**Speech for Arenberg Prize Award Ceremony 2015**

Benjamin van Soldt

It is a great honor for me to be here today to receive the Arenberg Prize 2015, and I am thankful towards the committee of the Coimbra Group and in particular to the Duke of Arenberg for nominating me as this year's awardee of the prize.

When I finished my undergraduate thesis at Leiden University under the supervision of Profs. Richardson and Poelmann, I knew three things: that I loved to study evolutionary relations of animal anatomies, that to do this well I'd need to incorporate other dimensions, and that anyway I would never get a job doing just anatomical research. As a second dimension, I found that adding either developmental biology or physiology would be the most interesting to me, so that I carefully structured my Master: first I did a study in developmental biology at Leiden University, again under the expert supervision of Profs Richardson and Poelmann. However, studying physiology at Leiden University was impossible since there is no department for it here. I therefor made use of the opportunity Erasmus presented me with, which enabled me to work at the zoophysiology section of the department of biosciences at the University of Aarhus under the supervision of Prof Wang and Danielsen. Thus the Erasmus exchange was a seamless part of my research plan, and served a strictly defined aim: to acquire a more holistic view of evolutionary processes and research. In addition I now have an international network of scientists to collaborate with (we are seeking to continue or start some new projects). Vitally, having done research in both developmental biology and physiology, I was able to assess what I would find most interesting to specialize for a PhD so that now I'm enrolled in the Genetics PhD program at Columbia University in the City of New York. Finally, we recently submitted for review one out of two manuscripts based on my research in Aarhus.

To now receive the Arenberg Prize on the basis of this research plan and the role Erasmus played in it is a great honor for me and a huge confidence boost, and I again want to express my deepest thanks to all parties involved in the selection process.

I didn't get to this stage by my own powers alone, however, and I am deeply indebted to several people. First of all my parents, whose support and help put me on the right path early on. Secondly my supervisors, Profs Richardson and Poelmann who could join us here today, and Profs Wang and Danielsen, who sadly weren't able to come to the Netherlands today, were also instrumental in my scientific development, offering valuable direction, consultation, explanation, help, support and supervision. Finally my friends who always offered comic or serious relief. Thank you.

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# Evolution through Erasmus

*Arenberg-Coimbra Group prize application*

*By Benjamin van Soldt*

In the course of billions of years, organisms have evolved from single cells to complicated multicellular beings with distinct anatomies, physiologies and behaviors. Nevertheless, all organisms, past and present, have shared characteristics through which their common origin can be inferred. For example, tetrapods have four limbs to assist them in locomotion. Through the identical "building plan" of the limbs, scientists can group tetrapod species and point at a single origin from which tetrapods likely evolved. It's through these instances of homology or analogy in structures that evolution can be reconstructed.

Of course such homologies need not be external, such as the limb: any organ may be used for evolutionary analysis. One organ system that shows dramatic conservation throughout the animal kingdom is the cardiorespiratory system: the heart and the lung. These two organs, which are highly interconnected with each other, can be found in as far back as insects. Here, the 'lungs' are an intricate network of tubes called the tracheal system. Ventilation (the entrance of air into the tracheal system) is a purely passive process, followed by passive diffusion of oxygen into the insect's haemolymph (analogous to blood in vertebrates). This carries it around the body. However, contrary to vertebrates, haemolymph is not contained in blood vessels throughout its journey around the insect's body. Instead, the haemolymph flows freely from anterior (the head) to posterior (the abdomen), and only here does it get collected in a vessel: the heart is a tube consisting of several pumping regions that thrust the haemolymph forward, into the aorta. The aorta then releases the blood anteriorly, in the head.

The basic plan of the cardiorespiratory system has been preserved: to this day oxygen-rich blood is pumped towards the head by the heart, then travels to the back of the animal, after which it returns to the heart. In addition, some basic elements have persisted throughout evolution: 1) the respiratory system consist of a (branched) network of tubes, 2) the heart consists of several, serially organized chambers that serve as pumps, and 3) an 'outflow tract' leads oxygen and nutrient-rich medium into an aorta, which guides it away from the heart and towards the head.

The evolution of the cardiorespiratory system from its relatively simple insect form to that of the much more complicated mammalian one proceeded in steps. When examining groups of organisms, such as fish, amphibians, reptiles and mammals, the previously mentioned basic plan and common characteristics are apparent, and show increasing complexity. Similar to insects, the fish heart is still a series of pumping chambers, though the chambers can now be formally regarded as being distinct subunits of a whole. They have distinct gene expression patterns concomitant with their respective morphologies and physiologies, and can thus be broken up and named individually: sinus venosus, atrium, ventricle and outflow tract.

The various subunits are mostly conserved. Amphibians feature a reorganization of the chamber topography: ventricle and atrium are positioned differently with respect to each other, and the atrium is split into two chambers, one of which accepts blood from the lungs, while the other accepts blood from the body. While snakes, turtles and lizards preserve the undivided heart of amphibians, the atrial division is further cemented in archosaurs (crocodiles and birds), where also a divided (septated) ventricle now appears. Mammals finally feature a four-chambered heart, in which both atrium and ventricle are divided. The respiratory system has undergone similar evolutionary steps, from the insect tracheal system to the gills of fish, the sac-like lungs of reptiles, and bifurcating system of tubular airways of mammals.

Of seminal importance is that with anatomical division of the heart in archosaurs and mammals, also the possibility of functional division developed: these hearts feature a low blood pressure (the right side of the heart) and high blood pressure system (the left side of the heart). The former perfuses the damage-prone lungs (the pulmonary circulation), whereas the latter perfuses the body (the systemic circulation). This division of blood pressures over systemic and pulmonary circulations shows the fundamental interconnectedness of the lungs and heart.

Of all so far described cardiorespiratory anatomies, the cardiorespiratory system of reptiles is arguably the most diverse. From the undivided ventricle of turtles to the divided ventricle of archosaurs, and from the simple sac-like lung of snakes to the complicated sheet-like arrangements in birds; the fundamental differences between some reptile cardiorespiratory systems are astounding, and are a testament to the many habitats reptiles inhabit. These range from oceans to tree canopies, and from humid swamps to deserts heats.

This diversity in morphology and concurrent habitats is a treasure trove for evolutionary biologists. In particular snakes are intriguing, for they inhabit virtually all habitats that reptiles were found to inhabit. In addition, their slim and long bodies, limblessness and use of venom, among other examples, make them into organisms that are highly adapted to their environments. For this reason herpetologists see snakes as a model organism for 'extreme adaptations': adaptations that surpass the 'norm'. Extreme adaptations are particularly desirable in evolutionary analysis: if you understand the most extreme adaptation, you'll likely have an easier time understanding intermediate adaptations.

The snake cardiorespiratory system is a beautiful example of one extreme adaptation: extreme asymmetry. Many tetrapod cardiorespiratory systems known to us are asymmetric. For example, the left lung in humans is smaller than the right lung, probably in order to provide room for the heart, which itself is positioned off-center. Snake lungs are heavily asymmetric, with the left lung being no more than 66% the length of the right lung. This is only in 'primitive snakes', which consist of the Pythons, among others. In 'advanced snakes', however, the left lung frequently is rudimentary or non-existent.

The various snake lung morphologies have consequences for the vascular patterns of the pulmonary arteries, which guide oxygen-poor blood from the heart to the lungs. I documented these vascular patterns in a thesis that concluded my BSc in Biology at Leiden University. In essence there are three lung morphology types (two big, though asymmetric lungs; one big and one small lung; and only one big lung), and the pulmonary arteries follow the patterns of the lungs almost exactly. In principal, if there are two functional lungs, there are two pulmonary arteries and if there is only one functional lung, there is only one pulmonary artery. In these cases, the heart is positioned above the lungs, so that pulmonary arteries can only run downwards, where they connect with the lung. However, the snake heart need not be positioned 'above' the lung; it may be positioned halfway the snake lung also. In these cases, there are also two pulmonary arteries: one follows the lung in an upward direction, while the other follows it downward.

At this point in time my attention was drawn to the evolutionary relations between the various morphology types that I had identified. How did these types arise in evolution? How are gene expression patterns conserved, or changed, to constitute the various types, and how do the types change the functionality of the cardiorespiratory system as a whole?

The above questions portray an important point: evolution is best studied by incorporating a variety of fields, genetics, developmental biology, ecology and physiology being the most prominent ones. Genetics and developmental biology represent previous rounds of natural selection, which have been eternalized in the genetic code that lies at the base of modern-day organisms; studying this shows us the genetic "road map" the animals took as they adapted to new habitats. In contrast, physiology (the expression of that genetic code in functional systems) is the subject of current natural selection and ecologists study the arena where these current rounds of selection play out in. Combining these fields can thereby give a fully-fledged understanding of previous selection events.

Understanding that attempting to specialize in all these fields will undoubtedly lead me to having to cut corners in the long term, I was faced with a decision: what field would I principally specialize in? I used my two-year MSc at Leiden University to find out the answer. The first year I spent in Leiden studying developmental aspects of the snake lung and pulmonary arteries, while the second year I spent at Aarhus University in Denmark via the Erasmus exchange program, analyzing functional aspects of the adult snake cardiorespiratory system.

In my Leiden thesis I worked on the development of the pulmonary arteries and lungs in a variety of snake species. Together with colleagues in Vienna, we scanned the embryos in a MicroCT scanner, after which I imported the scans into a computer program. Here I compiled 3D models, which I used to explain the development of the lungs and pulmonary arteries. I showed that the three types of lung morphology are principally distinguished by the speed of development of the left lung, constituting three general developmental patterns. The pulmonary arteries follow lung development such that if no left lung develops, no left pulmonary artery develops either. In addition we did molecular biology, to assess gene expression patterns, but this proved less successful. A follow-up study to troubleshoot problems we encountered is underway, though results obtained so far have been written up in a manuscript. This was recently accepted conditionally; some minor technical aspects have to be addressed.

In order to approach cardiorespiratory evolution from a new, fresh angle, I decided to also do a physiological study. However, Leiden University does not have a department of animal physiology. Therefore I decided to do an Erasmus exchange to work at the renowned section of zoophysiology of Aarhus University, Denmark, under the supervision of Profs. Tobias Wang and Carl Christian Danielsen, both well-respected scientists in their fields. In this thesis I tried to infer ancient evolutionary patterns by looking at current cardiorespiratory physiologies.

Pythons are unique among snakes in that they have ventricular pressure separation: though their ventricle lacks an anatomical division, they have a high-pressure systemic circulation and a low-pressure pulmonary circulation, like in mammals. Instead of an anatomical division, it turns out they have a moving anatomical structure that divides the heart at the right time at the right place. Thus, Pythons have a functionally divided ventricle despite having an anatomically undivided heart.

In my Aarhus thesis, I tested the strength of the pulmonary arteries and systemic arteries of Pythons, and compared them to the wall strength of arteries in the earth boa: a close relative of the Python, but without ventricular pressure separation. Typically, stronger artery walls will be employed to cope with higher pressures, so that Python's systemic artery walls can be expected to be stronger than its pulmonary artery walls. In the earth boa, however, we would have expected them to be of similar strength.

While our hypothesis proved correct for Python, the earth boa's systemic arteries showed unexpected strength, while the pulmonary arteries were unexpectedly weak. However, their respective diameters, which have a big influence on their strength, proved to vary in such a way that explained everything. Additionally, in both Python and earth boa the right pulmonary artery proved to be of bigger diameter and its wall stronger.

The different diameters and strengths of the pulmonary arteries are, in my opinion, the most interesting finding of this project and it poses new questions regarding cardiorespiratory evolution in snakes. That the left pulmonary artery would be smaller and weaker would fit perfectly with the finding that the left lung is the smaller of the two lungs: smaller lungs take up less oxygen, so that a smaller amount of blood, carried by a thinner vessel, will suffice to carry away the oxygen that is absorbed. However, this hypothesis only works if indeed the amount of oxygen that is taken up by the left lung is less than what is taken up by the right lung (the latter has a higher respiratory contribution). This is a point that I hope to investigate with colleagues at Aarhus University in the near future. If indeed we find a link between vessel diameter and lung respiratory contribution, an interesting evolutionary question rears its head: did the left lung decrease in size when the left pulmonary artery became thinner, or vice versa?

At the end of my MSc I had two theses about distinct subjects that served to increase my understanding of cardiorespiratory evolution; and gave me a host of new questions to answer. I'm convinced that had I not gone to Denmark by way of an Erasmus exchange, my horizons would not have broadened to the extent that they have been now. I think it's of significant importance to be aware of the contributions of myriad fields, and to combine this knowledge into one, comprehensive theorem. I believe that this Erasmus exchange has helped me do exactly that. Plus, It gave me the opportunity to work with top scientists in the field of reptile physiology and biomechanics, so that my network has broadened perhaps as much as my scientific horizon has.