? Evaluating the impact of a Rocket Flight on the Condition of honeybees (*Apis mellifera*). *Trans. Aerosp. Res.* **2020**, 36–46 (2020). <https://doi.org/10.2478/tar-2020-0009>
- STS-107: https://www.nasa.gov/mission_pages/shuttle/shuttlemissions/archives/sts-107.html
- Tarpy, D.R., Hatch, S., Fletcher, D.J.C.: The influence of queen age and quality during queen replacement in honeybee colonies. *Anim. Behav.* **59** (2000). <https://doi.org/10.1006/anbe.1999.1311>
- Thorp, R.W.: Chap. 2: Bumble Bees (Hymenoptera: Apidae): Commercial Use and Environmental Concerns. In: *For Nonnative Crops, Whence Pollinators of the Future?* (2023)
- Traynor, J.: A history of Almond Pollination in California. *Bee World*. **94** (2017). <https://doi.org/10.1080/0005772x.2017.1353273>
- Vandenberg, J.D., Massie, D.R., Shimanuki, H., Peterson, J.R., Poskevich, D.M.: Survival, behavior and comb construction by honey bees, *Apis mellifera*, in zero gravity aboard NASA shuttle mission STS-13. *Apidologie*. **16**, 369–384 (1985).
- Wietzke, A., Westphal, C., Gras, P., Kraft, M., Pfohl, K., Karlovsky, P., Pawelzik, E., Tschamtk, T., Smit, I.: Insect pollination as a key factor for strawberry physiology and marketable fruit quality. *Agric. Ecosyst. Environ.* **258**, 197–204 (2018). <https://doi.org/10.1016/J.AGEE.2018.01.036>
- Zhang, H., Zhou, Z., An, J.: Pollen release dynamics and daily patterns of pollen-collecting activity of honeybee *Apis mellifera* and Bumblebee *Bombus lantschouensis* in solar greenhouse. *Insects*. **10** (2019). <https://doi.org/10.3390/insects10070216>