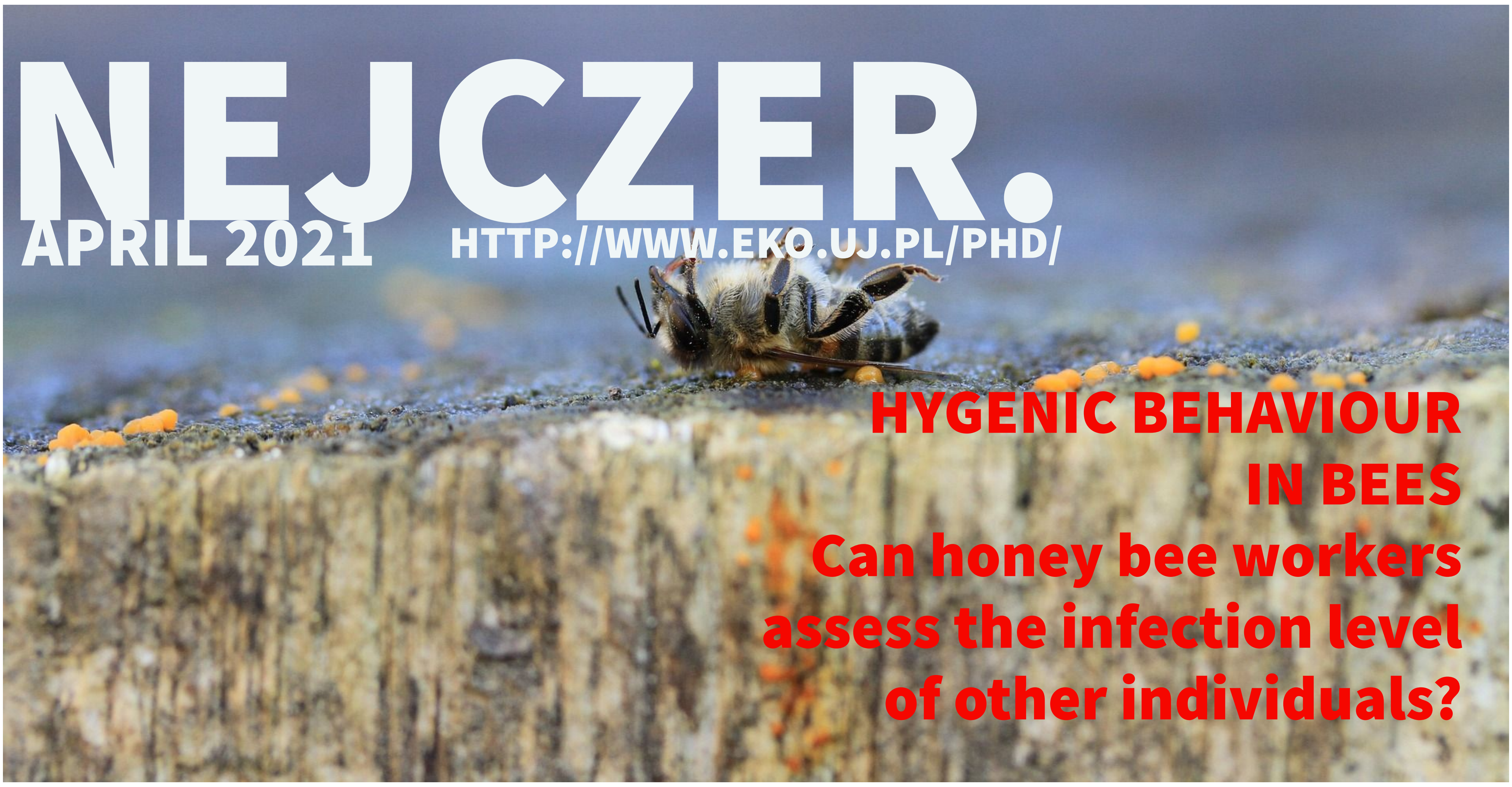

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APRIL 2021

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HYGENIC BEHAVIOUR IN BEES

Can honey bee workers assess the infection level of other individuals?

DECLINE OF POLLINATORS

Can ants replace bees as crop pollinators?

POLAR BEAR DIET

Feasibility of the alternative diets exhibited by polar bears aiming for conservation of this species

Methodological workshop in Evolutionary Biology – Grant writing
Microsoft Teams, 12th-19th April 2021

Cover made by Agata Burzawa

Table of contents

List of participants

ORGANISER:

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Prof. dr hab. Wiesław Babik	Institute of Environmental Sciences, JU
Dr hab. Piotr Nowicki, prof UJ	Institute of Environmental Sciences, JU

PARTICIPANTS:

Agata Burzawa	Institute of Environmental Sciences, JU
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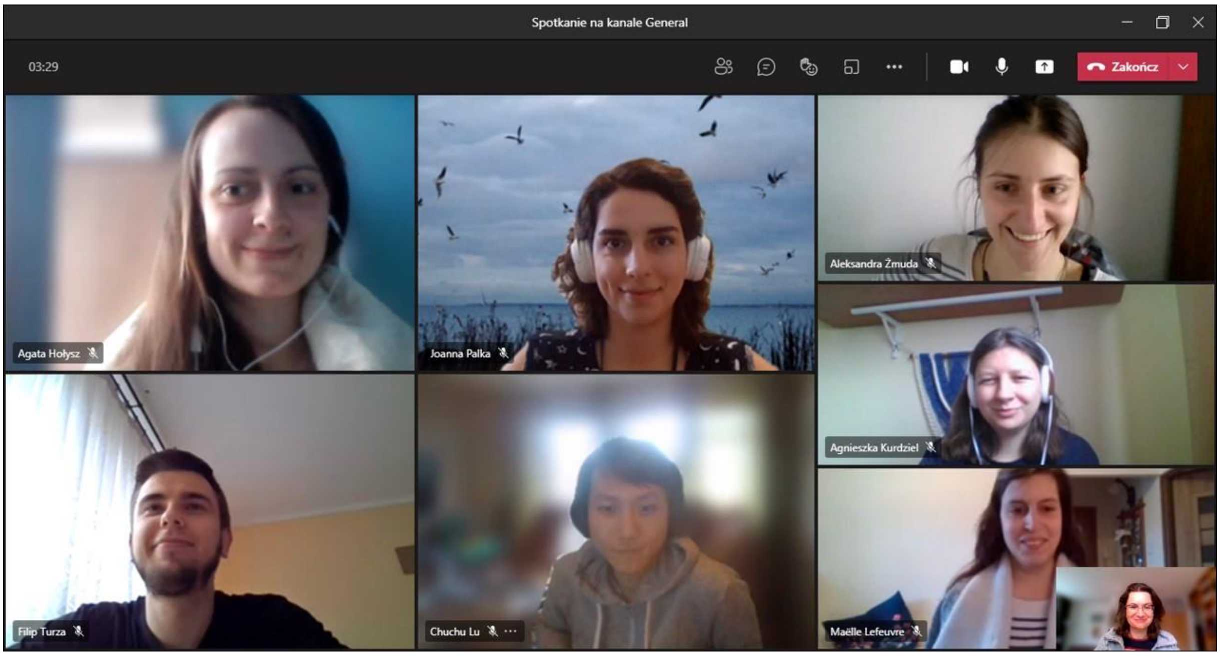
Maëlle Lefeuvre	Institute of Environmental Sciences, JU
Chuchu Lu	Institute of Environmental Sciences, JU
Joanna Palka	Institute of Environmental Sciences, JU
Agnieszka Kurdziel	Institute of Botany, JU
Aleksandra Żmuda	Institute of Botany, JU

Group photo



Group photo taken on Microsoft Teams,
Dr. Joanna Rutkowska bottom right

Research topics proposed and chosen by participants

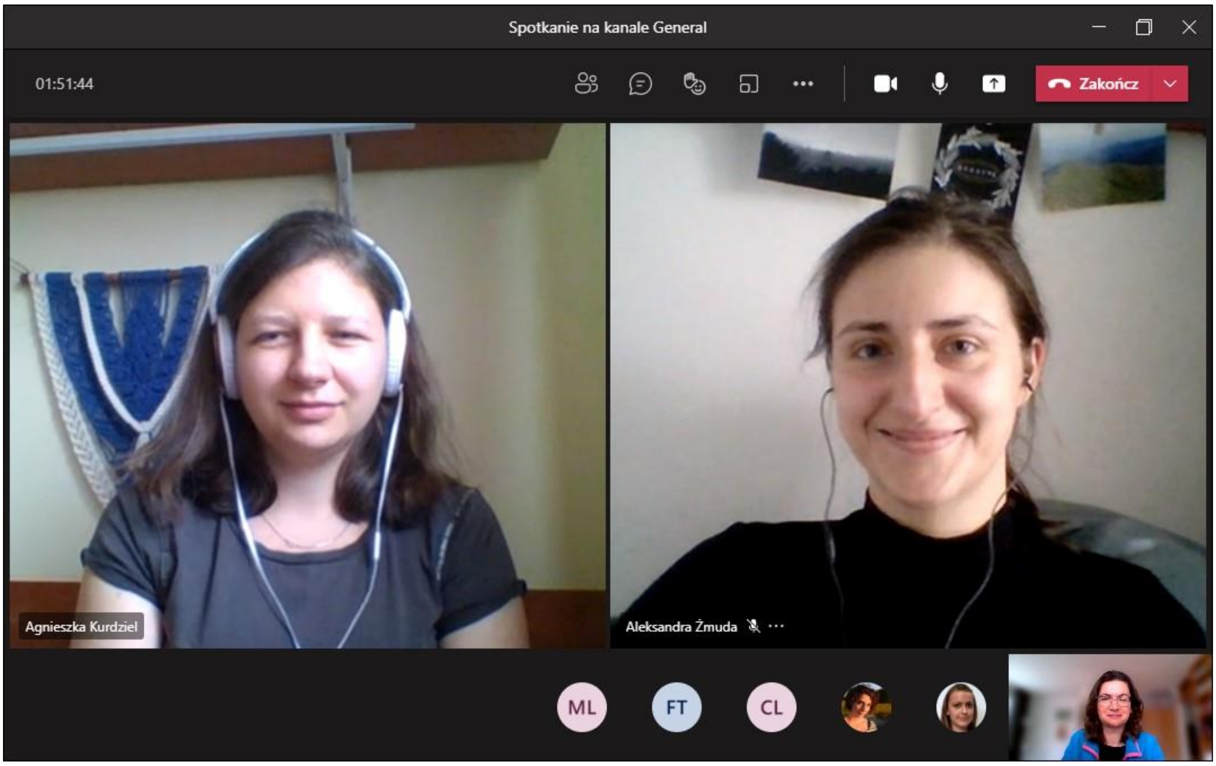
1. Does medical honey have better antibacterial properties than other types of honey? (FT)
2. **Can ants replace bees as flower pollinators? (FT)**
3. Why rats save their companions? (FT)
4. Does using GFP marker affect fitness related traits of animals? (JP)
5. How does number of herbivorous enemies affect evolution of camouflage in plants? (JP)
6. Horizontal transfer of gens responsible for resistance to toxic metabolites from plants to insects (JP)
7. How reduction of mobility during Covid pandemic affects spinal problems? (AK)
8. Cat and owner communication - is it better in old breads? (AK)
9. What is the base of Arabidopsis tolerance to heavy metals – genetic or epigenetic? (AK)
10. Choice of copulation place in honey bees (AŻ)
11. **Can honey bee workers assess the infection level of other individuals? (AŻ)**
12. Is microplastic accumulated in pollen and nectar and how does it affect solitary bees? (AŻ)
13. Does the amount of CO_2 increases in the exhaled air as a result of wearing face mask? (AH)
14. Local effects of pandemic on carbon footprint and air quality (AH)
15. Whether and how does genome stends behind temperature-driven cell size rule? (AH)
16. **Feasibility of alternative diets in polar bears (CL)**
17. Are ZOOs providing enough education to justify their existence? (CL)
18. The effect of pandemic on food availability for urban-adapted species (CL)
19. The influence of personality of Przewalski horses on their movement behaviour at herd and individual level (ML)
20. Potential of successful cultivation of southern crops varieties in northern areas (ML)
21. What are basis of adaptation of sea star to extreme cold? (ML)
22. Evolution of Genus *Paramacrobiotus (Tardigrada) (PD)*
23. Diversity assessment of Indian Limno-terrestrial Tardigrades (PD)

*Projects in bold were later chosen as research topics

Project 1: **Can honey bee workers assess the infection level of other individuals?**

Agnieszka Kurdziel, Aleksandra Żmuda

Photo of team members



Draft project proposal

Title: Can honey bee workers assess the infection level of other individuals?

Applicants: Agnieszka Kurdziel, Aleksandra Żmuda

Summary:
Honey bees play an important role both in the functioning of the natural environment and in human life. An essential element of removing pathogens from bee colonies is their hygienic behavior. Bees clean the hives of dead and infected individuals, sick bees may also self-remove themselves from a colony. It is a well-known fact that workers can identify infected individuals. We want to check if they can determine the severity of the disease. Additionally, we aim to identify whether factors such as age and breed of bee are important. For this purpose, for two years we will conduct field studies in cooperation with local beekeepers. we will observe the natural behavior of bees and check whether the strength of the colony improves the cleaning of the hive and whether the bees differentiate the threats by taking sick individuals by removing them on different distances to hive. In the laboratory part, we will focus on the sensitivity threshold for infections detection. We hope that this research will help to better understand the underlying basis of bee hygiene behavior and improve bee immunity by including our studies in breeding plans of beekeepers.

SHORT DESCRIPTION OF THE RESEARCH PROJECT

1) Scientific goal of the project

(description of the problem to be solved, research questions and hypotheses)

Honey bee, the best-known insect pollinator species, is s model organism for many research problems. As social insects, they show many behaviors related to the protection of their own family, for example altruistic self-removal of health-compromised workers from their hive. Hygienic behavior of honey bees (*Apis mellifera*) has been studied for over 80 years with the aim of understanding mechanisms of pathogen and parasite resistance and colony health. The term hygienic behavior was coined by Rothenbuhler (1964) to describe the process of detection and elimination of diseased brood by adult honey bees. (Spivak & Danka 2021). In this project, we will focus on the ability of honey bee workers to detect disease. We want to find out if they can assess the infection level of other individuals.

Bees' ability to recognize odors is well researched in honey bee communication (Robertson & Wanner 2006). Additionally, it has been confirmed that bee diseases such as the wing deformation virus can affect the presence of certain compounds in their bodies, such as proteins (Erban et al. 2019). There are known cases of bees identifying infections in adults and removing them from the hive (Baracchi et al. 2012). We want to better understand the ability of workers to detect pathogens. Are they able to distinguish the scale of the threat? How does the colony strength affect their way of dealing with the threat? Does the ability to detect the presence of pathogens change with age or is it breed-dependent?

The first part of the project aims to test if the reaction of the workers depends on the degree of threat and/or the strength of the family. We assume that dead and more infected individuals are carried out from the hive farther than less infected ones. We also want to confirm if strong colonies detect faster bees infected with pathogens which would result in the earlier removal from the nest. This part is based on observations and a field experiment.

The second part of the project complements the first. Under controlled conditions, we plan to determine how high the infection level must be (measured e.g. by the number of spores in an infected individual) so that other bees can detect it. Additionally, we want to answer the question of whether this ability changes with the breed of bees and age of workers (a change in their function in the colony), or not. Workers derived from wild bee breeds (Apis mellifera mellifera) are characterized by higher aggressiveness, which is directly related to a greater number of hygienic behaviours (Uzunov et al. 2014). We predict that it is associated with greater sensitivity in pathogen detection and the effectiveness of their elimination. We assume that the younger bees that work inside the hive recognize sick individuals better than the older workers collecting food.

2) Significance of the project

(state of the art, justification for tackling a specific scientific problem, justification for the pioneering nature of the project, the impact of the project results on the development of the research field and scientific discipline);

Honey bees (*Apis mellifera*) provide important pollination services in agricultural settings worldwide and in many natural ecosystems. Honey bees and other pollinating insects are under threat from a variety of natural and anthropogenic causes, ranging from viruses and bacteria to other insects and even mammals (Yañez et al. 2020). Thanks to the cultural importance of honey bees during much of modern human history the study of honey bee disease is an ancient topic, discussed in the literature since the ancient Greeks. The advent of modern microbiology and methods for culturing and observing microbes led to the first formal confirmation of several honey bee pathogens. Bee pathology has grown substantially in the past 50 years, with the identification of additional bacterial, fungal, and viral disease agents. Also bee responses toward those pathogens are better known (Yanes et al. 2019, 2006). Research efforts to understand honey bee resistance mechanisms are motivated by desires to breed and manage bees that are naturally resistant to parasites and, more generally, to better understand how an insect host interacts with a diverse set of pathogens. As an example of the former, beekeepers and researchers have long tried to develop lineages of bees with traits that enable colonies to survive attacks from their pathogens and parasites (Kefuss et al., 2004).

That is why we want to focus on gathering data about individual response on sick bee by other which work is to remove source of infection from the colony. Most of the communication between bees take place in dark interior of crowded hive. The best way of information transfer is chemical communications. Because of the disease in honey bee proteome is chemically change what have influence on the smell of that individual (Erban et al. 2019).

Every year millions of euros are spent by beekeepers and European Union to protect honey bee from diseases. Better understanding of honey bee biology in terms of infections identification can help to maintain this important pollinator species in good condition without chemical influence on colonies and honey consumers. We think that passible colleration between removing distance of dead body and infectious level of the bee can bee easy predictor for beekepers to notice ongoing infection in the hive. Basing on the distance that dead bodies We also hope that our research on the wild breed of honey bees in combination with contact with beekeepers will contribute to the popularization of wild varieties of bees.

3) Concept and work plan (Agnieszka)

(general work plan, specific research goals, results of preliminary research, risk analysis);

In the first two years of the project, we will focus on learning about the natural response of bees to various diseases. To achieve that, we are planning to do the following:

0) In the period between seasons (from mid-September to mid-April) we will contact new beekeepers to improve our knowledge and better understand bee diseases. We also plan to establish cooperation needed for the field stage of the project.
1) In the next two seasons we intend to conduct observations of infected apiaries. We plan to obtain information about the pathogen occurrence from already known beekeepers who have agreed to our presence in their apiary for 2-3 days in order to collect data.
- With the help of purchased cameras we will record both the removal of dead and infected bees as well as the self-removal from the hive performed by sick bees.
- By making use of the light-colored mats placed at the entrance to the nest we want to measure the distance from the hive to the bees placed outside. We will collect insects from the mats during the regular hive rounds and preserve the material in order to further determine the degree of contamination. In this way, we want to examine the relationship between the degree of infection and the distance to which insects are moved.
-We want to conduct an experiment consisting of placing micro transmitters on some of the ejected dead insects. These insects will be thrown back into the hive. Based on the observation of their location, we want to determine how quickly they are going to be found and removed from the hive. For this, the breed of bees and strength of the family will be considered as variables. (We aim to rent a set which enables tracking and recording the location while using this type of transmitters, since such a set is used during one of other ongoing projects: Preludium 17 Registration no.: 2019/33 / N / NZ8 / 02864).
- In addition, we will gain practical knowledge and experience in the field of bee breeding and maintain contact with many experienced beekeepers.
In the time between visiting apiaries and between seasons, we will execute laboratory work and analyse collected materials. As an example, we will determine the number of Nosema spores (one of the pathogens) or check recordings to see which bees removed themselves and which have been removed by others.
Having two seasons at our disposal, we have a better chance to observe phenomena and dependencies related to infections in apiaries and have enough time to try to identify factors affecting them.
2) In the second part, we want to conduct a series of experiments under laboratory conditions.
- As the first step, we will buy and breed two types of bees: a variety closer to the wild one, characterized by e.g. increased amount of hygienic behaviours and sensitivity to stimuli and a more gentle variety, eg *Apis mellifera carnica*.
-We will select and mark a group of one-aged workers (just after pupation). These insects will be collected from the hive to form experimental groups.
- We will put transmitters on the individuals to accurately monitor their position and collect detailed numerical data.
- We will place a Zander cage with an infected bee locked inside in the confined space (healthy-control). We want to compare the reaction of two bee breeds to infected individuals from their own hives.
The observed variables in the experiments will be the pathogen type (selected on the basis of field studies) and the degree of infection, as well as the age of healthy workers. We want to see if the age and the associated type of their work affects their ability to detect infections.
In autumn and winter we will process and analyse the collected data and present the results obtained during the project.

Risk analysis:
Insufficient amount of available data due to the lack of consent of beekeepers to conduct research in their apiaries. Multi-pathogenic infections, interfering with outcomes. If there is no possibility to work with micro transmitters, only cameras will be used.

4) Research methodology
(underlying scientific methodology, methods, techniques and research tools, methods of results analysis, equipment and devices to be used in research)

Collection of data. Apiary fieldwork.
During the first two years of the project we will focus on working in apiaries infected by four main honeybee colony diseases caused by pathogens from different taxonomic groups:

- Nosemosis (*Nosema ceranae*) - microsporadian
- Deformed wing virus (DWV) - viral
- Chalkbrood (*Ascosphaera apis*) - fungal
- American fouldbrood (*Paenibacillus larvae*) - bacterial

In cooperation with 24 local apiaries (six locations for each pathogen group) we will collect dead bees which were carried out of the hive by worker bees or in the act of suicide separation. Observation will be conducted on sunny warm days during the whole beekeeping season, respectively to occurrence of different pathogenic diseases.
To detect how the body got outside the colony, we will use a camera which will be recording a 1,5-meter area in front of the hive. We will put rubber mat with small tentacles to protect bees' bodies from being moved by wind (Fig.1. point 1). Before recording, all hives we be opened to estimate colony's strength by counting all frames with worker bees and number of individuals on one frame (Delaplane et al. 2013). In each apiary on one day 6 cameras will be recording 6 different colonies. We are planning to be in one apiary for 3 days to collect data from 18 colonies. Every experimental day will last from 11 a.m. to 2 p.m. in the time of highest colony activity. In every 1 hour we are going to note down how many bees were taken out and how far from the hive they were left behind (Fig.1. point 2). After that to the thorax of dead bee we are going to glue a micro transmitter which can be detected by radio radar. We will collect 4 bees with visibly different level of infections. The equipped bee will be return to the colony (Fig.1. point 3). Localisation data will be read from the micro transmitter in 3 demotions (Fig.1. point 4). Numerical data will be storage in a computer (Fig.1. point 4). As far as dead bee will be removed again, we are going to collect it, detach antenna and freeze body in -20°C for the laboratory investigation. Using the already developed methodologies, we will identify the type and number of pathogenic pathogens in the bee's body (Brettell et al. 2017; Chen et al. 2009; De Graaf et al. 2006; Chorbinski & Rypula 2003) (Fig.1. point 5).

Fig. 1. Schema on apiary fieldwork. Description in a main text.

Collection of data. Laboratory glass experiment.

This part will have place in laboratory of Behavioural Ecology Group, Institute of Environmental Sciences, Jagiellonian University in Kraków. We will take worker bees from 10 colonies in the Jagiellonian University Apiary. Half of them is going to be from wilder and more aggressive breed *Apis mellifera mellifera* and other 5 hives of *Apis mellifera carnica*. In glass box we will put one healthy and one artificially infected bee from the same colony to observe and record by a camera their behaviour to each other. Sick individuals will be infected with 3 types of pathogens (*Nosema ceranae*; Deformed wing virus; *Ascosphaera apis*). In each of pathogen bees will show 4 different stages of each disease: just infected, weakened less active one, bees with strong symptoms; dead one. As a control will use heathy individual. Sick individuals will differ in number of given spores/viruses and time from the beginning of infection. Infected individual will be place in the center of glass box in a little plastic cage to limit movements of the bee. In the cage will be holls to make passible chemical communication between experimental bees. Healthy individuals will be breed to be in the same age using queen isolator in the colony. We will use them in the experiment in 1st, 10th, 20th and 30th day after leaving cells. Before putting healthy bee to the glass box, we will equip it with micro transmitter on its thorax to record its position in relation to the sick bee. In each experimental subgroup it will be 10 healthy individuals recorded for 10 minutes. After that

Data analysis. Apiary fieldwork.

From collected data will be at least 2 statistical analyses. All of them will be linear model. In first dependent variable will be distance from the hive and 4 factors (strength of colony, infection level of the bee, colony, suicidal/removed individual, type of pathogen). Second analysis depend variable time of removing dead bee from the hive and 4 factors (strength of family, infection level of the bee, colony, type of pathogen).

Data analysis. Laboratory glass experiment.

Data recorded by micro transmitter will be worked out by computer program to show how much time healthy bee spent in a chosen distance range in relation to sick bee. From camera recordings we will note type and number of different behaviours directed to infected individual. We will use linear model analysis. Dependent variable will be time that healthy bee spent on the area 5 cm radius from the diseased bee and 5 factors (age of healthy bee, infection level of ill bee, type of breed, family, type of pathogen).

5) Project literature

(a reference list for publications included in the project description, with full bibliographic data).

Baracchi, D., Fadda, A., & Turillazzi, S. (2012). Evidence for antiseptic behaviour towards sick adult bees in honey bee colonies. *Journal of insect physiology*, *58*(12), 1589-1596.

Brettell, L. E., Mordecai, G. J., Schroeder, D. C., Jones, I. M., Da Silva, J. R., Vicente-Rubiano, M., & Martin, S. J. (2017). A comparison of deformed wing virus in deformed and asymptomatic honey bees. *Insects*, *8*(1), 28.

Chen, Y. P., Evans, J. D., Murphy, C., Gutell, R., Zuker, M., Gundensen-Rindal, D. A. W. N., & Pettis, J. S. (2009). Morphological, Molecular, and Phylogenetic Characterization of Nosema ceranae, a Microsporidian Parasite Isolated from the European Honey Bee, Apis mellifera 1. *Journal of Eukaryotic Microbiology*, *56*(2), 142-147.

Chorbinski, P., & Rypula, K. (2003). Studies on the morphology of strains Ascosphaera apis isolated from chalkbrood disease of the honey bees. *Electron. J. Pol. Agric. Univ*, *6*. (wykrywanie grzybicy)

De Graaf, D. C., Alippi, A. M., Brown, M., Evans, J. D., Feldlaufer, M., Gregorc, A., & Ritter, W. (2006). Diagnosis of American foulbrood in honey bees: a synthesis and proposed analytical protocols. *Letters in applied microbiology*, *43*(6), 583-590.

Erban, T., Sopko, B., Kadlikova, K., Talacko, P., & Harant, K. (2019). Varroa destructor parasitism has a greater effect on proteome changes than the deformed wing virus and activates TGF-β signaling pathways. *Scientific reports*, *9*(1), 1-19.

Kefuss, J., Vanpoucke, J., De Lahitte, J.D., Ritter, W., 2004. Varroa tolerance in France of Intermissa bees from Tunisia and their naturally mated descendants: 1993– 2004. *American Bee Journal* 144, 563–568

Spivak, M., & Danka, R. G. (2021). Perspectives on hygienic behavior in Apis mellifera and other social insects. *Apidologie*, *52*(1), 1-16.

Robertson, H. M., & Wanner, K. W. (2006). The chemoreceptor superfamily in the honey bee, Apis mellifera: expansion of the odorant, but not gustatory, receptor family. Genome research, 16(11), 1395-1403. (wykrywanie zapachów)

Yañez, O., Piot, N., Dalmon, A., de Miranda, J. R., Chantawannakul, P., Panziera, D., Asmiri D. , Smagghe G., Schroeder D., & Chejanovsky, N. (2020). Bee viruses: Routes of infection in Hymenoptera. *Frontiers in microbiology*, *11*.

Uzunov, A., Costa, C., Panasiuk, B., Meixner, M., Kryger, P., Hatjina, F., & Büchler, R. (2014). Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. Journal of Apicultural Research, 53(2), 248-260.
Delaplane, K. S., Van Der Steen, J., & Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of Apis mellifera colonies. *Journal of Apicultural Research*, *52*(1), 1-12.

6. Table with budget of the project.

	Amount in PLN
Direct costs, including	167,500
- personnel costs and scholarships	54,000
- research equipment/device/software cost	48,400
- other direct costs	65,100
Indirect costs, including:	36,850
- indirect costs of OA	3,350
- other indirect costs	33,500
Total costs	204,350

7. Breakdown of project costs including justification and relevance for the tasks in the project)

Name	**Justification**	**Total cost [PLN]**
Direct costs		
- personnel costs and scholarships		**54,000**
mgr Agnieszka Kurdziel	750 PLN additional monthly salary for 3 years (12 x 3 x 750 PLN)	27,000
mgr Aleksandra Żmuda	750 PLN additional monthly salary for 3 years (12 x 3 x 750 PLN)	27,000
- research equipment/device/software cost		**48,400**
Camera	6 cameras with tripods for video recording of bees taking out sick individuals (6 x 4,500 PLN)	27,000
Freezer	300 litres freezer for storaging taken bee samples (1 x 1,400 PLN)	1,400
Honey bee colony in hives	10 hives with strong colonies (10 x 2,000 PLN)	20,000
- other direct costs		**65,100**
Conferences and business trips	This cost in this category (49,000 PLN) include: • Bisness trips (34, 000 PLN) to 24 apiaries (around 100 km in one way) each for 3 days (travels 3,000 PLN x 2 years = 6,000 PLN and stay costs 2 people x 7,000 PLN x 2 years = 28,000 PLN) • Abroad conference (15,000 PLN) costs	49,000

	as fees (2000 PLN x 2 people = 4,000 PLN, "Eurbee 2024"; fee = 450€ = 2,000 PLN), travel costs (3,000 PLN x 2 people = 6,000 PLN) and stay costs (2,500 PLN x 2 people = 5,000 PLN).	
Materials and small equipment	• Laboratory materials (glass, reagents): 5,000 PL • Office materials for field work (permanent fine-tipped Staedler markers for marking bees / paper folders / pens / sheets/ rubber mats for collecting bees/plastic cages for glass experiment): 2,100 PLN • Cameras waterproof protection (6 x 100 PLN) • Apiary equipment (frames, sheets of beeswax, empty hives for cure therapy, sugar for winter feeding: 1,900 PLN)	9,600
Biological material	3 types of pathogens for the glass experiment (3 x 500 PLN)	1,500
Outsourced services	The costs of outsorcing and subcontracting (5000 PLN) include poster printing for conferences along with linguistic correction of the manuscripts.	5,000

Reviews

Piotr Nowicki

1. **Assessment of scientific quality of the research project** (scientific relevance, importance, originality and novelty of research or tasks to be performed; quality ought to be evaluated in an international context)

The proposal is centred around a rather specific but important issue of bee ability, and factors affecting it, to detect the level of infection in fellow bee workers. The rationale behind the proposal is well explained and trustworthy. The specific research questions to be asked are interesting and clearly outlined, although the latter becomes evident only after reading the workplan description, since several crucial terms for defining the research questions (e.g. scale of threat, colony strength) are specified only there. A minor concern in this respect is that against its intention the envisaged research will not allow to assess if strong colonies faster detect and remove infected bees, because in this case it is impossible to distinguish the driver from the predictor (i.e. it could equally well be that colonies with better worker ability to detect infections are prone to grow stronger).
On the other hand, the large fragments of the proposal are somewhat chaotically (possibly hastily) written, which makes it impossible to fully appreciate the quality and relevance of the proposed study. The proposal suggests a serious of potentially useful experiments under a single umbrella topic, but fails to prove that they will be successfully integrated with one another to create an overall comprehensive approach.

2. **Assessment of potential impact of the research project** (the potential for substantial international impact on the research field(s) and for high quality research publications and other research outputs, taking into account the specifics of the research field and the variety of forms of impact and output; impact ought to be evaluated using an international context)

The proposal has a capacity to lead to advancements in honey bee research. However, it is broader importance beyond the focal study system, e.g. for the understanding of the evolution and ecology of insect societies is not demonstrated convincingly. Similarly, the potential implications for improved apiculture (which could be a great achievement of the project), while claimed in the proposal, are not adequately substantiated to support the claim. In particular, the utility of the distance at which dead workers are removed as a simple index of the colony health is unconvincingly explained.

3. **Assessment of feasibility of the research project** (the feasibility of the proposed project, including the appropriateness of the research methodology to achieve the goals of the project, the risk management description, research facilities and equipment, international cooperation (if any), other factors affecting the feasibility of the project)

The methodology is only broadly elaborated on and it lacks sufficient level of detail in places. The description of the prospective analyses is written in bad English, and it is necessary for readers to assume that all the variables other than those mentioned as dependent ones will be used as predictors. More importantly, the description of the envisaged aviary experiment does not explain how the dead individuals not removed from the hives will be considered (if at all): while no removal distance or duration can be determined in such cases their frequencies – which may vary between different dead workers groups – seem much more informative for assessing the disease detection abilities in workers. Besides, it should be noted that the recordable removal distances are inevitably constrained by the maximum distance at which the mats placed, and thus a preliminary pilot investigation to establish the likely range of expected removal distances seems necessary. Concerning the laboratory experiment, it is not clarified how different types of worker behaviour towards infected individual will be categorised, and how (again, if at all) this information will be analysed.

The feasibility of the apiary experiment is somewhat undermined by uncertain willingnessof beekeepers to get involved in the project. It is highly useful that this issue is correctly identified as a potential risk to the successful implementation of the project, but regretfully no mitigation measure is suggested as normally expected from the risk management plan. In this respect is also unconvincing that contacting beekeepers in order to acquire better understanding bee diseases and to establish cooperation with them is planned as the initial task of the project, because such preliminary activities should be completed already at the proposal drafting phase.

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

The proposed budget is very well detailed and with thoroughly justified costs. A minor remark is that ten bee hives to be purchased should most likely be regarded as consumables rather than as equipment.

5. **Strengths of the proposal**

(i) well explained rationale and clearly outlined research questions;
(ii) very well detailed and thoroughly justified budget.

6. **Weaknesses of the proposal**

(i) a relatively narrow scope with little effort to demonstrate a broader importance of the proposal;
(ii) rather chaotic presentation of the proposed research and the methodology description lacking sufficient level of detail on some key issues.

<u>Chuchu Lu</u>

1. **Assessment of scientific quality of the research project** (scientific relevance, importance, originality and novelty of research or tasks to be performed; quality ought to be evaluated in an international context)

This project poses questions that are relevant to populations of bees which can potentially be applied to other species. With bees providing important ecosystem services to the world, this study will be contributing to the maintenance of bees at an international scale.

Novelty of this project lies in the desire to understand the mechanisms behind the hygienic behaviour carried out by the removal of infected individuals. However, the approach of the experimental design is somewhat lacking in providing direct information on the relationship between this behaviour and the strength of the colony. Instead of assessing the effect of distance removed on the strength of the colony, perhaps the stronger the colonies, the quicker and farther they remove the infected. This in the sense will not give evidence on the mechanisms in which the worker bees are able to detect them at different efficiency.

2. **Assessment of potential impact of the research project** (the potential for substantial international impact on the research field(s) and for high quality research publications and other research outputs, taking into account the specifics of the research field and the variety of forms of impact and output; impact ought to be evaluated using an international context)

This project has the potential for substantial impact on the pathogenic research of bees internationally. However, specific plans to producing publications or the research outputs this study can lead to are not clearly stated in the proposal. More consideration should be given to the potential strength of this project and the practical application it may bring.

3. **Assessment of feasibility of the research project** (the feasibility of the proposed project, including the appropriateness of the research methodology to achieve the goals of the project, the risk management description, research facilities and equipment, international cooperation (if any), other factors affecting the feasibility of the project)

The feasibility of the proposed project is explained appropriately with thorough description of the experimental design and clear plan of the methodology, equipment, and facilities. Risk assessment and cooperation with public beekeepers were described, though not at an international scale. The research project is well-planned with minor things to point out as follow,

- At the end of the paragraph "collection of data", the sentence/paragraph appeared to be incomplete.
- One would assume that the infected bees are carried out and dropped just outside of the hive entrance. Will the platform provide accurate measures to interpret the distance removed? If this is not the case, a preliminary measure of the distance removed found on the ground could be conducted beforehand.
- Reaction of the worker bees looking at infected bee inside glass box is not described clearly. With only the position of the healthy bee in relation to the infected bee may not be enough. Some more specific behaviours could be identified before the experiments.
- Judging from the risk assessment, if micro-transmitter is unavailable, how do the authors plan to account for the time the infected body is discovered until removed? Same situation can be said even with a micro-transmitter. How will the distance/location from the entrance after putting in be standardized for all 4 infected dead bodies?

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

Yes.

5. **Strengths of the proposal**

Strength of the proposal is the great potential it has to be practically applied to the bee diversity at a larger scale. The experimental design and methodologies are described clearly overall.

6. **Weaknesses of the proposal**

Further consideration can be given to some minor points in the experimental set-up. With the potential of the project, the authors ought to account for the broader implications it can provide.

<u>Agata Burzawa</u>

1. **Assessment of scientific quality of the research project**

Pollinators act important role in all terrestrial ecosystems. Production of the majority of human food relies directly or indirectly on pollinators services. Approximately three quarters of the world's crop require pollinating by insects. Relating to this information this is extremely important to maintain hives in good health and condition. The aim of the project is to find if bees can assess the infection in their hive. Investigating the hygienic behaviour naturally used by the swarm will help bee keepers to adjust their assistance in order to obtain commonly benefits for all mankind.

I have found this proposal project essential, original, and interesting. In my opinion the subject of the study is very important and worth deeper investigation. On the other hand it is really interesting research.

I'm not an expert in that field, but the potential scientific output will be on high quality as it is well planned.

I really like the second experiment witch is quite novel to me.

2. **Assessment of potential impact of the research project**

Although, honey bees is a very popular study subject there are still a lot of significant problem to be solved. According to the project proposal the are still much unknown on bees pathogens. As the pollinators are in decline their well-being is crucial for the agriculture and world's economy. The feedback gained from the research will provide high quality data with can be used in international publication. Subject field has been broadly studied and supported by specialized scientific literature.

3. **Assessment of feasibility of the research**

All parts of proposal project are reasonable, easy to obtain and justified. The cooperation of local bee-keepers is beneficial for both parties. The researchers will obtain most up today information about local environment, beekeepers will gain solution for future problem.

In my opinion, the technic of using light-colored mats placed at the entrance to the nest is a novel resolution as it helps the researcher to find infected bodies easier than in grass. Maybe it is not so clear why it should be light-colored mats. I'm only guessing that on light colour the dead bees body will be better to observed.

In laboratory glass experiment the behavioural aspect will be difficult to be measured.

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

The budget might be underestimated. Additional costs for local bee-keepers should be included or information about the nature of researcher- beekeeper cooperation ought to be added to the context. Also there is no information about costs of the digital signature (JU regulations). However, all budget is justified. Also costs connected with transports between locals beekeepers aren't mentioned. In my opinion it should be, especially that in project proposal those trips are planned between April and September. I assumed that during this 5 months there will be more than one visit for the locals. It is good idea to add also the costs including linguistic correction of the manuscripts in the budget.

5. **Strengths of the proposal**

The work plan is precised and detailed prepared. In my opinion the strongest point can be found in the study subject itself. Also the thesis statement is very interesting. The bees will be infected by different pathogens witch will be grouped into different classes. This is a witty and reasonable approach as it provides clarity and order. Also the future for this field research is developmental. So this particular experiment could be use as a background for the further investigation. All mentioned equipment are easy accessible and commonly use in similar experiments. In general the proposal project has logical structure and visually clear form of text. In my opinion this project is possible to perform and all data are easy to achieve. For me it was really good job!

6. **Weaknesses of the proposal**

I found the budget as the weakest point. The costs could be more precisely planned. More factors should be taken into consideration. In my opinion infecting bees is on the verge of the ethics. In experiment the model organism is Apis mellifera mellifera and Apis mellifera carnica. This is only a representation of two species. In order to make the sample more significant the research could be conducted on bigger number of species. Unless the applicants are sure referring to given literature that those species are the best experimental model of hygienic behaviour. Probably the number of individuals of Apis mellifera mellifera and Apis mellifera carnica in experiment number 2 should be also mentioned. It is worth to know how big the sample will be. The methodology of data analysis could be more preciously described including the statistical model. Either the researchers should be assured that mentioned statistical analysis is appropriately performed. The aspect of natural mortality is omitted; however, this is very important factor that also should be taken into consideration. I don’t know why in the headline number 3 (concept and work plan) there is a name of one researcher. Probably it is only an oversight. If not, they should consequently put names of researchers involved in each part in every headline.

Final project proposal

Title: Can honey bee workers assess the infection level of other individuals?

Applicants: Agnieszka Kurdziel, Aleksandra Żmuda

Summary:
Honey bees play an important role both in the functioning of the natural environment and in human life. An essential element of removing pathogens from bee colonies is their hygienic behaviour. Bees clean the hives of dead and infected individuals. It is a well-known fact that workers can identify infected individuals. **We want to check if they can determine the severity of the disease.** In first step we will establish threshold of infection detection for 3 common bee diseases caused by different type of pathogens. We will also compere hygienic behaviour of workers from two kind of bees (more wild -*Apis mellifera mellifera* and popular in apiary *Apis mellifera carnica*). **We will measure time of removal of 'odour ball' (sample of 'sick' odour) from hive to check if it may be a good to predictor of hygienic behaviour level**. In second part of project, we will focus on looking answer for our main question. For this purpose, we will conduct field studies in cooperation with local beekeepers. We will observe the natural behaviour of bees and **check whether the bees differentiate the threats by taking more sick individuals by removing on further distances to hive**. We hope that this research will help to better understand the underlying basis of bee hygiene behaviour and improve bee immunity by including our studies in breeding plans of beekeepers.

SHORT DESCRIPTION OF THE RESEARCH PROJECT

1) Scientific goal of the project

Honey bee (*Apis mellifera*) , the best-known insect pollinator species, is s model organism for many research problems. As social insects, they show many behaviours related to the protection of their own family, for example altruistic self-removal of health-compromised workers from their hive. To understand health of bees colony and mechanism of pathogens resistance, hygienic behaviour has been studied for over 80 years. The term hygienic behaviour was coined by Rothenbuhler (1964) to describe the process of detection and elimination of diseased brood by adult honey bees. (Spivak & Danka 2021). In this project, we will focus on the ability of honey bee workers to detect disease. We want to find out if they can assess the infection level of other individuals.

Bees' ability to recognize odours is well researched in honey bee communication (Robertson & Wanner 2006). Additionally, it has been confirmed that bee diseases such as the wing deformation virus can affect the presence of certain compounds in their bodies, such as proteins (Erban et al. 2019). There are known cases of bees identifying infections in adults and removing them from the hive (Baracchi et al. 2012). We want to better understand the ability of workers to detect pathogens. Are they able to distinguish the scale of the threat? How does the colony strength affect their way of dealing with the threat? Does the ability to detect the presence of pathogens change with age or is it breed-dependent?

During the first year of our project under controlled conditions, we plan to determine how high the infection level must be (measured e.g. by the number of spores in an infected individual) so that other bees can detect it. Additionally, we want to answer the question of whether this ability changes with the breed of bees or not. Workers derived from wild bee breeds (*Apis mellifera mellifera*) are characterized by higher aggressiveness, which is directly related to a greater number of hygienic behaviours (Uzunov et al. 2014). We predict that it is associated with greater sensitivity in pathogen detection and the effectiveness of their elimination. **We hypothesise that worker bees can distinguish individuals with low and high infection level.** We plan to create a simple test to assess the hygiene behaviour levels of bees. We want to use the bee's sensitive receptors to develop an odour ball that will contains compounds that bees will recognize as a disease threat (clove oil, extract from thorax cuticula of infected bees). **We expect that time of detection and removing of 'odour ball' from a colony is a good predictor of hygienic ability of a colony.**

The second part (year 2 and 3) of the project aims to test if the reaction of the workers depends on the degree of threat and level of hygienic behaviour of the family. We know some bees (like wild one) are faster in detecting bees infected with pathogens which would result in the earlier removal from the nest. **We assume that dead and more infected individuals are carried out from the hive farther than less infected ones.** We also want to confirm effectiveness of hygienic behaviours using 'odour ball' test in field study. This part is based on observations and a field experiment.

2) Significance of the project

Bees and other pollinators play a crucial role in approving mankind in food. Globally income coming from pollination service was assessed on 153 billion EUR of pollination in 2005. (Gallai et al. 2009). The honey bee is widespread and the most abundant bee species because of beekeeping. The high number of introduced colonies increase the risk of transmitting diseases pathogens. Every year European Union spend more than 9,7 mln EUR to protect honey bee from diseases and 16,5 mln EUR for recompensation of colonies losses. The second as much money is spent by EU member states (Report from the commission to the European Parliament and the council 2019). At the same time, human activity, and climate changes raise the risk of pests and pathogens spreads and increased infection level which leads to a rapid drop in colony number (Vanbergen & Initiative 2013). In our study, we want to understand better the behaviours of bees to individuals infected by 4 diseases agents which cause major mortal infections in Europe.

Bee pathology is a rapidly developing branch of science, scientist identify the additional bacterial, fungal, and viral disease agents (Evans & Spivak 2010) and study individual immunity responses with the growth of new technologies and molecular-genetic techniques (Yañez et al. 2020). Social behaviours allowing them to protect the colony from sick individuals. Developing of hygienic behaviours might be the reason for a reduction in the number of immune genes in bees in comparison to other insects (Larsen et. al. 2019). Furthermore, they have three times more genes responsible for scent identification. Chemical communication is the best way for information transfer in the dark interior of a crowded hive. In the case of disease honey bee proteome is chemically change, and it influences the smell of that individual (Erban et al. 2019). Basing on that knowledge we assume that bee workers remove individual with different infections levels to protect their colony. Research on honey bee colony representing social structure can allow us to better understand the evolution and ecology of insect societies. Especially knowledge in infections identification can help to maintain this important pollinator species in the good condition without chemical influence on colonies and honey consumers.

We have established cooperation with the Provincial association of beekeepers of Małopolska region in the second and third year of our project. The currently used method of detecting level of hygienic behaviours is basing on freezing parts of larvae and pupae in a frame with help of liquid nitrogen (Spivak & Gilliam 1998). It requires danger and expensive regents what makes this technique hard to use by beekeepers. We expect that the 'odour ball' hygienic test can be easily used by beekeepers as a fast tool to state the hygienic status of a colony as well as observe on removing distance of dead bees. Conducting our study in local apiaries will allow us to collect data about infections and identify bees with higher levels of hygiene behaviours. What is also important we want to perform an educational program on bee diseases and their prevention. In the last part of the project, we want to create a website to popularise our findings of high hygienic breed bees.

3) Concept and work plan

Our general work plan is presented in Table 1. Thanks to the experience of one of the applicants in working in an apiary, we will perform work in the university apiary ourselves, using available protective clothing.

Table 1. General work plan.

Lp	Research task	Time
1	Glass experiment in JU laboratory	April – September 2022
2	Pilot study on 'odour ball' removing	April – September 2022
3	Apiary works preparing colonies for winter	End of September 2022
4	Analysis of collected data, preparing for the next season	October 2020 – February 2023
5	First year of apiary experiment	March – August 2023
6	Analysis of collected data, preparing for the next season	September 2023 - February 2024
7	Second year of apiary experiment	March – August 2024
8	Statistical analysis of collected data, conference, educational website for beekeepers	September 2024 - April 2025

In the first year of the project, we will focus on the threshold of disease detection and the preparation of a test for comparing hygiene behaviour in a bee family. The goal of the first season is to gather experimental data on the sensitivity of infection detection and to compare them.

During the next two seasons, we will visit local apiaries. In cooperation with beekeepers, we will determine the level of hygienic behaviour in their bees' colonies. Based on observations with the use of mats and laboratory analysis of the collected material, we will determine the relationship between the distance at which the bees are taken and the degree of infection. This data will also be linked to the breed of bees (information from beekeepers).

In addition to conducting research and elaborating the results, our work will include searching for new contacts and exchanging information with beekeepers. We will collect information on infections also on diseases that do not require mandatory reporting. We want to spread the knowledge about hygienic behaviour of bees and its practical importance for colony health and breeding selection.

Results of preliminary research:

- Based on previous observations, we know that the bees are carried out of the hive at an average distance of 50 cm. The maximum distance is 120 cm, hence the length of the mats in our tests will be 150 cm.
- Thanks to contacts with beekeepers from Provincial association of beekeepers of Małopolska region, we have selected a preliminary list of apiaries in which we can conduct our research.
- We aim to borrow a set which enables tracking and recording the location of bees, with the help of Aleksandra Łoś -the IP of ongoing projects: Preludium 17 Registration no.: 2019/33 / N / NZ8 / 02864.

Risk analysis:

Bad weather conditions, not suitable for field testing. Due to the variability of the weather, we will spend two seasons to the part in local apiary to collect a sufficient body of data. Multi-pathogenic infections, interfering with outcomes. In the case of high intensity of more than one pathogen in the apiary visited, we will not analyse the results in terms of the pathogen, and we will complete the data by visiting an additional apiary.

4) Research methodology

Collection of data. Laboratory glass experiment

This part will have a place in the laboratory of Behavioural Ecology Group, Institute of Environmental Sciences, Jagiellonian University in Kraków. We will take worker bees from 10 colonies in the Jagiellonian University Apiary. Half of them is going to be from wilder and more hygienic breed *Apis mellifera mellifera* and the other 5 hives of *Apis mellifera carnica*. In the glass box, we will put one healthy and one artificially infected bee from the same colony. The sick individuals will be infected with one of four known doses of a pathogen and not infected individuals as a control. We will conduct this study for 3 types of disease agents (*Nosema ceranae*; Deformed wing virus; *Ascosphaera apis*). The infected individual will be placed in a incubator for 24 hours, 6 days and 12 days in temperature of 32 – 34°C. After that time they will be put in a little plastic cage to limit their movements and placed in the centre of the circle glass box of radius 15 cm. In the cage, there will be holes to enable chemical and physical communication between experimental bees. Before putting the healthy bee to the glass box, we will equip it with an antenna on its thorax to record its position in relation to the sick bee (Geffre et al. 2020). We divide glass box for 3 circular zones with the same centre: 0- 5cm, 5-10 cm, and 10- 15 cm radius. Basing on localisation of healthy a computer programme will calculate time spent by health bee in each of those zones. Bees will be also recorded by the camera to capture and then count summary duration of their social behaviours as feeding, grooming or aggressive reaction. The healthy individuals will be bred to the same age using a queen isolator in the colony. We will use bees between 15 and 21 days after emerging from cells because they have the biggest experience in performing tasks inside the hive and the highest olfactory sensitivity (Gramacho & Spivak 2003). In each experimental subgroup, there will be 10 healthy individuals recorded for 10 minutes (3 types of pathogens x 5 infection dose x 3 variants of incubation time x5 families x 2 honey bee breed lines x 10 individuals = 4500 individuals).

Data analysis. Laboratory glass experiment

To analyse obtained data we will perform 3 linear model analysis. In each depend variable will be time which healthy bee spend in one of 3 zones. As a factors we will put: type of pathogen, dose of pathogen, type of breed, family, type of pathogen.

Preliminary study – 'odour ball'

Our idea bases on hygienic behaviour of removing dead and sick individuals form a colony. We do not want to spread diseases and infect colonies what prompted us to look for an alternative way of measurement hygienic level of the colony. To each colony we will put 5 soft balls soaked with clove oil or an extract from a thorax cuticula of infected bee (Fig.1. point 1). Antenna tracking will give as information about time of detection and removing balls from the hive (Fig.1. point 2). After establishing the substance and optimal dose of it we will perform measurements on detection and removing time of 'odour ball' from healthy colonies.

Collection of data. Apiary fieldwork

During next two years of the project we will focus on working in apiaries infected by four main honeybee colony diseases caused by pathogens from different taxonomic groups:

- Nosemosis (*Nosema ceranae*) - microsporadian
- Deformed wing virus (DWV) - viral

- Chalkbrood (*Ascosphaera apis*) - fungal
- American fouldbrood (*Paenibacillus larvae*) - bacterial

In cooperation with 24 local apiaries (six locations for each pathogen group), we will collect dead bees which were carried out of the hive by worker bees or in the act of suicide separation. Observation will be conducted on sunny warm days during the whole beekeeping season, respectively to the occurrence of different pathogenic diseases. To detect how the body got outside the colony, we will use a camera that will be recording a 1,5-meter area in front of the hive. We will put a rubber mat with small tentacles to protect bees' bodies from being moved by wind (Fig.1). It will be light-coloured to make a good contrast with dead individuals.

Before video recording, all hives will be opened to estimate the colony's strength by counting all frames with worker bees and the number of individuals on one frame (Delaplane et al. 2013). Then we will perform the 'odour ball' hygienic test with the previously described methodology (Fig.1. point 1 and 2).

In each apiary, on one day 6 cameras will be recording 6 different colonies. We are planning to be in one apiary for 3 days to collect data from 18 colonies. Every experimental day will last from 11 a.m. to 2 p.m. at the time of greater colony activity. In every 1 hour, we are going to note down how many bees were taken out and how far from the hive they were left behind (Fig.1. point 3). After that to the thorax of the dead bee, we are going to glue an antenna which can be detected by radio radar. We will collect 6 bees with visibly different level of infections. The equipped bee will be returned to the colony (Fig.1. point 5). Localisation data will be read from the antenna in 3 demotions. Numerical data will be stored in a computer (Fig.1. point 8). As soon as the dead bee will be removed again (Fig.1. point 6), we are going to collect it, detach the antenna and freeze the body at -20°C for the laboratory investigation. Using the already developed methodologies, we will identify the type and number of pathogenic pathogens in the bee's body (Brettell et al. 2017; Chen et al. 2009; De Graaf et al. 2006; Chorbinski & Rypula 2003) (Fig.1. point 7).

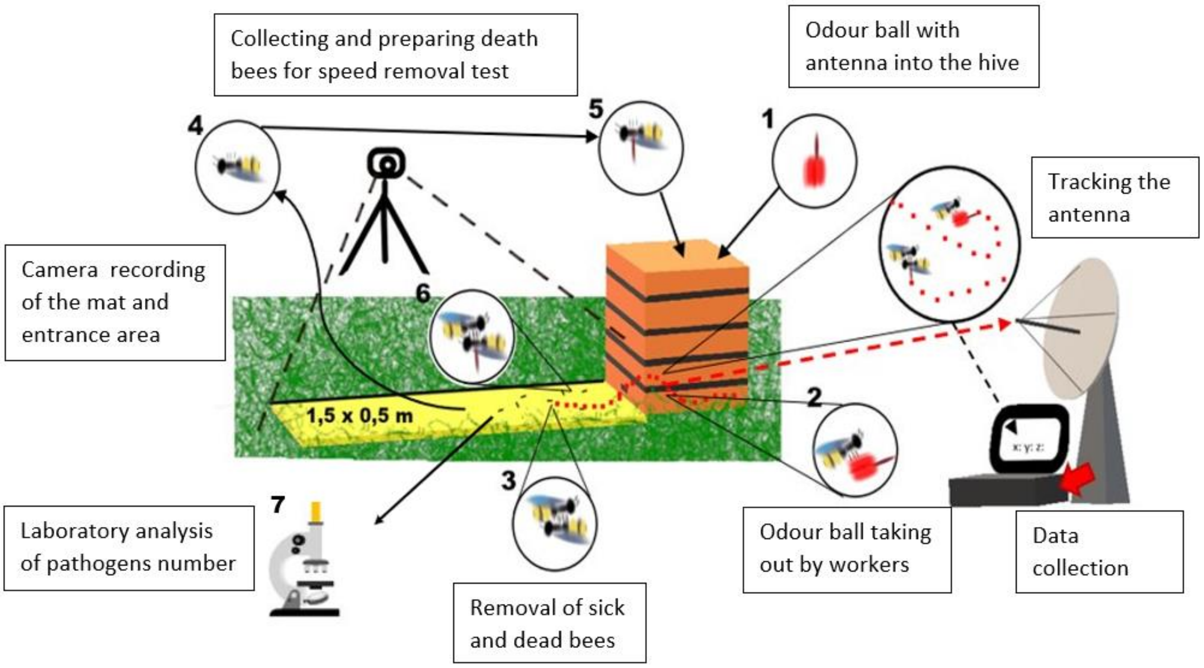


Fig. 1. Schema on apiary fieldwork. Description in a main text.

Data analysis. Apiary fieldwork

To check if 'odour ball' removing time will be a good predictor of colony hygienic behaviour we will conduct a correlation analysis of that parameter in comparison to the number of pathogens found in honey bees bodies.

Collected data will be analysed with at least two statistical tests. All of them will be linear models. In the first test dependent variable will be the distance from the hive and 4 factors (strength of the colony, infection the level of the bee, colony, suicidal/removed individual, type of pathogen). In the second analysis, the dependent variable will be the time of removing dead bee from the hive and 4 factors will be the strength of family, infection level of the bee, colony, type of pathogen.

5) Project literature

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6. Table with budget of the project

	Amount in PLN
Direct costs, including	167,500
- personnel costs and scholarships	54,000
- research equipment/device/software cost	28,400
- other direct costs	85,100
Indirect costs, including:	36,850
- indirect costs of OA	3,350
- other indirect costs	33,500
Total costs	204,350

7. Breakdown of project costs including justification and relevance for the tasks in the project)

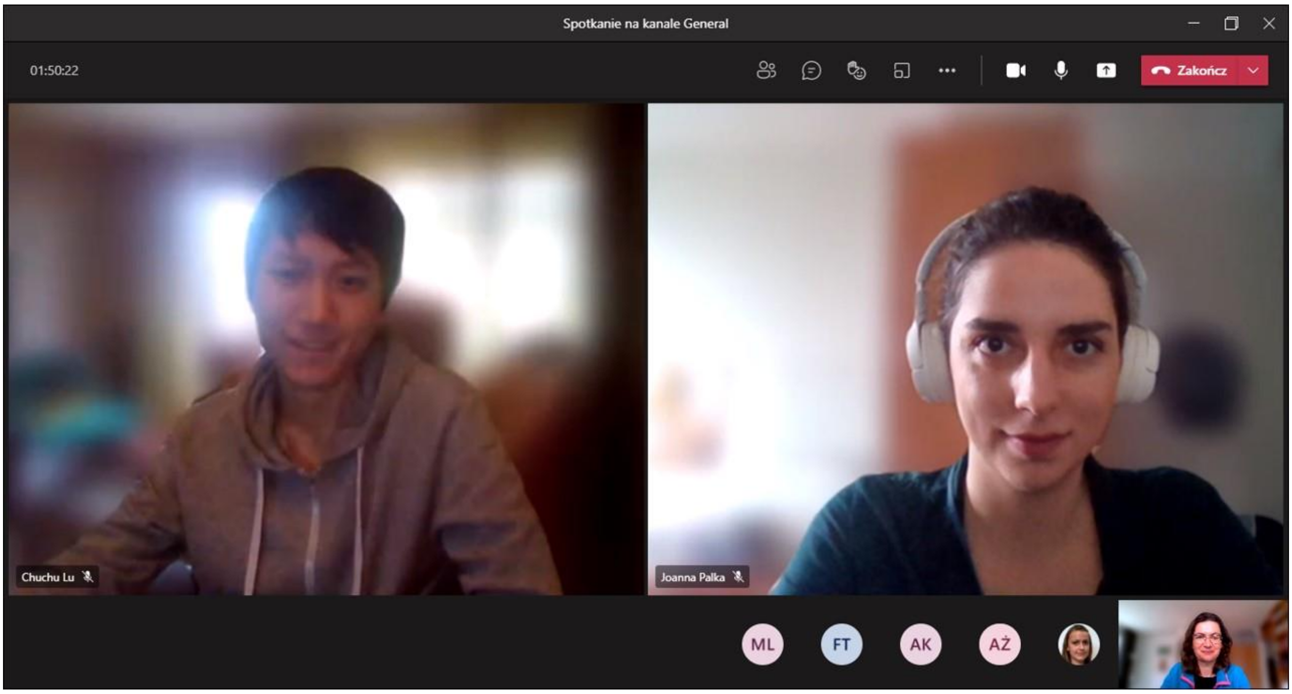
Name	**Justification**	**Total cost [PLN]**
Direct costs		
- personnel costs and scholarships		**54,000**
mgr Agnieszka Kurdziel	750 PLN additional monthly salary for 3 years (12 x 3 x 750 PLN)	27,000
mgr Aleksandra Żmuda	750 PLN additional monthly salary for 3 years (12 x 3 x 750 PLN)	27,000
- research equipment/device/software cost		**28,400**
Camera	6 cameras with tripods for video recording of bees taking out sick individuals (6 x 4,500 PLN)	27,000
Freezer	300 litres freezer for storaging taken bee samples (1 x 1,400 PLN)	1,400
- other direct costs		**85,100**
Conferences and business trips	This cost in this category (49,000 PLN) include: • Business trips (34, 000 PLN) to 24 apiaries (around 100 km one way) each for 3 days (travels 3,000 PLN x 2 years = 6,000 PLN and accommodation costs 2	49,000nt

	people x 7,000 PLN x 2 years = 28,000 PLN) • Foreign conference (15,000 PLN) costs as fees (2000 PLN x 2 people = 4,000 PLN, "Eurbee 2024”; fee = 450€ = 2,000 PLN), travel costs (3,000 PLN x 2 people = 6,000 PLN) and stay costs (2,500 PLN x 2 people = 5,000 PLN).	
Honey bee colony in hives	10 hives with strong colonies (10 x 2,000 PLN)	20,000
Materials and small equipment	• Laboratory materials (glass, reagents): 5,000 PL • Office materials for field work (permanent fine-tipped Staedler markers for marking bees / paper folders / pens / sheets/ rubber mats for collecting bees/plastic cages for glass experiment): 2,100 PLN • Cameras waterproof protection (6 x 100 PLN) • Apiary equipment (frames, sheets of beeswax, empty hives for cure therapy, sugar for winter feeding: 1,900 PLN)	9,600
Biological material	3 types of pathogens for the glass experiment (3 x 500 PLN)	1,500
Outsourced services	The costs of outsourcing and subcontracting (5000 PLN) including poster printing for conferences along with linguistic correction of the manuscripts.	5,000

Project 2: **Feasibility of the alternative diets exhibited by polar bears aiming for conservation of this species**

Chuchu Lu, Joanna Palka

Photo of team members



Draft project proposal

Title: Feasibility of the alternative diets exhibited by polar bears aiming for conservation of this species

Applicants: Lu Chuchu, Palka Joanna

Summary:
Environmental disruptions driven by climate change are a major contributing factor to the loss of biodiversity. Constantly shrinking ice cape in the Arctic is causing a decline in the population of polar bears at an unprecedented scale. Majority of animals, especially large vertebrates, are not able to adapt to such quick and drastic changes. The goal of this project is to check whether the alternative diet that the polar bears are shifting to can lead to the recovery of polar bear populations. To achieve this, we will carry out experimental manipulation by supplementing their diet with caribou or snow goose meat. We will estimate the nutritional value of the current diet of those animals and compare it with the newly adapted diet. Analysis of animal feces will help us understand the assimilability of the used food. Change in animal condition will be monitored and analyzed by comparisons of the body parameters made twice a year before and after the summer period. Indicators such as body condition index, body temperature, and hormone levels will be used. Additionally, using GPS, activity measures such as distance covered and movement rate will be calculated. The adult individuals will be measured, but most importantly we will also keep track of cub mortality. We will also compare those results to the ones made at the population level, on which we will gather the data of trans-seasonal survival rate. Multi-seasonality of this research will help to shed a light on the condition of the animals foraging on different prey and how it affects their offspring survival. Which, in a long term, will contribute to the preservation of this vulnerable species.

SHORT DESCRIPTION OF THE RESEARCH PROJECT

1) Scientific goal of the project

(description of the problem to be solved, research questions and hypotheses)
Global warming is affecting wildlife throughout the whole globe. The rate of change is significantly faster than the rate of evolution and adaptation towards new environments. Some of the animals are especially vulnerable for those changes. One of the most drastic examples concerns polar bears (*Ursus maritimus*), which diet has been changing due to sea ice melting. The diet of this species is mostly carnivorous, consisting primarily of ringed seals and less frequently on bearded seals (Gormezano & Rockwell 2013a). Currently, they are spending more time on land because of early ice melts and later freezes in the Arctic. This limits the amount of prey (ringed seal pups) they can hunt on ice over winter, which is their main source of annual fat reserve. Surviving these extended periods on land without access to seals is believed to be critical to the persistence of polar bears in western Hudson Bay (Molnár et al. 2010). A previous study has investigated berries as an alternative option for polar bears on land using stable carbon isotopes (Hobson et al.2009). The authors found that bears fed on berries received an insignificant amount of energy and did not compensate for the insufficient fat reserve. Other researchers more recently found shifts in polar bear diet with an increase in proportion of animal contents while plant composition did not change. We will investigate two of the main animal sources utilized by polar bears on land, caribous and snow geese. Number of caribou has increased by ca.50-fold since the 1960s and the snow goose population has also increased by ca.20-fold (Gormezano & Rockwell 2013a).

Traditional models used to predict predatory behaviour were based on balanced and optimized energy intake. On the contrary, polar bears are starting to exhibit an energy inefficient foraging style in the midst of the climate crisis. Whether the alternative diet the polar bears are adapting to can actually cover their energy expenditure on land over the long summer season remains unclear. A study showed that based on carbon isotopic models, diets based entirely on terrestrial food such as berries would not compensate for the loss of fat reserve overwinter (Iles et al. 2013). However, this has not been tested experimentally to compare experimental groups of fasting bears exposed to terrestrial diet and the fasting control group. Another study estimated that based on oxygen consumption measures, a polar bear would have to catch a snow goose in under 12 seconds to have a net gain in energy (Lunn and Stirling. 1985). Further analyses on the energetic values provided by various alternative food sources will give better understanding of the change in foraging efficiency. Currently, there are no official supplemental feeding efforts for polar bears due to logistic and legislative difficulties. Maintaining a long-term supplemental feeding program is time consuming and comes at a great cost. This project will be able to provide greater knowledge on the potential implementation of the supplementation programs for polar bears.

Within the present project we intend to fill in the existing knowledge gap by investigating the ongoing process of dietary adaptation in an environment affected by climate change. First we will quantify and qualify the content within the alternative diets of the polar bears in the western Hudson Bay population through the use of stable carbon isotopes and fatty acid signatures. Secondly, we assess the energetic values of the alternative food sources experimentally by artificially manipulating the diets. Finally, we will determine whether some of the resources within their alternative diets can be increased or safely introduced to their natural habitat on land. The main focus is to facilitate the conservation efforts of the unique polar bear species. The following **hypotheses** will be tested:

1. Individuals that exhibit greater change in their foraging behaviour have greater survival rate and fitness than those unable to adapt.
2. The alternative diet provides enough energetic value for the polar bears to survive until the next ice freeze.
3. Individuals that exhibit changes in foraging behaviour to pursue alternative prey will receive net energy gain.
4. Experimental groups with supplemented diets had greater survival rate and fitness than the control fasting group.

2) Significance of the project

(state of the art, justification for tackling a specific scientific problem, justification for the pioneering nature of the project, the impact of the project results on the development of the research field and scientific discipline)

Individual variation in habitat selection can influence survival and population dynamics. At the speed of current habitat loss for polar bears in the Arctics, it is crucial to evaluate the alternative diets in hopes of applying the knowledge to the conservation efforts. Prior to global warming, male polar bears relied predominantly on their fat reserve for 4 to 5 months until the next sea ice freeze while pregnant females could spend up to 8 months fasting in their maternity den (Ramsay and Stirling. 1988). Recent increase of temperature has greatly impacted the sea ice dynamics to which the polar bears are experiencing greater nutritional stress resulting from earlier ice breakups (Regehr et al. 2007). This indicates that the bears are required to rely on their stored fat for a longer period of time in summer, which is forcing them to adjust their foraging and fasting behaviours. Previous research has found that the overall proportion of animals in the diet has increased while the proportion of vegetation has not. So far, studies have been limited to observational, population-monitoring, and fecal composition analyses. Lack of empirical research on the effects of change in dietary composition on polar bear survivability and fitness poses concerns to further implementation of conservation programs. This project will experimentally assess the energetic values to the polar bears from recently observed, non-plant resources such as caribou, snow geese. By manipulating the dietary supplementation, we will have precise measures on how much energy supply the bears gain from these alternative resources. Outcome of this study will have broader implications toward conservation efforts, where suitable resources could be increased or supplemented within their known habitats.

This change in foraging behaviours of polar bears contradicts with the predictions made by energy-optimizing foraging models (Iles et al. 2013). Understanding the trade-offs between the energy gain and the energy inefficient pursuits of prey can give further insights into the nutritional needs and the survivability of polar bears on land. The novelty of the project lies in the experimental approach as well as the large scale monitoring and video recordings of the individual foraging behaviours. Other studies have suggested that cub survival in the first year is closely related to the maternal body condition. Survival of the cubs beyond 8 months is predominantly affected by the current year environmental condition (Regehr et al. 2007). In order to ultimately support the conservation of the polar bear population, we will pay close attention to the extent of which the alternative dietary composition can be beneficial. Additionally, fitting camera collars on every individual in all the subpopulations in the Hudson Bay region will produce unprecedented detailed results compared to traditional census and monitoring methods. This knowledge on the adaptation of foraging patterns will not only apply to polar bears but can have broader application towards other endangered species that are threatened by the loss of habitats and food sources. With the amount of data we will collect during this project, we expect numerous impactful publications which will have practical contributions when engaging the public and applying the results to government programs.

3) Concept and work plan

(general work plan, specific research goals, results of preliminary research, risk analysis)

Based on previous studies performed by McCall et al. 2016 we have the data about the spatial distribution of 18 subpopulations of polar bear in this area. We would like to collaborate with this group in terms of animal capturing and tracking. Regular tracking and monitoring will be performed on all the populations present in the region. Based on those recordings we will gain the knowledge of their natural diet and it will help us with calculations of movement rate or hunting efficiency needed for further estimations of energy balance. Also, at population level survival rate will be estimated. Four experimental groups differing in diet manipulation will be established and 25 individuals per group will be chosen (10 adult males and 15 adult females), making sure that additionally in each group at least 5 cubs are present in order to monitor their survival. Those 100 (25x4) individuals will be tracked using collars, equipped with GPS and cameras. This will enable us to document their foraging behaviour on land and track their movements to estimate their activity and places of food searching. Additionally the samples of blood will be collected to estimate the fatty acid and hormonal composition and level. Samples of feces will also be collected in order to evaluate the compositions of the diet. The survival rate and physical condition of the population, will be estimated at the start and the end of the summer, for three seasons. Physical condition evaluation will be based on changes in weight of the animals and changes in composition of nutrients found in the blood samples. Additionally, we will gather data about the sex of individuals their reproductive status and litter size. The same individuals will be traced throughout the three experimental seasons, with the exception of dead animals, including cubes. We will make sure that in every season the number of mothers with cubes will not be lower than 5 (per group).

Possible risk includes danger of camera/gps tracker destruction, but rate of such failures was not high in previous studies (McCall et al. 2016).

4) Research methodology
(underlying scientific methodology, methods, techniques and research tools, methods of results analysis, equipment and devices to be used in research)
Study area: Populations at the coast of Hudson Bay in Manitoba, Canada, between La Pe´rouse Bay (58430N, 93240W) and Cape Churchill (58460N, 93140W).

Capture and handling: Polar bears will be captured and released on the sea ice during spring (mid-May to end-June) and during autumn (September to November). Polar bears will be located using their GPS tracker on their collar which was placed there by the McCall team. Bears spotted from a helicopter will be immobilised with a rapid-injection dart (Palmer Cap-Chur Equipment, Douglasville, GA) containing zolazepam–tiletamine (Telazol® or Zoletil®) (Rode 2014).

Experimental group assignment: We will use the previously gathered data and combine it with RSF (resource selection functions) models to evaluate habitat selection and predict the relative probability of habitat use (Manly et al. 2002). Based on those predictions, we will assign ~25 adult bears into each of the four groups.

1. (control) group - non eating or eating sporadically during the season
2. supplemented with caribou meat
3. supplemented with snow geese meat
4. supplemented with both meat types

Diet supplementation: By checking the routine paths of bear movement we will choose a spot in which we will be dropping the dietary supplements. In order to obtain the meat for the needs of this experiment we will contact local caribou farms and local hunters, which will provide us fresh caribou and snow geese meat.

Fecal collection and analysis: Scats will be collected by walking through the tracks of polar bears near coastal regions from the end of May to the first of August. Polar bears tracks will be identified by the location data from the trackers and we will walk through those tracts to search for the scats. Similarly, we will collect the scats from the habitats near den from the end of May to mid June. Scats will be kept frozen for further analysis.

GPS tracking data: Collars equipped with a global positioning system (GPS) receiver and transmitted data through a satellite link.

Hormone analysis: Blood samples will be collected from the femoral vein into evacuated, heparinized containers and stored cool and dark until centrifugation within 8 hr of collection. Obtained plasma will be stored at -20°C until analysis. Plasma concentrations of the steroid hormones P4 and E2 and the glucocorticoid cortisol will be analyzed using radioimmunoassay (RIA) (Haave et al. 2003).
Body condition measurements: We will adopt a noninvasive method that uses photographs to measure the body condition of wild brown bears (Shirane et al. 2020). The authors have validated the accuracy against real measurements of the captured bears and suggested wider application for monitoring large size free-ranging animals. Body mass (kg) and straight-line body length (cm) will be measured upon capture. Torso height (cm) will be measured as the distance from the lowest point of the abdomen to the spine. Age will be estimated by counting

the cementum annuli of the teeth (Medill et al. 2009). We will calculate body condition index (BCI) as described by Cattet et al. 2002. Lateral photographs of each individual captured will be taken. We will use ImageJ software to extract morphometric measurements from the lateral photographs of polar bears as described in Shirane et al.2020. Differences in body condition among age and sex classes will be taken into account when analyzing the measurements. In addition, we will assess the effects of experimental supplementation on BCI. Finally abdominal temperature will also be measured during capture as an indicator of fasting behaviour. These procedures will be repeated for 3 years at the beginning and at the end of the season.

Statistical analyses: Analyses will be carried out in R with packages of lme4 and emmeans. Effects of experimental groups on the response variables including BCI, body temperature (°C, lower temperature represents energy expenditure typical of fasting), female hormone concentration will be analyzed with linear mixed-effect models (LMEM) and generalized mixed-effect models (GLMM). The models will include fixed factors of experimental groups, sex, distance covered (m, collected at the end of the season), movement rate (m/s), percent time active. Recordings from each of the bears will be scored to determine the foraging behaviour and the dietary sources. Individual pursuit of animal prey will be used to estimate the energy spent by the bear and the net energy gain from the hunt. Adult and cub overwinter survival rate and the reproductive success of the populations will be documented over 3 years. Finally, we will determine whether the dietary supplementation was beneficial in improving the population growth.

5) Project literature

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6. Table with budget of the project.

	Amount in PLN
Direct costs, including:	**2 473 000**
• personnel costs and scholarships	504 000
• research equipment/device/software costs	475 000
• other direct costs	1 494 000
Indirect costs, including:	**544 060**
• indirect cost of OA	49 460
• other indirect costs	494 600
Total costs	**3 017 060**

7. Breakdown of project costs including justification and relevance for the tasks in the project)

Direct costs include:

Remuneration for the research team

- the principal investigator: 5 000 zł per month x 36 months = 180 000 zł - be actively involved in all aspects of the project, in particular: managing the team (including supervising the PhD student involved in the project), planning and supervising research, trouble-shooting, analysing data, writing manuscripts and promoting results.
- technician - 4 000 zł per month x 36 months = 144 000 zł - will be involved in all laboratory aspects of the project, especially sample handling. responsible for the proper flow, as well as all kinds of analysis. This lab manager will also be responsible for the continuous supply of consumables and reagents required for the lab work. The Technician will be employed throughout the project
- the PhD student: 3 000 zł per month x 36 months = 108 000 zł - will help the technician but also will participate in article writing (at least two international publications are expected)
- part time employees (3): 4 000 zł per month x 6 (2 month x 3 seasons) x 3 people = 72 000 zł - will be hired form Canada, to help us with the feeding of animals

Equipments

- Collars with GPS tracking loggers fitted with minimal size cameras: 500 zł x 800 bears = 400000 zł. This will include large size bear collars with GPS transmitter and receiver. Additionally, minimal size camera recorders will be customized to be fitted inside the loggers.

- Blood sample collection kits and equipment for RIA analyses for 600 samples: 6 000 zł
- Cameras for body condition photographs: 2000 zł x 4 = 8000 zł
- Small laboratory equipment including needles, capillary tubes, pipette tips: 1 000 zł per year x 36 months = 36 000 zł
- rapid-injection darts: 10 zł x (100 bears x 6 (2 times per 3 seasons) + 700 additional bears at the population level) = 13 000 zł
- Computers with software needed for data analysis: 12 000 zł

Other costs:

Food for bears

- 7 zł/kg of caribou meat x 37.5 individuals (25 + 12.5 of mixed treatment) x 240 kg of meat in one season (per individual) x 3 seasons = 189 000 zł
- 25 zł/kg of snow geese x 37.5 (25 + 12.5 of mixed treatment) individuals x 240 kg of meat in one season (per individual) x 3 seasons = 675 000 zł

Estimated by calculating 30% of caloric intake.

Helicopter rental

180 000 zł per season x 3 = 540 000 zł

The helicopter with trained pilot/pilots will be needed throughout the whole experimental part performed in Canada, especially for spotting individuals needed for sample collecting and food dropping

Business trips, visits and consultations: 90 000 zł - for team members trips to Canada twice a year (plus stay expenses - including diet) ~70 000 zł + 10 000 zł for consultations with collaboratory team + conferences ~10 000 zł

Reviews

Wiesław Babik

1. **Assessment of scientific quality of the research project** (scientific relevance, importance, originality and novelty of research or tasks to be performed; quality ought to be evaluated in an international context)

   This is potentially a high-quality project: it is timely, has an appropriate scale, combines observational and semi-experimental approach in a novel way. There are however several issues that affect its overall quality. First, although the overall goals are stated explicitly, the purpose of the project is not entirely clear to me. Is the project intended to help in practical management, i.e., does it try to assess whether food supplementation works, or is it more basic science, trying to answer, through partially controlled experiments, whether the suggested changes in polar bear behaviour in the terrestrial phase are sufficient to prevent starving the bears to death? I think these doubts are related to the way the project is presented, and how the title is formulated. In particular background information, scattered in sections on goals and significance, is not sufficient – for example, there's no information on how bears utilise the two resources (caribou and geese) in nature. As a more concrete example, the info in l. 37-40 is a good start, but the thread breaks abruptly before presenting a valid hypothesis based on this information
2. **Assessment of potential impact of the research project** (the potential for substantial international impact on the research field(s) and for high quality research publications and other research outputs, taking into account the specifics of the research field and the variety of forms of impact and output; impact ought to be evaluated using an international context)

   The potential impact of the project, if completed successfully, is high. The project has the potential to provide important information at the interface of basic and applied conservation science, the results may be published in major journals and inform practical conservation. As the description of state of the art is focused on the polar bear, there is insufficient information to judge its broader significance.
3. **Assessment of feasibility of the research project** (the feasibility of the proposed project, including the appropriateness of the research methodology to achieve the goals of the project, the risk management description, research facilities and equipment, international cooperation (if any), other factors affecting the feasibility of the project)

   Overall, the project is feasible and the proposed methodology is appropriate. However, risk analysis is minimal and restricted to simple technical issues. In my opinion, this is a high-risk project because of potential problems with logistics, collaboration and the overall unpredictability of working with large animals in difficult conditions in remote areas.
4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

   The costs are well explained and justified, although overall they are high. In particular, the cost of meat is high, especially in the perspective of using the proposed approach as a management strategy.

5. **Strengths of the proposal**
An interesting and timely topic. Charismatic model. Large scale of the project. Combination of observational and experimental approaches

6. **Weaknesses of the proposal**
The summary does not present explicitly formulated research hypotheses. Goals mixed with state of the art. Not sure that all specific hypotheses can be tested with the proposed approach. Terms, such as "adaptation" are used throughout the text in various, sometimes incompatible contexts . An overall imbalance in project description – procedures are described in detail, while significance, a broader context and risk analysis are a bit superficial

<u>Agnieszka Kurdziel</u>

1. **Assessment of scientific quality of the research project** (scientific relevance, importance, originality and novelty of research or tasks to be performed; quality ought to be evaluated in an international context)

I really like the idea of helping polar bears, especially in the face of current climate change. I appreciate author's willingness to learn more about their eating habits and to see how changes in nutrition can affect the condition of the animals. However, my concerns are what will happen after the end of the project and how the animals will cope later. I have not been able to find out what part of the normal diet will be the additional meat, so it is difficult for me to assess the level of interference with the eating habits in the project. I also assume that there will be a continuation of the project, including at least further animal observation. The issue of the project is very interesting and consistently written. Polar bears are currently in a very difficult situation, there is even information about the possibility of their extinction by 2100. In order to prevent this from happening, it is necessary to take action. The problem of periodic starvation among polar bears is very serious and also dangerous for humans. Research to answer the question of how changes in nutrition affect animals is very important. I also think the project highlights the importance of acting to reverse climate change. Due to the selected species, it is very media-friendly. It can contribute to raising public awareness and creating a more positive image of Poland as a country which, despite its energy policy based on fossil fuels, pays attention to the global climate problem.

2. **Assessment of potential impact of the research project** (the potential for substantial international impact on the research field(s) and for high quality research publications and other research outputs, taking into account the specifics of the research field and the variety of forms of impact and output; impact ought to be evaluated using an international context)

For the reasons mentioned above like species, global, media, research results have a chance to find their way to leading scientific journals. Also, the possibility of obtaining data is very large and sufficient to prepare several publications. The project is very developmental towards continuing research and even introducing specific actions in the most endangered polar bear populations that may result in further results and research.

3. **Assessment of feasibility of the research project** (the feasibility of the proposed project, including the appropriateness of the research methodology to achieve the goals of the project, the risk management description, research facilities and equipment, international cooperation (if any), other factors affecting the feasibility of the project)

I am missing a lot of information here, so I will focus more on questions than on evaluation.

Notes: I had a problem with finding the research area on the map and determining its size, I even asked other people to check it and the effect was similar, so please be clear about the information.

Questions: **1 Experiment**) How much, in what form, how often, how, when and in how many places should the meat be placed? The costs include fairly accurate amounts of meat, but there is very little in the materials and methods, and this is the main part of the experiment. **2 Observation**) How it will be estimated how many bears ate, I found information about only 4 cameras, I remind you that the camera needs electricity, so I do not count those on collars (such cameras can be used only for short observations). I suggest photo-traps in places where food is left. How will the bears be weighed? **3 Logistics**) These 4 cameras also need electricity, will there be a base near the place where the bears occur, there is very little information in the project about the organization, where the laboratory will be - I did not find anything about the cost of transporting the samples, will all this be done by a helicopter? Where will it get fuel from, is it included in the rental costs? **4 Job**) There is information about the employment of several people, which is understandable in this type of project, but I still do not know exactly what the participation of the authors themselves will be. I assume the temporary staff will help with the sampling, but isn't there a need for a vet? Will he handle it the principal investigator?

In conclusion: The research sample and the information to be collected are described very well, similar the statistical analysis, but there is a lack of a large amount of organizational and technical information, so it is difficult for me to assess the feasibility of this project. I assume that more information will be included in the detailed description, but it would be good to mention at least a part, e.g. whether the material will be analyzed in Canada or Poland.

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

As I wrote in the previous section, there is a lack of a lot of organizational information, which also makes it difficult to assess the entire cost estimate.

Collars with GPS…-why 800 bears? did I miss something?, rapid-injection darts: 10 zł (I suspect only for darts, I found information that putting a 1 bison to sleep with such a met hod costs about 200$) … tips: 1 000 zł per <u>year</u> x 36 <u>months</u> = 36 000 zł

I am afraid there is much room for improvement in the cost estimate.

5. **Strengths of the proposal**

Topics, innovation.

An interesting research problem.

Mediality and a chance for good publications.

Developmental nature of research.

Well-described research groups, information gathering and analysis.

6. **Weaknesses of the proposal**

Description of the organization and research facilities , cost estimate and costs

<u>Filip Turza</u>

1. **Assessment of scientific quality of the research project**

The scientific goal of the grant proposal is to check whether the alternative diet that the polar bears are shifting to can lead to the recovery of polar bear populations. The presented project is understandable and the structure of the text is logical. The choice of model organism as polar bear is justified. The project proposes a sensible hypotheses: that (1) individuals that exhibit greater change in their foraging behaviour have greater survival rate and fitness than those unable to adapt, (2) the alternative diet provides enough energetic value for the polar bears to survive until the next ice freeze, (3) individuals that exhibit changes in foraging behaviour to pursue alternative prey will receive net energy gain and that (4) experimental groups with supplemented diets had greater survival rate and fitness than the control fasting group. The hypotheses proposed are interesting and worth investigating. The authors describe correct scientific methodology. I highly appreciate the scientific quality of the research project.

2. **Assessment of potential impact of the research project**

Significance of the project is well justified. It is incredibly interesting and addresses important aspects of polar bears as victims of the effects of environmental disruptions driven by climate change. It addresses an important issue of species conservation in times of the effects of global warming that we have to deal with. I fully agree that it is crucial to evaluate the alternative diets in hopes of applying the knowledge to the conservation efforts. The knowledge gap is well defined and innovative potential of the project is emphasized by the authors and supported by appropriate argumentation. If the hypotheses are properly tested, the results are likely to be published in high impact factor journals. Proposed study have profound potential to be a pioneering project in broadening knowledge about other endangered species that are threatened by the loss of habitats and food sources. The potential impact of a research project is very high.

3. **Assessment of feasibility of the research project**

The problem to study is very ambitious and feasible. It addresses important aspects of threatened polar bear species. Undoubtedly, it is a promising field of study which require considerable commitment. Risk analysis in the project has been done. Indeed, authors pointed out the possible problem, however, I have add two more doubts. First of all, one about the stress caused by the use of helicopters and bear catching, which can may affect their food preferences as well as increase their mortality. Secondly, I am not sure if the authors took into account how they will obtain consent for research on an endangered species such as polar bears. Probably such consent is difficult to obtain. Regarding other aspects of assessment of feasibility I believe that scheme of subsequent tasks and division of tasks for each year (e.g. in the work plan section) could make the project more clear. Thanks to them, it would be easier to assess the feasibility of the project and at a later stage to verify the progress of the project.

4. Are the costs to be incurred well justified with regards to the subject and scope of the research?

The costs to be incurred are well justified with regards to the subject and scope of the research. However, I have two notes on funding. First of all, the authors did not take into account the specific number of computers (this is a detail, but with the main equipment it is important for the financing institution). Secondly, I suggest that small laboratory equipment including needles, capillary tubes, pipette tips and rapid-injection darts should be included in other costs as materials.

4. **Strengths of the proposal**

The presented project is written well. The problem to study is very ambitious. It focuses on interesting topic of great scientific importance. Additionally, proposal has applicable character and results can help in nature con servation. Novelty of the idea makes it a strong grant proposal. Hypotheses and their predictions are properly formulated. Budget is well planned. The strongest point of this project is that knowledge on the adaptation of foraging patterns of polar bears can help in the planning research about other threatened species. The results of the project can have broader application towards other endangered species that are threatened by the loss of habitats and food sources.

5. **Weaknesses of the proposal**

The weakness of the application is the lack of bulleted the most important research tasks. Additionaly, the literature section needs some minor corrections. Sometimes the authors put a doi link, sometimes they don't put it but I think it is not necessarily in the application form. The same goes for journal names and article titles (italics or not italics). Moreover, the source: "Gormezano, LJ, & Rockwell, RF (2013) b. Dietary composition and spatial patterns of polar bear foraging on land in western Hudson Bay. BMC ecology, 13 (1), 1-14" is not included in the project description. One of the most serious things which I consider to be a problematic is obtaining approval for research, and if successful another problem is inducing stress in animals, which I mentioned in the section "Assessment of feasibility of the research project".

Maëlle Lefeuvre

1. **Assessment of scientific quality of the research project** (scientific relevance, importance, originality and novelty of research or tasks to be performed; quality ought to be evaluated in an international context)

As mentioned and well-illustrated in the project proposal, the situation of polar bears is critical. In addition to the reduction of their habitat, their predominant prey is available during a shorter time and it induces lack of fat reserves for winter. It is easy to imagine which detrimental effects it can have on polar bears survival and the future of this species. It is worth mentioning also that the human activities on the entire planet are the source of the disappearance of the bears' habitat. Thus, the relevance of this study project is evident, and its outcomes are obviously needed at the international level.

Polar bears are already in the sights of ecologists, but this project adds an ambitious dimension to the study of one of the largest terrestrial predators, by the number of studied individuals, the implied equipment and the amount of data expected. The results would promise to bring reliable evidence on diet adaptation, predictions for the future of polar bears and support for conservation actions.

2. **Assessment of potential impact of the research project** (the potential for substantial international impact on the research field(s) and for high quality research publications and other research outputs, taking into account the specifics of the research field and the variety of forms of impact and output; impact ought to be evaluated using an international context)

The first thing that stands out from this project is the amount of data which would be generated: survival and reproduction, movement rate, diet, foraging behaviour and success, blood and feces composition, physical condition, measured for a hundred of bears. All together, these data should draw a consequent picture of the current situation from different perspectives. In addition, one has to notice the substantial sample size of this project compared to other studies on large mammals, even more in a solitary species such as polar bears.

I am convinced that this project would generate high quality results, supported by the features mentioned above and modern and high-tech techniques and equipment. Publications would bring new support for conservation programs and awareness of the climate change consequences on northern fauna.

3. **Assessment of feasibility of the research project** (the feasibility of the proposed project, including the appropriateness of the research methodology to achieve the goals of the project, the risk management description, research facilities and equipment, international cooperation (if any), other factors affecting the feasibility of the project)

As mentioned above, this study is ambitious, and the workload would probably be very important, on account for the tremendous amount of samples and data from an impressive number of individuals. Moreover, according to the proposal, only three people would be fully involved in this huge project. They would be dealing with capture, samplings, preparation and analysis of the organic samples, body condition measurements, video tracking viewing, analysis of movements of bears, for a hundred of individuals and within a year only, because those tasks have to be repeated every year. In addition, 700 additional bears have to be found and equipped with collars, and video tracks have to be watched and analysed, which represent a substantial amount of time. It is also mentioned in the scientific goal that diet content will be quantify and qualify, and that food introduction feasibility will be studied, but it is not included in the research plan. These additional parts will take time and probably money to be achieved.
Nevertheless, the sampling periods seem wisely organized to me, spread over the warm season, and avoiding sampling overloading.
My second concern is about the legislative difficulties that were mentioned at the line 53. If I understood well, some legislative barriers unable the establishment of regular food supplementation for polar bears. Unfamiliar with these laws and obstacles, I wonder to what extent this would affect this project and the possibility for meat distribution.

Research in the field is prone to external risks due to the various parameters that we cannot control. I can think about two potential risks for this study, which may be considered. First, I imagine that polar bears are not the unique predator in North Canada. This means that meat provided for bears might attract other carnivorous species who would reduce the amount of food available for the focal species. In addition, other polar bears living in the food dropping area could consume this meat. The second risk concerns the situation after the end of the study. Bears will be habituated for three years to come eating on carcasses at particular places, maybe even at regular time periods. After the end of the project, as supplementation is not organized by Canadian authorities, food will probably suddenly stop to be provided. Depending on the proportion of the diet covered by this meat, the first year without supplementation might be more or less difficult for polar bears. The consideration of this issue is not included in this proposal.

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

All the listed costs are directly related to this project, with all the necessary equipment to conduct this study. Due to the amount of data which are planned to be collected, including videos, costs of online or external memory could be added. I would finally advice the authors to revise their budget to spot and correct mistakes. For instance, the costs of laboratory material should be reduced, as 1 000 PLN per year * 36 months (3 years) is not equal to 36 000 PLN.

5. **Strengths of the proposal**

This topic is of great international interest, and the results would bring a lot of information about polar bears' current diet adaptation and the situation of the species. This project involves a consequent number of measures covering different aspects (biological, behavioral, ecological) of the foraging strategy and efficiency. Hence, it would draw a broad overview of the topic which should emphasize the crucial issue of sea ice melting on survival of polar bears.

6. **Weaknesses of the proposal**

It is a very complete project but all the measures and analyses would probably need more than three years to be completed. Maybe it is worth considering reducing the number of measurements, hiring more people, or splitting this project into two projects.

Proposed corrections:

Line 26: vulnerable <u>to</u> those changes

Lines 35-36: I would start the sentence by ‘’More recently’’
Line 38: The number of caribous
Lines 47-49: This sentence is quite long just to say that no experiment was conducted.
Line 31: we will assess, and this sentence is quite hard to follow.
Line 72: I would not use the past tense.
Line 79: in the Arctic
Lines 90-92: This sentence is not clear.
Lines 125-126: Additionally, blood samples will be collected
Line 131: a coma is missing, this is a list of things
Lines 132-133: including cubs. / mothers with cubs
Line 136: GPS trackers
Line 162: will provide us with meat
Line 171: and transmitting data
Budget regarding food for bears: would be easier for the reader to write the formulas with exactly the same pattern

Final project proposal

Title: Title: Feasibility of the alternative diets exhibited by polar bears aiming for conservation of this species

Applicants: ChuChu Lu, Joanna **Palka**

Summary:
Environmental disruptions driven by climate change are a major contributing factor to the loss of biodiversity. Constantly shrinking ice cape in the Arctic is causing a decline in the population of polar bears at an unprecedented scale. Majority of animals, especially large vertebrates, are not able to adapt to such quick and drastic changes. The goal of this project is to check whether the alternative diet that the polar bears are shifting to can lead to the recovery of polar bear populations. To do this we will perform observation on both populations and perform experiments with altered diets on smaller animal groups. Thanks to that we will be able to estimate the impact of change of foraging behaviour on reproductive success and survival rate at population level. Additionally, experiments in which we will supply animals with alternative diets will answer the question, if such a diet could potentially replace previous dietary strategies. Condition of animals will be measured before and after the diet alteration throughout the three-year time period. Multi-seasonality of this research will help to shed a light on the condition of the animals foraging on different prey and how it affects their offspring survival. Which, in a long term, will contribute to the preservation of this vulnerable species.

SHORT DESCRIPTION OF THE RESEARCH PROJECT
1) Scientific goal of the project
(description of the problem to be solved, research questions and hypotheses)
Global warming is affecting wildlife throughout the whole globe. The rate of change is significantly faster than the rate of evolution and adaptation towards new environments. Some of the animals are especially vulnerable for those changes. One of the most drastic examples concerns polar bears (*Ursus maritimus*), which diet has been changing due to sea ice melting. The diet of this species is mostly carnivorous, consisting primarily of ringed seals and less frequently on bearded seals (Gormezano & Rockwell 2013). Currently, they are spending more time on land because of early ice melts and later freezes in the Arctic. This limits the amount of prey (ringed seal pups) they can hunt on ice over winter, which is their main source of annual fat reserve. Surviving these extended periods on land without access to seals is believed to be critical to the persistence of polar bears in western Hudson Bay (Molnár et al. 2010). A previous study has investigated berries as an alternative option for polar bears on land using stable carbon isotopes (Hobson et al.2009). The authors found that bears fed on berries received an insignificant amount of energy and did not compensate for the insufficient fat reserve. More recently, other researchers found shifts in polar bear diet with an increase in proportion of animal contents while plant composition did not change. We will investigate two of the main animal sources utilized by polar bears on land, caribous and snow geese. Number of caribou has increased by ca.50-fold since the 1960s and the snow goose population has also increased by ca.20-fold (Gormezano & Rockwell 2013).

Traditional models used to predict predatory behaviour were based on balanced and optimized energy intake. On the contrary, polar bears are starting to exhibit an energy inefficient foraging style in the midst of the climate crisis. Whether the alternative diet the polar bears are adapting to can actually cover their energy expenditure on land over the long summer season remains unclear. A study showed that based on carbon isotopic models, diets based entirely on terrestrial food such as berries would not compensate for the loss of fat reserve overwinter (Iles et al. 2013). However, this has not been tested experimentally to compare experimental groups of fasting bears exposed to terrestrial diet and the fasting control group. Another study estimated that based on oxygen consumption measures, a polar bear would have to catch a snow goose in under 12 seconds to have a net gain in energy (Lunn and Stirling. 1985). Further analyses on the energetic values provided by various alternative food sources will give better understanding of the change in foraging efficiency. Currently, there are no official supplemental feeding efforts for polar bears due to logistic and legislative difficulties. Maintaining a long-term supplemental feeding program is time consuming and comes at a great cost. This project will be able to provide greater knowledge on the potential implementation of the supplementation programs for polar bears.

Within the present project we intend to fill in the existing knowledge gap by investigating the ongoing process of dietary adaptation in an environment affected by climate change. First we will quantify and qualify the content within the alternative diets of the polar bears in the western Hudson Bay population through the use of stable carbon isotopes and fatty acid signatures. Secondly, we assess the energetic values of the alternative food sources experimentally by artificially manipulating the diets. Finally, we will determine whether some of the resources within their alternative diets can be increased or safely introduced to their natural habitat on land. The main focus is to facilitate the conservation efforts of the unique polar bear species. The following **hypotheses** will be tested:

1. Individuals that exhibit greater change in their foraging behaviour have greater survival rate and reproductive success than those unable to adapt.
2. The alternative diet provides enough energetic value for the polar bears to survive until the next ice freeze.
3. Individuals that exhibit changes in foraging behaviour to pursue alternative prey will receive net energy gain.
4. Experimental groups with supplemented diets will have greater survival rate and fitness than the control fasting group.

2) Significance of the project

(state of the art, justification for tackling a specific scientific problem, justification for the pioneering nature of the project, the impact of the project results on the development of the research field and scientific discipline)

Individual variation in habitat selection can influence survival and population dynamics. At the speed of current habitat loss for polar bears in the Arctic, it is crucial to evaluate the alternative diets in hopes of applying the knowledge to the conservation efforts. Prior to global warming, male polar bears relied predominantly on their fat reserve for 4 to 5 months until the next sea ice freeze while pregnant females could spend up to 8 months fasting in their maternity den (Ramsay and Stirling. 1988). Recent increase of temperature has greatly impacted the sea ice dynamics to which the polar bears are experiencing greater nutritional stress resulting from earlier ice breakups (Regehr et al. 2007). This indicates that the bears are required to rely on their stored fat for a longer period of time in summer, which is forcing them to adjust their foraging and fasting behaviours. Previous research has found that the overall proportion of animals in the diet has increased while the proportion of vegetation has not. So far, studies have been limited to observational, population-monitoring, and fecal composition analyses. Lack of empirical research on the effects of change in dietary composition on polar bear survivability and fitness poses concerns to further implementation of conservation programs. This project will experimentally assess the energetic values to the polar bears from recently observed, non-plant resources such as caribou, snow geese. By manipulating the dietary supplementation, we will have precise measures on how much energy supply the bears gain from these alternative resources. Outcome of this study will have broader implications toward conservation efforts, where suitable resources could be increased or supplemented within their known habitats.

This change in foraging behaviours of polar bears contradicts with the predictions made by energy-optimizing foraging models (Iles et al. 2013). Understanding the trade-offs between the energy gain and the energy inefficient pursuits of prey can give further insights into the nutritional needs and the survivability of polar bears on land. The novelty of the project lies in the experimental approach as well as the large scale monitoring and video recordings of the individual foraging behaviours. Other studies have suggested that cub survival in the first year is closely related to the maternal body condition. Survival of the cubs beyond 8 months is predominantly affected by the current year environmental condition (Regehr et al. 2007). In order to ultimately support the conservation of the polar bear population, we will pay close attention to the extent of which the alternative dietary composition can be beneficial. Additionally, fitting camera collars on every individual in all the subpopulations in the Hudson Bay region will produce unprecedented detailed results compared to traditional census and monitoring methods. This knowledge on the adaptation of foraging patterns will not only apply to polar bears but can have broader application towards other endangered species that are threatened by the loss of habitats and food sources. With the amount of data we will collect during this project, we expect numerous impactful publications which will have practical contributions when engaging the public and applying the results to government programs.

3) Concept and work plan

(general work plan, specific research goals, results of preliminary research, risk analysis)
Based on previous studies performed by McCall et al. 2016 we have the data about the spatial distribution of 18 subpopulations of polar bears in this area. We would like to collaborate with this group in terms of animal capturing and tracking. Regular tracking and monitoring will be performed on all the populations present in the region. Based on those recordings we will gain the knowledge of their natural diet and it will help us with calculations of movement rate or hunting efficiency needed for further estimations of energy balance. Also, at population level survival rate and reproductive success will be estimated. Four experimental groups differing in diet manipulation will be established and 25 individuals per group will be chosen (10 adult males and 15 adult females), making sure that additionally in each group at least 5 cubs are present in order to monitor their survival. Those 100 (25x4) individuals will be tracked using collars, equipped with GPS and cameras. This will enable us to document their foraging behaviour on land and track their movements to estimate their activity and places of food searching. Additionally, blood samples will be collected to estimate the fatty acid and hormonal composition and level. Samples of feces will also be collected in order to evaluate the compositions of the diet. The survival rate and physical condition of the population, will be estimated at the start and the end of the summer, for three seasons. Physical condition evaluation will be based on changes in weight of the animals and changes in composition of nutrients found in the blood samples. Additionally, we will gather data about the sex of individuals, reproductive status, and litter size. The same individuals will be traced throughout the three experimental seasons, with the exception of dead animals, including cubs. We will make sure that in every season the number of mothers with cubes will not be lower than 5 (per group).

In order to avoid the focal polar bear groups being habituated to the food supplementation after the end of this project, we will keep track of those populations in collaborations with government sections, which take care of those lands. Also, with the effort of that study we hope to find an alternative diet, which later on may translate into increase of geese and/or caribou populations (used as a source of food in this study). Additionally, we want to advocate future research on other alternative sources to be introduced or supplemented until we find a stable source of energy for the polar bears during their terrestrial period.

Data collection in the field will be distributed among all members of the project including, 2 PIs, one PhD student, 1 Master student, technicians, and other part time employees at the facility. Additionally, an internship program will be established prior to the field season to acquire support for the large amount of data required. Most blood samples will be processed and analyzed on site at the Churchill Northern Studies Centre. Videos and GPS tracking data will be stored and brought back to Poland for further analyses.

Possible risk includes danger of camera/GPS trackers destruction, but rate of such failures was not high in previous studies (McCall et al. 2016). Potential difficulties concerning the legislative regulations on the manipulation of the dietary supplementation. Local laws and regulations will be assessed carefully prior to the experiments and based on previous research, the risk of affecting the experiment is low.

4) Research methodology

(underlying scientific methodology, methods, techniques and research tools, methods of results analysis, equipment and devices to be used in research)

Study area: Populations at the coast of Hudson Bay in Manitoba, Canada, areas within the study area will extend from the town of Churchill, Manitoba (58 ° 46 ′ N, 94 ° 12 ′ W), east to Cape Churchill (58 ° 47 ′ N, 93 ° 15 ′ W) and south to Rupert Creek (57 ° 50 ′ N, 92 ° 44 ′ W).

Capture and handling: Polar bears will be captured and released on the sea ice during spring (mid-May to end-June) and during autumn (September to November). Polar bears will be located using their GPS tracker on their collar which was placed there by the McCall team. Bears spotted from a helicopter will be immobilised with a rapid-injection dart (Palmer Cap-Chur Equipment, Douglasville, GA) containing zolazepam–tiletamine (Telazol® or Zoletil®) (Rode 2014).

Experimental group assignment: We will use the previously gathered data and combine it with RSF (resource selection functions) models to evaluate habitat selection and predict the relative probability of habitat use (Manly et al. 2002). Based on those predictions, we will assign ~25 adult bears into each of the four groups.

1. (control) group - non eating or eating sporadically during the season
2. supplemented with caribou meat
3. supplemented with snow geese meat
4. supplemented with both meat types

Diet supplementation: By checking the routine paths of bear movement we will choose a spot in which we will be dropping the dietary supplements. In order to obtain the meat for the needs of this experiment we will contact local caribou farms and local hunters, which will provide us with fresh caribou and snow geese meat. Placement of the food supplementation will be organized in accordance to the GPS tracking record of the group paths. Food will be dropped shortly prior to the polar bears arrive in nearby locations to avoid other carnivorous species reaching for the food before our groups. Camera traps set up around the food stations will be recording the real time consumption of the supplements.

Fecal collection and analysis: Scats will be collected by walking through the tracks of polar bears near coastal regions from the end of May to the first of August. Polar bears tracks will be identified by the location data from the trackers and we will walk through those tracts to search for the scats. Similarly, we will collect the scats from the habitats near den from the end of May to mid June. Scats will be kept frozen for further analysis.

GPS tracking data: Collars equipped with a global positioning system (GPS) receiver and transmitting data through a satellite link.

Hormone analysis: Blood samples will be collected from the femoral vein into evacuated, heparinized containers and stored cool and dark until centrifugation within 8 hr of collection. Obtained plasma will be stored at -20°C until analysis. Plasma concentrations of the steroid hormones P4 and E2 and the glucocorticoid cortisol will be analyzed using radioimmunoassay (RIA) (Haave et al. 2003).

Body condition measurements: Calibrated hanging scale similar to what Shirane et al 2020 used for brown bears will be used in this study. We will adopt a noninvasive method that uses photographs to measure the body condition of wild brown bears (Shirane et al. 2020). The authors have validated the accuracy against real measurements of the captured bears and suggested wider application for monitoring large size free-ranging animals. Body mass (kg) and straight-line body length (cm) will be measured upon capture. Torso height (cm) will be measured as the distance from the lowest point of the abdomen to the spine. Age will be estimated by counting the cementum annuli of the teeth (Medill et al. 2009). We will calculate body condition index (BCI) as described by Cattet et al. 2002. Lateral photographs of each individual captured will be taken. We will use ImageJ software to extract morphometric measurements from the lateral photographs of polar bears as described in Shirane et al.2020. Differences in body condition among age and sex classes will be taken into account when analyzing the measurements. In addition, we will assess the effects of experimental supplementation on BCI. Finally abdominal temperature will also be measured during capture as an indicator of fasting behaviour. These procedures will be repeated for 3 years at the beginning and at the end of the season.

Statistical analyses: Analyses will be carried out in R with packages of lme4 and emmeans. Effects of experimental groups on the response variables including BCI, body temperature (°C, lower temperature represents energy expenditure typical of fasting), female hormone concentration will be analyzed with linear mixed-effect models (LMEM) and generalized mixed-effect models (GLMM). The models will include fixed factors of experimental groups, sex, distance covered (m, collected at the end of the season), movement rate (m/s), percent time active. Recordings from each of the bears will be scored to determine the foraging behaviour and the dietary sources. Individual pursuit of animal prey will be used to estimate the energy spent by the bear and the net energy gain from the hunt. Adult and cub overwinter survival rate and the reproductive success of the populations will be documented over 3 years. Finally, we will determine whether the dietary supplementation was beneficial in improving the population growth.

5) Project literature

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6. Table with budget of the project.

	Amount in PLN
Direct costs, including:	2 440 500
• personnel costs and scholarships	504000
• research equipment/device/software costs	442500
• other direct costs	1 494 000
Indirect costs, including:	536910
• indirect cost of OA	48810
• other indirect costs	488100
Total costs	2 977 410

7. Breakdown of project costs including justification and relevance for the tasks in the project)

Direct costs include:

Remuneration for the research team

- the principal investigator: 5 000 zł per month x 36 months = 180 000 zł - be actively involved in all aspects of the project, in particular: managing the team (including supervising the PhD student involved in the project), planning and supervising research, trouble-shooting, analysing data, writing manuscripts and promoting results.
- technician - 4 000 zł per month x 36 months = 144 000 zł - will be involved in all laboratory aspects of the project, especially sample handling. responsible for the proper flow, as well as all kinds of analysis. This lab manager will also be responsible for the continuous supply of consumables and reagents required for the lab work. The Technician will be employed throughout the project
- the PhD student: 3 000 zł per month x 36 months = 108 000 zł - will help the technician but also will participate in article writing (at least two international publications are expected)
- part time employees (3): 4 000 zł per month x 6 (2 month x 3 seasons) x 3 people = 72 000 zł - will be hired form Canada, to help us with the feeding of animals

Equipments

- Collars with GPS tracking loggers fitted with minimal size cameras: 500 zł x 800 bears = 400000 zł. This will include large size bear collars with GPS transmitter and receiver. Additionally, minimal size camera recorders will be customized to be fitted inside the loggers.
- Blood sample collection kits and equipment for RIA analyses for 600 samples: 6 000 zł
- Cameras for body condition photographs: 2000 zł x 4 = 8000 zł
- Calibrated hanging spring scale for up to 500 kg: 250 zł x 2 = 500 zł
- Small laboratory equipment including needles, capillary tubes, pipette tips: 1 000 zł per year x 3 years = 3000 zł
- rapid-injection darts: 10 zł x (100 bears x 6 (2 times per 3 seasons) + 700 additional bears at the population level) = 13 000 zł
- Computers with software needed for data analysis: 12 000 zł

Other costs:

Food for bears

- 7 zł/kg of caribou meat x 37.5 individuals (25 + 12.5 of mixed treatment) x 240 kg of meat in one season (per individual) x 3 seasons = 189 000 zł
- 25 zł/kg of snow geese x 37.5 (25 + 12.5 of mixed treatment) individuals x 240 kg of meat in one season (per individual) x 3 seasons = 675 000 zł

Estimated by calculating 30% of calorie intake.

Helicopter rental

180 000 zł per season x 3 = 540 000 zł

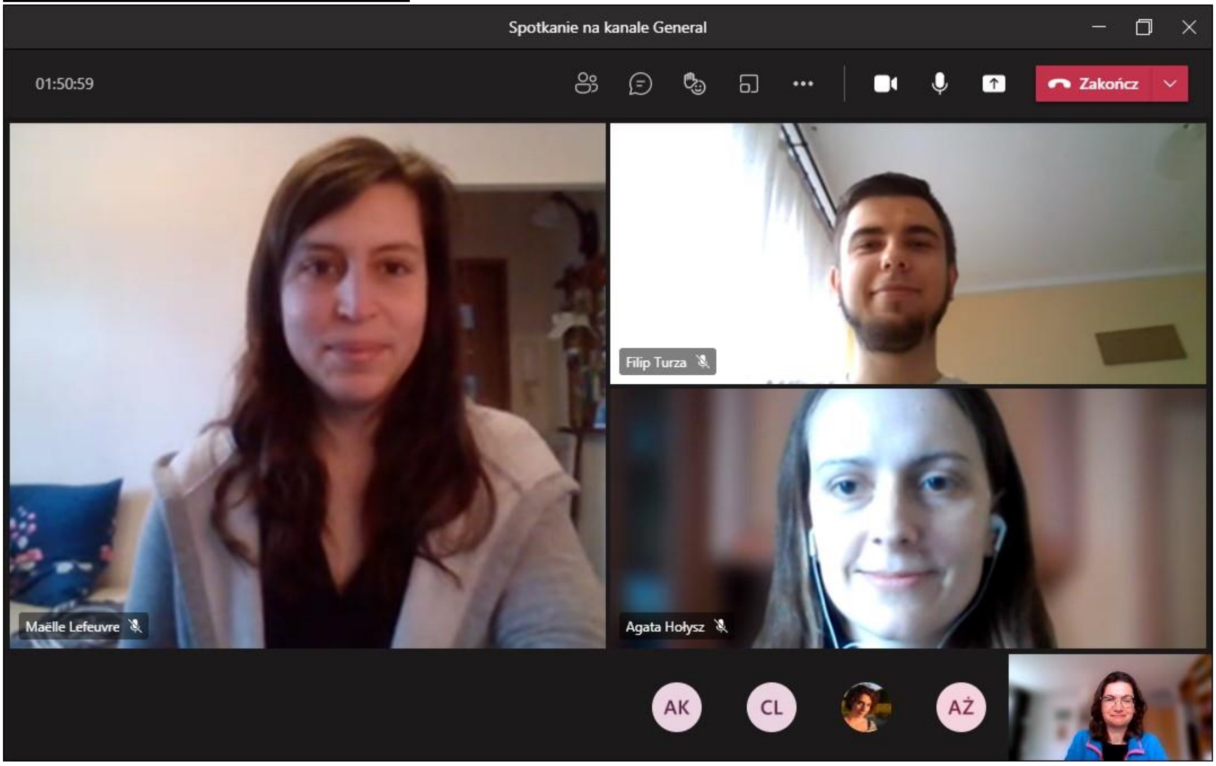
The helicopter with trained pilot/pilots will be needed throughout the whole experimental part performed in Canada, especially for spotting individuals needed for sample collecting and food dropping

Business trips, visits and consultations: 90 000 zł - for team members trips to Canada every year (plus stay expenses - including diet) ~70 000 zł + 10 000 zł for consultations with collaboratory team + conferences ~10 000 zł

Project 3: **Can ants replace bees as crop pollinators?**

Maëlle Lefeuvre, Filip Turza, Agata Burzawa

Photo of team members



Draft project proposal

Summary
In this project, we attempt to evaluate the role of ants as effective pollinators of crops in comparison to bee-pollination. Despite previous research, the potential of ants has not been studied in depth. Therefore, our main goal is to analyze the effectiveness of ant-pollination on different crop species. **We hypothesize that (1) ant-pollination will be at least as efficient as bee-pollination, and that (2) efficiency of ant-pollination will vary between different crop species according to their characteristics**. We will measure crop products parameters to assess the efficiency of pollination such as the number of seeds and fruits produced, their size and weight. The outcomes of this study will contribute to a better understanding of the role of ants as pollinators of crop species belonging to different families, and we hope that it will arouse interest of environmental biologists and encourage further research in this field.

SHORT DESCRIPTION OF THE RESEARCH PROJECT

1) Scientific goal of the project

Production of the majority of human food relies directly or indirectly on pollinators services, as pollinators support 9,5% of global food production (Garibaldi et al. 2013, Ollerton, Winfree and Tarrant 2011). Intensive agriculture (especially pesticide use), urbanization, habitat loss, fragmentation and climate change lead to insects extinction. A recent review estimates that 40% of insect species are dramatically declining, including bees (Sanchez-Bayo and Wyckuys 2019). Bees are commonly known as major pollinators, as they are responsible for 20% of pollination in human food production (Losey and Vaughan 2006). However, the number of species dropped significantly since last century, and agricultural intensification is still responsible for worrying reduction of bees populations (reviewed in Sanchez-Bayo and Wyckuys 2019).

A challenge for environmental biologists is to identify alternative pollinators. In this area of research, ants are reported as a potential alternative to bees. Indeed, ants use the nectar of plants as a food source that increases ant colonies size and their survivorship (Byk and Del-Claro 2011). For instance, fruit, seed sets and seed size of crop *Jatropha curcas* have been shown to be relatively similar between ant- and bee-pollinated flowers (Samra et. al 2014). On the other hand, the fruit set of the grass species *Euphorbia seguieriana* decreased by about two thirds compared to pollination by bees (Rostás et al. 2018). Thus, the ant-pollinators idea has supporters (Del-Claro et al. 2019, Delnevo et al. 2020) and sceptics (Beattie et al. 1985, Rostás ant Tauts 2010).

Nevertheless, ant pollination is a rarely studied phenomenon, limited to a few studies (Samra et. al 2014, Kuriakose et al. 2018, Rostás et al. 2018, Del-Claro et al. 2019). Knowledge about potential crops pollinated by ants is desperately needed, because so far, pollination by ants has been neglected due to insufficient evidence (Del-Claro 2019, Delnevo et al. 2020). In this project, we plan to observe the ant-pollination of plant species with a nutritional interest for humans. The comparison with bee-pollination will allow us to better understand the implication of ants in our food production and their potential as substitute pollinators in case of bees extinction.

We will base our work on two hypotheses: first, related to the ants-bees comparison, we expect that **crops will be pollinated at least with the same efficiency by ants than by bees**. Secondly, focusing on ant-pollination, we assume that **the characteristics of the different crop species will affect the efficiency of pollination**. Indeed, we expect more ants visits of short than high plant species, as well as scentful flowers more than scentless flowers.

2) Significance of the project

In the prospect of bees' extinction, pollination by human hand is investigated, but the tremendous time and money necessary for this task, and its complexity, are not very encouraging for our future. If we are not capable of replacing bees and the ecosystemic services they provide, other substitutes must be considered, such as other pollinator species.

Ants are among the most evolutionarily successful insects groups (Hölldobler and Wilson 1990). More than 16000 species of ants are known globally (Bolton 2020), they constitute a huge group of insects that have a great potential to replace the role of other pollinators. The mutualistic interactions of some of those species with plants are promising. In addition, ants have a better detoxification system and are able to survive in more unfavorable conditions (Schläppi et al. 2020).

The impact of previous studies about ant-pollination is limited for different reasons. First, they focus on one (Rostás et al. 2018, Samra et al. 2014) or two plant species only (Delnevo et al. 2020) which are not cultivated and consumed by humans. Then, these studies were conducted in the field, which do not ensure the control of all the parameters. The project we propose will start covering those gaps, with the use of four different crop species in a controlled environment. We hope that this study will bring pieces of solution regarding the bees' extinction issue, and will encourage other studies in the field of alternative pollination strategies.

3) Concept and work plan

In the presented project we selected a few crop species (Table 1). The criteria for selection were the possibility of growing in greenhouse conditions, ease of purchase, and high probability of visit by ants and bees (Hossain et al. 2018). We also selected potential bee species (Table 2) and ant species (Table 3) for the study. The criteria for selection were the possibility of survival in greenhouse conditions and ease of purchase, commonness of the species and type of habitat in which these insects live under natural conditions.

Table 1. Crop species potentially pollinated by bees and ants.

Common name	**Family**	**Species**	**Flowering period**
Oilseed rape	Brassicaceae	*Brassica napus*	March to June
Cucumber	Cucurbitaceae	*Cucumis sativus*	July
Tomato	Solanaceae	*Solanum lycopersicum*	Spring to Autumn
Peach	Rosaceae	*Prunus persica*	March to May

Table 2. Bee species as potential pollinators (based on Banaszak 2000).

Family	**Species**
Megachilidae	*Osmia rufa*
Megachilidae	*Osmia cornuta*
Megachilidae	*Chelostoma florisomne*
Colletidae	*Hylaeus variegatus*

Table 3. Ant species as potential pollinators (based on Czechowski et al. 2012).

Subfamily	**Tribe**	**Species**
Myrmicinae	Myrmicini	*Myrmica rugulosa*
		Myrmica sabuleti

		Manica rubida
Formicinae	Formicini	*Formica fusca*
		Formica cinerea
		Formica cunicularia
	Lassini	*Lasius alienus*
		Lasius niger
		Lasius emarginatus

The research will be carried out according to the following plan (see Table 4).

Table 4. General work plan.

Lp.	Research task
1.	Pilot study: Collecting ants and bees on crops previously selected for the project (season 2023)
2.	Identification of collected species of ants and bees
3.	Preparation of experimental set up for the season 2024
4.	Growing plants and breeding insects in the greenhouse - pollination (season 2024)
5.	Harvest and measurements (for season 2024)
6.	Preparation of experimental set up for the season 2025
7.	Growing plants and breeding insects in the greenhouse - pollination (season 2025)
8.	Harvest and measurements (for season 2025)
9.	Statistical analysis of collected data

4) Research methodology

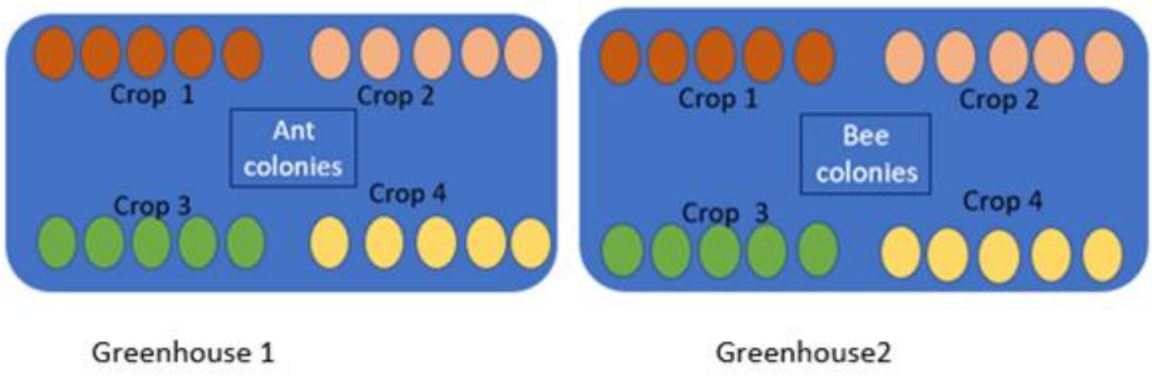
Pilot study: Choosing the species of pollinators

Before starting the actual study, we wish to use the most adaptive species of ants and bees to ensure reliable results. Ants and bees will be collected on already implanted crops, during blooming season, approximately every 10 days in each field, at two different periods of the day: 10:00-12:00 and 13:00-15:00, during their maximum diurnal activity. Pollinators will be caught with sweep nets during sunny or partially cloudy days following the methods described by Hahn et al. (2015) and Czechowski et al. (2012) and will be identified before releasing. We will select only one species of bee and one species of ant for our experiment, which will be the predominant species caught during this pilot study.

Experiment: Comparing ants- and bees-pollinated crops

The experiment will be conducted during two years in greenhouses belonging to the University of Agriculture in Kraków, Poland. All the agricultural equipment necessary for crops growing and harvest, such as seeders, tractors and combined harvesters will be provided by the faculty of Horticulture of University of Agriculture as a result of inter-universities collaboration.

Two greenhouses of 0,4ha each, one for bees and one for ants, will be physically divided into 20 units of 200m^2. Each crop species will be cultivated in 5 units in each of the greenhouses (Scheme 1).



Scheme 1. Experimental set up for each year of study.

Conditions will be similar in the two greenhouses and for the two years of experiment. In each unit, a nest of insects will be placed in the middle, with access to the crop, supplementary food to cover insects' nutrients needs, and water. According to Linsley (1958) and Czechowski et. al. (2012) studies, 50 solitary bees and 1000 ants are enough to pollinate one unit area (200m^2).

Crops will be cultivated and harvested without chemical input, with techniques avoiding damages for bees and ants. After harvest, we will measure seeds/fruits number, size and weight. It has been shown that pollination services can increase fruits and vegetables production by 9% to 112% (Sharman et al. 2015). The size of seeds will be measured using the application ImageJ, based on photographs. The size of fruits will be measured with a caliper. The weight of plant products will be measured with a scale.

Statistical analysis

Our data will be analyzed with the R software. Statistical analysis of the experimental data will be performed using Nested ANOVA model. We have two insect species, for each insect species we have 4 crop species, and for each crop species we have the 3 parameters: number of seeds/fruits produced, size and weight of seeds/fruits.

5) Project literature

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6. Table with budget of the project.

	Amount in PLN
Direct costs, including	**815 490**
- personnel costs and scholarships	576 000
- research equipment/device/software cost	100 000
- other direct costs	139 490
Indirect costs, including:	**179 407.80**
- indirect costs of OA	16 309.80
- other indirect costs	163 098
Total costs	**994 897.80**

7. Breakdown of project costs:

<u>Personnel costs and scholarships:</u>

Salaries for three Principal Investigators: 36 months x 4000 PLN per person (in total 432 000 PLN), justification: planning and conducting research tasks, statistical analyses of results and manuscript writing (related to the tasks 1-9).

Salaries for two technical assistants: 36 months x 2000 PLN per person (in total 144 000 PLN), justification: caring for crops and insects in greenhouses (related to the tasks 4 and 7).

<u>Research equipment/device/software cost:</u>
Dividers to separate the units in greenhouses (100 000 PLN) (related to the tasks 3 and 6).

<u>Other direct costs</u>
Catching nets: 150 PLN, justification: equipment needed to catch insects in the field during the pilot study (related to task 1).
The cost of the hives and solitary bees: 1770 PLN (570 PLN and 20 hives at 60 PLN each) (related to the tasks 4 and 7).
The cost of the ants' nests: 10000 PLN, justification: ants will be collected in the wild but nests are needed for housing in the greenhouses (related to the tasks 3 and 6).
The cost of the crop seeds and trees for 2 years: 1370 PLN (rape: 50 PLN; tomato: 50 PLN; cucumber: 70 PLN; peach: 1200 PLN), justification: crop seeds and trees needed to carry out our experiment (related to the tasks 3 and 6).
Greenhouse maintenance costs: 100 000 PLN, justification: financial participation for the use of the greenhouses (related to the tasks 3, 4, 5, 6, 7 and 8).
Transport: 3600 PLN, justification: greenhouses are situated 4 km away from our institution, price was calculated for abonnement to public transport (related to the tasks 3, 4, 5, 6, 7 and 8).
Purchase of materials and minor equipment:
Special diet for ants and bees: 1000 per year (in total 2000 PLN), justification: the need to provide insects with nutrients in greenhouse conditions (related to the tasks 3 and 6).
Outsourced services: 5000 PLN, justification: the costs include linguistic correction of the manuscripts.
Conferences: 5000 PLN per person (in total 15 0000 PLN), justification: the costs include the European conference fee, transport, daily allowances and accomodation.
Costs of the digital signature (JU regulations): 600 PLN, justification: according to the internal regulations of the university there is obligation to pay costs of the digital signature.

Reviews

Piotr Nowicki

1. **Assessment of scientific quality of the research project** (scientific relevance, importance, originality and novelty of research or tasks to be performed; quality ought to be evaluated in an international context)

The proposal deals with an important topic of recent pollinators decline and potential solutions to mitigate it. It is neatly aimed at evaluating the effectiveness of ants vs. bees as alternative pollinators of crop plants. However, while the overall aim of the prospective project is well defined, its specific objectives are not. In particular, the two hypotheses adopted are not particularly appealing. The first one (that ant-pollination will be at least as efficient as bee-pollination) is rather vaguely formulated. Its formulation implicitly suggests that the hypothesis could be considered confirmed in the case of no significant differences detected between ant and bee pollination effectiveness, which is easy to achieve e.g. with small sample size (fortunately, this is not necessarily the case given the proposed extent of the study). Hence, it would much more useful if the authors defined their objective as quantifying the relative effectiveness of ants and bees as pollinators (since a result that, let say, ants are ca. 80% as good bees in pollinating crop X appears a much more valuable outcome, especially for applied purposes, than proving that they are significantly better/or worse as pollinators). In turn, the second hypothesis (that the efficiency of ant-pollination will vary between different crop species according to their characteristics) is not fully addressed by the proposed research. The proposal does not sufficiently explain how the effects of selected plant traits (apparently: height, and flower scent) will be analysed, other than through simple between-species comparisons, but in such a case the plant species selection should have been better justified, and it should optimally include many more species.

2. **Assessment of potential impact of the research project** (the potential for substantial international impact on the research field(s) and for high quality research publications and other research outputs, taking into account the specifics of the research field and the variety of forms of impact and output; impact ought to be evaluated using an international context)

The proposal suggests a generally well-designed applied research, with a very good capacity to provide publishable results even if of far more narrow scope than the claimed goals (as explained in the following section). Moreover, the expected results could be of potentially strong implications for further studies in its domain, and ultimately for agricultural practice. Nevertheless, a minor shortcoming of the proposal is that it does relatively little to advertise its potential in the latter field; for instance it might have indicated how the role of ant pollination could be supported in agricultural practice.

3. **Assessment of feasibility of the research project** (the feasibility of the proposed project, including the appropriateness of the research methodology to achieve the goals of the project, the risk management description, research facilities and equipment, international cooperation (if any), other factors affecting the feasibility of the project)

As presented, the proposed research appears perfectly feasible, since the study design is relatively simple, straightforward and reliable. Some specific methodological issues are some overlooked, e.g. how the absence of other pollinators (especially ants) in the greenhouses will be ensured or how plant seeds and fruits will be counted or sampled for measuring or weighting, but these seem to be of rather minor importance for the success of the planned study, and are possible to be decided at a later stage.

Regardless of the above, it must be stressed that the proposed workplan is very narrowly focused as compared with the claimed ambitious goals of the project as it basically offers assessing the effectiveness of a single ant pollinator species vs. a single bee pollinator species. It is easy to imagine that the outcome of such a comparison will depend solely on the ant and bee species choice, which thus makes the species selection a critical issue for the whole project. Unfortunately, the proposal does not provide adequate details on how this selection will be done, because the description of the pilot study is rather underdeveloped. Specifically, it is not clarified (i) in what kind of cropland the pilot will be conducted; (ii) on what basis the sampling hours were decided (which is an important factor for ant sampling, with many species known to differ strongly in their activity hours); and (iii) what will happen if the clearly most common pollinators turn out to include other species than those in the pre-selected list (e.g. the honey bee).

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

The overall cost of project seems relatively high which not a problem in itself, but when you request several times higher funding than you competitors then you have to demonstrate that your project is also several times better than that of your competitors, which with current proposal you fail to do. More specifically, it appears that the personnel costs are exaggerated. It is not justified (i) why 3 Principal Investigators (PIs) are needed for the mostly technical work probably doable by a single PI with the help of two technical assistants (and in the case of more labour required it would be cheaper to employ additional technical assistants than additional PIs); and (ii) why technical assistants are to be employed for the total duration of the project, if they are only to be involved in the tasks restricted to two growing seasons (thus presumably for ca. 12-18 months in total). Besides, the relatively high cost of dividers to separate the units in greenhouses is not explained in adequate detail.

5. **Strengths of the proposal**

(i) well-defined overall aim of the study, which is strongly positioned within the scope of a very hot topic for applied ecology and agricultural science;
(ii) well-designed applied research of potentially strong implications for further studies in its domain, and ultimately for agricultural practice.

6. **Weaknesses of the proposal**

(i) poorly specified research hypotheses;
(ii) inadequate detail provided on the selection of the study species, which is critical issue for the project;
(iii) relatively high costs, which in parts could apparently be substantially reduced.

Joanna Palka

1. **Assessment of scientific quality of the research project**

The topic is very relevant and important, especially in the light of ongoing changes in the environment. The decline in bee pollinators creates a serious risk for a future of humanity because it will drastically reduce the crop quantity (and probably quality). Research proposed here is quite a novel approach toward this topic, which could help to resolve this problem in the future. On one hand, some similar studies were conducted before but on the other, important thing which distinguish this research from the others is the scale. Suggested research would bring a world-wide implication and benefit and indeed encourage more scientist into elaborate on that topic.

2. **Assessment of potential impact of the research project**

This research is having a big potential in terms of impact on international research fields. Bee decline problem is spreading worldwide, not only in certain countries. This means, that such research is needed on an international scale urgently. Publications based on this research have a potential to be high quality because proposed methodology is quite well established, although have some drawbacks (this topic is elaborated in the next section). Impact of publications based on this research should be high, concerning the importance of this topic. Moreover, I think this research could not only bring high-impact publications, but also it could be applied in everyday life in the future. These quantities are rendering this research highly valuable.

Although beginning of the research proposal strongly suggests that the authors of described project will perform their experiment on a wide range of ant and bee families. That is why, I would suggest removal of table 2 and 3 from this proposal.

3. **Assessment of feasibility of the research project**

Providing a pilot study is a very good idea for research which was not previously studied, so its presence is highly appreciated. Although, I do not understand what does it mean that ants and bees will be collected on the already implanted crops? When are you planning to plant the crops are you planning to use already existing crops? Does the pilot study have to take the whole year?

Regarding the methods section. Is it necessary to place all the 5 units of crops in the one place? If not, I would suggest to randomization of a crops. In current setup, the localization of certain crops could influence the preferences of crop pollination. Also, in your aims, the hypothesis was to compare efficiency of ant and bee pollinators. Is measuring the size of seeds and fruit weight enough to answer your question? Basing on my knowledge, I think that the majority of variance in size and weight of fruits is based on their natural differences. Information that fruits or vegetable production can be significantly increased by pollinators in mentioned in the text, but has there been any research showing such difference in fruit or vegetable mass between different pollinators? Maybe adding observations of efficiency of pollination based on number of pollinated flowers/plants would be a good idea? Or at least some king of estimation based on randomly chosen number of flower petals which will be pollinated.

In current form of the project the part including risk assessment and management is lacking. What protective measures are the authors of the project going to use if some parasite or other fungal or bacterial disease will attack the animals? Are they considering usage of pesticides in that case? How will it affect their research?

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

The costs seem to be well justified although a bit over simplified. My suggestion would be to also include expenses of collaboration (of course if the authors will consider it necessary). The money should be shared among the people helping in the establishment of the project (and most probably also the data analysis). One of the weak sides of the budget section is lack of clarity, how the yearly costs were estimated. Like for example diet costs or yearly expenses of the greenhouse maintenance cost are not considered in terms of monthly maintenance costs. Beside those small things, I would consider that budget plan is well established and explained.

5. **Strengths of the proposal**

The research question is definitely very strong side of this proposal. This topic seems to be extremely important and such research is needed. Also, multi seasonality of this research is a good idea. Based on that we could interfere if indeed the ants could potentially replace the bee pollinators (in certain cases). Also, I appreciate the fact that different species of crops will be used in this research. Especially, that the species belong to different families. These results could bring us some very exciting news, which will be appreciated worldwide. In general, I would classify this proposal as a good and well thought over.

6. **Weaknesses of the proposal**

The main weakness is not including any risk assessment in this research. Additionally, the muliti seasonality of the research could be increased. It would be better to plan in for three years, but on the other side I understand that the presence of pilot study in needed. Also, it would be nice if the research could be done on more pollinator species, but I understand, that not everything can be done in those time restrictions.

Aleksandra Żmuda

1. **Assessment of scientific quality of the research project**

Chosen project topic is relevant according to pollinator declines. Intensive agriculture, pesticides use and climate change may decrease gain from bee-pollinated crops. Idea to compare pollinating efficiency of bees and ants is not quite new but recently performed researches were conduct only on a few plant species. That is not enough compering with variety of plants commonly eaten by people around the world. What is novel in the project is used methodology in case of greenhouse part of experiment.

Feeding people with crop plants is a world-wide interests. Rapidly growing number of people make farmers and researchers to look for new techniques to protect plants, increase yields and increase the quality of harvested vegetables and fruits. If as a results of this project authors find relatively cheap in maintenance and safe ants species able to efficient pollinate crops it will be beneficial for big customer group.

2. **Assessment of potential impact of the research project**

This project present method to verify if one species of ants can be as efficient pollinator as bee-pollinator. The pilot research will bring scientific world answer about different potential ant and bee species which can pollinate 4 species of crops. This might only partially fill knowledge gap. On the same time it can focus biologist and agriculture scientists attention on looking for possible artificial pollinators. As pollinators are likely to decline further, the prospect of ants as pollinators may become more and more interesting.

It is worth to mention that cooperation with specialist from University of Agriculture in Cracow will cause that used agricultural method will reflect current agricultural trends and new technologies.

Chosen crop plant species origin from different plant families. That create opportunity to find which plant families might be favorable to pollination by ants.

3. **Assessment of feasibility of the research project**

Authors plan to choose only one ant and one bee species to greenhouse part of the experiment. It is seen to be caused by high cost of greenhouse maintenance and separation of each plot. On the other hand it is crucial for the project to be sure that plants have only one possible pollinator to eliminate the risk of other insects affecting the harvest.

I am wondering if authors are prepare to situation that during pilot study you might find out that the most common bee-pollinator on one of the crops is species that is not available to buy. Are authors going to try establish their own breeding colony?

I did not find information abut number of visited fields during pilot study. In my point of view it is crucial to look in different areas, because they can significantly differ in biodiversity of

ants and bees between regions (variety of crop planted in neighborhoods, different wild plants, trees).
Tomato flowers are difficult to pollinate by little bees species, because it is needed special pollination technique - buzz pollinating. Only bees from some genus as *Bombus*, *Anthophora* and *Lassioglossum* are able to perform buzz pollination and be efficient pollinators for tomatoes. In a given bee-pollinator species there is any species from that genus. In citated work of Hossain et al. (2018) there is no information about *Solanum lycopersicum* as good crop to be pollinated by ants.
As a other risk factor I want to point crop protection. Authors assured that there will be any chemical plant protection. It is crucial to maintain good condition of pollinators the obtain reliable data. In the same time is necessary to maintain optimal condition to growth and fruiting of plants, because the output will be measurements of fruits. I recommend to think about other that chemical ways of growing plants. Especially in greenhouses where temperature and humidity are high bacterial dieses spread quickly. Also I am curious about fertilization method – is it planned as a one of the main factors of growth and development of fruits? Given plant species have different nutritional requirements – are you planning to take measurements of soil properties to establish optimal soil conditions?

4. **Are the costs to be incurred well justified with regards to the subject and scope of the research?**

According to Preludium 20 project regulations: "remuneration of up to 1,500 PLN per month for the research team, i.e. the principal investigator and (optionally) co-investigator" 4000 PLN per month for each of PI is not justified cost. It could be maximum 54000 PLN for 3 years project for all of PI.
Big part of the budget are dividers to separate the units in greenhouses (100 000 PLN). What are they made from? Are they permanently or temporary?
Costs of cucumber seeds are underestimated – for 2 years cultivation it is around 400 PLN.
Other cost are well justified with regards to the subject and scope of the research.

5. **Strengths of the proposal**

- The topic is really interesting because of pollinators decline. It might be needed to find alternative pollinators. Ants may be one of them.
- Structure of this research project is clear. Proposal is well written – subsequent sentences of the argument follow from each other. There is no repetitions of given information. Thanks to tables it is easily visible which plant, bees and ants are going to be investigate in this study.
- Table 4 with briefly general work plan make possible to fast connecting budget cost with exact tasks.
- I appreciate that hypothesis are bold – that allowed to find them easily.

6. **Weaknesses of the proposal**

- Line 91 – given reference Hahn et al. (2015) is about collecting caterpillars of butterflies. As I understood you want to catch ants and flying bees. Are you sure that this methodology will fit to mobile insects?
- Line 92 – From my experience is not possible to identify majority of bees species without stereoscopic microscope so during field work it might be impossible.
- There is no information about risk assessments
- Some technical details which were mention in 3. part

Final project proposal

Comparison of ant- and bee-pollination efficiency: study of ants' potential as crops pollinators

Agata Burzawa, Maëlle Lefeuvre, Filip Turza

Summary

In this project, we attempt to evaluate the role of ants as effective pollinators of crops in comparison to bee-pollination. Despite previous research, the potential of ants has not been studied in depth. Therefore, our main goal is to analyze the effectiveness of ant-pollination on different crop species. **We hypothesize different pollination outputs according to the crop species in comparison to bees and between crops pollinated by ants.** We will measure crop products parameters to assess the efficiency of pollination such as the number of seeds and fruits produced, their size and weight. The outcomes of this study will contribute to a better understanding of the role of ants as pollinators of crop species belonging to different families, and we hope that it will arouse interest of environmental biologists and encourage further research in this field.

SHORT DESCRIPTION OF THE RESEARCH PROJECT

1) Scientific goal of the project

Production of the majority of human food relies directly or indirectly on pollinators services, as pollinators support 9,5% of global food production (Garibaldi et al. 2013, Ollerton et al. 2011). Intensive agriculture (especially pesticide use), urbanization, habitat loss, fragmentation and climate change lead to insects extinction. A recent review estimates that 40% of insect species are dramatically declining, including bees (Sanchez-Bayo and Wyckuys 2019). Bees are commonly known as major pollinators, as they are responsible for 20% of pollination in human food production (Losey and Vaughan 2006). However, the number of species dropped significantly since last century, and agricultural intensification is still responsible for worrying reduction of bees populations (reviewed in Sanchez-Bayo and Wyckuys 2019).

A challenge for environmental biologists is to identify alternative pollinators. In this area of research, ants are reported as a potential alternative to bees. Indeed, ants use the nectar of plants as a food source that positively affects their colonies size as well as their survivorship (Byk and Del-Claro 2011). For instance, fruit, seed sets and seed size of crop *Jatropha curcas* have been shown to be relatively similar between ant- and bee-pollinated flowers (Samra et. al 2014). On the other hand, the fruit set of the grass species *Euphorbia seguieriana* decreased by about two thirds compared to pollination by bees (Rostás et al. 2018). Thus, the ant-pollinators idea has supporters (Del-Claro et al. 2019, Delnevo et al. 2020) and sceptics (Beattie et al. 1985, Rostás ant Tauts 2010).

Nevertheless, ant pollination is a rarely studied phenomenon, limited to a few studies (Samra et. al 2014, Kuriakose et al. 2018, Rostás et al. 2018, Del-Claro et al. 2019). Knowledge about potential crops pollinated by ants is desperately needed, because so far, pollination by ants has been neglected due to insufficient evidence (Del-Claro 2019, Delnevo et al. 2020). In this project, we plan to observe the ant-pollination of plant species with a nutritional interest for humans. The comparison with bee-pollination will allow us to better understand the implication of ants in our food production and their potential as substitute pollinators in case of bees extinction.

We will base our work on two aspects: the comparison of ant- and bee-pollination and the ant-pollination efficiency according to the crop species. For the first aspect, we have hypothesis for each crop species involved in our project:

- Rape: we expect ants to pollinate 40% as much as bees.
- Tomato: we expect ants to pollinate 30% as much as bees.
- Cucumber: we expect ants to pollinate 20% more than bees.
- Peach: we expect ants to pollinate 80% as much as bees.

For the second aspect, we assume that **the characteristics of the different crop species will affect the efficiency of pollination**. Indeed, we expect more ants visits on short than high plant species, as well as scentful flowers more than scentless flowers.

2) Significance of the project

In the prospect of bees' extinction, pollination by human hand is investigated, but the tremendous time and money necessary for this task, and its complexity, are not very encouraging for our future. If we are not capable of replacing bees and the ecosystemic services they provide, other substitutes must be considered, such as other pollinator species.

Ants are among the most evolutionarily successful group of insects (Hölldobler and Wilson 1990). More than 16000 species of ants are known in the world (Bolton 2020), they constitute a huge group of insects that have a great potential to replace the role of other pollinators. The mutualistic interactions of some of those species with plants are promising. In addition, ants have a better detoxification system and are able to survive in more unfavorable conditions (Schläppi et al. 2020).

The impact of previous studies about ant-pollination is limited for different reasons. First, they focus on one (Rostás et al. 2018, Samra et al. 2014) or two plant species only (Delnevo et al. 2020) which are not cultivated and consumed by humans. Then, these studies were conducted in the field, which do not ensure the control of all the parameters. The project we propose will start covering those gaps, with the use of four different crop species in a controlled environment. We hope that this study will bring pieces of solution regarding the bees' extinction issue, and will encourage other studies in the field of alternative pollination strategies.

3) Concept and work plan

In the presented project, we selected a few crop species (Table 1). The criteria for selection were the possibility of growing in greenhouse conditions, ease of purchase, and high probability of visit by ants and bees (Hossain et al. 2018). We also selected potential bee species (Table 2) and ant species (Table 3) for the study. The criteria for selection were the possibility of survival in greenhouse conditions and ease of purchase, commonness of the species and type of habitat in which these insects live under natural conditions.

Table 1. Crop species potentially pollinated by bees and ants (based on Hossain et al. 2018).

Common name	**Family**	**Species**	**Flowering period**
Oilseed rape	Brassicaceae	*Brassica napus*	March to June
Cucumber	Cucurbitaceae	*Cucumis sativus*	July
Tomato	Solanaceae	*Solanum lycopersicum*	Spring to Autumn
Peach	Rosaceae	*Prunus persica*	March to May

Table 2. Bee species selected to study as potential pollinators (based on Banaszak 2000).

Family	**Species**
Apidae	*Bombus terrestris*
Megachilidae	*Osmia rufa, Osmia cornuta, Chelostoma florisomne*
Colletidae	*Hylaeus variegatus*

Table 3. Ant species within the Formicidae family selected to study as potential pollinators (based on Czechowski et al. 2012).

Subfamily	**Tribe**	**Species**
Myrmicinae	Myrmicini	*Myrmica rugulosa, Myrmica sabuleti, Manica rubida*
Formicinae	Formicini	*Formica fusca, Formica cinerea, Formica cunicularia*
	Lassini	*Lasius alienus, Lasius niger, Lasius emarginatus*

The research will be carried out according to the following plan (see Table 4).

Table 4. General work plan.

Lp.	**Research task**
1.	Pilot study: Collecting ants and bees on crops previously selected for the project (season 2022)
2.	Identification of collected species of ants and bees
3.	Preparation of experimental set up for the season 2023
4.	Growing plants and breeding insects in the greenhouse - pollination (season 2023)
5.	Harvest and measurements (for season 2023)
6.	Preparation of experimental set up for the season 2024
7.	Growing plants and breeding insects in the greenhouse - pollination (season 2024)
8.	Harvest and measurements (for season 2024)
9.	Statistical analysis of collected data

The major risk of our study is the failure of crops growing. To limit our impact on ants and bees in the greenhouses, crops will be managed without chemical input and losing crops because of diseases is a possibility. However, we will use organique substitutes which proved their efficiency, and we hope that the physical separation between the units will reduce diseases spreading. One other risk is the introduction of other pollinators in the greenhouses. Here again, we hope that dividers will limit this issue. In addition, safety and hygiene protocols will be applied at the entrance of the greenhouses.

4) Research methodology

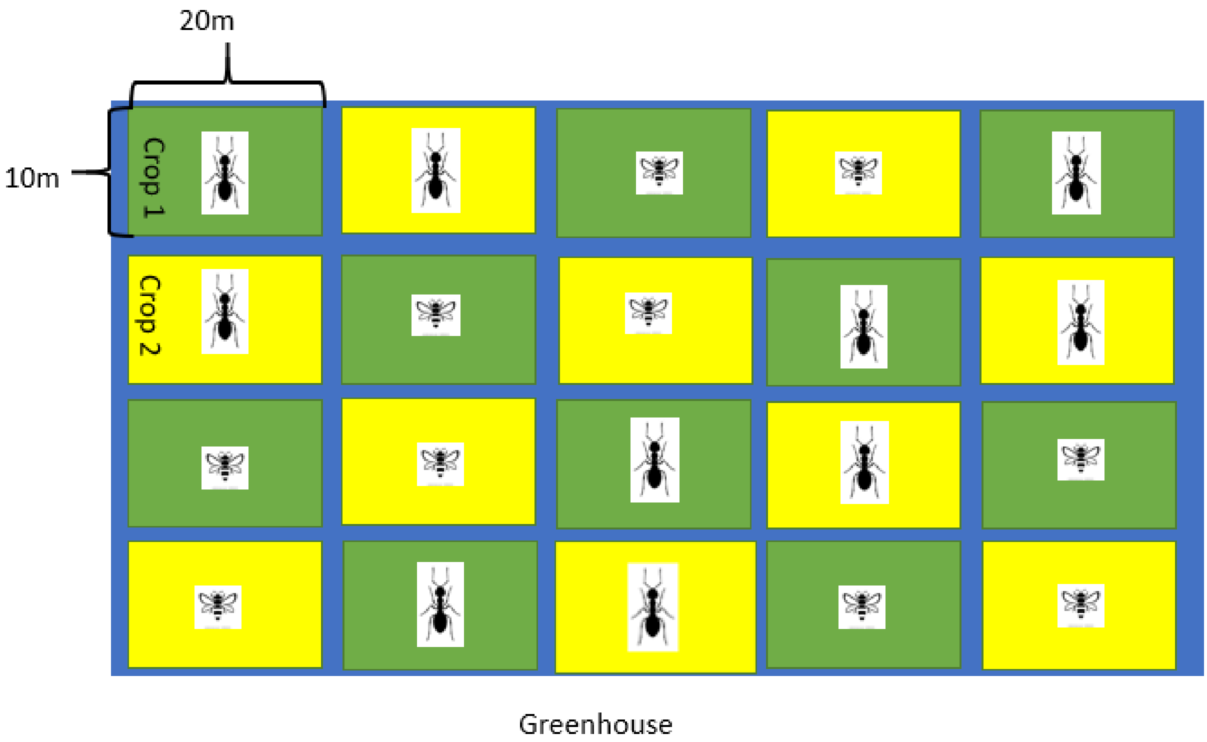
<u>Pilot study: Choosing the species of pollinators</u>

Before starting the actual study, we wish to use the most adaptive species of ants and bees to ensure reliable results. Ants and bees will be collected on already implanted crops from Table 1. For this purpose, we will set up the cooperation with local farmers. Samplings of pollinators will occur during the blooming season, approximately every 10 days in each field (4 times on each crop), at two different periods of the day: 10:00-12:00 and 13:00-15:00, during the maximum diurnal activity of pollinators (Hahn et al. 2015). Ants and bees will be caught with sweep nets during sunny or partially cloudy days following the methods described by Hahn et al. (2015) and Czechowski et al. (2012), and will be identified with the aid of an entomology expert in the field and marked before releasing (to avoid recounting the same individual). The sample fields will be approximately 0,01 ha for each crop. We will select only one species of bee and one species of ant for our experiment, which will be the predominant species caught during this pilot study.

Experiment: Comparing ants- and bees-pollinated crops

The experiment will be conducted during two years in greenhouses belonging to the University of Agriculture in Kraków, Poland. All the agricultural equipment necessary for crops growing and harvest, such as seeders, tractors and combined harvesters, as well as dividers, will be provided by the faculty of Horticulture of University of Agriculture as a result of inter-universities collaboration.

Two greenhouses of 0,4ha each, equipped with rigid partitions, will be physically divided into 20 units of $200m^2$. Each crop species will be cultivated in 10 units in each of the greenhouses. The position of the experimental fields in the greenhouse will be randomised to avoid a bias of exposition to the sunlight (Scheme 1).



Scheme 1. Example of the experimental setup for one year of study in one of the greenhouses.

Conditions of humidity and the availability of sun will be similar in the two greenhouses. In each unit, a nest of insects will be placed in the middle, with access to the crop, supplementary food to cover insects' nutrients needs, and water. According to Linsley (1958) and Czechowski et. al. (2012) studies, 50 solitary bees and 1000 ants are enough to pollinate one unit area ($200m^2$).

During the first year, we will plant tomatoes (*Solanum lycopersicum*) and peaches (*Prunus persica*), and during the second year we will plant cucumbers (*Cucumis sativus*) and oilseed rape (*Brassica napus*). Crops will be cultivated and harvested without chemical input, with techniques avoiding damages for bees and ants. We will use natural pests protection and fertilizers. An industrial substrate with compost, adapted to organic agriculture, will be used, and mulching will cover the ground around the roots to limit pests infection, development of weeds and draining of soil.

During the blooming season, plants' height will be measured and 20 sain flowers in each greenhouse unit will be randomly chosen for scent measurements using the headspace solid-phase microextraction method (HP-SPME, reviewed in Stashenko and Martinez 2008). Without cutting the flower, volatile molecules are trapped under a glass flask and captured on extraction fiber. The samples are then analysed by gas chromatography (Bartàk et al. 2003).

At harvest, in each unit, 50 plant products (rape seeds, tomatoes, cucumbers or peaches) will be randomly collected for measurements. It has been shown that pollination services can increase fruits and vegetables production by 9% to 112% (Sharman et al. 2015). To assess pollination efficiency, we will measure seeds/fruits number, size and weight. The size of seeds will be measured using the application ImageJ, based on photographs. The size of fruits will be measured with a caliper. The weight of plant products will be measured with a scale.

Statistical analysis

Our data will be analyzed with the R software. Statistical analysis of the experimental data will be performed using Nested ANOVA model. We have two insect species, for each insect species we have 4 crop species, and for each crop species we have the 3 parameters: number of seeds/fruits produced, size and weight of seeds/fruits.

5) Project literature

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6. Table with budget of the project.

	Amount in PLN
Direct costs, including	**367 224**
- personnel costs and scholarships	242 400
- research equipment/device/software cost	30 000
- other direct costs	94 824
Indirect costs, including:	**80 789.28**
- indirect costs of OA	7 344.48
- other indirect costs	73 444.80
Total costs	**448 013.28**

7. Breakdown of project costs:

Personnel costs and scholarships:

Salaries for Principal Investigator: 36 months x 4000 PLN (in total 144 000 PLN), justification: planning and conducting research tasks, statistical analyses and manuscripts writing (related to the tasks 1-9).

Salaries for two technical assistants: 24 months x 2000 PLN per person (in total 96 000 PLN), justification: caring for crops and insects in greenhouses (related to the tasks 4 and 7).

Salary for entomology expert: 150 PLN per hour x 4 hours per crops x 4 samplings per crops (in total 2 400 PLN).

<u>Research equipment/device/software cost:</u>
Gas chromatograph: 30 000 PLN, justification: material for the analysis of flower scents (related to tasks 4 and 7).

<u>Other direct costs</u>
Catching nets: 50 PLN, justification: equipment needed to catch insects in the field during the pilot study (related to task 1).
The cost of the hives and solitary bees: 570 PLN for bees + 20 hives x 60 PLN each (in total 1770 PLN) (related to the tasks 4 and 7).
The cost of the ants' nests: 500 PLN x 20 nest (in total 10 000 PLN), justification: ants will be collected in the wild but nests are needed for housing in the greenhouses (related to the tasks 3 and 6).
The cost of the crop seeds and trees for 2 years: 2 300 PLN (rape: 50 PLN; tomato: 550 PLN; cucumber: 500 PLN; peach: 1 200 PLN), justification: crop seeds and trees needed to carry out our experiment (related to the tasks 3 and 6).
Greenhouse maintenance costs: 32 500 PLN per year (in total 65 000 PLN), justification: financial participation for the use of the greenhouses (related to the tasks 3, 4, 5, 6, 7 and 8).
Transport: 46 PLN per month x 2 years (in total 1 104 PLN), justification: greenhouses are situated 4 km away from our institution, price was calculated for abonnement to public transport (related to the tasks 3, 4, 5, 6, 7 and 8).
Purchase of materials and minor equipment:
Special diet for ants and bees: 1 000 per year (100 PLN per 2 kg of a protein-carbohydrate diet, 20 kg for 2 years, in total 2 000 PLN), justification: the need to provide insects with nutrients in greenhouse conditions (related to the tasks 3 and 6).
Laboratory material for flower scent analysis: 2 000 PLN (products for gas chromatography).
Outsourced services: 5 000 PLN, justification: the costs include linguistic correction of the manuscripts.
Conferences: 5 000 PLN for the PI, justification: the costs include the European conference fee, transport, daily allowances and accomodation.
Costs of the digital signature (JU regulations): 600 PLN, justification: according to the internal regulations of the university, there is an obligation to pay costs of the digital signature.