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Arbeitgruppe: Spezielle Zoologie: Funktionelle Morphologie und Biomechanik unter der Leitung von Prof. Dr. Stanislav Gorb

Betreuer: Dr. Lars Heepe

Thema: Comparison of adhesion force testing in *C. septempunctata*

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Klassenstufe 12

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1. Personal Information

My name is Chantal Martin. I recently graduated from the „Carl Zeiss Gymnasium" in Jena, a high school specializing in Maths, Sciences and Technology. I'm interested in Biology and Chemistry and plan to continue my studies of science during university.

I took part in the German qualifying rounds of the International Biology Olympiad. During the nationwide round, there was the possibility to apply for a summer internship sponsored by the VBIO and the Förderverein BiologieOlympiade e.V. Goals of this internship were to experience scientific research firsthand and to write a report accompanying this. Prior to this internship, I had no experience in the field of biological research. The internship placement specifically interested me because of the link between mechanics and physics to biology.

2. Introduction

The internship I applied for and got selected for took place at the Zoological Institute of the University of Kiel (Christian-Albrechts-Universität zu Kiel). Under the chairmanship of Prof. Dr. Stanislav Gorb, the research group "Functional Morphology and Biomechanics" specializes in underlying functional principles in nature, interactions between organisms and their environment, such as the compositions of friction and adhesion systems of arthropods and vertebrates. Other topics of research include composition and role of functional biological materials and implementing and testing discoveries as synthetic materials.

The one-month internship took place from the 25.06.2018 to the 20.07.2018. Under the direct supervision of Dr. Lars Heepe, I was able to conduct an independent study on the attachment forces of the seven-spotted ladybird beetle *Coccinella septempunctata*.

3. Abstract

In research of attachment of organisms to different surfaces, the quantifiable values supporting theses are gained through friction and adhesion force testing. In general, two methods are being used in the study of adhesion of arthropods, such as insects: friction force testing on a horizontal centrifugal force measurement (Gorb E.V., 2010) and traction force testing with a traction force sensor (Heepe, Wolff, & Gorb, 2016).

In this study, the individual performance of specimens of the seven-spotted ladybird beetle *C. septempunctata* in three different force measurement techniques, the standard horizontal centrifugal and traction force sensor measurements, as well as the less common vertical centrifugal measurement, was examined. During the duration of these tests, the same beetles and surfaces with the same material and polish were used to ensure greatest comparability. The objective of this study was to acquire data for the three force testing methods and to assess their comparability. Data of this study will expectedly offer assistance to the planning of other studies and experimental set-ups and the choice of force testing method in further *Coleoptera* attachment research.

4. Project Information

The attachment systems of arthropods and vertebrates have been a research focus of the group Functional Biomechanics and Morphology and other researchers in the field of biomimetics for many years because of their high diversity of adhesion enhancing structures.

The seven-spotted ladybird beetle *C. septempunctata* was chosen for this study, due to commercial availability, large size, the display of sexual dimorphism in adhesion and previous studies of attachment systems in this species.

To attach to a surface, all species must generate friction between their appendages and the surface they attach to. Studying the attachment systems can lead to finding out about the lifestyle and interaction of the subjects with an ecosystem. Attachment structures of insects being researched are usually located on the distal segments of an insect leg, the tarsomers. Many species also display claws (*ungues*) on the pretarsus, the most distal segment of the leg. There are two systems found with insect attachment to smooth surfaces: smooth pads or hairy, setose surfaces (Gorb, 2002). Through both mechanisms, the resulting real contact area is increased, while providing flexibility and adaptive ability to attach to and detach from different surfaces.

C. septempunctata posess soft lobes, also called pulvilli, covered with tiny cuticular hairs, which are called setae, on their tarsomers (Figure 1). Claws on the pretarsus are only useful while attaching to rough or interlockable surfaces.

The attachment of *C. septempunctata* on smooth surfaces is achieved through different shapes of hair-like setae on their tarsomers. Both males and females display setae with “pointed, filamentous tips” (Figure 1E), “lanceolate tips” (Figure 1F) and “rounded, spatula-shaped tips” (Figure 1G) (Heepe, Wolff, & Gorb, 2016). Only males display a fourth kind of setae with “discoidal terminal elements” (Figure 1K), which can be seen in the middle of tarsomers on the male legs (Figure 1H-J) as opposed to the female legs (Figure 1B-D) (Heepe, Wolff, & Gorb, 2016). Similar structures were found in another order of *Coleoptera*: the Colorado potato beetle *Leptinotarsa decemlineata*, resulting in sexual dimorphism of the attachment ability to smooth surfaces with males generating a much higher adhesion force than females (Voigt D., 2008).

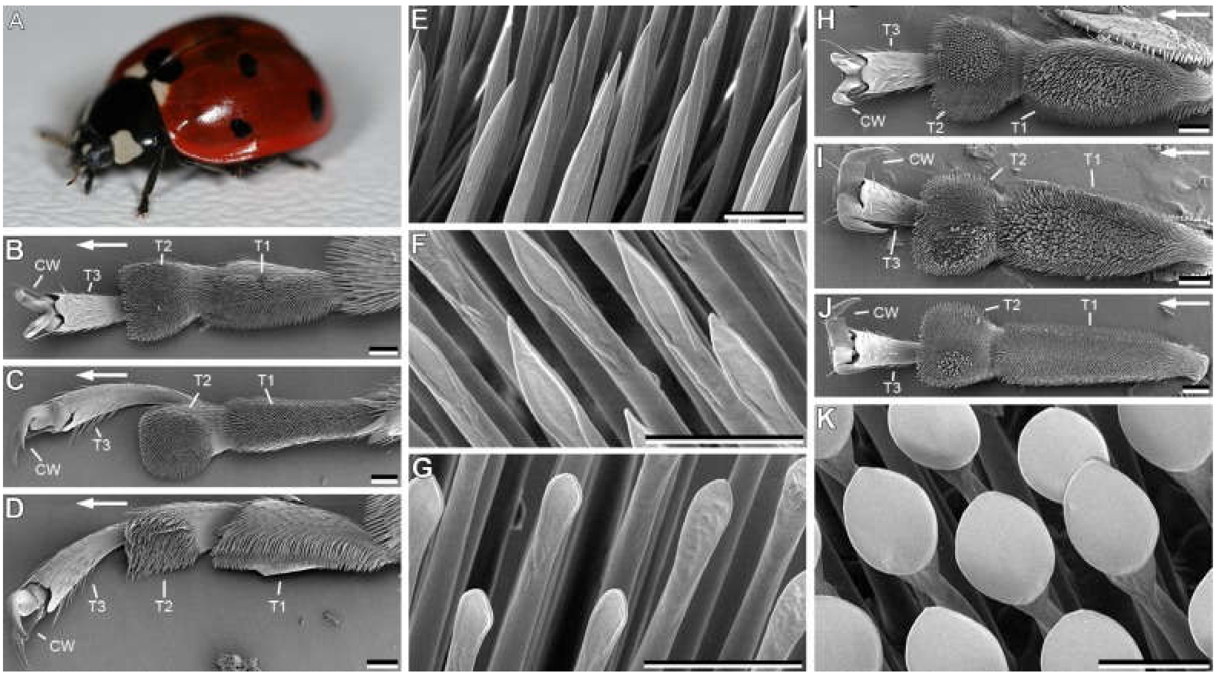

Figure 1: Attachment devices on fore- and hindlegs of female and male Coccinella septempunctata (A): “Tarsi of forelegs (B), midlegs (C), and hindlegs (D) in females are ventrally covered by different types of tenent setae (E–G). Tarsi of forelegs (H), midlegs (I), and hindlegs (J) in males. Tarsi of males were also ventrally covered by tenent setae types shown in (E, F), but have an additional type, which is terminated with discoidal terminal elements (K). CW, claws; T1, first proximal tarsomer; T2, second proximal tarsomer; T3, third proximal tarsomer. The arrows in (B–D) and (H–J) indicate distal direction. Scale bars in (B–D, H–J), 100 µm. Scale bars in (E–G, K), 10 µm.” (Heepe, Wolff, & Gorb, 2016)

Previous experiments to the attachment strength *of Coccinella septempunctata* under different conditions, i.e. humidity (Gorb E.V., 2010), roughness (Gorb E.V., 2010), etc. have been performed, but none directly comparing the performance of individual beetles for three different force measurement methods. A difference in the forces measured by varying technique is presumed. The passive force testing of the centrifuge will most likely result in higher attachment forces than the active pulling of a force sensor. If a comparability between the force measurement techniques is observed, this can be used in future studies. This would allow one measurement technique to be sufficient for a study and a choice of measurement technique for optimal set-up for a force measurement in a specific experiment. For example, results of a traction force measurement conducted on a surface unable to be attached to the centrifuge would hypothetically be comparable to results measured on this centrifuge.

1 Methods

1.1 Insects

A total of 68 adult *C. septempunctata* beetles (34 male, 34 female) were obtained from a commercial supplier (Katz Biotech AG, Baruth, Germany). Beetles were held separately in ventilated tubes throughout the experiment. Water was changed every two days, beetles were fed a sucrose solution and aphids gathered on the campus of the University of Kiel, Schleswig-Holstein, Germany. Specimens were held at temperatures of 10 °C overnight and were brought out to room temperature (22 – 25 °C) about 30 min before testing to ensure full activity. Each specimen undertook multiple runs of all three force measurement techniques.

The average mass of a male beetle was 34.81 mg (σ = 3.23), while the average female weighed 41.71 mg (σ = 3.24) without the wax sealing on the elytra.

A droplet of wax sealing the elytra together was used to prevent the specimens from flying away during the experiments. To apply the wax, specimens were anesthetized by CO_2. Specimens were weighed daily using a Mettler Toledo AG204 balance with a precision of 0.1 mg. If a wax seal had broken or fallen off, it was replaced and the specimen was reweighed.

The specimens were tested in a two-week period, first both centrifugal experiments alternating randomly and then the force sensor experiment. To assess if this time lapse had an effect on their performance, the horizontal experiment was repeated after the span of force testing. These results were not included in the results of the initial performance of the horizontal runs and were separately compared to these.

Each specimen underwent three different force tests. The testing sequence was randomized before each test. If a specimen did not complete any runs of a test, it was taken out of the evaluation of this test. If a specimen did not complete all runs of a test, the maximum of the runs it completed was noted and taken into the evaluation.

1.2 Force Measurements

Three different methods were used to test the attachment force of the beetles. A centrifugal device was used for two different measurements of passive attachment, while a force gauge was used to test the maximal pull force a beetle can create moving on a specific surface. In addition to the attachment force, the safety factor was calculated. This is the quotient of attachment force divided by weight force and shows how many times its own weight the individual can withstand.

1.2.1 Centrifugal force measurements

The general set-up of the method of centrifugal force measurement can be seen in Figure 2.

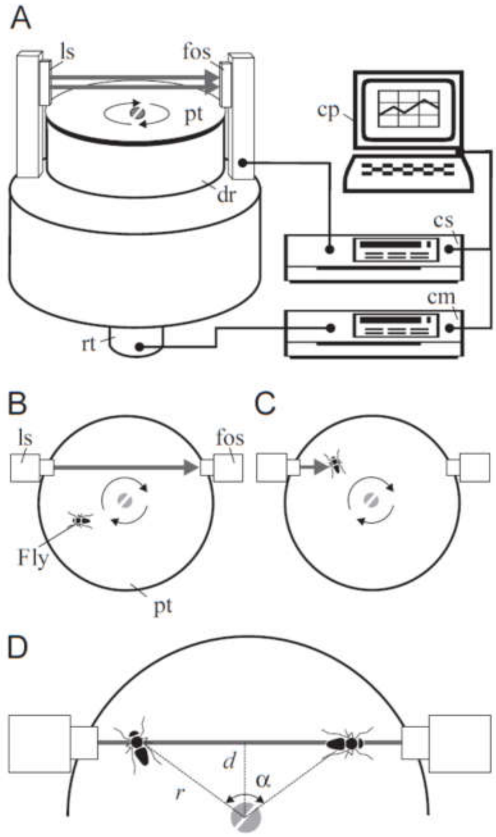

Figure 2: Centrifugal force measurement (A) Layout of the centrifuge. Plexiglas disc (pt) attached to a metal drum (dr),rotated by the motor and a laser (ls) and fibre-optic sensor (fos), monitored by a computer (cp) (B–D) Detection of insect position (view from above). The fly passes the laser beam twice per rotation, interrupting the sensor signal twice. Given the speed of the motor, the time between signal interruptions and the distance between the sensor placement and centre of the drum (d), the angle α and position of the fly on the disc can be calculated (D (Gorb S, 2001)

1.2.1.1 Horizontal attachment forces

For the horizontal centrifugal force measurement, a specimen was placed on top of a centrifugal drum at a starting rotational speed of 50 rotations per minute, RPM, (i.e. slow speed). The rotational speed was accelerated to a speed of 3000 RPM in 20 s. Light sensors recorded the radius and the rotational speed at which the specimen lost contact with the centrifugal drum. The "break-away" force or resistance force is considered the adhesion force of the insect. Not only does it depend on the rotational speed of the drum, but also of the distance of the beetle to the rotational axis.

Each specimen was tested five times (5 runs).

1.2.1.2 Vertical attachment forces

During the vertical centrifugal force measurement, a specimen was placed on the side of a standing centrifugal drum. This was first accelerated to the rotational speed of 50 RPM (i.e. slow speed). The rotational speed was accelerated to a speed of 3000 RPM in 20 s. Light sensors recorded the radius and the rotational speed at which the specimen lost contact with the centrifugal drum.

Each specimen was tested five times (5 runs).

For both centrifugal tests, the maximum RPM (and therefore the acceleration at which the insect lost contact with the drum) of each run was determined. With this, the resistance force was calculated.

Both centrifugal drums were made out of the same material (Plexiglas) and had the same polish and therefore a surface free energy of 41.1 mN m^{-1}.

1.2.2 Calculations of the Centrifugal Force Testing

With the fibre-optic sensors and data acquisition software PC Fly (Tetra GmbH, Ilmenau, Germany) linked to the centrifuge, the insect's position on the drum could be recorded and used to calculate the resistance or friction force. For the vertical force testing, the radius of the drum was used as the insect's position (r = 5 cm).

All calculations were taken from a previous experiment with the centrifuge (Gorb S, 2001). The resistance forces of horizontal and vertical testing were calculated from the tangential as well as centripedal forces determined as follows.

F (r, w) = $$F = \sqrt{{F_t}^2 + {F_c}^2}$$

1.2.3 Traction force measurements

For the force sensor measurement, specimens were attached with a human hair to a force transducer (10 g capacity; FORT10, World Precision Instruments, Inc., Sarasota, FL, USA) connected to a BIOPAC TCI-102 system and a BIOPAC Model MP100 (BIOPAC Systems, Inc., Goleta, CA, USA). Force-time curves were visualized using Acq Knowledge 3.7.0 (BIOPAC Systems, Inc., Goleta, CA, USA). The hair was glued to the elytra and connected to the force sensor by hanging a loop in the end of the hair on the hook of the sensor. The specimen was placed on the Plexiglas horizontal drum of the centrifuge to ensure material and polish did not differ. This drum was fixed in place so that specimens were walking perpendicularly to the force sensor. They continued walking until the hair was taut. Force exerted on the sensor by specimens by pulling was measured.

2 Results

For each specimen and each test, the maximum achieved attachment force calculated with the maximum achieved RPM (in case of the centrifugal tests) or maximum achieved force (in case of the force sensor test) was chosen as the result for each beetle in the respective test. For the centrifugal experiments, the "safety factor" of the resistance force was calculated by dividing the resistance force through the weight of the insect.

The data obtained, were analyzed according to the force dependence of the beetle sex (comparing male to female) as well as the comparison of achieved force between different measurement set-ups (comparing horizontal centrifugal force testing, vertical force testing and traction force sensor testing).

2.1 Dependence of sex

During the horizontal force testing (insects spinning perpendicular to rotational axis), males reached a higher attachment force than females. Here and later, data will be shown as median [minimum, maximum]. Figure 3Figure 3 shows the range and mean as well as the average of the horizontal forces measured for male and female beetles. In the horizontal centrifugal force testing, males reached a median force of 28.8 mN [1.1 mN, 55.8 mN], while females reached a median force of 19.3 mN [2.2 mN, 43.7 mN]. The box-plot shows that the median holding force for male beetles is higher than female beetles and that the distribution of the forces measured is wider than for the female beetle. The weakest female beetle generated a higher force than the weakest male beetle. On average, the male safety factor for the horizontal centrifuge was 83.9, while the female safety factor was 48.4.

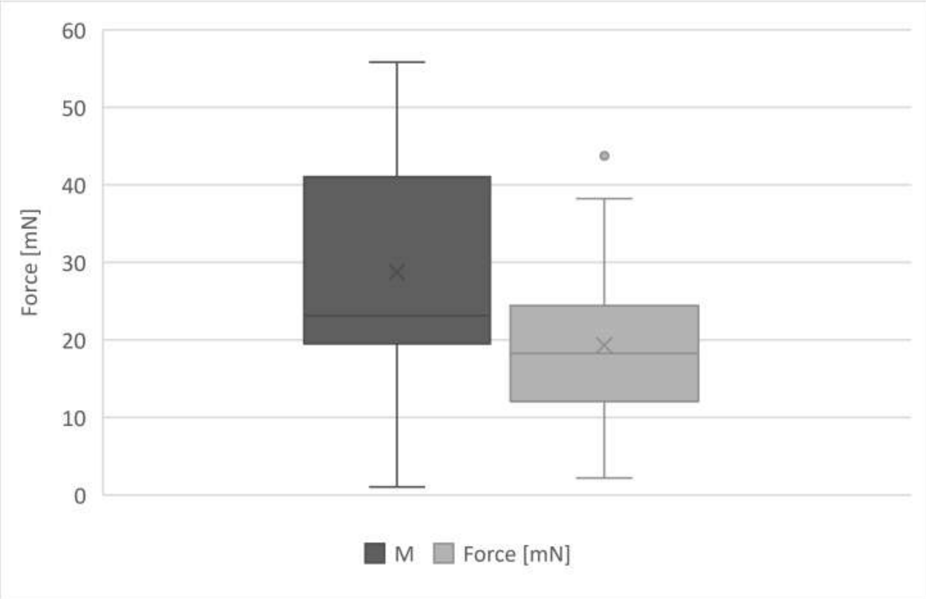

Figure 3: Box-and-whiskers-plot of the maximum forces measured for male and female beetles on centrifuge running horizontally.
The ends of the boxes show the 25th and 75th percentile of the data set, while the line in the box marks the median. The cross is marking the average of the data set. The lines show the minimum to maximum range of the data. The small circle denotes an outlier of the data set.

In Figure 4 the results for the vertical horizontal force testing (insects spinning parallel to rotational axis) can be found. In the vertical centrifugal force testing, males reached a median force of 4.4 mN [1.9 mN, 11.0 mN], while females reached a median force of 1.9 mN [0.5 mN, 4.2 mN]. In this test, males also show a higher performance than females, their respective percentile boxes do not overlap. On average, the male safety factor for the vertical centrifuge was 12.9, while the female safety factor was 4.8.

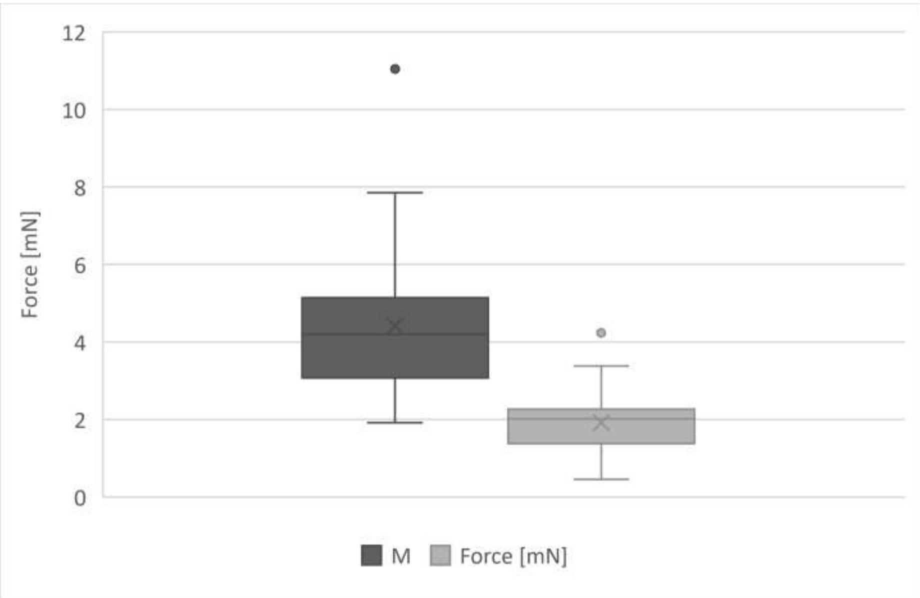

Figure 4: Box-and-whiskers-plot of the forces measured for male and female beetles on centrifuge running vertically. For more details on the box-and-whisker-plot please refer to Figure 3.

Figure 5 presents the results for the traction force measurement testing, in which the beetles were mechanically connected to a force sensor, the highest force exerted on the force sensor by the beetle pulling is evaluated. In the traction force testing, males reached a median force of 9.7 mN [4.4 mN, 20.1 mN], while females reached a median force of 4.4 mN [1.8 mN, 7.6 mN].

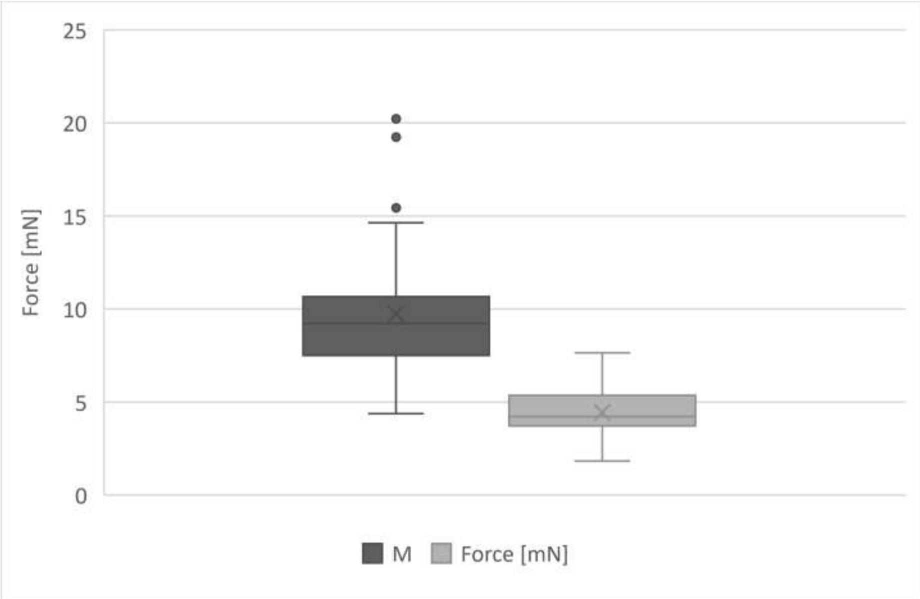

Figure 5: Box-and-whiskers-plot comparing force measurement results for male and female beetles for the traction test. For more details on the box-and-whisker-plot please refer to Figure 3.

2.2 Dependence of testing method

A comparison of the forces measured in the three different tests (horizontal, vertical and traction force testing) for male beetles can be found in Figure 6. This highlights the fact that forces achieved during the horizontal test (28.8 mN [1.1 mN, 55.8 mN]) are much higher than forces achieved during the vertical or traction force testing. Forces generated during the traction test (9.7 mN [4.4 mN, 20.1 mN]) are higher than the forces achieved during vertical centrifugal testing (4.4 mN [1.9 mN, 11.0 mN]).

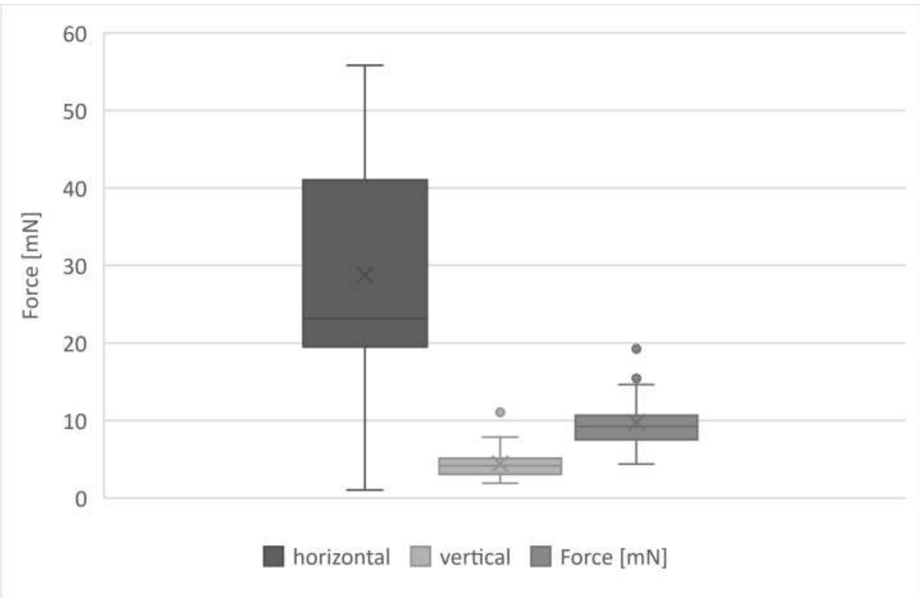

Figure 6: Comparison of force tests on the male beetle. For more details on the box-and-whisker-plot please refer to Figure 3.

A comparison of the forces measured in the three different tests (horizontal, vertical and traction force testing) for female beetles can be found in Figure 7. The data of these tests behave similarly to the data of the males' testing, in the sense that the highest forces are achieved during horizontal centrifugal testing (19.3 mN [2.2 mN, 43.7]) and the lowest during vertical centrifugal testing (1.9 mN

[0.5 mN, 4.2 mN]). While the generated traction force (4.4 mN [1.8 mN, 7,6 mN]) is higher than the force achieved in vertical centrifugal testing, the data are more similar in range than the horizontal and traction testing.

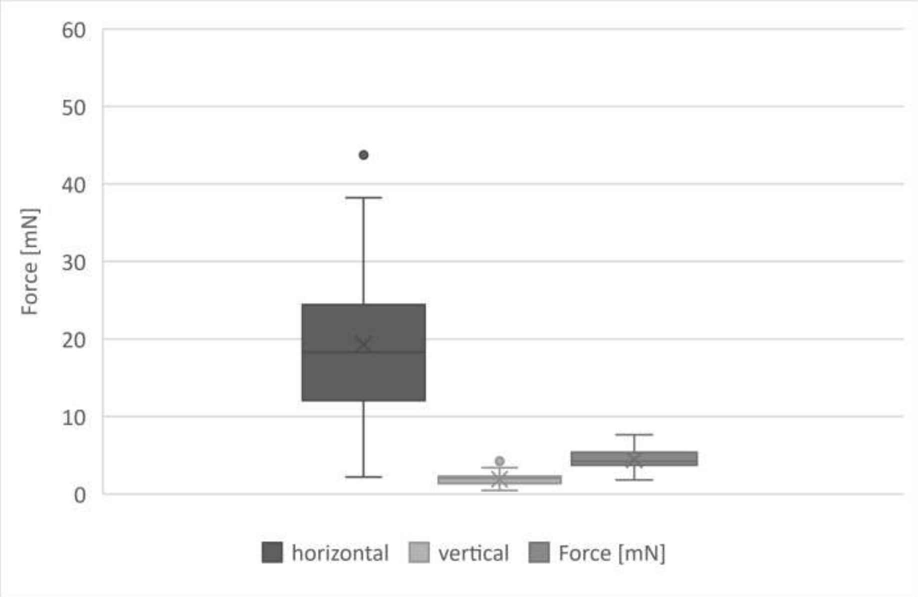

Figure 7: female beetle. For more details on the box-and-whisker-plot please refer to Figure 3.

In general, the average measured force values of the vertical centrifugal force measurement are approximately only 1/9 of the forces exhibited in the horizontal centrifugal force measurement, both for males and females.

3 Discussion

In all in all three test set-ups, males showed higher maximum forces than females. This confirms previous data gathered in other testing (Gorb E.V., 2010), although the methods of force testing have mostly been used singularly up to now. Males exhibit a higher force than females, especially when being measured on a relatively smooth surface, such as Plexiglas. This is said to result from sexual dimorphism in the beetles, in which males exhibit an additional type of tenant setae that enhance the adhesion to a female's elytra during copulation (Gorb E.V., 2010). The measured data ranges are also confirmed by values measured in previous studies. This is the first time measured forces of all three experimental set-ups used to measure forces have been compared with such a large group of subjects. Not only does this give an overview of the range and median of achieved forces in the different measurements useful for reference in further research, the set-up also allows a direct comparison of an individual's achieved forces in the different testing methods.

All three methods of measurement are used to determine attachment forces in the research of attachment. It is crucial to note the differences, especially between the centrifugal force measurements and the traction force sensor measurements, regarding the behavior of the examined organism. During the traction force sensor measurements, a specimen must actively pull on the force sensor for a notation of force to be achieved, while the forces measured with a centrifugal set-up are passive or involuntary.

The highest forces were measured during the horizontal centrifugal test, the lowest during the vertical centrifugal test. This is of interest, because a first assumption would most likely link a higher measured force to both involuntary centrifugal force experiments, leaving the traction force experiment, in which a specimen is actively pulling away from the force sensor, with presumed lower forces. This is not the case.

It is also important to note that the data used in the evaluation is always the maximum value achieved by each individual. This was actively chosen to show the capable force range and must be kept in mind while comparing other experimental data to this study.

4 Conclusion

Through the internship I gained many new experiences and knowledge on academic and personal levels. Before the internship, I had strongly considered a career in research in the scientific fieId due to my interests and educational path. Through my work in the research group I can confirm that scientific research, especially lab work such as planning and conducting experiments, is something I am interested in and enjoy. The extreme detail of biomechanical research was of immense interest to me, being a topic that Biology in school does not even cover. An aspect I had never regarded was how macroscopical relationships, that exist in nature and we presuppose, actually stem from microscopical, quantifiable interdependencies. Serious research in the field of Biomimetics and implementing these findings in synthetic systems will continue to be relevant in the future.

I decided to write my report in English because this was the primary language I came in contact with during my research. Not only was all of the literature (e.g. research papers) in English, but it was also the language spoken by the diverse research group with scientists from many different countries. In addition to my own study, I got to look over the shoulders of members of the Gorb group and was fascinated by the many different projects and studies running. I also had the chance to listen to talks by members of the group in preparation for a conference and a presentation of a bachelor thesis, from which I learned skills for my own future presentations.

As a prospective university student it was also very useful to have a trial of “student life”, i.e. living alone in a shared flat with all the respective time-management and housekeeping aspects but also navigating a new social setting. In the research group, I got the chance to interview Bachelor, Master and PhD students on everything from their choice of university to topic of research and motivation. This was extremely helpful while choosing and applying to different universities.

One of the most promising conclusions I can draw from my placement in the research group, is the importance of interdisciplinary research. In the Gorb group, there were not only biologists, but also other scientists with backgrounds in Physics, Chemistry and Mechanical Engineering conducting research on biological topics. As a person with broad interests in Biology as well as in Chemistry I find this quite inspiring and must negate my previous assumption that choosing one science as a major limits you to a very specific and narrow field of application. This realization facilitated my final university course choice. I have started studying Chemistry at the TU Dresden (Technische Universität Dresden) and hope to later combine this with Biology, either choosing Biochemistry as a Master or taking part in similar interdisciplinary research in Biomechanics or other fields as in my internship.

5 Acknowledgements

First off, I would like to thank Prof. Stanislav Gorb for offering the internship and admitting me into the research group as well as personally checking up on me and the experiment and Angela Veenendal for the organization and helping me get situated. I would also like to thank my supervisor Dr. Lars Heepe for his time, motivation, patience and problem-solving, as well as Dennis Petersen for his assistance with the measurement methods and the revision of this report. I would also like to thank Patricia Scholz for supervising me from the side of the Förderverein Biologieolympiade e.V. and editing of this report.

Finally, I want to thank the whole research group “Functional Morphology and Biomechanics” for such a positive reception, discussions, explanations and letting me look over their shoulders while conducting experiments and any other interesting things.

I would also like to thank the Förderverein Biologieolympiade e.V. and the VBIO for this amazing opportunity and the sponsorship of this internship.

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