

EFFECT OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT AND FUNCTIONING OF ANT COLONIES OF THE SPECIES MESSOR BARBARUS

The aim of my interdisciplinary project this year was to investigate how specific environmental factors, such as temperature or humidity changes, would impact the development and functioning of colonies of Messor Barbarus ants. In the experiment, my focus was primarily on observation and drawing conclusions. My intervention was limited to changing the environmental factors I investigated and potentially influencing the experiment's structure, not its course. This limitation was due to the participation of living organisms, namely ants.

I also intend to examine the adaptive abilities of ants to changes in their environment, referencing Charles Darwin's evolutionary theory of natural selection.

My main premise was to create optimal conditions for the studied ants by replicating their natural environment. Without this, the experiment would lack significance. I concentrated on providing suitable conditions throughout their life cycle, from birth to death. I simulated all possible natural changes in their environment, such as potential threats like the presence of other organisms – flour beetles and field crickets. This was crucial because, during drawing conclusions, adjustments had to be made for these variables.

In the experiment, I mainly played the role of an observer, although, as mentioned earlier, I intervened in environmental factors affecting the ants. Importantly, I did not interfere directly with their behavior, development, or life cycle. I only altered the conditions in which they lived, changes that did not directly threaten their lives.

The entire experiment was documented in the form of a photo report. The taken photos depict the entirety of the experiment.

In summary, the above introduction illustrates the general principles I followed during the experiment. My goal was to examine the influence of environmental factors on the development and functioning of Messor Barbarus ant colonies.

Preparing for this year's interdisciplinary project, I decided to conduct an experiment related to ants. I chose this topic because I have always been fascinated by insects, especially ants, whose behavior often resembles that of humans. Similar to us, they live in vast communities we call colonies, exhibiting a strict social hierarchy, independent thinking, and the potential for conflicts. I decided to explore how a colony of Messor Barbarus ants

develops under various environmental factors. To start the experiment, I needed to expand my knowledge about ants. I chose *Messor Barbarus* as the ideal species because it is easy to breed and commonly selected for establishing new colonies. I'll share more about these ants shortly. Next, I had to acquire a formicarium, a glass box with corridors specially carved for ants to establish their nests. Additionally, I needed an arena, a second glass box connected to the formicarium, allowing ants to move in an open space. In the arena, I placed sand and soil as the substrate, along with some dried pieces of plants found in the forest floor, creating an imitation of the forest litter—the natural habitat of these ants. I added pine cones and branches to complete the simulation. I also included a plastic object, a water feeder made of plastic and glass, containing water. The setup looked like this:



After preparing the living space, I still needed to acquire ants. They are sold as young colonies consisting of 30 to 40 individuals and a queen. My colony initially had a total of 34

individuals, the smallest number throughout the experiment, as it remained unchanged. Once everything was ready, I could officially introduce the ants to the formicarium. The colony's inception date was November 24, 2023, with 34 ants, including the queen. *Messor Barbarus* is easy to breed and originates from Southern Europe, including Spain, Portugal, Italy, and France. Recommended for novice breeders due to being a perfect combination of affordable, easy-to-keep, and intriguing ants. They exhibit significant size diversity among worker ants within a colony, with a distinct caste division. The smallest ants, nurses, may be only 3 millimeters, while the largest, soldiers, can reach 14 millimeters or even more. Soldiers have massive heads, and sometimes, a larger-headed soldier is referred to as a supermajor, though opinions vary among ant keepers on whether a separate caste designation is necessary. Soldiers effortlessly crush large seeds.

Ants of this species prefer relatively low humidity, high sunlight exposure, and a diverse vegetation around their nest. They commonly nest under stones, logs, or in tree branches. Soldiers primarily serve as seed huskers, but in the face of danger, they release a special liquid for combat and pheromones that trigger a rapid influx of more soldiers. Colonies are often described as monogynous (with one queen), yet there are numerous instances where someone accidentally released multiple colonies or queens, leading these ants to merge and establish a colony together. Such colonies often reach tens of thousands of workers, and additional queens are not killed, suggesting the possibility of polygynous colonies occurring in nature. Confirming or excluding this is challenging as queens typically reside about 50 cm underground. *Messor Barbarus* can create foraging trails up to approximately 40-70 meters in length, with varying figures provided by different sources.

The *Messor* genus, including the species *Messor Barbarus*, distinguishes itself not only through polymorphism but primarily by their diet, which largely consists of seeds. While some ant species occasionally consume seeds (e.g., *Pheidole pallidula* or the Polish *Tetramorium caespitum*), for most ants, seeds are merely a supplementary part of their diet, typically small and oily. In contrast, the diet of *Messor* ants is heavily reliant on seeds. They consume almost all seeds that come into their mandibles, making them a popular choice among ant keepers, with *Messor Barbarus* being a frequent choice due to its affordability, widespread availability, and abundant sources of information. The fact that these ants rely extensively on seeds in their diet is a significant advantage in keeping them. A large *Messor* colony can survive unattended for several weeks, during which time ants relying on honey would likely perish or suffer significant colony losses. *Messor* ants can simply access their seed storage and consume reserves. Speaking of seeds, these ants selectively choose seeds from the mix based on their preferences and consume them first. Within the nest, there are specialized chambers designated for seed storage. Ants secrete a white, milk-like substance that covers the chamber walls. This substance surprisingly exhibits strong fungicidal properties, safeguarding the seeds from mold and other fungi in case excess moisture enters the nest. It's noteworthy that soldiers crush the seeds, while smaller ants grind them into what's known as ant bread, consumed by the entire colony, including the queen.

In this particular ant species, each ant belongs to a specific caste from birth to death. While it is typical for ants to have distinct castes, there are instances where an individual ant may belong to multiple castes. Based on my observations, I have identified the following caste hierarchy from the lowest to the highest:

- a) Larvae - the lowest caste, even though they are developing ants, still require care, including feeding and hydration.
- b) Nurses - belonging to the worker caste, their primary role is serving as midwives to the queen, feeding larvae, and, if necessary, becoming the first to be consumed by the colony if food is scarce.
- c) Workers - handle various tasks, organize the colony, manage food supplies, collect food, and actively participate in defending against invaders when needed.
- d) Soldiers - the largest ants in the colony after the queen. Their mandibles are adapted not only for crushing like workers but also for tearing meat. They serve as scouts and are the first to engage in combat if there's a threat. Interestingly, their anatomical structure prevents them from eating independently, so they must be fed by workers.
- e) Males (king) - this caste consists of only one individual at a time. He fertilizes the queen, but after a few weeks, the king is replaced by another individual. In *Messor Barbarus*, the king always belongs to the worker caste.
- f) Queen - has priority in everything, from food and water to choosing the king. She doesn't directly manage everything happening in the colony since ants are programmed for their tasks even as larvae.

As mentioned earlier, the colony was established on November 24, 2023, with 34 individuals. However, before delving into environmental factor studies, I allowed the colony a month for development under optimal conditions, serving as the control phase of the experiment. During this time, they received a diet of poppy, sunflower, and rye seeds, along with meat from deceased flour beetle individuals. The air humidity was maintained at 48%, and the temperature was kept at 26 degrees Celsius. They also had constant access to water. Under these conditions, the colony expanded to 52 individuals in a month, confirmed by daily counts of new larvae. The larval developmental stages vary significantly, facilitating the identification of the same larva on consecutive days. Now, it was time to investigate the first factor: temperature. *Messor Barbarus* ants thrive in temperatures ranging from 21 to 31 degrees Celsius. I reduced the room temperature where the colony was situated to 20 degrees Celsius, slightly below the recommended minimum. Initially, there were no observable changes, but by the end of the first week, I noticed that the queen had not laid any new larvae. Normally, the colony produced 4-6 larvae within a week, but this time, there were only 2. Subsequently, I observed reduced ant activity; they moved slower and seemed lethargic, sleeping four hours longer than usual, totaling 13 hours a day. Additionally, uneaten seeds were left on the arena. The ants received one flour beetle every three days, and previously, it took three days to consume it. Now, they finished it in just one day. The conclusions are apparent: lowering the air temperature below the recommended limit resulted in ants moving more slowly, having less energy. The queen laid fewer larvae, and the ants consumed fewer seeds but preferred more meat, likely because meat is richer in energy. However, an interesting phenomenon occurred where some ants remained motionless inside a deceased flour beetle's body after creating a large opening. They didn't feed on the meat but seemed to use it as a source of warmth. While I couldn't find information confirming this hypothesis, I also found nothing contradicting it.

Before proceeding with further observations, I restored the temperature to 26 degrees and waited for two weeks until the colony returned to normal. During this time, the number of individuals increased to 56. Following this period, it was time to do the opposite and increase the temperature to 30 degrees Celsius. The results were quickly apparent. By the second day, the ants started behaving differently. This time, the situation was the opposite of the previous experiment: ants consumed more seeds than usual from their reserves but showed minimal interest in eating meat. A flour beetle lay on the arena for as long as 6 days before being consumed. Regarding the queen, she laid exactly the same number of larvae as under normal conditions. Workers and soldiers, on the other hand, increasingly visited the water feeder. An unusual phenomenon was observed as ants attempted to insert themselves into a cotton swab placed in the water feeder soaked with water. It seemed as if they were trying to cool themselves down. This can be clearly seen in the image below:



The conclusions are evident. Excessive air temperature causes ants to have a greater need for substances found in seeds than in meat. These substances include simple

sugars and fats stored in the seed's endosperm, which surrounds the seed embryo. This endosperm is the target for ants. During heat, there is no disruption in the larval developmental cycle, and the queen produces almost identical quantities of larvae. Ants' behavior shows an increased need for water, but their motor skills remain unaffected.

After concluding the temperature-related studies, it was time to investigate the impact of humidity on the development and functioning of the ant colony. However, before doing so, I gave the ant colony a month to acclimate to standard conditions. At the end of this period, the colony comprised approximately 78 individuals. I also had to increase the amount of food provided, both seeds and the quantity of dead flour beetles, to 2 every three days, due to the increased population. For technical reasons, I couldn't decrease the ants' humidity, but I could increase it. For two weeks, I sprayed the entire arena and formicarium with water each day, maintaining a dampness level suitable for movement. Messor Barbarus ants are recommended to live in an environment with a humidity level of up to 70%, but I increased it to 86%. The first results were visible on the third day when the stored seeds in the ant colonies started germinating. Most of them were carried to the arena, where I removed them. A problem arose because theoretically, by removing potentially germinating seeds, I disrupted the experiment, directly intervening in the ant environment. However, I considered that potentially germinating seeds might disrupt the experiment, so I decided to remove them from the colony. I returned to observations and noticed that the queen became much more active than usual. She frequently moved throughout the colony, seemingly walking aimlessly, but what caught my attention the most was that her entire entourage, including larvae, followed her. From my observations, it appeared that larvae were being relocated to lower-humidity areas to facilitate proper development. For insects, including ants, larvae represent the colony's future, making it a priority to provide suitable conditions for their development. Such relocation of offspring in insects is termed larval dispersal. This was not the only unnatural occurrence in the colony. Almost two weeks after increasing humidity, worker ants started moving in a highly uncoordinated manner. Their walking resembled that of an individual under the influence of alcohol, bumping into obstacles, colliding with walls, and having difficulties carrying anything. Ants communicate using pheromones, chemical substances they produce. Workers have poor eyesight, so soldiers leave a pheromone trail for them, allowing them to navigate terrain and find their way back to the colony. Increased humidity led to pheromonal agnosia, causing problems in recognizing and detecting ant pheromones. Consequently, workers walked in an uncoordinated manner. Pheromones attach to surfaces, but increased water in the air caused them to diffuse. Chemical compounds dissolved in water couldn't be detected by the ants. Another surprise was the strange growths that covered some sunflower seeds. It turned out that increased humidity led to the development of fungi on the seeds. I couldn't determine the fungus's species, but the most surprising fact was that instead of removing fungus-covered seeds from the formicarium, ants began storing these seeds in the most humid areas. Apparently, the fungus prevented the seeds from germinating, but ants started consuming this fungus along with the seeds. This can be clearly seen in the photo below:



The conclusions from this experiment are that high humidity significantly adversely affects ant functioning. Their movement and coordination are disrupted, and the ants themselves do not function correctly. It is surprising how quickly the ants adapted to these changes. They were able to utilize humidity to start cultivating a fungus, which they could later consume. Additionally, they used it to preserve seeds. After two weeks, I decided to conclude this part of the project. Due to the regular relocation of larvae, as mentioned earlier, there might be a margin of error in counting. However, I estimated that the ant population was around 89 individuals at that time. This time, I gave the ants a full three weeks for reorganization.

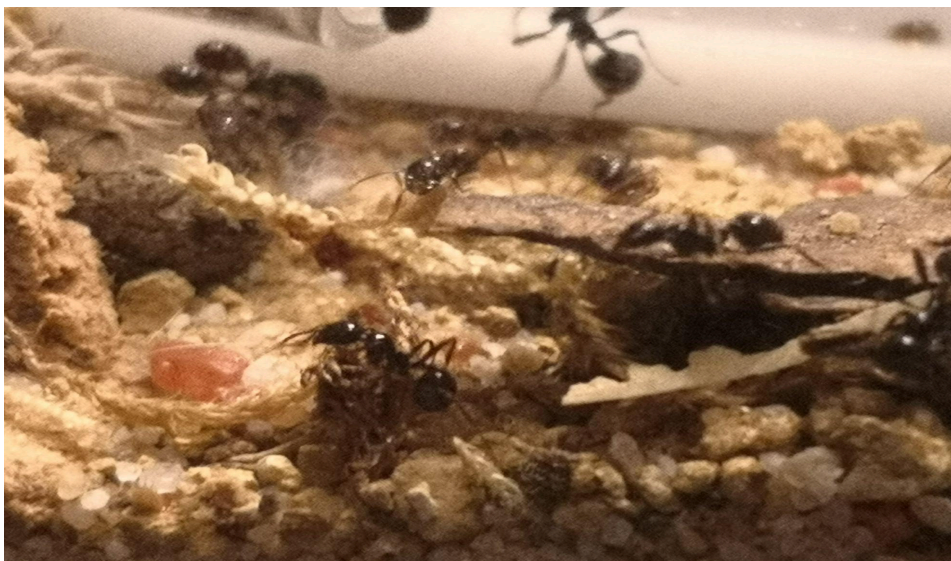
After this period, it was time to explore the last conventional factor: food. On a weekly scale, the colony required 20 grams of seeds and 4 flour beetle larvae. Most seeds were stored in the formicarium, but each time, the ants consumed all the flour beetles down to the last piece. The diet of *Messor Barbarus* is mainly based on other organisms, often even other ant species. In their natural environment, they frequently attack termite colonies or wasp nests. Termites and ants have been at war for about 40 million years, and it is a fair

fight. Both species, ants and termites, are of similar sizes and lead similar lifestyles. Wasps, on the other hand, are entirely different. While it may seem that theoretically, ants should fear wasps, it is quite the opposite. The average wasp nest of the most popular species, Vespidae, consists of up to 200 individuals, with a size of up to 3 centimeters. Meanwhile, as mentioned at the beginning, the size of a soldier ant from the *Messor Barbarus* species is up to 14 millimeters. It seems that wasps surpass ants in size, but ant colonies consist of several thousand individuals, rendering wasps defenseless. In the case of an ant attack on a wasp nest, wasps usually attempt to fight but quickly start losing. Wasp workers evacuate larvae, which are a common part of the ant diet. Due to ethical reasons, I couldn't conduct such experiments, but I could introduce variations into the ant colony's diet. For a week, I did not provide any flour beetle larvae to the ants, but instead, I tripled the number of seeds over the week. So, instead of 20g, they received 60g. From my observations, it emerged that the ants' life cycle practically did not change. Workers performed their duties as they should, and soldiers continued patrolling the arena, just as they should. The usefulness of soldiers in the colony increased because they had to crush all the seeds, as worker ants have too small mandibles for this task. On the other hand, soldiers, with their exact mandibles, cannot consume food on their own, so they have to be fed by worker ants. Such situations had occurred in the colony before, as the ants had access to seeds from the beginning, but now it happened much more frequently. The absence of meat caused worker ants to start producing ant bread from poppy seeds. It had a brown color and consisted mainly of carbohydrates and fats. Information I read revealed that, for ants, this food is simply tastier than regular seeds. Indeed, I observed that they crowded around it in groups, as seen clearly in the photo below:



The complete removal of meat from the ants' diet had one significant drawback. Throughout the week when insects consumed only grains, the queen gave birth to only 2 larvae. Since about 3-5 ants died every week, with only 2 being born, the natural increase in the colony was at best -0.3%. The conclusion is straightforward: the total removal of meat from the ant diet results in significantly fewer larvae being born than those that die. This is because the queen needs protein to form new larvae, which is abundant in meat but scarce in seeds. However, there is a solution to this problem. Some plant species, such as soybeans, contain large amounts of protein in their seeds. Therefore, diversifying the ant diet with soy seeds could lead to more larvae being born. The second conclusion from this experiment is that ants have adapted perfectly to the new diet and have easily ensured their food supply. The phenomenon of insects adapting to changes in nutrition caused by environmental changes is known as dietary adaptation.

Unfortunately, I couldn't conduct an experiment in which ants consumed only meat because the ant stores were filled to the brim with grains, and I would have to wait several months until their supplies ran out. Instead, before the last tested factor, I gave the ants a month to adapt to their previous diet. When the designated time passed, I counted 112 ants in the colony. They were receiving 6 flour beetle larvae and 30g of seeds weekly. Over the last 2 months, the colony had grown to almost four times its initial size. It was time to check its ability to respond to threats. Until now, the ants had only received dead food that they didn't have to catch themselves. Now, I intended to introduce a live flour beetle larva into the colony. It is entirely harmless to ants. Although it is about 6 times larger than them, it is a herbivore and cannot harm the ants. Its protection lies in a thick chitinous armor that covers its entire body and head. The only vulnerable point is its exposed legs. On the 71st day of the colony's existence, the first foreign organism appeared in its territory. I placed a live flour beetle larva on the arena. The ants immediately went into action. A few seconds after placing the larva on the arena, it began crawling toward the entrance to the ant nest. So far, the entrance had been blocked by pieces of plants and wood that I initially placed on the arena. It wasn't a tight barricade, but until now, the ants hadn't needed one. It looked like this:



The flour beetle larva easily breached the ants' barricade and, lured by the scent of the grains it fed on, entered the colony. The ants seemed uncertain about what to do at first, but eventually, they swarmed the larva. Its chitinous armor protected it so well that the ants couldn't harm it. The corridors were too narrow for it to turn around, so it crawled deeper into the colony. Eventually, it reached the grain storage area where it could have turned back, but it was too late. Surrounded, the larva was devoured alive by the ants after about 12 minutes of battle. The entire incident concluded with the larva being eaten on the spot. Following this event, the ants reorganized the arena, mainly rearranging some pieces of wood. They also covered the entrance to the colony with sand and soil, but in a way that allowed them to freely enter and exit. The ants decided to adapt their environment in case of another such attack. This phenomenon is known as behavioral defense against predators. Below are photos illustrating key moments from this experiment, and a complete photo report is included in the attached file:





In summary, considering all the data and observations, I deem the entire experiment successful. The *Messor barbarus* ant species I investigated displayed tremendous resilience

to the environmental factors I tested. They exhibited adaptability, changing their habits and responding to threats. The primary conclusion drawn from the experiment is that ants of the studied species are capable of adapting to a changing environment, demonstrating what is known as species adaptation. This supports the concept proposed by Charles Darwin over two centuries ago – the idea that each species must adapt or face extinction. The ants subjected to too low temperatures moved slower, and their queen laid fewer larvae, but they adapted by utilizing the warmth from the flour beetle larva's insides. When facing excessively high temperatures, they had to alter their diet, yet they adapted. When humidity exceeded the permissible norm for these ants, they managed to survive despite disruptions in their pheromone communication. When provided only seeds as food, they created nutritious ant bread. Moreover, when a live organism entered their colony, they were capable of defense, followed by adaptation and preparation for similar threats. The impact of environmental factors on the development and functioning of Messor Barbarus ant colonies is such that they have learned to adapt, indirectly affirming Charles Darwin's theories on natural selection, where every organism must adapt, or it will perish.

As of writing this work, the colony has already grown to 132 individuals, increasing its population every day. The main challenges in this year's project were creating suitable conditions for the ants, ensuring good and simultaneously optimal air temperature and humidity. Documentation was also challenging due to capturing images of such small organisms. I consider my project successful, as it provided a better understanding of how insect communities, such as ant colonies, function. It was a difficult yet intriguing experience.

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