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- How does the sunlight affect plant height and biomass production?
- Does terrain morphology affect the density of earthworm populations?
- Does ambient temperature influence ants walking speed?
- Influence of grasshopper's size on the jumping length

CONTENTS

1. PARTICIPANTS.....	3
2. RESEARCH TEAMS.....	4
3. RESEARCH TOPICS PROPOSED BY PARTICIPANTS	5
4. PROJECTS.....	6
4.1 How does the sunlight affect plant height and biomass production?	6
4.1.1 Project proposal	6
4.1.2 Research report – first version	8
4.1.3 Reviews	12
4.1.4 Research report – final version	17
4.2 Does terrain morphology affect the density of earthworm populations?.....	21
4.2.1 Project proposal	21
4.2.2 Research report – first version	23
4.2.3 Reviews	26
4.2.4 Research report – final version	31
4.3 Does ambient temperature influence ants walking speed?	35
4.3.1 Project proposal	35
4.3.2 Research report – first version	37
4.3.3 Reviews	40
4.3.4 Research report – final version	45
4.4 Influence of grasshopper’s size on the jumping length	49
4.4.1 Project proposal	49
4.4.2 Research report – first version	51
4.4.3 Reviews	55
4.4.4 Research report – final version	61
GALLERY.....	66

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1. PARTICIPANTS

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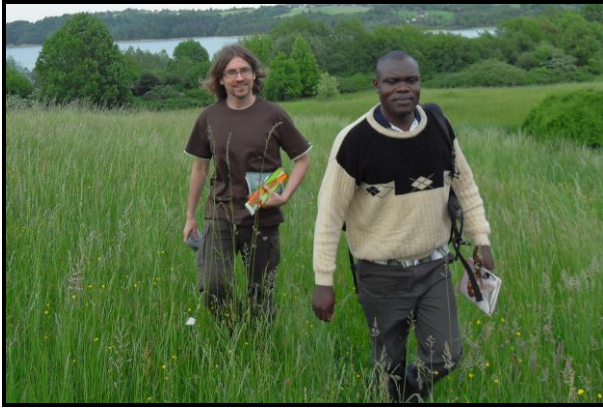
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3. RESEARCH TOPICS PROPOSED BY PARTICIPANTS

- 1) Do the ecological niches exist? (MF)
- 2) Wasps nests in relation to aphids colonies (MF)
- 3) Does farmlands near the Dobczyce Lake affect water quality? (MF)
- 4) Does number of inflorescence in pink clover depend on length of stem? (AM)
- 5) Does insect biodiversity depend on the distance from the lake? (AM)
- 6) Goats preferences for grass species (AM)
- 7) The number of inflorescence in buttercup depends on amount of sunlight (KW)
- 8) The number of inflorescence in buttercup depends on the distance from the road (KW)
- 9) Does the height of the grass bromus depend on amount of light? (KW)
- 10) Does the regrowth rate of different grass species depend on amount of light? (AR)
- 11) Do aphids produce more honeydew if exposed to alcohol? (AR)
- 12) Do ants are able to differentiate colors? (AR)
- 13) Does number of plants of a given species differ in different light conditions? (GD)
- 14) Can we find adaptations to specific light conditions in specific plant species? (GD)
- 15) Are adaptations to light conditions reflected in biomass production? (GD)
- 16) Does the aggressiveness of spiders depend on familiarity and sex? (IG)
- 17) Does the number of earthworms depend on the shape of the terrain? (IG)
- 18) Does the activity of ground walking invertebrates depend on vegetation height? (IG)
- 19) Does the goat milk taste depend on stress invoked by human disturbance? (MS)
- 20) Cricket body length and jumping distance (MS)
- 21) Does the speed of an ant depend on ambient temperature? (MS)
- 22) Do meadows and forests differ in the number of ticks species? (MP)
- 23) Does the shape of leaves differ in respect to distance from the lake? (MP)
- 24) Are spiders attracted by sugar, caffeine and alcohol? (MP)

Chosen projects:

- 1) Does number of inflorescence in pink clover depend on length of stem? (AM)
- 2) Are adaptations to light conditions reflected in biomass production? (GD)
- 3) Does the aggressiveness of spiders depend on familiarity and sex? (IG)
- 4) Cricket body length and jumping distance (MS)

Finally done projects:

- 1) Does ambient temperature influence ants walking speed? (MS)
- 2) How does the sunlight affect plant height and biomass production? (GD)
- 3) Does terrain morphology affect the density of earthworm populations? (IG)
- 4) Influence of grasshopper's size on the jumping length (MS)

4. PROJECTS

4.1 How does the sunlight affect plant height and biomass production?

Geoffrey Dheyongera & Michał Filipiak

4.1.1 Project proposal

Does sunlight affect plant morphology and biomass production?

Summary

Wide variations in light conditions occur in different habitats. These variations may affect plant phenotype and, consequently, biomass production. We will use *Ranunculus lanuginosus* as a model organism to address the question of how different light conditions impact on plant phenotype. We will compare individuals inhabiting two ecosystems differing in sunlight intensity: a meadow and a forest. We will compare biomass and vigour of individuals inhabiting those ecosystems to see if the sunlight intensity may influence their morphology and biomass production.

Background

The interaction between organisms and abiotic factors characterizing their environment is vital for maintenance of ecosystems. Light is the most important factor for primary production that sustains energy flow to higher trophic levels through photosynthesis. Different light conditions affect energy flow in ecosystems. Differences in light conditions are documented to affect species richness and specimens phenotype. Particular species specimens can vary in productivity and morphology associated with limited vital factors in particular type of ecosystem.

Productivity not always means investing in biomass production. Our knowledge concerning aspects of life histories of particular species in different environments and phenotypic differences driven by limiting important abiotic factors is scarce. It is not obvious

how the sunlight abundance or its lack can affect biomass production of plants in their natural habitats.

The study aims at exploring how light variation can affect plant production. We will focus on two characteristics of plant production: the influence of the sunlight on plant phenotype and biomass. This is motivated by the fact that under limited light, competition for faster access to light might involve faster growth in height but may not necessary mean an increase in biomass.

Our preliminary results showed a significant difference ($t=6.409$, $df=25$, $p<0,0001$) between the mean height of the *Ranunculus lanuginosus* inhabiting a forest (representing low of light intensity) and a meadow (representing high light intensity). In a forest specimens are taller. We measured a vigour as a biomass of individuals in relation to their height. The mean biomass divided per height turned out to be higher in a meadow ($t=-3,077$, $df=35$, $p=0,004$), but we observed that forest specimens are much more branched. It is unclear why biomass divided per height turned out to be higher on a meadow. We think it may be due to too simple measurement method. In a meadow individuals are more expansive and more frequently have a tendency to sprout side shoots, which are hard to distinguish from the maternal plant. Our result may reflect measuring few individuals biomass instead of one individual biomass. We need to develop the method to cope with this obstacle.

The proposed research

We will work on the *Ranunculus lanuginosus*, which is a common and abundant species inhabiting various ecosystems, such as light patches in forests, brushes, and sunny but humid meadows in forest surroundings. *R. lanuginosus* is easy to recognize.

We aim at testing two hypotheses:

1. The sunlight affects the *R. lanuginosus* phenotype.
2. *R. lanuginosus* specimens are characterized by higher biomass production in sunlight richer habitat.

Species from two study sites will be compared. One site will be the open meadow, which should reflect full light conditions, and the other will be forest patches, which will reflect a partial exposure to light. We won't control other environmental factors. We will sample 5 patches per study site. 10 specimens per patch will be used to estimate biomass as a measure of production, whereas their vigour and height will be used as a predictor of

morphology. The height will be measured as the height of the tallest stem from the ground to the last node of the highest branch. Vigour will be measured as the biomass of one specimen in relation to its height and to the number of main (flowering) stems outgrowing measured rosette. This will eliminate the problem with more expansive meadow individuals.

Further perspectives

This project is an introduction for more detailed studies concerning the influence of the sunlight on plants as primary producers.

4.1.2 Research report – first version

How do sunlight affect plant morphology and biomass production?

Abstract

We investigated the influence of light intensity and exposure duration on plant biomass and height. Comparing *Ranunculus laginosus* in a forest and meadow, we found that forest individuals had 1.5 times greater height and 2 times greater biomass. That means that increase in sunlight intense in moderate climate not always results in higher biomass production. Resources allocation to vegetative organs in shady ecosystems may be an important factor driving biomass production.

Introduction

In general, plant production is influenced by abiotic and biotic factors. But we don't know how plants in different ecosystems would respond to various abiotic factors. Organisms' phenotypes can be shaped by a number of abiotic factors. Exposure to sunlight is among the most important ones as far as plants are concerned. Differences in light conditions are documented to influence plant biomass, species richness and phenotype. These changes may reflect a plant's adaptability, an important factor for survival. Adaptability can thus influence plant population structure and function. In this paper we discuss the issue of shaping plant production by light in a moderate climate.

Our knowledge on adaptive strategies of particular plant species in different environments is scarce. It is not obvious how sunlight intensity and exposure duration can

affect biomass production and shape of plants in their natural habitats in a moderate climate.

This study aims at exploring how light variation can affect various aspects of plant production in a moderate climate. We will focus on two characteristics of plant production: influence of the sunlight on plant morphology and biomass production. This is motivated by fact that under limited light, competition for faster access to light might involve faster growth in height but may not necessary mean an increase in biomass. We tested the hypothesis that:

1. *Ranunculus lanuginosus* has higher biomass in meadows than forests.
2. *Ranunculus lanuginosus* species are shorter in meadows than forests.

Some previous works (Morawska-Płoskonka et al., 2010 and literature cited there) showed that environmental factors may be more important in organisms phenotype shaping, than biotic interactions between specimens. Plants can shaping their morphology in the sense of resources allocation in relation to different abiotic factors. Taking this into consideration we want to find tendencies in plant biomass production in response to the sunlight intensity.

We used *Ranunculus lanuginosus* as a model. It is an abundant species, easy to recognize and habiting various environments.

We expect that the sunlight will be constraining biomass production, which should be manifested in specimens exuberance.

Materials and methods

Study sites

We carried on the research in two ecosystems: a meadow and a forest. The ecosystems represented different sunlight conditions: high light intensity through whole day (the meadow) and low light intensity (the forest). The forest individuals inhabited small gaps, where intensity and duration of sunlight through the day were limited by trees surrounding the gaps. The meadow individuals were exposed to maximum sunlight intensity all day. Vegetation was much denser on the meadow.

Both sites were situated near Gaik-Brzezowa field station of the Institute of Geography of Jagiellonian University, near the south part of Dobczyckie Lake. They were located south-east from the Station. The forest was adjacent to the meadow.

Model organism

We were working on *Ranunculus lanuginosus*. It is a common, abundant species inhabiting both ecosystems and is easy to identify. Our observations indicate that it is affected by difference in light conditions.

Field work

We were sampling in 5 sites on the meadow and 10 sites in the forest. Larger sample size in the forest was necessary, to reduce variation in the data. We collected 5 specimens per site on the meadow. Due to lower abundance in the forest, 3 to 6 specimens per site were collected. We randomized meadow sites by throwing a piece of wood behind and sampling individuals surrounding the wood. In the forest randomizing was hindered because individuals were inhabiting gaps only. Therefore in the forest we randomized individuals in gaps by dividing the gap to the parts and collecting the nearest individual from each part. During sampling we omitted ecotone between the meadow and the forest.

At each site we measured height and biomass of every sampled individual. We measured the height as the distance between the ground and the highest node on the main (flowering) stem. We measured the biomass as a fresh weight of aboveground part of every individual. Thus we did not take into consideration differences in water content between the forest individuals and the meadow individuals.

We decided to treat as an individual one rosette containing only one main (flowering) stem surrounded by stems with leaves.

Statistics

We compared study sites using a one-way ANOVA. We compared mean height, mean biomass and mean standardized biomass. The standardized biomass means:

$$sB = \frac{b}{h \times S}$$

sB – standardized biomass of an individual,

b – mass of the aboveground parts of an individual,

h – height of an individual,

S – number of individuals (number of main (flowering) stems outgrowing measured rosette).

We divided b per h to indicate a vigor of an individual, which means the size of its leaves the number of vegetative stems (containing leaves only) and width of individual in relation to its height. We included S, because of difficulties in precise recognizing of an

individual. Sometimes two individuals were connected very closely and their division was impossible.

Results

We analyzed data on 52 samples from the forest ecosystem and 25 samples from the meadow. A one-way ANOVA demonstrated that mean height, mean biomass and mean standardized biomass were significantly higher for plants from the forest compared to the meadow. For the height: $F=72$, $df=1$, $p<0.05^{***}$, for the biomass: $F=29,6$, $df=1$, $p<0.05^{***}$, for the standardized biomass $F=14,7$, $df=1$, $p<0.05^{***}$. Means are shown in the table 1.

	height (cm)	biomass	standardized
Forest	46,39 ± 1,34	25,53 ± 1,88	0,46 ± 0,03
Meadow	28,15 ± 1,24	9,53 ± 1,46	0,28 ± 0,03

Table.1 Means and a standard error of mean for height, biomass and standardized biomass.

To establish the relationship between biomass and height, we performed Pearson's correlation analysis. No significant correlation could be demonstrated ($p=0.064$).

Discussion

Height, biomass and standardized biomass turned out to be higher in the forest. That is an evidence for affecting plant production by the sunlight. But the way of this affecting is unexpected. It is clear, that forest plants need to reach greater height. It ease the access the sunlight in shady habitats. But why should forest individuals invest more resources in a vigor? Instead of showed here significances, we observed that forest individuals have much bigger leaves, more leaves and are much more luxuriant, than the meadow ones.

Our results showed than sunlight intensity influences biomass and height in *R. lanuginosus*. However, contrary to our hypothesis, species in forests had higher biomass than in meadows. Two explanations are possible for this result. It is likely that forest species develop copying strategies to efficiently capture the limited light. Such strategies would be related to organs that capture light. From our field observations, we noted that forest plants have more and larger leaves than meadow plants. So, maybe vegetative organs, especially leaves, are of the most importance for fitness in shady conditions. Thus it is good to invest as much resources, as possible in growing not only height, but width as well, when the light is a limiting factor. That lead to conclusion that sometimes less light means higher biomass.

Secondly, the higher light intensity in the meadows might have caused higher rates of transpiration thus reducing biomass. Further still, possible degradation of chloroplasts which slows down the process of photosynthesis is also likely under high light intensity. It is known that high light intensity destroys chloroplasts which are vital in capturing sunlight energy and converting it to biomass. Meadow individuals are smaller and have little number of small leaves. They are hidden between specimens of other species. We think it may be an adaptation for less water loss.

Our data confirm that light intensity influences plant biomass and phenotype in this species. However greater height is not directly related to biomass. Biomass is more related to a vigor of individuals. Too intense light may provide to become stunted.

4.1.3 Reviews

→ **Prof. Tadeusz Kawecki** – Review of the project “Does sunlight affect plant morphology and biomass production?”

This study finds, surprisingly, that ramets of a buttercup species are taller and have a greater biomass (both raw and relative to plant height) when they are growing in a forest rather than in a meadow. The authors conclude that the meadow plants receive too much light, which is somehow harmful for them. I believe a simpler explanation is that light is not a limiting factor in the meadows; the authors should consider this alternative explanation.

The main limitation of the study is the fact that it did not manipulate sunlight; rather, two environments have been chosen that differed in total sunlight amount, but also in other ways (soil and air humidity, temperature, soil composition etc.). One thus cannot be sure if the systematic differences between the meadow and forest sites are due to sunlight rather than something else. Unless the authors can convincingly argue that it is indeed sunlight, they should acknowledge this caveat and tone down their conclusions.

Another caveat concerns the fact that only the above-ground biomass was measured. The authors should discuss the possibility that the meadow plants face stiffer competition for nutrients in the soil and thus invest a greater part of their resources in the roots than forest plants.

I also believe the following statistical issues should be addressed:

First, the design followed a stratified sampling scheme, with multiple plants measured for each of several sites within each environment. However, the different sites within an environment seem to have been pooled for the ANOVA. This is not quite appropriate; rather, sites should be treated as a random factor nested within the environment.

Second, the authors mention testing for correlation between biomass and height and finding none; however, the reported P is very close to significance. The details are not given (please give them), but this correlation seems to have been performed on data pooled across environments. However, given that the relationship between height and biomass seems to differ between environments, it would seem more appropriate to control for the environment type by including it in the analysis as a categorical explanatory variable. This new analysis would be an analysis of covariance.

Minor points:

Abstract: the first sentence misleadingly suggests that two light-related factors will be studied.

M&M: I am confused about the S variable; the definition seems to be circular. Are the authors trying to say that sometimes their individuals consisted of several ramets; in that case the entire "cluster" of ramets was weighed together and the total mass divided by the number of ramets?

Results: the actual P values should be given (rather than $p < 0.05$); if the asterisks are supposed to indicate the significance level, they will become superfluous. Also, please report both numerator and denominator degrees of freedom for F-tests.

Throughout: the authors should use a spell checker and grammar checker.

→ **Iwona Giska** - Review of the project *"Does sunlight affect plant morphology and biomass production?"*

In general I like the paper because it is clear and logical. Authors wanted to answer a question that at the beginning seemed to be quite predictable but they came with opposite results

than they had expected. It is important, if it is a matter of finding an interesting phenomenon or a mistake in making predictions. What are my more detailed remarks:

- The abstract is good as it is precise, clear and contains crucial information about the results of the research.
- The introduction is relevant to the research topic. It would be better if the future and present tense in third paragraph would be changed into past tense.
- I would suggest to write a bit more information about the species to have better base for the predictions because according to some sources *R. lanuginosus* is found mostly in forest habitat. It would change the hypotheses into opposite. Is the identification of the species proper?
- The description of the table should be above not under the table.
- I do not like the sentence in the first paragraph of discussion "It is *clear*, that forest plants need to reach greater height" . If it is *clear* why authors asked about that? And why is it clear if there are different strategies to obtain more light when it is limited? As mentioned later by authors increase of the surface of leaves that in forest could be more efficient as herbs will be never so high as trees that create the shadow.
- Except of previously mentioned sentence, I really like the discussion as authors fully described possible explanation of their findings.
- I think it is very difficult to forgive that at the end of the manuscript there is no detailed reference to the publication of Morawska-Płoskonka et al. 2010 mentioned in the introduction, especially as it comes from such a good journal like Nejczer!
- I suggest to check the title.

→ **Agata Miska** - Review of the project "Does sunlight affect plant morphology and biomass production?"

In general submit project is interesting and worth taking into consideration. Discussion which explains how obtained data refers to reality is the best part. On the other hand, it is possible to find some language mistakes.

In Introduction (line 16) you use contraction, which are inexcusable in formal language.

In material and methods (line 66) you describe how you obtain samples, but it is not clear for me, how does it look like in forest area... I wonder if you take a nearest individual, aren't they exposed to different conditions, than that from central part?

You measured (Line 71) fresh weight from ground to the highest node of plant -but fresh weight could be different in plants from meadow and forest. Better solution would be to measure dry masses and compare them.

In discussion (line 103) you write that "*It is clear, that forest plants need to reach greater height*". For me it is not so clear - in meadow species competition is huge, and some kind of solution for plants would be to produce longer stem in order to get more sunlight.

→ **Agata Rudolf** - Review of the project "*Does sunlight affect plant morphology and biomass production?*"

The structure of the paper is logic and clear. Each new thought begins in new paragraph which make the structure more visible. However, the language is a bit heavy so reading the whole paper is kind of tiring.

The thesis are strongly underlined which I liked, whereas the general concept is a bit confusing.

You mention about the possible role of accumulation the nutrients in the forest plants but you forget about it later in the discussion.

I think that finding possible explanation of your results could be little hard, because of plenty of other possible traits which could affect the experiment.

I like the explanation that large vegetative parts of the plant (leaves) in forest could be adaptation to "catching more light" and the small leaves on the meadow are adaptation to less water loss, but I think that in case of forest plants that could be also the adaptation to more water respiration instead of light influence. In forests there is more water in the air than in meadow, so plants have to be able to get rid of it when it is needed to avoid destroying their cells.

What is more I think that maybe the competition between the plants could also be affected. In meadow the competition between various plants such as grass species is very high, so maybe the limited factor for *Ranunculus* is the absence of some chemical

supplement which has been used out by other species in the meadow. Whereas, in the forest the composition of plants is different and is not including so many species of monocotyledons so, there is no need to “fight” for nutrients so desperately and Ranunculuses can grow larger. Whereas in meadow the plants have to compete much harder so they are not able to grow large. Maybe this doesn’t have anything to do with the light.

Another thing is that sometimes meadows could lose their quality if they are not cultivated by humans, because monocultures (or just wrong composition) of monocotyledons can cause that some nutrients will be used up. The forest is more “balanced” ecosystem.

The last thing that I have in mind is that maybe some toxic substances soak into the meadow soil from the human houses placed nearby and affect the Ranunculuses growth.

Of course these are only ideas which would have to be tested to find the right answer.

Also to give more clear view of your results one meadow and one forest is not enough, but of course we had only one day to conduct the field work.

Although, the project is interesting and ambitious. It has been written very nicely and I like it.

→ **Katarzyna Wężowicz** - Review of the project “Does sunlight affect plant morphology and biomass production?”

Authors undertook very interesting topic. It describes an adaptation of plants to growing in two sites with different amount of light.

Project is logical planned and carried out. The aims and predictions are clearly presented. Very interesting are results and discussion.

Unclear is statement “the nearest individuals in each part” in Material and Methods. The Latin name of tested species should be written in italics each time. Using the second time the Latin species name it is allowed to write the Latin generic name in a shorter form. There are some language mistakes.

4.1.4 Research report – final version

How does the sunlight affect plant height and biomass production?

Abstract

We investigated the influence of light intensity on plant height and above ground fresh weight using *Ranunculus lanuginosus* as a model species. Forest patches were chosen to represent low light intensity as compared to open meadows. We found that forest individuals were 1.5 higher and 2 times heavier than meadow ones. We concluded that an increase in sunlight intensity does not always result in higher plant weight. Other factors could be limiting. Resources allocation to vegetative organs in shady ecosystems may play a role in the above-ground biomass production. The influence of other environmental factors remains ambiguous.

Introduction

In general, plant production is influenced by abiotic and biotic factors. But we don't know how plants would respond to various abiotic factors in different ecosystems. Organisms' phenotypes can be shaped by a number of abiotic factors. Exposure to sunlight is among the most important factors, as far as plants are concerned. Differences in light conditions are documented to influence plant phenotype, such as biomass or morphology. Such influence may reflect a plant's adaptability, an important factor for survival. In this paper we discuss the issue of shaping a plant morphology and biomass by light.

Our knowledge on adaptive strategies of particular plant species in different environments is scarce. It is not obvious how sunlight intensity and light exposure duration can affect biomass production and shape plants in their natural habitats.

This study aimed at exploring how light variation can affect various aspects of plant production. We focussed on two characteristics of plant production: the influence of the sunlight on plant morphology and fresh weight. Our choice is motivated by the fact that under limited light competition for access to light might involve faster growth in height but may not necessarily mean an increase in biomass. We tested the hypotheses that: *R. lanuginosus* has higher above ground fresh weight but a shorter height in meadows than forests.

Forest specimens inhabit small light patches, penetrated by the sunlight because of gaps in canopies. Taller individuals have a greater chance to gain as much light as they need for the development and reproduction. We used *Ranunculus lanuginosus* as a model species. It is an abundant species, easy to recognize, habiting various environments.

Materials and methods

We carried out the research in two ecosystems: a meadow and a forest. The ecosystems represented different sunlight conditions: high light intensity through the whole day (the meadow) and low light intensity (the forest). The forest individuals inhabited small gaps where intensity and duration of sunlight through the day were limited by trees surrounding the gaps. The meadow individuals were exposed to maximum sunlight intensity all day. Vegetation was much denser on the meadow. Both sites were situated near Gaik-Brzezowa field station of the Institute of Geography of Jagiellonian University, near the south part of Dobczyckie Lake. They were located south-east from the Station. The forest was adjacent to the meadow.

Samples were collected from 5 sites on the meadow and 10 sites in the forest. Larger sample size in the forest was necessary to reduce the variation in the data. Our preliminary research showed much greater variation in the forest. On all the meadow the conditions were the same. We collected 5 specimens per site on the meadow. Due to the lower abundance in the forest, 3 to 6 specimens per site were collected. We randomized meadow sites by throwing a piece of wood behind and sampling individuals surrounding the wood. In the forest randomizing was hindered because individuals inhabited gaps only. Therefore in the forest we randomized individuals in gaps by dividing the gap to the parts and collecting the nearest individual from each part.

At each site we measured height and fresh weight of every sampled individual. We measured the height as the distance between the ground and the highest node on the main (flowering) stem. We measured the fresh weight of aboveground part of every individual. We did not take into consideration the differences in water content between the forest individuals and the meadow individuals.

We decided to treat one rosette containing only one main (flowering) stem surrounded by stems with leaves as an individual.

We compared study sites using a one-way ANOVA. We compared mean height, mean biomass and mean standardized biomass. The standardized biomass means:

$$sB = \frac{b}{h * S}$$

sB – standardized biomass of an individual,

b – mass of the aboveground parts of an individual,

h – height of an individual,

S – number of individuals (number of main (flowering) stems outgrowing measured rosette).

We divided b per h to indicate a vigor of an individual, which means the size of its leaves, the number of vegetative stems (containing leaves only) and the width of individual in relation to its height. We included S because of some difficulties in the precise recognition of an individual. Sometimes two individuals were connected very closely and their division was impossible.

Results

We analyzed 52 samples from the forest and 25 samples from the meadow. A one-way ANOVA demonstrated that mean height, mean biomass and mean standardized biomass were significantly higher for plants from the forest compared to the meadow. Means and statistics are shown in the table 1.

Table1. Height and Fresh weight (Means ± SE) of plants from the two sites.

	Height (cm)	Fresh weight (g)	Standardized biomass (g/cm)
Forest	46,39 ± 1,34	25,53 ± 1,88	0,46 ± 0,03
Meadow	28,15 ± 1,24	9,53 ± 1,46	0,28 ± 0,03
F	72	29,6	14,7
df	1	1	1
p	<0.0001	=0.001	=0.001

To establish the relationship between height and fresh weight, we performed a correlation analysis. No significant relationship could be demonstrated.

Discussion

Height, biomass and standardized biomass turned out to be higher in the forest. But the main force affecting plant production remains ambiguous. We didn't control all environmental factors acting in both ecosystems. Instead of the sunlight, soil structure and

stoichiometry, humidity and temperature may be important factors shaping plant phenotype.

Sunlight intensity may influence fresh weight and height in *R. laginosus*. However, contrary to our hypothesis, species in forests had higher fresh weight than in meadows. Two explanations are possible for this result. It is likely that forest species develop the coping strategies of the efficiency in capturing the limited light. Such strategies would be related to organs that capture light. From our field observations, we noted that forest plants have more and larger leaves than meadow plants. So, it may be that vegetative organs, especially leaves, are of the most importance for fitness in shady conditions. Thus it is good to invest as much resources as possible in a growth in not only height but width as well, in a case when the light is a limiting factor. This leads us to the conclusion that sometimes less light means higher biomass. Secondly, the higher intensity of the light in the meadows might have caused higher rates of transpiration thus reducing biomass. Furthermore, still possible degradation of chloroplasts, which slows down the process of photosynthesis, is also likely under the high light intensity. It is known that high light intensity destroys chloroplasts, which are vital in capturing sunlight energy and converting it to biomass. Meadow individuals are smaller and have little number of small leaves. They are hidden between specimens of other species. We think it may be an adaptation for less water loss.

We measured above-ground fresh weight only. But below-ground fresh weight should be measured as well in order to see if the meadow plants are forced to allocate their resources in below-ground fresh weight rather than in above-ground.

Our data confirm that light intensity may influence plant fresh weight and morphology in this species. However greater height is not directly related to biomass. Biomass is more related to a vigour of individuals. The role of the sunlight in relation to other environmental factors remains uncovered. Thus, more detailed studies are needed to eliminate other environmental factors' influence. The use of fresh weight on assumption that both ecosystems have same soil water content is a limitation in this study. Future studies need to consider measuring both above ground and below ground biomass in addition to soil nutrient studies for better results.

4.2 Does terrain morphology affect the density of earthworm populations?

Iwona Giska & Marcin Plech

4.2.1 Project proposal

Does topography have impact on distribution and the density of earthworm populations?

Summary

Earthworms are commonly distributed through various habitats all over the world, including forests, meadows and crop fields, but the population density and their number vary. Although numerous studies have revealed earthworm dependence on the chemical characteristics of the soil and its moisture, most of them ignored the geographical and topographical factors. We assume that the type of area may be of prolific importance and want to check if the shape of terrain can affect the abundance of earthworms. Epigeic earthworm species are expected to be more vulnerable to the changes of ruggedness of the terrain therefore this group will be the object of this study. Earthworms will be counted in the samples of soil taken from slopes of hills differing in the type of habitat. Mean number of earthworms between sampling sites will be compared between sampling sites. Obtained results may help to establish additional measures of discriminating between areas suitable for agriculture.

Aims

The aim of this study is to check if topography is a relevant factor for the earthworms when choosing habitat. We specifically want to check how population distribution and density vary between flat and hilly sites and how population density, calculated as the number of specimens per square meter area, change in relation to increasing ruggedness of slopes.

The hypothesis that will be tested is that the number of earthworms is lower at slopes than on the base of hills. Additionally it will be checked whether it is a general or habitat dependent phenomenon.

Methods

The study will be carried out in the area of Little Poland in the vicinity of Dobczyce Lake. It is a hilly area, rich in various ecological habitats. Among these habitats are meadows and crop

fields, both inhabited by earthworms. Hills differed in habitat will be chosen as the study sites. The habitats will be two meadows and two crop fields. At each study site there will be three sampling points (A, B, C) located at different height of the hill (Fig.1). At each point five replicate samples of soil will be taken. Soil will be dug from the area of 50 x 50 cm to a depth of 20 cm. It will be hand sorted at the site. Earthworm specimens, both adults and juveniles, will be counted.



Fig. 1. Location of sampling points along the hill slope.

To check if density of earthworms depends on the height of sampling point on the slope and habitat type two-way ANOVA will be performed with habitat type and the height of sampling point as the factors and mean number of found earthworms as dependent variable.

Expected results

Soil pH, organic matter content and moisture are one of the most important environmental factors that influence the distribution of earthworms (Edwards and Bohlen, 1996). It has been already reported that number of earthworms is larger on meadows than on crop fields. It was explained by less intensive soil cultivation and high level of organic matter on meadows (Didden, 2001). Furthermore denser vegetation on meadows stabilizes in comparison to crop fields. Considering these facts, it is predicted that on meadows the number of recorded earthworms will be the highest among all studied habitats. Regarding the height of the slope, it is expected that the disturbances in surface layer of the soil will increase with increased height. It will be stressful for epigeic earthworms species such as e.g. *Lumbricus rubellus* or *Dendrobaena sp.* Based on that, it is expected that at higher location the number of recorded earthworms will be lower than at the base of the hills.

References

- Didden W.A.M. (2001)** Earthworm communities in grasslands and horticultural soils. *Biol Fertil Soils* (2001) 33: 111–117
- Edwards CA, Bohlen PJ (1996)** Biology and ecology of earthworms. Chapman and Hall, London

4.2.2 Research report – first version

Does topography affect distribution and the density of earthworm populations?

Abstract

Earthworms communities can be affected by multiple factors related to the characteristics of the soil and environment, however there was no research on the impact of topography, meaning the steepness of the terrain. As the steep hills are more affected by the wind and water flow they seem to be less stable. We checked if these possible disturbances on steep slopes affect earthworms population sizes. We counted earthworms present in soil samples collected from sites differing in steepness and habitat sites. We did not find any influence of steepness on the density of earthworms populations. We confirmed that meadows, where earthworm populations are seven times larger than in forests, are more suitable type of habitat for this group of soil invertebrates

Introduction

Many studies concerning the distribution of earthworms in relation to environmental conditions have already been done but none has asked whether topography affects earthworms communities. We assume that topography, meaning the steepness of the terrain, has an influence on earthworms abundance. Earthworms are present in various habitats all over the world but their number depends on environmental conditions. The pH, moisture and organic matter content are main soil properties that influence earthworms distribution (Edwards and Bohlen 1996, Didden 2001). These properties can be affected by other environmental factors, which additionally may create more stressful conditions. Steep hills, exposed to strong wind or intensive water flow, can disturb the soil structure. Vegetation type and human activity are among the factors that can influence intensity of these disturbances. Dense vegetation protects surface layer of soil from erosion caused by wind or water. The erosion processes are also affected by soil cultivation practices that make soil less stable. These factors seem to be more important on steep hills where erosion often occurs. In this case the type of habitat is important for soil stabilization.

When the surface layer of the soil is disturbed, it is stressful for earthworms living there. Among all earthworm species, epigeic earthworms are expected to be strongly affected as

they are abundant close to ground surface. Endogeic and anecic earthworms that live deep in the soil do not seem to be vulnerable to the disturbances observed on rugged terrain.

The aim of the study is to check whether steepness of the terrain together with habitat type affects earthworms communities. For this purpose we conducted research on hills differing in habitats. We took samples of soil at sites with different steepness on hills in the forest and on the meadow. Subsequently we counted earthworms present there. According to our predictions the type of habitat is important for the size of earthworm population. We did not find any effect of topography on the density of earthworms.

Materials and methods

We performed the study near Dobczyce Lake in the region of Little Poland. We picked there one hill in the forest and one on the meadow. At three various heights of the slope differing in steepness we designated sampling points: on the top, in the middle, at the bottom. We collected three soil samples from each point (Fig.1). Soil was dug from the area of 40 x 40 cm to a depth of 20 cm. We counted earthworms in each sample and calculated their density per square meter.

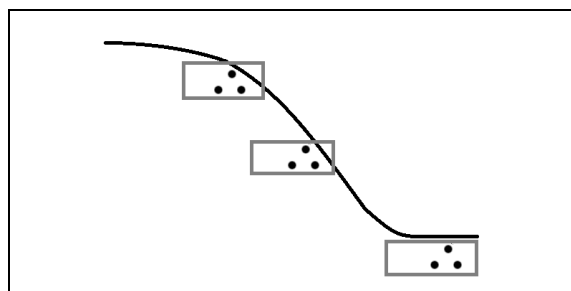


Fig. 1 Schematic representation of sampling method. Particular sampling points are marked with grey frames, each sample is represented by a black spot.

Our hypotheses concerning the impact of the habitat type and the steepness of the terrain on the density of earthworms were tested using two-way ANOVA. We used SPSS software (SPSS Statistics, ver. 17.0.1, WinWrap).

Results

Number of earthworms that we found at each sampling site in different habitats is shown on Figure 2. On meadows the number of earthworms was about six times higher than in forests

with the average density 106.9 ± 37.2 specimens/m² for all meadows sites and 15.3 ± 11.7 specimens/m² for forests.

The two-way ANOVA results show that the steepness of the terrain does not have significant impact on the density of earthworms ($p=0.723$ for forest, $p=0.197$ for meadow).

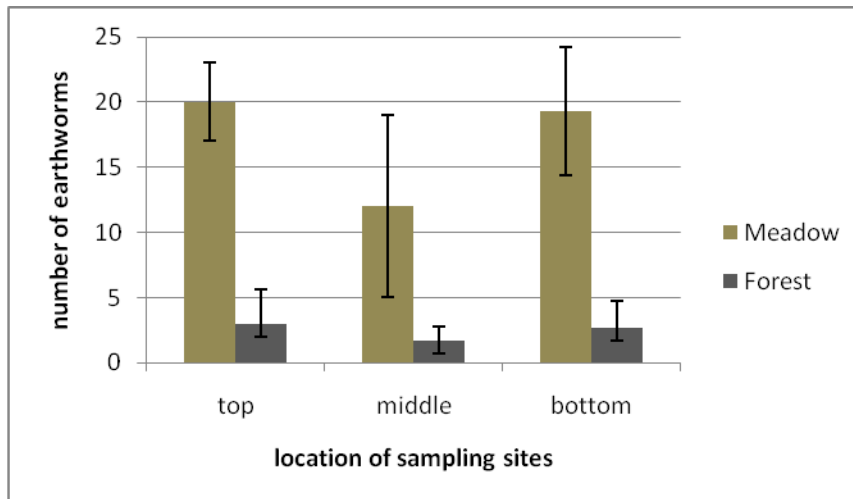


Fig. 2 Number of earthworms present on the top, middle and bottom of hills differing in habitat type.

Discussion

The results of the study show that steepness of terrain does not affect earthworms density. It can be explained by dense grass and herbs cover on meadows. This vegetation protects soil on steep slopes and animals living there are not more stressed than these from flat terrain. Forest vegetation, especially trees also act as protecting layer. Differences that we found between meadows and forests are in agreement with the literature. According to previous work earthworms are more abundant on meadows than in different habitats (Didden 2001). We assume that the results might be affected by the type of soil. At all sampling points we sampled clay soil which has dense structure generally more stable. To fully answer the research question more hills should be analyzed taking into account also different types of soil.

References

- Didden W.A.M. (2001)** Earthworm communities in grasslands and horticultural soils. *Biol Fertil Soils* (2001) 33: 111–117
- Edwards CA, Bohlen PJ (1996)** Biology and ecology of earthworms. Chapman and Hall, London

4.2.3 Reviews

→ **Prof. Tadeusz Kawecki** - Review of the project *“Does topography affect distribution and the density of earthworm populations?”*

The authors make a good case why the steepness of a slope should have consequences for the ecology and thus presumably the population density of earthworms, so the study is well justified. The writing is generally lucid and clear.

The greatest weakness of the paper is the fact that only one slope transect in each of the two environments has been included. As a consequence of the small sample size, the negative results are not sufficiently convincing. At least, confidence intervals for the differences between elevations should be reported to let the reader know what the size of the effect that could have been missed is.

Furthermore, while the introduction makes the case for the steepness of the slope as being important, the methods and results are formulated in terms of sites at different elevations along a slope. The relationship between the two is never explained. Is the middle part the steepest? The steepness of the terrain at each site should be estimated and reported.

Finally, I have a couple of comments on the analysis:

First, the statistical model used is not clear; 2-way ANOVA is mentioned, but then the results report separate analysis for each environment. The data show some parallels between the two environments in that in both the middle elevation tends to have the lowest densities, so it would make sense to analyze the data together.

Second, the data are counts, so the error is expected to follow the Poisson distribution. Therefore it would have been more appropriate to analyze the data with a generalized linear model with a Poisson error distribution. ANOVA is also problematic because the variances are not expected to be independent of the means.

Third, the meaning of bars in the figure and the \pm values in the text should be clarified: are these standard deviations or standard errors?

→ **Geoffrey Dheyongera** - Review of the project "Does topography affect distribution and the density of earthworm populations?"

In your abstract and introduction you mention that you looked at disturbance in terms of erosion by wind and water flow. To me, in a forest and meadow, I don't think wind and water flow can have an impact on an earthworm some centimeters or so under soil.

- What were your reasons for assuming that densities of earthworms some distance under soil would be different?
- Would wind or water flow redistribute these worms? I can't imagine such erosion in a meadow and forest.
- If wind and water flow redistribute worms they would not operate here since the soil is well covered, the result of no effect of steepness as found by the study would in this case be expected even before the research.
- The higher densities in the meadows might reflect a sampling error or chance event. The 7 times magnitude of difference appears too big to be explained by the small forces of wind and water flow in these habitats.
- To me the bolded sentence is not clear. It seems to imply that there are seven times more populations in meadows than forests. Is this the case or you mean population size?

The assumption of that type of soil might be the issue for higher densities in meadows as stated in discussion would hold if in your field work you observed differences in soil type between the meadows and forest. Therefore you want to think that this might be the source of variation. Is this soil type variation a reality? What types did you observe? I think soil stability is not the point causing differences. In fact both study sites have stable soil. It is highly likely that soil structure and chemistry are more important than soil stability. I see that you consider some of these factors in your next plan. These may be more informative.

→ **Michał Filipiak** - Review of the project *“Does topography affect distribution and the density of earthworm populations?”*

I have one general reservation: topography means position of elements forming particular area. You were asking about terrain morphology – that means a landform features. You use clear and simple language. The paper is pleasant to read.

In more here:

Abstract: Not steepness but inclination.

Introduction: Nice, logical, easy to read. I can't find unnecessary information, every sentence is closely related to the study topic. I like it.

Materials and methods: Simple and clear. I like it. Figure 1 is not necessary in my opinion.

Results: In previous section you mentioned about one meadow. Now I see: meadows. In the abstract the number of the earthworms is 7 times higher, now – 6.

Results: You write: **the** literature. I ask: what literature? “The” is not necessary here. In the next to last sentence sampling has another, funny, meaning. I would rather use “taking a soil sample” than “sampling”.

→ **Agata Rudolf** - Review of the project *“Does topography affect distribution and the density of earthworm populations?”*

It need to be said that authors show a large writing talent. The style in which the text is written is simple but precision, and make the whole text quite clear and easy to read.

There is, however, a problem with organization. There are some main titles of each part of the text, but then everything is written in one paragraph. The text wrote in such way is overloaded and it is difficult to keep order of this what you wanted to say. If each part is divided to paragraphs it is also much better for the reader to follow the text. Each new thought should be represented by new paragraph. For example like that:

“Introduction:

Many studies concerning the distribution of earthworms in relation to environmental conditions have already been done but none has asked whether topography affects earthworms communities.

We assume that topography, meaning the steepness of the terrain, has an influence on earthworms abundance.

Earthworms are present in various habitats all over the world but their number depends on environmental conditions. The pH, moisture and organic matter content are main soil properties that influence earthworms distribution (Edwards and Bohlen 1996, Didden 2001). These properties can be affected by other environmental factors, which additionally may create more stressful conditions.

Steep hills, exposed to strong wind or intensive water flow, can disturb the soil structure. Vegetation type and human activity are among the factors that can influence intensity of these disturbances. Dense vegetation protects surface layer of soil from erosion caused by wind or water. The erosion processes are also affected by soil cultivation practices that make soil less stable. These factors seem to be more important on steep hills where erosion often occurs. In this case the type of habitat is important for soil stabilization.

When the surface layer of the soil is disturbed, it is stressful for earthworms living there. Among all earthworm species, epigeic earthworms are expected to be strongly affected as they are abundant close to ground surface. Endogeic and anecic earthworms that live deep in the soil do not seem to be vulnerable to the disturbances observed on rugged terrain.

The aim of the study is to check whether steepness of the terrain together with habitat type affects earthworms communities. For this purpose we conducted research on hills differing in habitats. We took samples of soil at sites with different steepness on hills in the forest and on the meadow. Subsequently we counted earthworms present there. According to our predictions the type of habitat is important for the size of earthworm population. **However**, we did not find any effect of topography on the density of earthworms.”

See? Now you can easily follow the text (It is the same, no changed text) and it is easily to decide if you still want to add something, and if so, than where exactly it should be added.

“Materials and methods:

We performed the study near Dobczyce Lake in the region of Little Poland. We picked there one hill in the forest and one on the meadow. (Should be more than one meadow and

one forest, but I know, I know- rain, cold, and you had only one day left to conduct the field work) At three various heights of the slope differing in steepness we designated sampling points: on the top, in the middle, at the bottom. (Was it three heights in the meadow and three heights in the forest? Because it is a bit confusing and not clear said here.) We collected three soil samples from each point (Fig.1). Soil was dug from the area of 40 x 40 cm to a depth of 20 cm. We counted earthworms in each sample and calculated their density per square meter.”

“The two-way ANOVA results show that the steepness of the terrain does not have significant impact on the density of earthworms ($p=0.723$ for forest, $p=0.197$ for meadow).

The results of the study show that steepness of terrain does not affect earthworms density.”

These 2 sentences says exactly the same, and they are directly one after another. When you read this it is like: “I have just read that, did I ? Or maybe it is something else and I misunderstood? I will go back and see... “ I know that for discussion you need to say it once again, but to avoid this repeat you should start your discussion on the observation that earthworms density is higher in meadows than in forests, and then go to that point about non significant impact of steepness of terrain on the density of earthworms.

Expect those things which I have pointed out I liked the report very much. Also the result showing that there is less earthworms in forest than in the meadow seem curious to me.

→ **Magdalena Surówka** - Review of the project “Does topography affect distribution and the density of earthworm populations?”

The text is written in quite clear way. The sentences are logically connected. However sometimes sentences are too long and too complicated what make it harder to understand.

In Introduction authors as an explanation for their studies put the fact that there weren't previous study about these specific conditions. Maybe there is more interesting reason to precede this study for example potential agricultural apply.

In Material and Methods there is defined location where experiment took place. Authors translate name of polish region: Małopolska. As far as I know there is no need to

translate the names of regions of Poland, except from Śląsk which have formalized translated name - Silesia.

In Discussion the third sentence isn't clear.

I prefer if text was justified.

At the end I would like to express my admiration about dedication for the project exposed by digging holes in very bad weather conditions.

4.2.4 Research report – final version

Does terrain morphology affect the density of earthworm populations?

Abstract

Earthworms communities can be affected by multiple factors related to the characteristics of the soil and environment, however there was no research on the impact of terrain morphology, meaning the steepness of the terrain. As the steep hills are more affected by the wind and water flow they seem to be less stable. We checked if these possible disturbances on steep slopes affect earthworms population sizes. We counted earthworms present in soil samples collected from sites differing in steepness and habitat sites. We did not find any influence of steepness on the density of earthworms populations. We confirmed that meadows, where earthworm populations are seven times larger than in forests, are more suitable type of habitat for this group of soil invertebrates.

Introduction

Many studies concerning the distribution of earthworms in relation to environmental conditions have already been done but none has asked whether topography affects earthworms communities. We assume that topography, meaning the steepness of the terrain, has an influence on earthworms abundance. Earthworms are present in various habitats all over the world but their number depends on environmental conditions. The pH, moisture and organic matter content are main soil properties that influence earthworms distribution (Edwards and Bohlen 1996, Didden 2001). These properties can be affected by other environmental factors, which additionally may create more stressful conditions. Steep

hills, exposed to strong wind or intensive water flow, can disturb the soil structure. Vegetation type and human activity are among the factors that can influence intensity of these disturbances. Dense vegetation protects surface layer of soil from erosion caused by wind or water. The erosion processes are also affected by soil cultivation practices that make soil less stable. These factors seem to be more important on steep hills where erosion often occurs. In this case the type of habitat is important for soil stabilization.

When the surface layer of the soil is disturbed, it is stressful for earthworms living there. Among all earthworm species, epigeic earthworms are expected to be strongly affected as they are abundant close to ground surface. Endogeic and anecic earthworms that live deep in the soil do not seem to be vulnerable to the disturbances observed on rugged terrain.

The aim of the study is to check whether steepness of the terrain together with habitat type affects earthworms communities. For this purpose we conducted research on hills differing in habitats. We took samples of soil at sites with different steepness on hills in the forest and on the meadow. Subsequently we counted earthworms present there. We found that terrain morphology can affect density of earthworms. According to our predictions the type of habitat is important for the size of earthworm population.

Materials and methods

We performed the study near Dobczyce Lake in the region of Little Poland. We picked there one hill in the forest and one on the meadow. At three various heights of the slope differing in steepness we designated sampling points: on the top, in the middle (the steepest point), at the bottom. We collected three soil samples from each point (Fig.1). Soil was dug from the area of 40 x 40 cm to a depth of 20 cm. We counted earthworms in each sample and calculated their density per square meter.

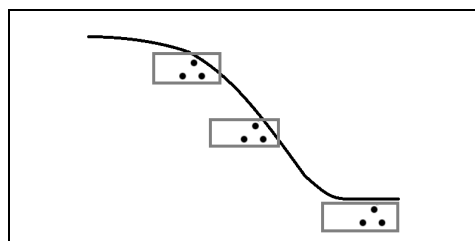


Fig. 2 Schematic representation of sampling method. Particular sampling points are marked with grey frames, each sample is represented by a black spot.

Our hypotheses concerning the impact of the habitat type and the steepness of the terrain on the density of earthworms were tested using regression analysis (generalized linear model with a Poisson error distribution). We used SPSS software (SPSS Statistics, ver. 17.0.1, WinWrap).

Results

Number of earthworms that we found at each sampling site in different habitats is shown on Figure 2. On meadows the number of earthworms was seven times higher than in forests with the average density 106.9 ± 37.2 specimens/m² for all meadows sites and 15.3 ± 11.7 specimens/m² for forests.

Statistical analysis revealed that:

- Steepness decreases the density of earthworm populations ($\chi^2=7,891$, $p=0.019$)
- Habitat type is a significant factor determining the density of earthworms ($\chi^2=72,891$ $p < 0.05$)

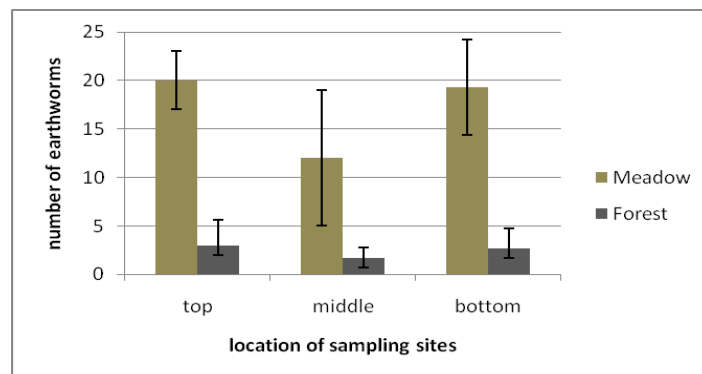


Fig. 2 Number of earthworms present on the top, middle and bottom of hills differing in habitat type (with bars representing means and lines SD values).

Discussion

Our results show that terrain morphology could affect earthworms density. At middle sites, which were considered as the steepest, number of earthworms was the lowest. It can be explained by increased soil disturbances caused by wind or water. The results should be considered as preliminary. Differences between sampling site varying in steepness are small. Dense grass protects soil on steep slopes and animals living there are not more stressed than these from flat terrain. It would be worth to examine this problem also in agriculturally cultivated areas where soil surface is bare and the effect of terrain morphology could be

easily visible. Forest vegetation, especially trees also act as protecting layer. Differences that we found between meadows and forests are in agreement with the literature. According to previous work earthworms are more abundant on meadows than in other habitats (Didden 2001). We assume that the results might be affected by the type of soil. At all sampling points we sampled clay soil which has dense structure generally more stable. To fully answer the research question more hills should be analyzed taking into account also different types of soil.



References

Didden W.A.M. (2001) Earthworm communities in grasslands and horticultural soils. *Biol Fertil Soils* (2001) 33: 111–117

Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms. Chapman and Hall, London

4.3 Does ambient temperature influence ants walking speed?

Agata Miska & Katarzyna Wężowicz

4.3.1 Project proposal

How ambient temperature influence on ants speed?

Summary

Ants are organisms which play essential role in terrestrial ecosystems. They spread plants seeds, become a part of food chains and could be in symbiosis with other species like aphids or butterflies. Because of the perfect ants' colony organization, it is possible for them to transport tree litter even for long distances. To fulfill this function high locomotory activity is required. The aim of the study is to assess the impact of different temperatures on black garden ant (*Lasius niger*) activity. The prediction is that low temperature slows down ants' movements, which are measured by their speed.

Aims

The key point of the study is to assess the impact of different temperature on ants' behavior. Ants are going to be tested in two different temperatures; daily temperature (19°C) and decreased temperature (around 4°C). As an indicator of their activity, running speed will be measured. Hypothesis: Ants move slower in low temperature.

Existing Knowledge

All insects have specific optimum temperature for living. Physiological processes are limited when deviations from the optimum temperature appear. Conditions (temperature, humidity, pH) inside ants nests differ from surrounding environment; differences of temperature inside and outside the nest can amount up to $\pm 20^{\circ}\text{C}$.

Methods

Ants (*Lasius niger*) are going to be gathered randomly in meadow in Gaik, near Dobczyce Lake between 5-7 p.m. Each individual will be tested once in a plastic tube (length 19cm). Time need to overcome the distance from one end of the tube to the other one will be measured.

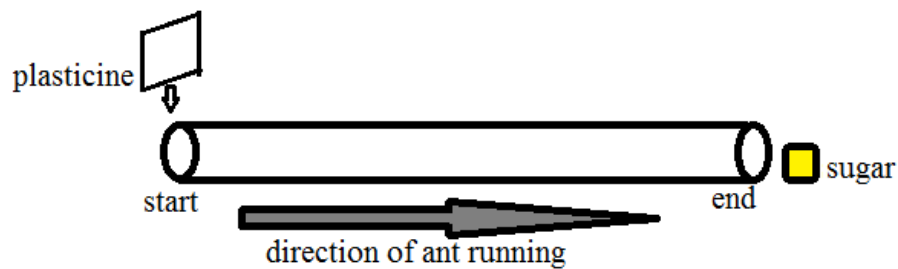


Fig. 1 Scheme of experiment.

The exit of the tube (called “end” – fig. 1) will be soaked in a sugar solution which will be used as a chemo attractant. After putting ants into the tube, the entrance will be locked by using plasticine. Experiment will be carry on two groups of ants, each composed of 50 individuals.

The first group will be tested in temperature of 19 °C. This temperature is in range of average daily temperature for April - June in Poland. Behavior of the second group will be analyzed in low temperature (around 4°C). During trial the tube will be placed in the ice. After each trial individuals will be collected in a box to avoid testing the same insect once again. Obtained data will be analyzed by using t-student test.

Expected results

It is presumed that activity of ants from the second group is limited. Decreased temperature will be affected as a stress factor, and in consequences the speed of movements will be lower.

4.3.2 Research report – first version

Do ambient temperature influence ants walking speed?

Summary

Ants are organisms which play essential role in terrestrial ecosystems. They are poikilothermic organisms what means changing body temperature, simultaneously with changing ambient temperature. The aim of this study is to assess the impact of different temperatures on black garden ant (*Lasius niger*) walking activity. We hypothesize that in low temperature ants activity is limited, because it disturbs chemical processes in ants' body. The prediction is that low temperature slows down ants' movements. We controlled ants speed in two different temperature conditions; diurnal temperature (20°C) - the range of average daily temperature for April - June in Poland and decreased temperature (around 4°C). This study shows that ants speed from low temperature is 85% lower than from the other group.

Introduction

All insects have specific optimum temperature for living. Physiological processes are limited when deviations from the optimum temperature appear. Ants are poikilothermic organisms, and because of their small size, body temperature may rapidly change in different ambient conditions. From chemical point of view high body temperature results in peptides denaturation. Decreased temperature results in lower activity of enzymes, what caused in disturb all chemical processes. In other insects species limited reproduction abilities appear. We want to check how various temperatures influence on ants physiological processes measured by activity. We hypothesize that in low temperature ants activity is limited, because it disturbs chemical processes in ants' body.

Ants are social insects, widespread all over the world. Their natural habitats are inter alia meadows, forests, cities. Their foraging targets are other insects, eggs of other species, honey dew of aphids and plant parts. They spread plants seeds, pollen, become a part of food chains, can be in symbiosis with other species like aphids or butterflies. Despite of small size they could be an important predator when they attack in group. Because of the perfect

ants' colony organization, it is possible for them to transport even for long distances much bigger thing than they are, like tree litter or animals body remains. Their main communicational signals are chemical substances. They play significant role in ecosystems. Mentioned abilities may be limited, because of changing in ants movements. We would like to check if there is any dependence between temperature and ants walking activity.

We predict that in low temperature ants movement will be limited. We are going to measure in two different temperatures time in which black garden ants will reach target. Obtained data will be transformed into speed.

Materials & Methods

Black garden ants workers (*Lasius niger*) gathered randomly in a meadow around research station in Gaik, near Dobczyce Lake. Experiment carried out simultaneously in two groups of ants, each composed of 15 individuals. The first group tested in room-temperature (around 20 °C). Walking speed of the second group analyzed in low temperature (around 4°C). Separated ant put on ice in for 45s before trial – longer time on ice resulted in constant lack of movements. During trial the tube placed in the ice. Each individual was assayed once.

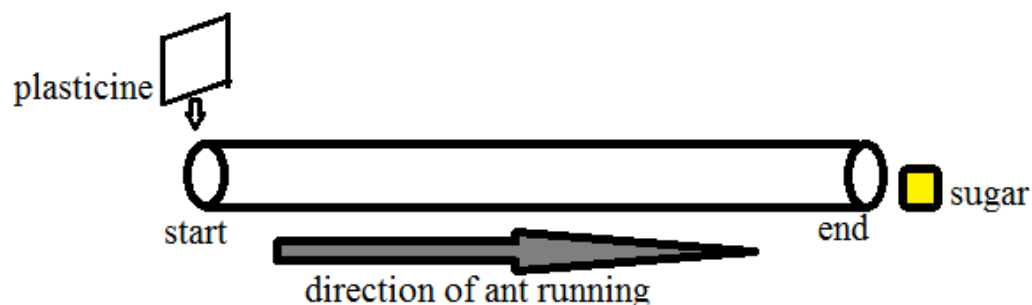


Fig. 1. Scheme of experiment.

Each individual tested once in a plastic tube (length 19cm, diameter 4mm). The exit of the tube (called "end" – fig. 1) soaked in a sugar solution which used as a chemo attractant. Simultaneously we put ant into the tube, started measure the time and lock the entrance. Time needed to travel the distance from one end of the tube to the other one measured. All ants walked straight, without changing direction. Pilot experiment with 50 cm long tube

showed that such a long tube isn't appropriate for this experiment. Far distance to chemo attractant resulted in ants backing off.

The obtained data calculated by using formula:

$$v=s/t$$

v-speed [cm/s]; s-distance [cm]; t-time [s]

Two obtained independent data sets analyzed by using t-Student test to compare their means. By using Two Way Anova test analyzed connection of each pairs tested in the same time. Also connection of each sample in order analyzed.

Results

In order to check differences in ants' activity we measured their speed in different temperature conditions. We found significant differences between measured speeds.

	low temperature	high temperature
mean value of speed [cm/s]	3,88	0,83
t=13,26, F=4,14, p=0,01		

Table 1. Mean value of speed in two groups of ants.

There is no connection between pairs, and between all pairs putted in order.

Discussion

This study has shown that there is significant difference between ants walking activity in two different temperatures. It is possible for ants to leave chemo – signals, in order to inform other about inter alia way. We rejected this option in this study by using Anova test.

Decreased temperature may influence on ants physiological processes. Temperature slows down chemical processes, what in consequences inter alia limit ants' movements. In broaden understanding it may disturb the most important process, that leads to species surviving. Reproduction in decreased temperature is limited in other insects.

Limitation of ants' moving activity also may influence on other species. Seeds and pollen spreading could be limited. Hyphae fungi may not change location, that in consequences disturb plant - fungi symbiosis.

It would be interesting in further studies to check if limited ants movement have impact on working of ecosystems. Also it would be interesting to select one line of ants to cold temperature and check the differences in their behavior and reproduction tasks.

4.3.3 Reviews

→ **Prof. Tadeusz Kawecki** – Review of the project *“Do ambient temperature influence ants walking speed?”*

This ms addresses the issue of how the well-known dependence of physiological processes on body temperature in invertebrates translates into changes in behavioral performance. I believe this is interesting; although we know that the rate of metabolic reactions is roughly proportional to temperature above 0 C, it is not well understood if behavioral performance (in this case running speed) also scales linearly with temperature. Yet, this thermal dependence of behavior has consequences for fitness-related traits, such as foraging or predator avoidance.

Unfortunately, focusing on just two experimental temperatures precludes an explicit test of the hypothesis that the relationship between the running speed and temperature is linear and possibly proportional. This is the single biggest weakness of this study. Instead, the authors test a more modest hypothesis that the speed is lower at 4 C than at 19 C; it is hardly surprising that this hypothesis is supported. I would strongly recommend that the authors repeat the experiments with 4-6 temperature points within the ecologically relevant range.

Nonetheless, the authors could still check if their results are consistent with running speed being proportional to temperature. In fact, the mean speed/temperature relationship for the two temperatures seems remarkably close to proportionality. I am surprised that the authors apparently missed this very interesting fact.

The general experimental approach is sound; while I would be worried about the reliability of the assay being compromise by lack of ants' motivation to cross the tube, this was apparently not a problem (nonetheless, the authors should be more precise; e.g., how often did the ants stop in the middle).

Several other points should be addressed:

(1) I think the introduction should emphasize/elucidate better the potential difference between the linear dependence of metabolic processes and the potentially more complex – and unknown – effect on behavioral performance.

(2) It is not clear what is the distribution of the speed measurements; I would also be surprised if variance of speed were the same at both temperatures. The authors should examine their data more closely and consider doing the analysis on log-transformed data (this would be particularly appropriate if variance were proportional to the mean and the distributions left-skewed).

(3) The means in Table 1 should be presented with standard errors; it would be more economical to report them in the text rather than in a table.

(4) It is not clear why both an t-test and F-test is reported – do they refer to the same hypothesis? If yes, they are redundant, if not, explain.

(5) Degrees of freedom should be reported for the statistical tests.

(6) The authors mention an additional test that took into account the order in which the measurements were taken. What is the rationale for this, why do the authors think the order of measurements might have mattered? If there is a good reason for this additional test, it should be reported.

→ **Michał Filipiak** - Review of the project *“Do ambient temperature influence ants walking speed?”*

I have one general reservation: you study something, what we already know. It's not interesting. So I don't like it. Apart from that you should abridge sentences and avoid uncommon words. Simplicity is what I like.

In more here:

Abstract: Your hypothesis is misleading - you don't study chemical processes in ants' bodies. This sentence is copied from the introduction – I don't like it.

Introduction: I think there is too much information related to various aspects of ants' biology but not connected with topic of this work. At the end of this chapter you say what you predict – that is repetition of your hypothesis - I already know that.

Materials and methods: You write that ants were put on ice in. So were they put on or put in? I don't understand what does constant lack of movements mean – death? I'm not sure about using the “assay” word. I think that it means testing quality (in the sense of purity) of something, but I may be wrong. Sometimes you use too many words, for example

“chemo attractant” should be reduced to “attractant”. Sometimes you forget about a subject (I mean: grammar subject). Table 1 is not necessary in my opinion.

Discussion: **You discovered that ants are faster in low temperature (tab.1). It’s an unexpected result, at variance with the common knowledge. Why you don’t concern this fact?**

You like complicated sentences too much. “Inter alia”s are unnecessary. The more so because chemical signals are used by ants’ workers first of all to inform about right path. I’m not sure what broader understanding is. You write that reproduction in decreased temperature is limited in other insects. So what?

The third paragraph is unjustified in my opinion, unless we expect an ice age soon. Proposed further studies are not important in my opinion.

I’m sure that most of these trivial errors was caused by lack of time. Best of luck!

→ **Iwona Giska** - Review of the project *“Do ambient temperature influence ants walking speed?”*

The paper is written clearly and it is easy to read. The research question does not seem to be novel. My general remark is about calling 20°C temperature as high for ants if it is in their optimal temperature range.

What is more:

- The word “insects” in the first sentence of introduction does not suit well as all organisms, not only insects, are characterized by their optimum temperature.
- In the second paragraph I do not understand the explanation of presented hypotheses as both high and low temperature affects chemical processes. Authors mentioned this in the first paragraph where they described influence of different temperatures on chemical processes in an organism. I would suggest to present what is the range of temperature tolerance for ants.
- I think that the third paragraph of the introduction is not fully connected to the study subject and there is too much not relevant information.
- In the last paragraph of the introduction present and future tense should be changed into past as the research has been already done.

- There are verbs lacking in multiple sentences. If passive voice is used there should be the form of “to be “ present so some sentences should be corrected. (For example: almost all sentences in Materials & Methods lack words were/was)
- So much space is not necessary to explain how speed is calculated as it is basic knowledge.
- I am not sure if the word “connection” is proper for statistical analysis. Relation could be better (last paragraph of Materials & Methods, last sentence in Results).
- In results there is a really meaningful mistake in the table. Values for high and low temperature seem to be put in wrong cells as they are different than their description in the text.
- The table should be described above not under the table.

→ **Marcin Plech** - Review of the project *“Do ambient temperature influence ants walking speed?”*

First of all discussed data, namely the abstract and discussion, are not compatible with presented results. At the beginning you indicate 87% decrease in ants' run speed after exposure to low temperature and the discussion presents conclusions reflecting such data. However results shown are completely opposite. Assumingly a mistake has been made while configuring the statistical test or while typing its outcome into the results section. Both mistakes probably have minor causes but major repercussions- please correct it urgently.

In abstract you indicate that you controlled ants' speed in two temperature conditions- is that really what you did? Think about that.

In the introductory part, first paragraph, you mention that the high body temperature causes denaturation of the proteins in the animal body. This study case is predominantly concerned with the effects of decreased temperature on performance of insects. You never even use temperature higher than the average met in the environments of this species and none of the known protein structures can be affected by 20 degrees Celcius. Mentioned phenomena is irrelevant in the context of presented study.

In the discussion part you mentioned of possible future studies to be conducted- do you really think that lowering the temperature would only affect the ants' movement and the latter changes of ecosystems would be subsequent to the lowered ant speed? If the

temperature of the environment would fall to 4 degrees ants' would probably vegetate and the environment itself would get strongly affected without ants involved. You can however think of other experiments testing the impact of temperature on ants in this context.

You also mention about selection experiment directed at developing a line of ants resistant to cold- do you really think that one line would be enough? Elaborate more on the advantages of developing such line.

These were the main flaws noticed while revision. Another thing that needs a little improvement is the language used. You could express some of the ideas and logics better, at this point various things are not clearly understood even after multiple reading.

In conclusion this idea has potential, but it would need some alteration of the method and reformulation of questions asked.

→ **Magdalena Surówka** - Review of the project *“Do ambient temperature influence ants walking speed?”*

In general the paper has clear aims, methods and results but language used is sometimes confusing. For example in abstract in third sentence the word “it” refers to temperature but the word order suggests “ant activity”. Further in abstract authors explained why they use 20 °C maybe it is too detailed for abstract – I suggest moving it to the Material and Methods chapter.

In Introduction the sentence of “reproduction of other species” has no connections with the project’s topic. I would also reconsider the order of sentences in next paragraph, because there are mixed two topics: role in ecosystem and ants abilities.

In Material and Methods sentence that each individual was tested once was written twice. Maybe there is no need to write that 50 centimeters long tube wasn’t taken - wouldn’t be better to say why 19 cm long was taken using the same argument.

In Results in table 1. is written that in low temperature the ants speed is 3,88 cm/s and in high temperature is 0,83 cm/s. I can’t find in the text in which temperature ants walk faster but from the hypothesis I suppose that ant walk faster in high temperature what doesn’t feet with data in table.

In Discussion I don't clearly understand how Anova test can tell that ants don't leave chemo-signals. In next paragraph there is similar sentence as in Introduction about other insects reproduction which is not directly connected with previous sentences. Author may consider developing Discussion chapter to increase clarity of messages.

Also it would be easier to read it if the text was justified and had pages numbered.

4.3.4 Research report – final version

Does ambient temperature influence ants walking speed?

Summary

Ants are organisms which play essential role in terrestrial ecosystems. They are poikilothermic organisms what means changing body temperature, simultaneously with changing ambient temperature, so their activity should depend on ambient temperature. The aim of this study is to assess the impact of different temperatures on black garden ant (*Lasius niger*) walking activity. We hypothesize that in low temperature ants activity is limited, due to lower metabolism. We controlled ants walking speed in two different temperature conditions; diurnal temperature (20°C) and decreased temperature (around 4°C). This study shows that ants speed from low temperature is 85% lower than from the other group.

Introduction

All insects have specific optimum temperature for living. Physiological processes are limited when deviations from the optimum temperature appear. Ants are poikilothermic organisms, and because of their small size, body temperature may rapidly change in different ambient conditions. High body temperature results in peptides denaturation while decreased temperature in lower activity of enzymes, what in consequences appears in lower animal activity.

We want to check how ambient temperatures influence ants walking time. We hypothesize that in low temperature ants activity is limited.

Ants are social insects, widespread all over the world. Their natural habitats are inter alia meadows, forests and other terrestrial habitats. They forage on insects, eggs of other species, honey dew of aphids and plant parts. They spread plants seeds, pollen, become a part of food chains, can be in symbiosis with other species like aphids or butterflies. Despite of small size they could be an important predator when they attack in group. Because of the perfect ants' colony organization, it is possible for them to transport even for long distances much bigger thing than they are, like tree litter or animals body remains. Their main communicational signals are chemical substances. They play significant role in ecosystems. Mentioned abilities may be limited, because of changing in ants movements. We would like to check if there is any dependence between temperature and ants walking activity.

We predict that in low temperature ants movement will be limited. We are going to measure in two different temperatures time in which black garden ants will reach target.

Materials & Methods

Black garden ants workers (*Lasius niger*) gathered in a meadow around research station in Gaik, near Dobczyce Lake. Experiment was carried out simultaneously in two groups of ants, each composed of 15 individuals. Whole experiment was performed using equipment showed on figure 1. The first group tested in room-temperature (around 20 °C). Walking speed of the second group analyzed in low temperature (around 4°C). Separated ant put on ice in for 45s before trial – longer time on ice resulted in constant lack of movements. During trial the tube placed in the ice (fig. 2). Each individual was assayed once.

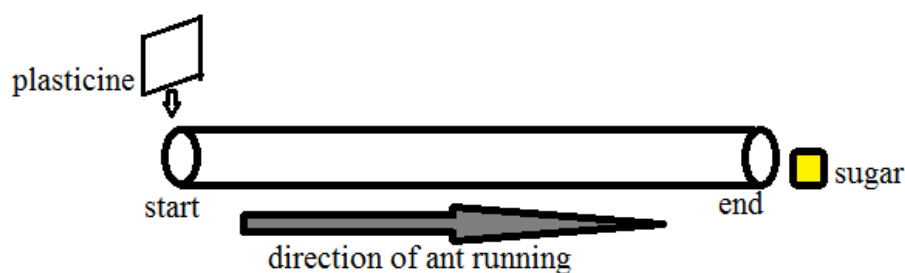


Fig. 1 Scheme of experiment.



Fig. 2 Scheme of experiment in low temperature.

Each individual tested once in a plastic tube (length 19cm, diameter 4mm). The exit of the tube (called “end” – fig. 1) soaked in a sugar solution which was used as a chemo attractant. However, the effect of closing the entrance probably was enough stimuli to make ant moving. Simultaneously we put ant into the tube, started measure the time and lock the entrance. Time needed to travel the distance from one end of the tube to the other one was measured. All ants walked straight, without changing direction.

The obtained data was calculated using the formula:

$$v=s/t$$

v-speed [cm/s]

s-distance [cm]

t-time [s]

The differences were tested with t-Student test for independent data. By using Two Way Anova we analyzed connection of each pairs tested in the same time. By using covariance, we analyzed connection of each sample in order.

Results

Speed in higher temperature was higher (mean = 3,88, standard error=0,22), then in lower temperature (mean=0,83, standard error=0,11 /t=12,6, df=28, p=0).

Additional covariant analyze, which allow us to see if there are any correlations between order in samples, showed negative result (as a variable were taken temperature, speed and order in each group) ($F_{1,27}=72,0912$, p=0, df=1). We checked if there is any dependence in pairs, and ANOVA analyze showed that there is no significant differences ($F_{1,27}=78,57$, p=0, df=1).

In addition we observed that the mean speed/temperature relationship for the two is remarkably close to proportionality.

Discussion

This study has shown that there is significant difference between ants walking activity in two different temperatures. It is possible for ants to leave chemo – signals, in order to inform other about way. We rejected this option in this study by using covariance analysis.

Decreased temperature may influence on ants physiological processes. Temperature slows down chemical processes, what in consequences limit ants' movements. In broaden understanding it may disturb the most important process, that leads to species surviving. In low temperature ants movements are slower what in consequences they may became a pray. Moreover in low temperature their foraging may be limited (Dornhaus *et al.* 2010).

Limitation of ants' moving activity also may influence on other species. Seeds and pollen spreading could be limited. Hyphae fungi may not change location, that in consequences disturb plant - fungi symbiosis.

It would be interesting in further studies to check ants behavior in different temperature points.

Biography

Dornhaus A, Powell S. Foraging and Defence Strategies. In:Ant ecology, Lach L, Parr C, Abbott K. New York, Oxford University Press, 2010, pp. 210 – 233.

4.4 Influence of grasshopper's size on the jumping length

Agata Rudolf & Magdalena Surówka

4.4.1 Project proposal

Summary

Body size is one of the traits that could affect the grasshopper's nymphs survival. The species develop jumping as the main strategy of escaping from predators. Based on biomechanical models, it could be predicted that jump distance depends on leg length and body mass. The aim of this study is to examine relationship between body size, leg length and jump distance. We expect positive correlation between leg length and jumping distance, whereas negative correlation between body size and jumping distance. Distance of the jump will be measured on population of grasshoppers collected on the Gaik - Brzezowa meadow and this data will be used to find a correlation to body mass and leg length. The study will shed light on physiology and the general fitness of grasshoppers as an ability to escape from predators.

General background and rationale

Grasshoppers are easy prey for predators. The way of defence that they develop to avoid meeting with their enemy is jumping for long distances. Reproduction strategy of grasshoppers is laying big amount of eggs. After hatching, youngs are left alone without parental care. It has been shown that most of them become an easy prey for such predators as spiders, birds or rodents. It is important for young grasshoppers to increase their ability to escape predator by achieving high body size possibly fast. By developing body size youngs simultaneity increase their leg length what directly effects physical abilities. That lead us to question how important is the correlation between body size (especially leg length) and jump distance. However in the same time growing body size cause increase of body mass which could have negative affect on aerobic abilities to escape from predators.

The study presented here is focused on investigating relations between body mass, leg length and jump distance in young grasshoppers which could have directly influence on species' survival. Our research group have experience in studying how various morphological and physiological traits affects adjustment and capability of survival among invertebrates

from different environments. The present study is a continuation of previous large project conducted by our research group in southern part of Poland. The project was dedicated to investigate the various evolutionary traits of vertebrates and invertebrates in relation to the populations fitness, and speciation in various environments of southern Poland. The meaningful results that we have achieved so far make us to continue the research in larger area.

The aim of this research is to test the hypothesis that leg length and body size of grasshoppers are related to jumping distance.

Proposed research, schedule, material and methods

The grasshoppers of two species (lac. *Omocestus viridulus* and *Chorhippus parallelus*) will be collected from the meadow of Brzezowa- Gaik near Dobczyce lake in the end of May. 16 (8 individuals of each 2 species) young animals will be catch and placed in laboratory in the Brzezowa field station.

The circle arena with 2 m diameter 1 m high barrier will be constructed to provide a proper condition to test jump distance. The arena will be useful to achieve repeatable results and to avoid escaping the animals during the test. The test will be conducted in 3 repeats for each individual in 5 minute intervals. The jump distance will be marked using colourful markers, where different colour will mark each jump. The distance will be measured using piece of string and ruler scale. The measurement will be based on observations of 2 researchers. The longest jump of each individual and the mean of three jumps will be taken to analysis.

To measure leg length the animals will be placed in the 4 °C for 5 minutes to decrease their metabolic rate. A calliper will be used to measure separately tibia and femur of the leg, and then these measures will be summed. The body size will be measured after conducting the individual performance test, from the top of the head to the end of the animal, using the same calliper.

The relationship between leg length, body size and jump distance among the two species, will be tested using analyses of covariance, whereas within the species using multi regression analyses.

We expect that leg length will positively correlate with jumping distance, whereas body size will correlate negatively with jumping distance.

Significance and perspective

This project will provide the background for future research aimed on evolutionary concepts answering the question if the jump distance depends on body size and leg length which could indirectly indicate if there is a negative or positive pressure of natural selection on grasshopper's body size. It has got scientific significance while this kind of approach was never proceeded.

4.4.2 Research report – first version

Influence of grasshopper's size on the length of jump

Summary

Body size is one of the traits that could affect the grasshopper's nymphs survival. The species develop jumping as the main strategy of escaping from predators. It could be predicted that jump distance depends on leg length and body size. The aim of this study is to examine relationship between body size and jump distance. We expect positive correlation between leg length and jumping distance, whereas negative correlation between body size and jumping distance. Distance of the jump was measured on population of grasshoppers collected on the Gaik - Brzezowa meadow and this data correlated to body size and leg length. The study will shed light on the general fitness of grasshoppers.

Introduction

The grasshopper's strategy of survival is to avoid predators by escape doing long distance jumps. Reproduction strategy of grasshoppers is laying big amount of eggs. After hatching, youngs are left alone without parental care. It has been shown that most of them become an easy prey for such predators as spiders, birds or rodents. It is important for young grasshopper to increase their ability to escape predator by achieving high body size possibly fast. By developing body size youngs simultaneity increase their leg length what directly effects physical abilities. That lead us to question how important is the correlation between body size (especially leg length) and jump distance. However in the same time growing body

size cause increase of body mass which could have negative affect on aerobic abilities to escape from predators. In this study we wanted to answer the question how the jump distance depends on body size and leg length of grasshoppers. We expected positive correlation between leg length and jumping distance, whereas negative correlation between body size and jumping distance. Our study confirmed our assumptions, but the results revealed presence of two kinds of grasshoppers species in our experiment. The statistical analysis confirmed by morphological overview led us to conclusion that collected species are *Omocestus viridulus* and *Chorhippus parallelus*. Having two analysed kind of grasshoppers exposed the question if there are significant differences in jump distance in relation to leg length and body size between species.

Results

To test association of jump distance in two measured species of grasshoppers we used analysis of covariance. The test showed that there are statistical differences between two species. The jump distance related to body length however, is not significant (Table 1).

Table 1. Covariance of jump distance depend on body length in two grasshoppers species

	SS	df	MS	F	P
Intercept	686,251	1	686,251	5,63007	0,041719
Body length	113,961	1	113,961	0,93494	0,358845
Species	1227,521	1	1227,521	10,07069	0,011303
Error	1097,014	9	121,890		

The analysis of covariance of jump distance related to leg length showed that there are statistical differences between two species but the jump distance is not related to leg length (Table 2).

Table 2. Covariance of jump distance depend on leg length in two grasshoppers species

	SS	df	MS	F	P
Intercept	391,737	1	391,737	2,931336	0,121032
Leg length	8,236	1	8,236	0,061631	0,809508
Species	1023,777	1	1023,777	7,660847	0,021831
Error	1202,738	9	133,638		

However, the covariance analysis showed correlations between measured traits within each grasshopper species (Table 3).

We find positive correlation between average jump distance and leg length ($r = 0,887$)(Fig. 1) but not between jump distance and body length (Fig. 2) in *Omocestus viridulus*. Positive correlation was also found between body and leg length ($r = 0,633$)(Fig. 3).

Table 3. Covariance of jump distance depend on leg length and body length in two

	SS	df	MS	F	p	Partial rta-	non	Observed
Intercept	391,737	1	391,737	2,93133	0,12103	0,245684	2,931336	0,334290
Leg length	8,236	1	8,236	0,06163	0,80950	0,006801	0,061631	0,055715
Species	1023,77	1	1023,77	7,66084	0,02183	0,459811	7,660847	0,693417
Error	1202,73	9	133,638					

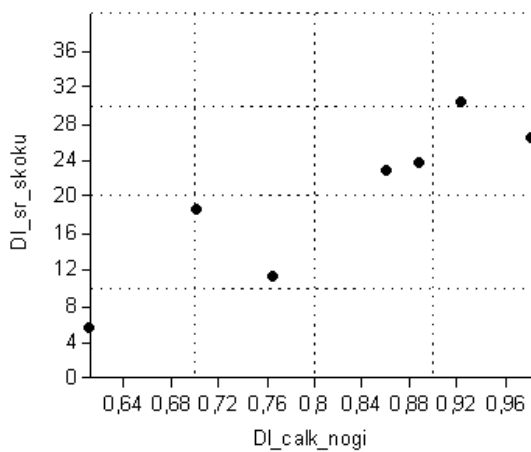


Fig.1

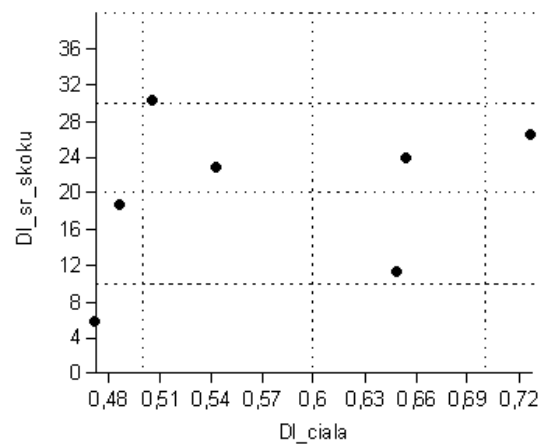


Fig.2

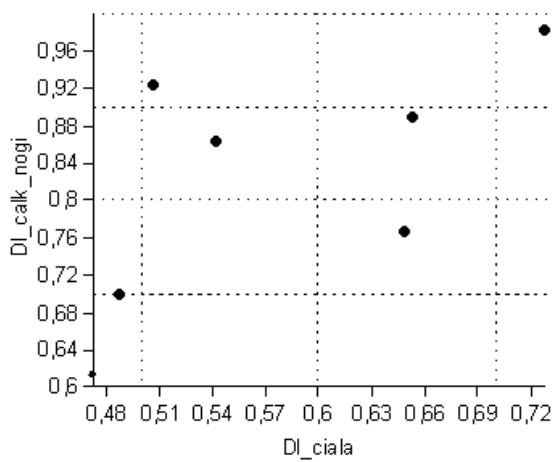


Fig.3

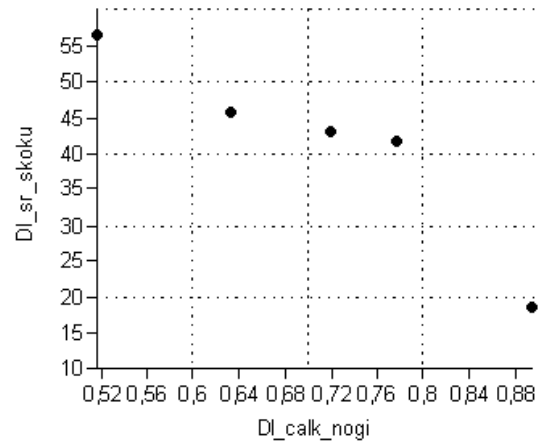


Fig.4

We find negative correlation between average jump distance and leg length ($r = -0,937$) (Fig. 4) and between jump distance and body length ($r = -0,986$) (Fig. 5) in *Chorhippus parallelus*. Positive correlation was found between body and leg length ($r = 0,869$) (Fig. 6).

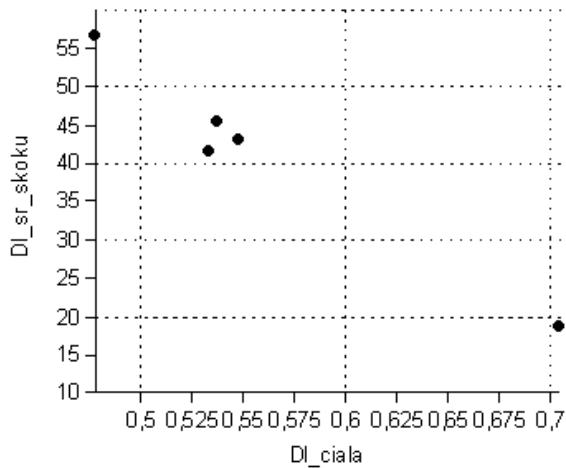


Fig. 5

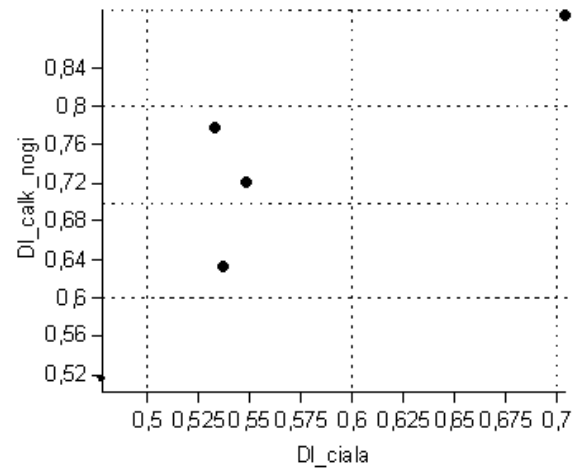


Fig. 6.

Similar results were obtained when the maximum jump distance was taken for analysis instead of average jump distance.

Discussion

The results indicate that grasshoppers *Omocestus viridulus* and *Chorhippus parallelus* have differences in morphology which include differences in leg morphology. *Omocestus viridulus* jumps further when the individuals become larger. It could have importance in their strategy of survival where young grow fast to achieve better abilities to escape from predators. Whereas in *Chorhippus parallelus* species young have ability to jump further when they are smaller what can increase their survival rate in nymph stage. Strong negative correlation between body length, leg length and jump distance in *Omocestus viridulus* and also strong positive correlation between leg length and body length indicate that in this species morphology is more homogenic whereas in *Chorhippus parallelus* we can find more individual variation in morphology.

Material and methods

We have collected the grasshoppers (lac. *Omocestus viridulus* and *Chorhippus parallelus*) from the meadow of Brzezowa- Gaik near Dobczyce lake in the end of May. 14 young animals were caught and placed in laboratory in the Brzezowa field station.

We have constructed the circle arena with 2 m diameter 1 m high barrier to provide a proper condition to test jump distance. The arena was useful to achieve repeatable results and to avoid escaping the animals during the test. The test was conducted in 3 repeats for each individual in 5 minute intervals. The jump distance were marked using colourful markers, where different colour marked each jump. The distance was measured using piece of string and ruler scale. The measurement was on observations of 2 researchers. To analysis was taken the longest jump of each individual and the mean of three jumps.

We measured body size after conducting the individual performance test, using calliper from the top of the head to the end of the animal. To measure leg length the animals were placed in the 4 °C for 5 minutes to decrease their metabolic rate. We used the same calliper to measure separately tibia and femur of the leg, and then these measures were summed up to get the whole leg length.

The relationship between the leg length, body size and jump distance, was tested using multivariate analyses instead.

4.4.3 Reviews

→ **Prof. Tadeusz Kawecki** – *Review of the project “Influence of grasshopper’s size on the length of jump”*

This ms addresses an interesting biomechanical problem. Assuming a ballistic trajectory, the main factors determining the jump length of grasshoppers should be the initial velocity and jump angle. While the jump angle is not investigated here (and so presumably assumed constant), it is reasonable to assume that the initial velocity increases with leg length and decreases with body mass. Additionally, larger body mass is presumably associated with slower deceleration due to air resistance. Interestingly, the hypothesis concerning the leg length seems to be supported in one species, but contradicted in the other.

While I find these results potentially exciting, the paper needs a substantial revision, including better motivation, possibly more precise hypotheses, and changes in the analysis (or at least better justification for the current analysis being used).

First, biomechanical considerations behind the study are not explicitly described in the paper, so the hypotheses lack justification. Furthermore, the hypotheses are rather vague; the tests assume a linear relationship between the measurements, but there is no reason elucidated as to why they should be linear. A simple model can be used to make predictions more firmly rooted in biomechanics. I have developed such a model at the end of this review.

The model considerations suggest a log-transformation of all measurements.

Irrespective of the log-transformation, the relationship between leg and jump length seems to be very different in the two species (this could be explicitly tested as heterogeneity of slopes). It would thus make sense to analyze the data for the two species separately (or focus on the 1st species for which more data are available).

Other points:

The Abstract does not give a hint of the results. The results and conclusion should be reported, in a highly condensed form. In turn, the initial part of the Abstract could be somewhat shortened; in particular the 1st and 2nd sentence are largely redundant to each other, and so are the 3rd and 4th sentence.

Introduction: I don't understand why the authors bring up aerobic abilities. Possibly this is a misunderstanding of the term; aerobic performance refers to the efficiency of oxygen use.

It would be worth indicating that larval stages do not have wings, so presumably the trajectory of the jump is to a good approximation ballistic, which simplifies the biomechanical aspect of the study.

While I find the Introduction and Material and Methods clearly written, the Results are a bit hard to follow, as it is not clear what the interpretation of the various tests are. Furthermore, there seems to be a problem with Table 3, it does not seem to contain what the caption says. Table 3 also lists values whose meaning is unclear to me; they should either be discussed or removed.

Significance tests for the various correlations should be reported.

Finally, the Discussion is rather terse. Could the authors comment on the broader significance of their study?

A biomechanical model:

Ignoring air resistance, the jump should follow a ballistic trajectory, with jump length S given by

$$S = V_s t \quad (1)$$

where V_s is the horizontal component of the initial velocity and t is the time between take off and landing, which consist of the time between take off and the moment when the apex of the jump is reached, plus the time from the apex to landing. If air resistance is ignored, both are equal to the time needed for the vertical component of the velocity V_h to reach zero under gravitational acceleration g :

$$t = 2 V_h/g. \quad (2)$$

If α is the constant jump angle, V_h and V_s are given by $\sin(\alpha)V$ and $\cos(\alpha)V$, where V is the total initial velocity in the direction of jump, so the jump distance

$$S = 2 \sin(\alpha) \cos(\alpha) V^2/g \quad (3)$$

The initial velocity V depends on the acceleration due to the leg muscles action a and the time over which they work T :

$$V = aT \quad (4)$$

where T is related to the length of the legs L by

$$L = aT^2/2 \quad (5)$$

Solving eqn (5) for T and substituting in eqn (4) we get

$$V = a\sqrt{2L/a} = \sqrt{2La} \quad (6)$$

The acceleration a depends on the force exerted by the muscles F and the body mass m

$$a = F/m \quad (7)$$

where the body mass is likely to scale with the cube of body length B

$$m = kB^3 \quad (8)$$

Assuming that the force exerted by muscles does not depend on their length but only on thickness, F can be held constant, so the initial jump velocity would be

$$V = \sqrt{2LF/kB^3} \quad (9)$$

Substituting (9) in (3) leads to the following model for the jump length

$$S = 4 \sin(\alpha) \cos(\alpha) FL/(kB^3 g) = c L/B^3 \quad (10)$$

where c is a proportionality constant equal to $4 \sin(\alpha) \cos(\alpha) F/(k g)$.

This simple model predicts that the jump length should be proportional to leg length and inversely proportional to the cube of body length. After log-transformation:

$$\log(S) = \log(c) + \log(L) - 3 \log(B) \quad (11)$$

which is a linear model. It would thus be more appropriate to carry out the multiple regression on log-transformed measurements, and it would be interesting to test if the slope of the partial regression of log jump length on log leg length is 1 and the slope of partial regression of log jump length on body length is -3.

→ **Agata Miska** - Review of the project *“Influence of grasshopper’s size on the length of jump”*

In general presented study is innovative, with interesting results. I can find some spelling/language mistakes, but for not native speakers this situation is understandable.

I would say that in introduction there is too much detail about history of collecting samples and designing experiment.

Grasshopper’s names are only in Latin. Do they exist in English? And if so, what are they?

In results figures are signed in polish! If whole paper is in English, you can’t just live it like that.

Authors could briefly tell us in discussion what might be the next step in next experiment.

In methods Authors describe whole equipment used in experiment. It would be easier if reader may see picture/drawing, which present it.

→ **Marcin Plech** - Review of the project *“Influence of grasshopper’s size on the length of jump”*

In general the report is a good one. There are however some minor flaws.

First of all you need to reformulate the abstract. You must stress the general idea standing behind this project and pose it in the first place, grasshoppers should be perceived only as a model and not the object of the study, which really is...?

The introductory part summarizes the whole idea very well. The logics described is simple and clear. There are some pitfalls in the use of language, but these do not disturb the whole structure of the chapter and it reads very good.

The results section would need the biggest alterations. Your results are presented in a very ordered way but still you get the impression of a messy approach. You do not need to put in all of the tables and figures simultaneously. In fact most of the results can be summarized in short statements concerning the very effect of measured factors and their statistical significance. You could show only the most important graphs and it would be as informative. This way it seems more like a waste of space.

Discussion is of particular interest. Its structure is appealing because, unlike most of the time, it is very short and it gives the right conclusions about obtained data. In this case however you probably should elaborate a bit more about the implications of these data as they have the potential. You could also propose some future studies that would add to the results of this study- it is not the end right?

→ **Geoffrey Dheyongera** - Review of the project *"Influence of grasshopper's size on the length of jump"*

The results really look interesting but few problems on language and redundant statements.

1. What is the reason for the first sentence in the abstract on body size affecting survival of g. hopper nymphs

2. You have already done this study and results are available, but you write the abstract in future tense. Rather than writing what you expect here, tell us what you found in past tense since the study is done.

3. I don't understand the context in which you use the word fitness in the last sentence of this abstract. Do you mean fitness in terms of inherited differential survival?

It is just common that a hungry individual will want a big portion of food. So you may need to exclude the alternative hypothesis that larger body size may as well increase predation

risk despite better jumping. This would be because many predators would prefer stocking larger prey. As such body size may correlate negatively with fitness.

You have various figures here showing the correlations and you report some of them as significant. Would you like to indicate the p-values? It appears what you report is not what is in table 3.

You set a study to find out the dependence of jump distance on body length and leg length. You argue that these traits are vital for survival via escape from predation but at the same time you found a negative correlations btm body length, leg length and jump distance. How would these findings affect adult survival? What is your conclusion? What is your future direction? Perhaps a future study to check if adult individuals with varying leg and body lengths have differential survival (densities) in addition to a predation experiment testing for predation risk among such adults would reinforce these findings.

→ **Katarzyna Wężowicz** - Review of the project *“Influence of grasshopper’s size on the length of jump”*

The topic of the project is interesting. Authors undertook the trial to describe relation between differences in morphology and jump distance in the grasshoppers.

The introduction, results and discussion are clearly presented. Very interesting is fact that there were two different species of grasshoppers in the experiment. The way of carrying out the experiment is interesting.

The weakness part of the project is lack of information about number of the tested grasshoppers belonging to each of the species. In the summary, in the aim of the study is needed to be mention about relation between jump distance and leg length. The descriptions under the figures should not be written in polish. There are some language mistakes in the project.

4.4.4 Research report – final version

Influence of grasshopper's size on the jumping length

Summary

The grasshoppers develop jumping as the main strategy of escaping from predators. It could be predicted that jump distance depends on leg length and body size. The aim of this study was to examine relationship between body size, leg length and jump distance in two species of grasshoppers. We expected positive correlation between leg length and jumping distance, whereas negative correlation between body size and jumping distance. Distance of the jump was measured in the population of grasshoppers collected on the Gaik - Brzezowa meadow and this data confirmed our predictions in species 1, whereas the relationship between leg length and jump distance in species 2 was reversed. Results of the study can reflect the differences in leg morphology and body proportion among the two species.

Introduction

The grasshoppers avoid predators by performing long distance jumps. After hatching, young grasshoppers are left alone without parental care. It has been shown that most of them become an easy prey for such predators as spiders, birds or rodents. It is important for young grasshoppers to increase their ability to escape predator by achieving high leg length possibly fast. To increase leg length young grasshoppers simultaneously grow body size. However in the same time growing body size cause increase of body mass which could have negative effect on physical abilities to escape from predators. That lead us to question how important is the correlation between body size, leg length and jump distance. This could indicate on survival strategy of invertebrates. In this study we wanted to answer the question how the jump distance depends on body size and leg length using grasshoppers as a model animal. We expected positive correlation between leg length and jumping distance, whereas negative correlation between body size and jumping distance. Our hypothesis are based on biomechanical model which assume a ballistic trajectory of jump (T. Kawecki, pers. comm.), where the initial velocity increases with leg length and decreases with body mass. In this model jump angle is assumed to be constant and the air resistance is ignored. Our study confirmed our hypothesis. The results exposed differences in jump distance in relation to leg

length and body size. We analyzed two species of grasshoppers and we exposed significant differences in analysed relations between species.

Material and methods

We have collected the grasshoppers (lac. *Omocestus viridulus* and *Chorhippus parallelus*) from the meadow of Brzezowa- Gaik near Dobczyce lake in the end of May. 7 individuals of *Omocestus viridulus* species and 5 of *Chorhippus parallelus* young animals were caught and placed in laboratory in the Brzezowa field station.

We have constructed the circle arena with 2 m diameter 1 m high barrier to provide a proper condition to test jump distance. The arena was useful to achieve repeatable results and to avoid escaping the animals during the test. The test was conducted in 3 repeats for each individual in 5 minute intervals. The jump distance were marked using colourful markers, where different colour marked each jump. The distance was measured using piece of string and ruler scale. The measurements were conducted by 2 researchers. For analysis we take the longest jump of each individual and the mean of three jumps.

We measured body size after conducting the individual performance test. To make measurement easier the animals were placed in the 4 °C for 5 minutes to decrease their metabolic rate and in consequence slow their reactions down. We measure the animal length from the top of the head to the end of the abdomen using calliper. We used the same calliper to measure separately tibia and femur of the leg, and then these measures were summed up to get the whole leg length.

To test association of jump distance in two measured species of grasshoppers we used analysis of covariance. Within each species the relationship between the leg length, body size and jump distance, was tested using linear correlation and for species 1 additionally multiple regression.

Results

Analysis of covariance show that interaction of body length, leg length and jump distance was significantly different between grasshoppers species ($P=0,021831$) (Table 1). In consequence the next analysis was conducted separately for each species. The simple linear regression was used separately within two species to show the relations between leg length and average jump distance (Fig.1 and 4), next body length and average jump distance (Fig.2 and 5), and also leg length and body length (Fig. 3 and 6).

Table 1. Interaction between leg length, body length and jump distance in 2 species of grasshoppers.

	SS	df	MS	F	P
Intercept	391,737	1	391,737	2,931336	0,121032
Leg length	8,236	1	8,236	0,061631	0,809508
Species	1023,777	1	1023,777	7,660847	0,021831
Error	1202,738	9	133,638		

We find positive correlation between average jump distance and leg length ($r = 0,887$; $n = 7$)(Fig. 1) but not between jump distance and body length ($r = 0,274$; $n = 7$) (Fig. 2) in *Omocestus viridulus*. Positive correlation was also found between body and leg length ($r = 0,633$; $n = 7$)(Fig. 3). The multiple regression analyses of *Omocestus viridulus* show no significance correlation between body size and jump distance connected with leg length ($b = -0,37$; $p = 0,208$) and significance correlation between leg length and jump distance connected with body size ($b = 1,12$; $p = 0,0104$).

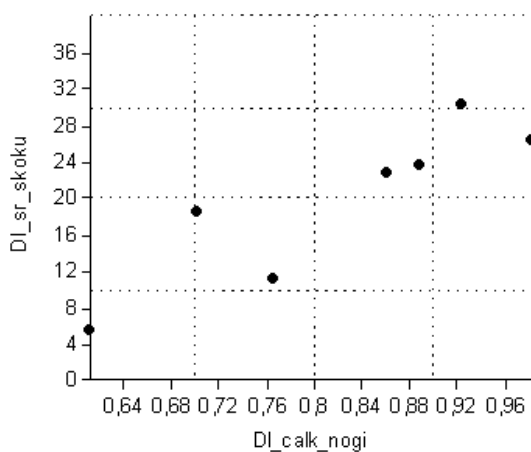


Fig.1

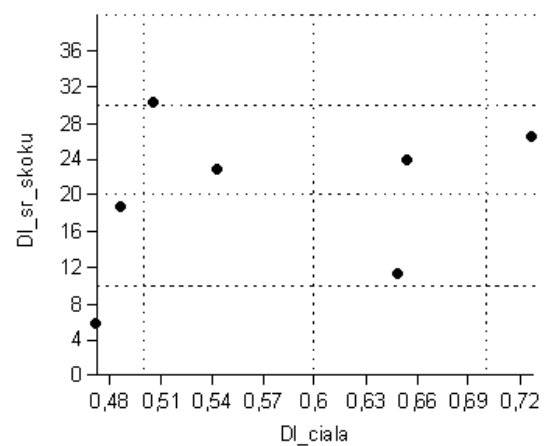


Fig.2

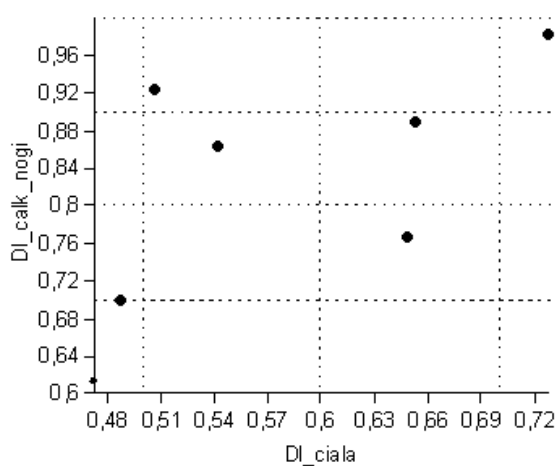


Fig.3

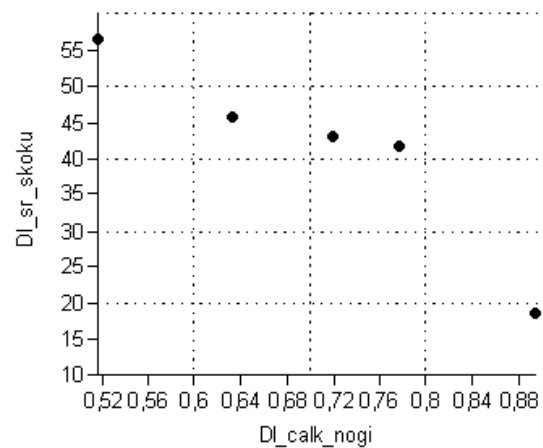


Fig.4

We find negative correlation between average jump distance and leg length ($r = -0,937$, $n = 5$)(Fig. 4) and between jump distance and body length ($r = -0,986$, $n = 5$) (Fig. 5) in *Chorhippus parallelus*. Positive correlation was found between body and leg length ($r = 0,869$, $n = 5$)(Fig. 6). The multi regression analyses were not conducted for *Chorhippus parallelus* because of small sample size.

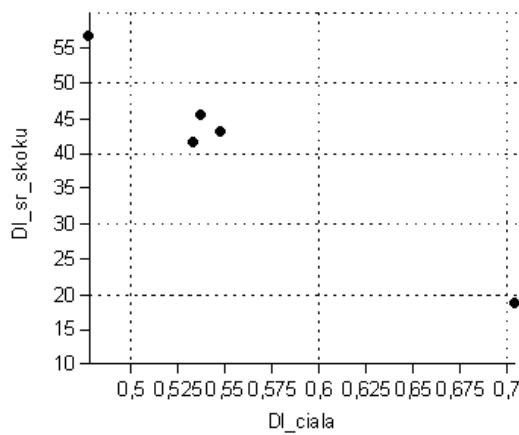


Fig. 5

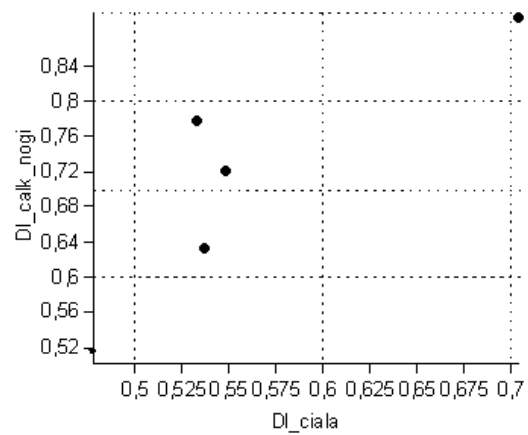


Fig. 6

Similar results were obtained when the maximum jump distance was taken for analysis instead of average jump distance.

Discussion

The results show that there is interaction between leg length and jump distance what confirms our hypothesis. It is also in agreement with biomechanical model. The relation between body size and jump distance is different in analysed species. In *Omocestus viridulus* species there is positive correlation but in the *Chorhippus parallelus* it is negative, what is in disagreement with the model. This results indicate that probably our model doesn't include other significant traits. One of them could be a difference in body morphology which include especially legs. *Omocestus viridulus* jumps further when the individuals become larger. It could have importance in their strategy of survival where youngs grow fast to achieve better abilities to escape form predators. Whereas in *Chorhippus parallelus* species young have ability to jump further when they are smaller what can increase their survival rate in nymph stage. To confirm this hypothesis it is necessary to precede more studies. One of the proposed further research is to study grasshoppers morphology in contexts of jump abilities.

Also survival rate in different development stage could tell more about the adaptation to escape from predators. More studies should include also other invertebrate species.

GALLERY

