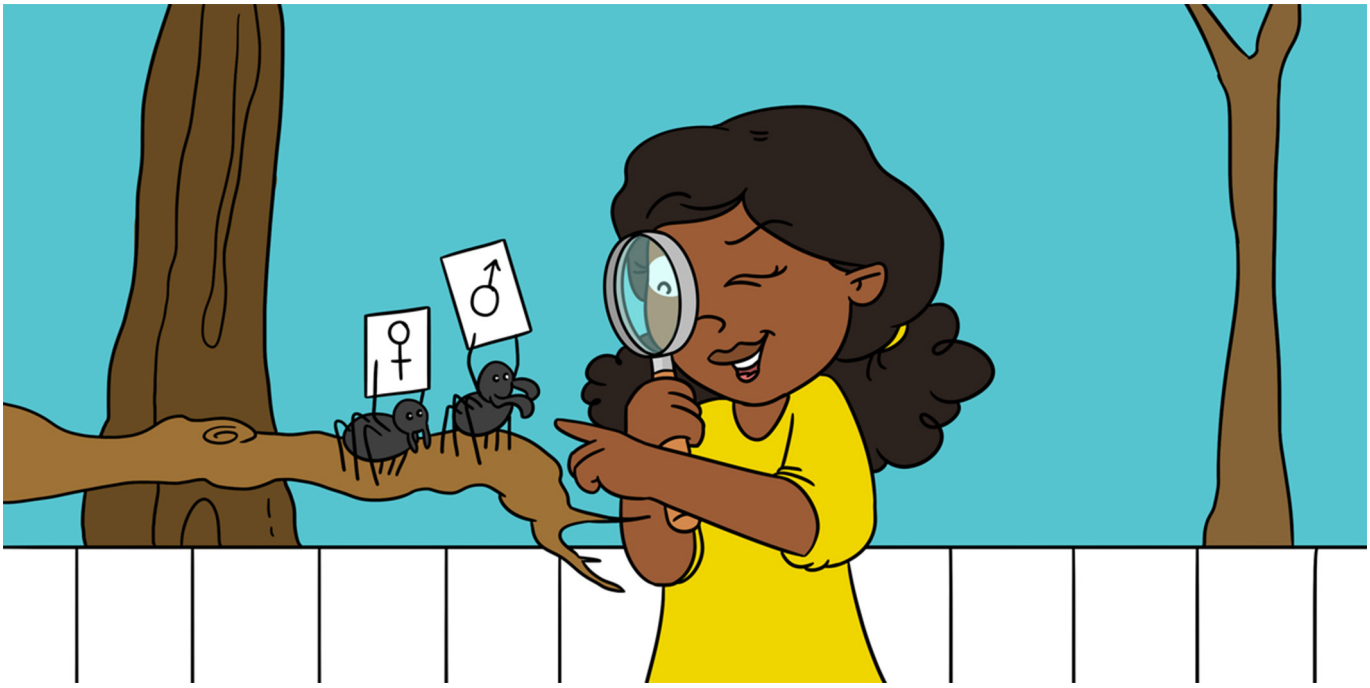
frontiers FOR YOUNG MINDS

BIODIVERSITY
Published: 26 June 2020
doi: 10.3389/frym.2020.00083



THE PROCESS OF BECOMING A "SPIDER-MAN"

Felix Simon Christian Quade [1*] ***and Natascha Turetzek*** [2*]

[1] *Molekulare Entwicklungsbiologie, Allgemeine Zoologie und Entwicklungsbiologie, Justus-Liebig-Universität Gießen, Giessen, Germany*

[2] *Ludwig-Maximilians-University Munich, Munich, Germany*

YOUNG REVIEWER:

JAMES

AGE: 11

Spider-Man gets his special powers from a spider bite. Real spider males do not have special powers, but they do have a specialized organ that female spiders do not have. It is called the bulbus organ, and it is needed for mating and thus really important. It is fascinating that the bulbus organ is absent in young spiders and has to develop inside of tiny appendages on the front of the spider, called pedipalps. The way organs develop inside already functioning body parts has puzzled researchers for years. Recently, scientists have found that the bulbus organ develops from a small set of cells, which are present early during the spider's development. Interestingly, during the development of the bulbus organ, other structures in the pedipalp are totally rebuilt from a mysterious material. Such processes usually only occur during metamorphosis in insects. This is the first example of a metamorphosis-like process happening in a species that does not have metamorphosis. So, maybe spiders have special powers after all!

DO YOU KNOW HOW TO TELL A SPIDER-MAN FROM A SPIDER-WOMAN?

Many people are so frightened of spiders that they never take a close look at these fascinating creatures. Spiders are really important! Thousands of different spider species exist, fulfilling many different roles. For example, preying on certain insects or other spiders helps to maintain a balanced ecosystem. Spiders are also fascinating because of their unique features, like the strong and elastic silk used to build complicated and beautiful webs, the production of venom, and their various advanced hunting and mating strategies. These features and skills differ between male and female spiders. But which structures make male and female spiders different?

You probably know of many animals in which the males and females look very different. Male lions, for example, have beautiful manes, while lioness do not. In birds, males tend to be beautifully colored, while the females are not—just think of ducks, chicken, or peacocks. But do you know how to distinguish spider-men and -women? Usually, female spiders are bigger, but only if they are well-fed. Females can also be a different color than the males of their species, but this is not true for all species. You can use another really safe trick to identify a male spider. In front of their four walking legs, both spider males and females have another pair of appendages, the **pedipalps**. The pedipalps are used for many of the spider skills explained above, like feeding, sensing, catching prey, and mating. And exactly on the tip of these pedipalps only adult male spiders have a specialized organ, which looks like boxing gloves. In Figure 1, you can see this specialized organ, which is called the **bulbus organ**. The adult male uses this bulbus organ to transfer its semen into the female during mating. Thus, the bulbus organ plays an essential role in spiders, because it ensures the production of offspring.

PEDIPALPS

Special leg-like appendages present in spiders, scorpions, and their relatives. In spiders, pedipalps are used for multiple purposes, like sensing the environment and transfer semen.

BULBUS ORGAN

Special organ on the male spiders pedipalp used to transfer the semen during mating.

ARTHROPODS

Group of animals including spiders, scorpions, mites, ticks, centipedes, crustaceans, and insects. Arthropods are characterized by their hard outer shell and many-jointed (arthro-) appendages (-pod).

CUTICLE

Hard, outer shell of arthropods, also called the exoskeleton.

HOW DO SPIDERS GROW UP?

Even though male and female spiders are both born with pedipalps, only the males form the bulbus organs as they become adults. So, how do spiders develop and, specifically, how does the bulbus organ form? Spiders initially develop inside eggs that their mothers laid in a cocoon. When they hatch from these eggs, the spiderlings are very small and fragile. The spiderlings look very similar to their parents but need to grow a lot. Growing is not that easy for spiders because, like other **arthropods**, they have a special outer covering. This covering is called **cuticle** (or exoskeleton) and protects the spider, just like a knight in shining armor. Like our bones, the hard cuticle gives the spiders their shape and bears their weight. Unfortunately for the spider, the cuticle cannot expand, so it must be shed allowing growth and body-shape changes. Therefore, when a spider builds a new cuticle, it must be made a bit large, so that there is room for the spider's growth and body

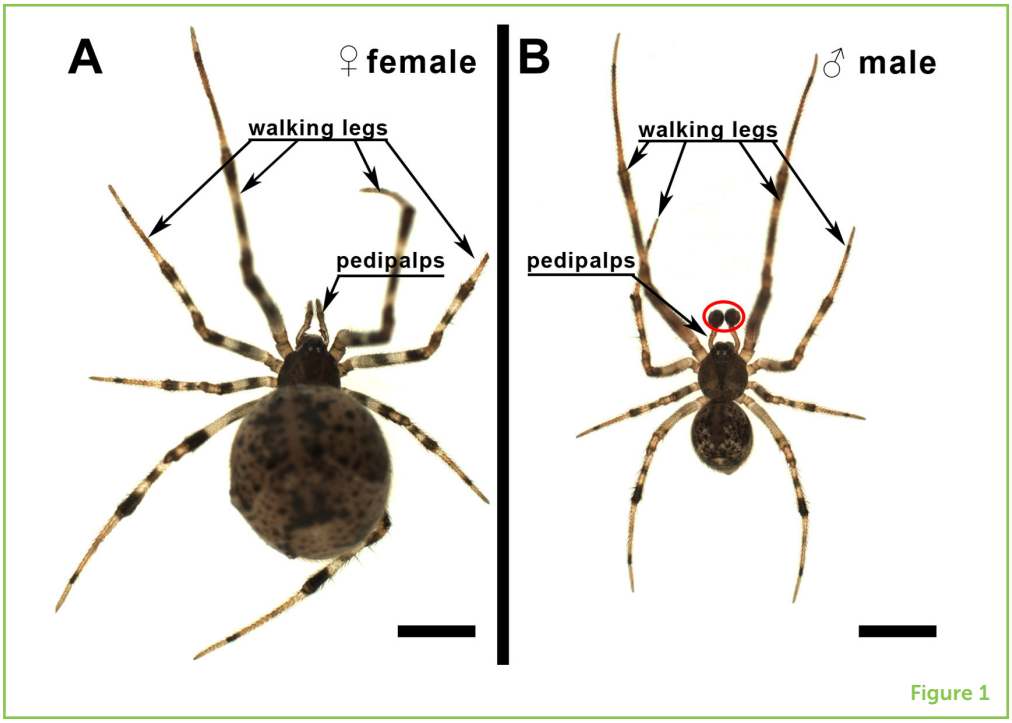


Figure 1

Comparison of female **(A)** and male **(B)** spiders. Females are generally bigger in size and may be a different color than the males. Both male and female spiders have small appendages in the front of their bodies called pedipalps. Males have a specialized organ called the bulbus organ on their pedipalps. The bulbus organs look like boxing gloves and are highlighted by a red circle on the male pedipalps.

changes to take place inside it. At some point, the spider slips out of its old cuticle and pumps itself up to its new size before hardening the new, bigger cuticle. This whole process is called **molting** and it happens several times in a spider's lifetime. Only toward the end of the male spider's growth does the bulbus organ begin to develop inside the pedipalp.

MOLTING

The process of shedding a hard outer shell in order to grow.

Scientists were puzzled for years over the fact that the complex structure of the male bulbus organ could develop inside the cuticle without disturbing the normal life of the animal. But, what exactly happens inside the pedipalps?

WHAT CAN WE SEE FROM THE OUTSIDE?

To understand how the bulbus organ is formed, scientists first investigated what can be seen on the outside of the cuticle of the male pedipalps. After hatching from the egg male and females spiderlings first look the same, because small male spiderling pedipalps are still lacking a bulbus organ (Figure 2, top left). After the molting of these spiderlings into an adolescent male spider the pedipalp is suddenly thickened at its tip and looks a bit like a blown-up balloon (Figure 2, top middle). Finally, after another molt this adolescent spider male becomes an adult male spider. The balloon at the tip of the pedipalp has transformed to the dark and complex folded bulbus organ. Although we can easily see changes in the size of the pedipalps as spiders develop, the crucial developmental processes that transform the "balloon" into the complex bulbus organs are not visible from the

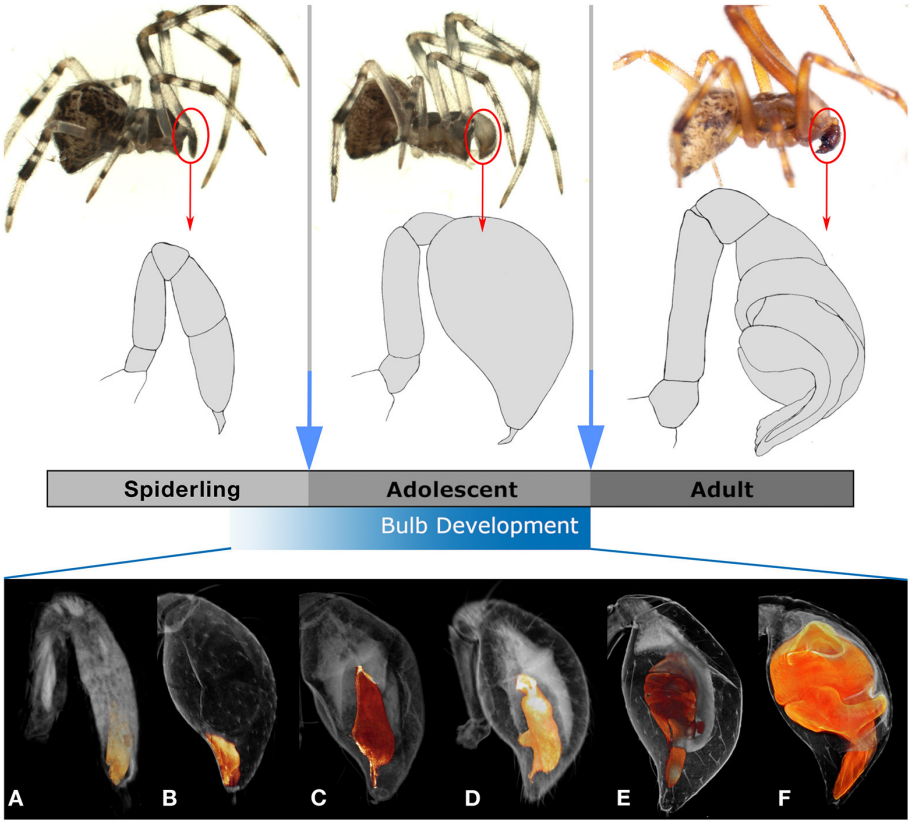


Figure 2

Development of the bulbus organ. Upper and middle rows: The pedipalp carrying the developing bulbus organ is highlighted by a red circle and enlarged in the drawing beneath each picture. You can see that the adolescent male pedipalp (middle) is growing, but no complicated outer structures are present. In the adult male (right), the bulbus organ is fully formed and has dark pigmentation. Bottom row (**A–F**): Internal view of bulbus organ development in spiderlings and adolescent males. The cuticle was bleached and the tissue was cleared so that 3D pictures could be reconstructed from images taken using a confocal laser scanning microscope. The part of the pedipalp which will become the bulbus organ is marked in orange. Derivative of Quade et al. (2019) under CC BY license.

outside. This is why, for many years, no one really knew how the bulbus organ formed inside the pedipalp and where the cells and tissues building this structure came from.

HOW DO WE LOOK THROUGH THE CUTICLE?

The cuticle of the transforming pedipalp is not only hard, but also non-transparent. So, we had to use some tricks to see what was happening inside. First, we used hydrogen peroxide to bleach the cuticle. You might know that bleaching can create bright spots on dark jeans or can make dark hair blonde. Both of these examples rely on the bleaching process removing the color pigments. We used the same effect to remove all the dark color from the cuticle, making it transparent.

However, we were still not able to clearly see the developing structures inside the cuticle, because the pictures we took were too blurry. This is because, like most tissues, pedipalps consist of a mixture of materials, for example water and tiny fat droplets. As the light travels through the different materials, it gets bent and scattered, making the images taken by the microscope camera blurry. You might have experienced these properties of light in your daily life. When you, for example, put a stick into water it looks crooked. Here the light gets bend on the border

between the water and the air on its way to your eye. This is similar to what happens with fog. Fog is actually just air containing many tiny water droplets. Because of all the little water droplets in fog, the light gets bent many times and therefore you cannot properly see what is behind the fog.

So, for our second trick, we needed to remove the "fog" in the tissue with a technique called tissue clearing. To clear the tissue in the pedipalps, we had to replace either the fat droplets or the water with another material. We chose to replace the water with a chemical with fat-like light properties called methyl salicylate, so that the light could move through the tissue without scattering, giving us a clear picture.

SLICING WITHOUT CUTTING

Once we bleached the tissue making it transparent, and replaced the water, we were ready to take pictures of the developing bulbus organ inside the pedipalp. To do this, we used a special microscope called a **confocal laser scanning microscope**, which uses special lasers to take pictures rather than using the white light from normal light bulbs. These lasers are extremely thin beams of light that can be focused on a very specific part of the pedipalp. We use this technique to take many pictures, at different levels from the bottom to the top of the sample. Each of these images is called a slice, because it is like taking a slice through the sample and taking a picture of the cut surface. These slices can be stitched back together on a computer, which ultimately reconstructs three-dimensional (3D) images of the entire pedipalp.

CONFOCAL LASER SCANNING MICROSCOPE

A complicated microscope which uses mirrors, pinholes and thin light beams, lasers. The laser beams can scan a sample at different levels producing various high resolution two-dimensional images. All these 2D images can afterwards be calculated together giving really sharp 3D images of complex tissues.

In these 3D pedipalp images we can mark all the different structures on every slice, so that we could highlight the structures in different colors on the 3D reconstruction (Figures 2A–F). This marking allowed us to figure out how the bulbus organ forms, all without ever cutting up the sample! Isn't that amazing?

WHAT IS NEEDED TO MAKE A SPIDER-MAN: SOME SURPRISES

The use of these advanced techniques revealed some surprises. First, we found that the development of the spiderling's bulbus organ starts even before the cuticle "balloon" blows up (Figure 2A). In the adolescent spider, the balloon then contains a small structure in its tip (Figures 2B–F) that grows and forms complex folds as it develops into its final shape inside the "balloon." The other surprise was that other structures in the pedipalp tip that do not belong to the bulbus organ were also rebuilt.

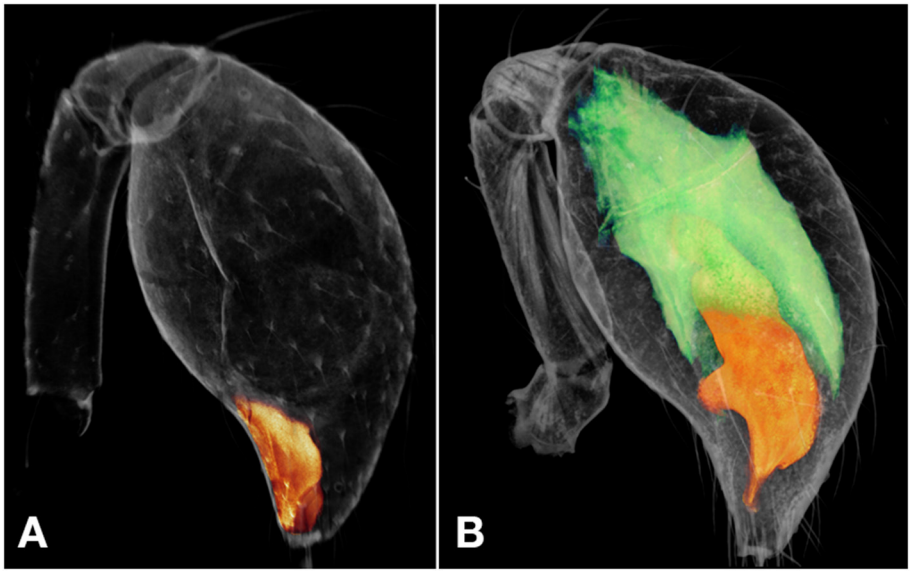


Figure 3

More detailed three-dimensional representation of early bulbus organ and pedipalp development as shown in Figures 2B,D. The structures becoming the bulbus organ are shown in orange, while the other structures of the pedipalp tip that are also rebuilding inside the cuticle are shown in green. Derivative of Quade et al. (2019) under CC BY license.

Usually, growing structures in spiders are built by reshaping the tissue lying directly under the cuticle. So, it was very surprising to realize that these other pedipalp structures were formed completely anew, by a process we do not fully understand yet. We saw that, right after molting, the cuticle "balloon" was empty except for the developing bulbus organ structures, but after some time, a new material appeared and rebuilt the other structures of the pedipalp tip (Figure 3). This material had some clusters of cells within it but did not appear to be made by these cells.

CONCLUSION

Although spiders and insects do not look much alike, they both belong to the arthropods and have to manage developing into an adult inside their hard outer cuticle. Some insects solved this problem in a special way. You probably know the book "the very hungry caterpillar." The caterpillar first transforms into an immobile pupa and finally hatches as the butterfly. This process is called **metamorphosis**. During this process many body parts of the caterpillar are "dissolved" and then reorganized to form the body parts of the adult butterfly. Other insects and spiders do not use such an immobile pupal stage and have to grow and develop all structures inside their cuticle, without disturbing the functionality of their outer body parts. Nobody knew how spiders and probably also other arthropods fulfill such astonishing tissue transformations underneath their cuticle without being protected in a pupa. Studying the developing spider male bulbus organ and using several tricks helped us to solve this mystery. Our findings indicate that the material for the developing structures can be set aside already really early during development, far before the structure is present on the outside. Also, we found that

METAMORPHOSIS

A process of dramatic transformation happening during the life of some animals, such as butterflies, which are born as caterpillars.

similar dissolving and reorganization processes known from insect metamorphosis could also take place during spider development without the transformation into a pupa. This discovery is very fascinating. Knowing this we are very curious to identify the origin and nature of this material and how the cells clustering in this material reorganize it.

ORIGINAL SOURCE ARTICLE

Quade, F. S. C., Holtzheimer, J., Frohn, J., Töpperwien, M., Salditt, T., and Prpic, N. M. 2019. Formation and development of the male copulatory organ in the spider *Parasteatoda tepidariorum* involves a metamorphosis-like process. *Sci. Rep.* 9:6945. doi: 10.1038/s41598-019-43192-9

SUBMITTED: 01 November 2019; **ACCEPTED:** 25 May 2020;
PUBLISHED ONLINE: 26 June 2020.

EDITED BY: Alistair P. McGregor, Oxford Brookes University, United Kingdom

CITATION: Quade FSC and Turetzek N (2020) The Process of Becoming a "Spider-Man". Front. Young Minds 8:83. doi: 10.3389/frym.2020.00083

CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWER

JAMES, AGE: 11
I am an 11 years old boy who loves reading. My favorite lessons at school are classics and history. My other hobbies are collecting rocks and fossils for my museum, and doing tournaments with my Hot Wheels collection.

AUTHORS

FELIX SIMON CHRISTIAN QUADE

As a small child, Dr. Felix Quade was already fascinated by nature's variety of shapes and how they evolved. He always was interested how things worked and wanted to look inside them. After school, he studied biology in Rostock and for his thesis, he worked on the claws of hermit crabs and their relatives using a technique called computer tomography. He did his Ph.D. in Göttingen, working on the development and adult morphology of the bulbus organ of spiders. Now he uses all kinds of microscopy and X-ray imaging to look inside animals and uses computers to make visible what is otherwise invisible. *felix.quade@allzool.bio.uni-giessen.de

NATASCHA TURETZEK

Dr. Natascha Turetzek was always fascinated by animal development and how an adult organism can be formed from one single cell. Therefore, she did her Ph.D. in evolutionary developmental biology, doing comparative analyses of embryonic development in spiders and different insects. Now she runs a lab at the LMU in Munich, working on the evolution and development of sense organs in different arthropods. *zhang@bio.lmu.de