Primer

Scaling in biology

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Ernest Rutherford is credited with the provocative assertion that all attempts to expand human knowledge are either physics or stamp collecting. Whatever your opinion of this statement, the two modes are not mutually exclusive, and some of the most interesting efforts to marry the two endeavours have looked at questions of scale in biology. Evolution may drive species to different sizes, shapes, energetic lifestyles and behaviours, but the laws of physics govern both the internal workings of life and its interaction with the external environment. For example, imagine taking a five centimetre tall shrew and multiplying each of its length dimensions by a factor of sixty to produce an elephant-sized shrew. What would happen? The weight that an animal's limbs must support scales with its volume, yet the forces that its bones can withstand or its muscles can produce scale with area. And so this animal would be in serious trouble, to say the least.

Comparisons between how a biological feature varies with predictions made by the laws of physics can offer explanatory power, testable hypotheses and guidance toward important open questions in biology. Scaling has been used, and must continue to be used, to shed light on these fundamental questions: How large can an animal be and still fly? Why do big animals have straight legs? How do bacteria propel themselves? This primer will provide an introduction to scaling, its mechanisms and limitations, and then focus on two fields — metabolics and biomechanics — for which there is both a rich history and thriving current research. Finally, I shall discuss a third area that appears ripe for future research in scaling: the neural control of locomotion.

Scaling in biology is an enormous subject, with a fascinating history. After Galileo's early strides toward understanding how geometry and size affect the mechanical soundness of structures, the method of dimensional analysis emerged during the industrial revolution. This new tool then found utility across biology: first in physiology (now metabolics) starting in the 1830s, then in the biomechanics of supporting structures (trees) in the 1880s, followed by a broad expansion into embryology and development, evolution and ecology, and animal locomotion in the early 1900s. Most recently, starting in the 1970s, scaling analyses have been applied to locomotion in microscopic environments. Overall, interesting scale effects appear in three different contexts: within the ontogenetic sequence of a single organism; within the members of a given species; and across species. I will focus on interspecific comparisons at the tissue and organismal levels; I do so, however, acknowledging the power of integrative approaches that consider the interaction across levels of biological organization.

Analysis of scaling

The analysis of scaling requires: first, measuring how a biological characteristic changes with size; second, understanding some useful properties of logarithms and statistical regression; and third, application of relevant physical laws. To get a feel for how it works, consider a cube of mass density ρ whose sides have length L. It has area proportional to L^2, volume proportional to L^3, a mass of ρL^3 and a weight of $\rho L^3 g$, both of which are also proportional to L^3. Now imagine manufacturing a set of such cubes in which each one has its linear dimensions multiplied by a random scale factor. One could then measure the weight, mass, length, area and volume of each, and plot the resultant quantities against each other. One would then expect to see, for example, that side length is proportional to $mass^{1/3}$, surface area is proportional to $mass^{2/3}$, and so on. This is referred to as isometric, or geometric, scaling, wherein the features of the object scale in the manner predicted by multiplication of all linear dimensions by a constant factor.

To make analysis easier, we can exploit the fact that taking the logarithm of a variable raised to some exponent brings that exponent down and makes it the slope of a line. If $y = ax^b$, then $\log y = \log a + b \log x$. Thus, we can take the logarithm of our measured variables, regress a line onto a plot of one against the other, and the slope of that fitted line is the exponent that relates the two variables. Figure 1A and B illustrate this: they show four curves with different values of a and b, plotted over four orders of magnitude, on linear and logarithmic axes. Part of the utility of this approach stems from the fact that a large number of biological features do in fact appear to scale according to a power law of the form $y = ax^b$. That makes these features amenable to comparison with the scaling that would be predicted from the equations that govern physical processes. The scaling need not be isometric, and frequently is not; for example, the cross sectional area of a bone might increase with body mass much faster than would be predicted by isometric scaling. This could be one way to make our elephant-sized shrew mechanically viable. It also happens to be the case with rhinoceroses and their kin, whose long bones have diameters that scale with body mass to the power 0.5 rather than 0.33. In cases like this, when a scaling exponent is different from that predicted by isometry, it is termed allometric.

Though scaling relationships are very powerful for illuminating biological function, they must be interpreted with caution. For example, examining the scaling of a feature across several species without considering how they are related to each other can lead to erroneous conclusions, because each species may not represent an independent sample of how that feature scales with the independent variable. As such, it is important to take into account the phylogenetic relationship between the examined species. This is illustrated in Figure 2A. It is also worth remembering that a statistically significant regression simply tells you something about the scatter of the points on your graph, and does not necessarily imply a biological cause. Scaling relationships are not, strictly speaking, biological laws, but rather are descriptions that may highlight underlying mechanisms. And one needs to be very careful when
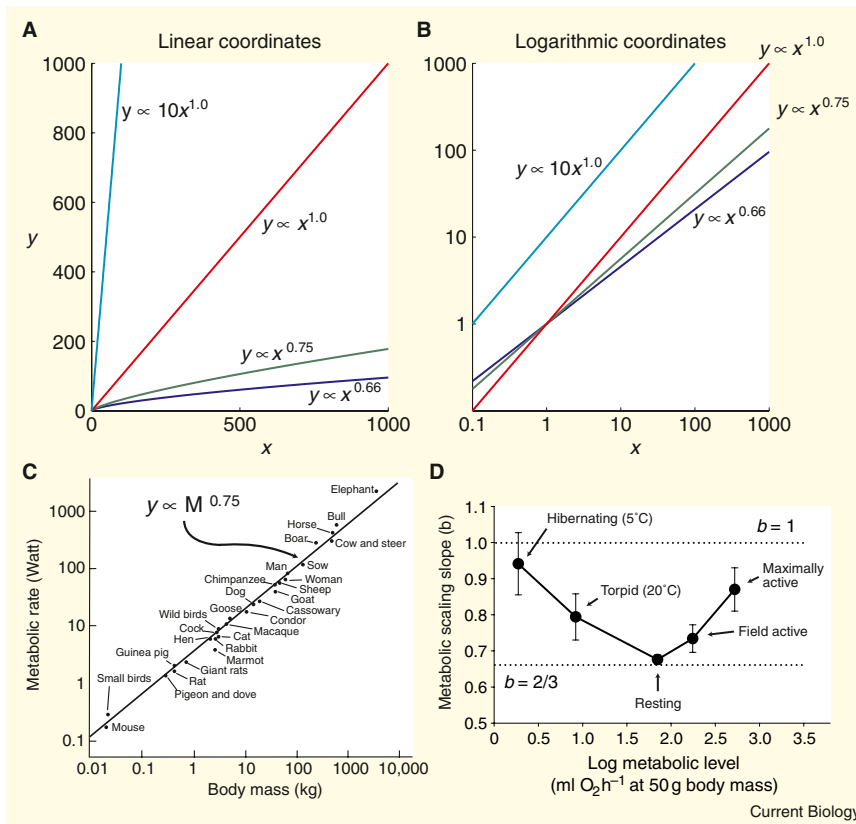
Figure 1. Examples of scaling relationships and scaling in metabolics.

Examples of scaling relationships of the form $y = ax^b$, for four different values of a and b, plotted over four orders of magnitude of x, on both (A) linear and (B) logarithmic axes. Since $\log y = \log a + b \log x$, plotting y against x on logarithmic coordinates brings the exponent b down and makes it the slope of the curve, whilst a becomes a constant offset. (C) Kleiber's law. Metabolic rate in birds and mammals scales with body mass to the ¾ power. This has been a long-standing puzzle, as *a priori* it could reasonably be expected to scale with surface area (⅔) or body mass (1), depending on whether it is limited by exchange through or over surfaces, or the volume of metabolic machinery under consideration. Adapted with permission from Schmidt-Nielsen (1984). (D) The metabolic-level boundaries hypothesis. Taking into account the physiological state of the animal, metabolic rate is found to vary systematically between ⅔ and 1 depending on the animals' activity level. It approaches ⅔ during states which are limited by surface area, such as resting, and 1 during those that are limited by volume, such as hibernation or maximal exertion. Adapted with permission from Glazier (2008).

drawing conclusions about ranges of body size that lie off the end of a measured scaling relationship: extrapolating to other size ranges without *a priori* knowledge of potential discontinuities or constraints is hazardous.

Metabolics

Some of the earliest biological investigations into scaling were done by French physiologists in the 1830s, who considered how lung surface area and volume scale with body mass in humans. This early work led to broader examination of how metabolic rate scales with body mass across species, which disclosed a ¾ power law named for its originator, Max Kleiber (Figure 1C). Kleiber's

law has remained one of the most persistent yet perplexing scaling relationships in biology. Because respiratory gasses are exchanged across epithelia, one might predict that metabolic rate would be governed by surface area and hence scale with body mass to the ⅔ power. Alternatively, it might be governed by the volume of metabolic machinery in an organism, and hence scale with body mass directly. Yet metabolic rate consistently scales between the two, specifically to the ¾ power of body mass.

The mechanistic basis for Kleiber's law is a source of on-going research and debate. Some have claimed a universal source of ¼ scaling laws in biology is the fractal nature of

respiratory branching patterns. Others have revealed systematic variation in the metabolic activity levels underlying Kleiber's law. Instead of measuring an average of metabolic scope, considering the state of the animal during metabolic measurements reveals a U- or V-shaped curve as the animal shifts from torpor, to rest, to field activity, to performing strenuous exercise (Figure 1D). This curve fits the 'metabolic-level boundaries' hypothesis which predicts that the exponent b for metabolic scaling should vary between ⅔ and 1 as metabolic level varies from being governed by heat flux through surfaces to being governed by heat production per unit volume (or mass) of an organism. At the two extremes of hibernation and strenuous exercise, volume effects predominate and b approaches 1. This is because in hibernation, the minimum metabolic level depends on body mass and surface area does not limit metabolic rate. Similarly, for brief periods of strenuous exercise the metabolic machinery throughout the body mass can be turned on. During rest, however, surface area effects dominate as excess heat is dissipated and b tends to ⅔, resulting in a U-shaped curve.

If scaling relationships such as Kleiber's law can be criticized as oversimplifications of more detailed, underlying mechanisms, then one might question the utility of describing broad ranges of life with power laws. But the 'metabolic-level boundaries hypothesis' grew out of considerations of how data varied systematically away from a ¾ rule. Therefore, it may never have been proposed without the starting point of Kleiber's law. This certainly highlights the utility of scaling for guiding and refining the iterations inherent in scientific research and discovery. In addition to answering broad questions about how life varies, the ways in which such analyses are wrong, which are termed the secondary signals of scaling relationships, can be just as important as the primary signal itself.

Biomechanics

Two of the critical roles of the metabolic machinery are the maintenance of support structures, such as tree trunks or appendages,
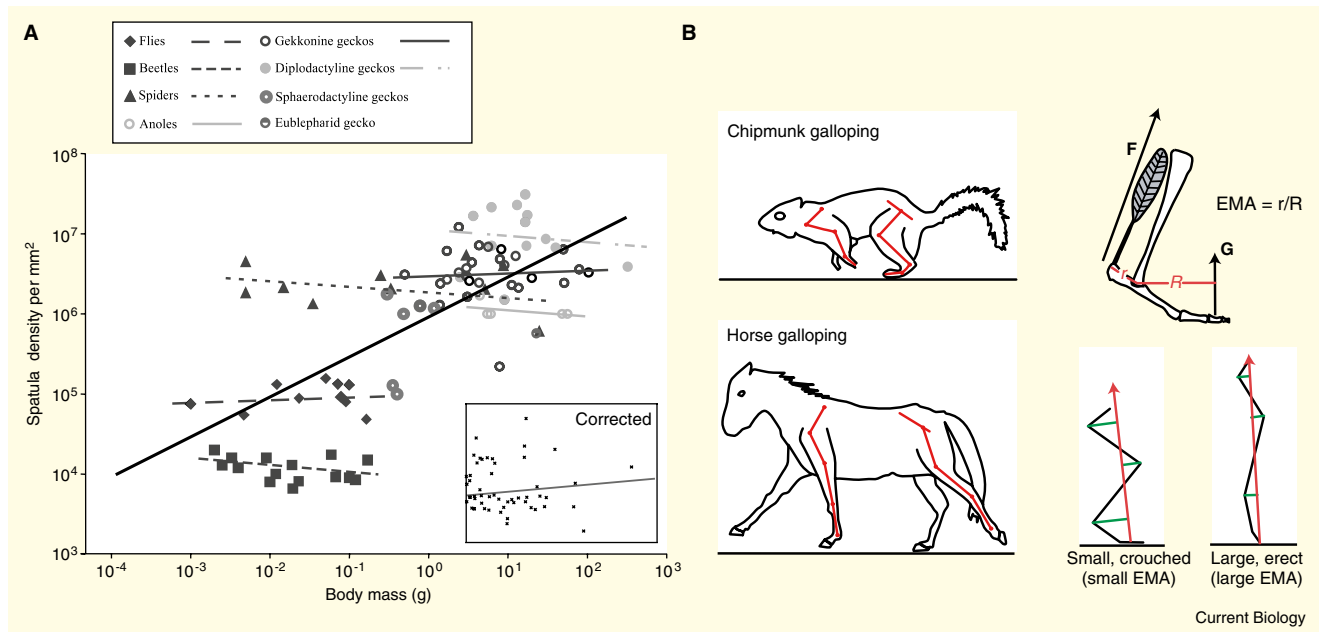
Figure 2. The importance of historical relatedness in scaling, and using novel design to overcome the effects of scale.
(A) The phylogenetic relationship between animals must be considered before invoking a scaling law. Raw data across species concerning the density of spatulae versus body mass in the adhesive structures of arthropods and reptiles results in statistically significant positive scaling with an exponent of 0.5 (solid black line). However, correcting the data for historical relatedness using the method of independent contrasts results in an insignificant relationship (inset). Because each species is more and less closely related to each other species, the data from all species cannot be considered to form a set of independent samples of how the dependent variable varies with body mass, and must be corrected. Adapted with permission from Peattie and Full (2007). (B) Leg posture and effective mechanical advantage (EMA) change with size. Small animals such as the chipmunk exhibit a crouched posture, while larger animals are more straight-legged. By altering the input (*r*) and/or output (*R*) lever arms of a muscle acting about a joint, modification of posture allows a muscle to produce more output force for a given input, and can result in safe muscle loads despite an increase in body weight. Adapted with permission from Biewener (1989, 2005).

and what is perhaps the signature behaviour of animals, locomotion. Within stable environments, the fossil record shows trends of animals evolving to larger size, a principle known as Cope's rule. With increasing size, however, isometrically scaled support structures can quickly become untenable, as we have seen in our shrew example. The stress that a supporting structure can withstand under bending scales with cross sectional area, or mass to the ⅔ power, yet the force of body weight scales directly with mass. Thus, a supporting structure with a safe cross sectional area in a tiny sapling could immediately buckle if scaled isometrically and placed inside a giant adult redwood. To compensate, a structure may adopt two different length scales, one for longitudinal length (*l*), and another for diameter (*d*), and then scale the diameter such that adequate cross sectional area is provided to support body weight. This is referred to as elastic similarity, and predicts allometry such that $d \propto l^{3/2}$. Indeed, both the diameter of

tree trunks and the midshaft diameter of bovid (cloven-hoofed mammal) long bones scale with length to the 3/2 (or equivalently, mass to the 0.5) power.

There are many ways in which to compensate for the effects of scale, including: allometric scaling of dimensions, novel design, changes in material properties, or changes in behaviour. The aforementioned allometric scaling of tree trunk and bovid humeri diameter are examples of the first method. An elegant example of the second, utilizing novel design, came from examination of leg posture in mammals (Figure 2B). The peak mechanical loads that muscle and bone endure depend on the input mechanical lever arm between a muscle and a joint, and the output lever arm between that joint and, for instance, the ground. These comprise the effective mechanical advantage of the muscle. Not only do larger animals adopt a more upright posture, such that a given muscle force produces greater output force, but the exponent at which the effective mechanical advantage of the muscle

scales is such that peak stresses are maintained to within a safety factor of two to four. This shift to straight-legged posture also ensures that bones are loaded more in compression (being squeezed along the long axis) and less in bending, which is advantageous as they are much stronger when loaded in compression.

A further power of scaling analysis lies in that it can explain how different physical forces become important at different sizes. A popular example is in swimming and flying locomotion. For small animals, viscous forces dominate their movement in a fluid, whereas for large animals, inertial forces take over. This determines whether the animal is moving in something that feels like honey, versus something that feels like air. This has huge consequences for the animal, and successful locomotion in these environments requires shaping everything from behaviour (the movement of wings, or undulation of the body) to morphology (the shape of the body, wings, or fins). Here dimensional analysis provides
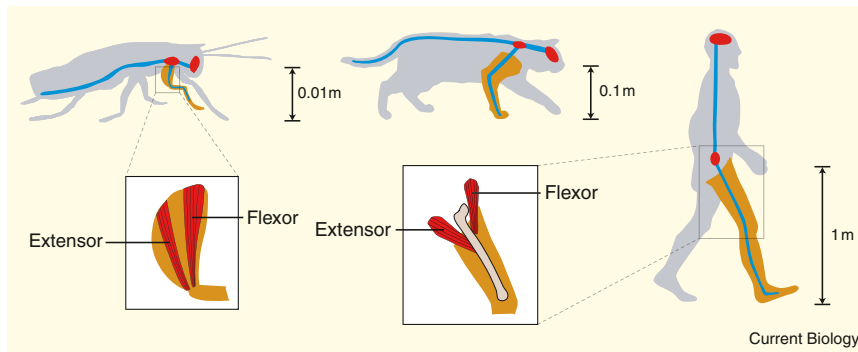
Figure 3. Consequences of scale for the neural control of locomotion.
An insect, cat, and human span three orders of magnitude in hip height, and have hierarchical neural control structures, with computation and integration centres (red) and transmission lines (blue). The nervous system activates muscles (insets) that exert forces about joints, moving appendages. With increasing size, neural control signals must travel further, with the potential to create long time delays that destabilize the animal. The physics governing the movement of appendages also changes with size. Whereas in small insects it is dominated by relatively strong muscle and tissue forces that easily overcome the inertia of limbs and the force of gravity on the appendages, cats and humans have more massive limbs with significant inertia and weight that must be controlled by relatively weaker muscles. This variation offers insight into how nervous systems work by showing us how they have evolved to produce stable locomotion under different physical regimes.

the Reynolds number, which takes in the properties of the fluid and the length of the animal, and determines which regime the animal will be in. The Reynolds number is one dimensionless parameter that describes a physical situation, and many others are used to describe how forces, lengths and times may scale. When all such parameters are made the same in two different systems, they are said to be dynamically similar.

A similar analysis applied to terrestrial locomotion leads to a reasonable prediction of when animals transition from a walk to a run. The Froude number — v^2/gl, where v is velocity and l is hip height — is a dimensionless parameter that can be thought of as a normalized speed. It compares the magnitude of centripetal acceleration about the pivoting leg to the acceleration due to gravity. As an animal speeds up, gravitational acceleration becomes unable to keep the animal 'stuck down' to the leg, and it begins to have an aerial phase, which is the start of running. This occurs at a Froude number of 1.0. Examination of the many different animals photographed in Eadweard Muybridge's 1887 book *Animal Locomotion* has confirmed that the Froude number is useful for separating walking and running gaits.

Neural control of locomotion
A future venue for scaling analyses is the neural control of locomotion. Integrative theories of how the nervous system controls movement are being formulated, the more ambitious of which reach from ion channels to center of mass forces. Scaling analyses will be useful to confirm or refute these frameworks and to guide us toward general principles. In order to understand how neural circuits have evolved and adapted, we can study how they vary in the face of changes in scale. The physics of appendages and the external environment will change with size, as will the delays inherent in the transmission of neural signals, and the volume available to the nervous system (Figure 3). These differences with size are important for the developing individual as well, whose proportions may change dramatically throughout ontogeny. In addition to the consequences of size, we can study how nervous systems have adjusted to the different time scales required for diverse behaviours. Each of these variations offers a window for future research into how nervous systems work to control biological motion.

The neural control architecture must reflect the physical forces that are governing locomotion, by sending commands to muscles that produce the desired outcome,

and by responding to unexpected perturbations in an appropriate manner. For small animals, forces developed by muscles and connective tissue outweigh the effects of inertia and the gravitational force, such that appendages are highly damped, over-actuated, and have rest positions that don't depend on orientation with respect to gravity. In large animals, inertial effects become important, and a limb that is set in motion will remain in motion, because its inertia overcomes the damping losses of joints, and the resistance to stretching of inactive muscles. These consequences of scale will be reflected in the commands sent to muscles, for example in the timing of bursts of motor neuron spikes. Thus, we can use size as a control parameter to look for principles of how nervous systems control limbs and bodies working in different physical regimes.

The time it takes for neural signals to travel throughout the body has important consequences for control, and is also affected by animal size. As animals get larger, the distance over which neural signals must travel increases, and long transmission delays will result if conduction velocity remains constant. To interpret potential consequences of these delays, we can employ an interdisciplinary approach by turning to control theory. Control theory tells us that a delay in the signal coming from a sensor is critical to stability — to the point where even perfectly accurate sensory information is useless if it is outdated. The control engineer will tell you that a conduction delay of no more than 1/10th of the response time of the structure that is being controlled — in this case the musculoskeletal system — is acceptable. Beyond that, and you may be reacting to perturbations that have already happened, and could drive the system even further into instability. Hypothesizing about the consequences of neural transmission time within a larger control theoretic framework gives a way to make predictions about how conduction velocity might vary across animals and behaviours, and about how neurobiological control structures might adapt their strategies for different sizes.

Conclusion

Questions of scale in biology have a rich history, and an exciting future. The investigation of how life copes with changes in size has unquestionably advanced our understanding of basic biology. Nanotechnology, microfabrication, and microelectronics are providing new tools for biological investigation. They make it possible to sense and perturb previously inaccessible microscopic life in more and more sophisticated ways. Less appreciated but equally important is that for larger organisms they enable sensing and perturbation of multiple parts of intact, freely behaving animals, in complex or even native habitats. As we move towards integrated measurement of metabolism, biomechanics, and neural control in freely behaving animals, the future for questions of scale in biology looks extremely bright.

Acknowledgments

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Erroneous quadruped walking depictions in natural history museums

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Since the work of the photographer Eadweard Muybridge in the 1880s [1,2], experts know well how quadruped animals walk. All walking tetrapods advance their legs in the same sequence, and only the timing of supporting feet may differ [3–6]. Given the long time since Muybridge's work, one would assume that this knowledge should be reflected in the depictions of walking quadrupeds made by work of painters, taxidermists, anatomists and toy designers. The postures of legs of walking horses, however, are frequently erroneously illustrated in the fine arts [7]. To see if this also applies to museums, veterinary books and toy shops, we collected hundreds of walking depictions and tested whether or not they correctly display limb positions. We found that almost half of the depictions are wrong. This high error rate in walking illustrations in natural history museums and veterinary anatomy books is particularly unexpected in a time where high-speed cameras and the internet offer ideal possibilities to obtain reliable quantitative information about tetrapod walking.

Although humans have observed walking quadrupeds for thousands of years, the exact characterization of the walking of tetrapods had to wait for the advent of photography [1,2]. The usual sequence by which the legs of walking quadrupeds contact the ground, the so-called 'foot-fall formula', is: left hind leg–left foreleg–right hind leg–right foreleg (LH–LF–RH–RF). The biophysical reason for this uniformity is that this gait confers maximal static stability to the body [6].

To study how correctly this foot-fall formula is represented in natural history museums, veterinary books
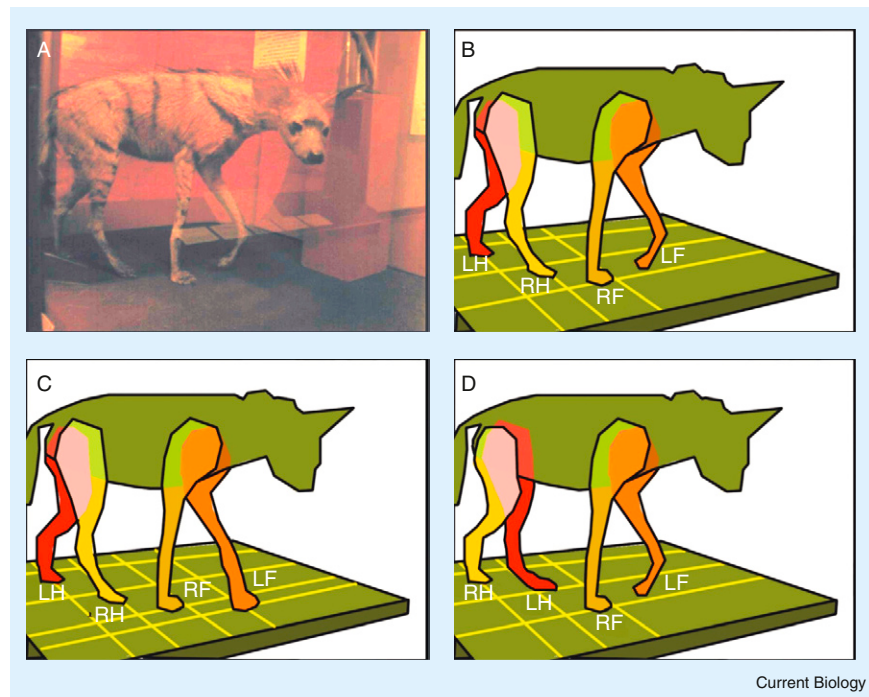
Current Biology

Figure 1. Erroneous three-foot-supported walking depiction of an aardwolf (*Proteles cristatus*).

(A) Sample at the Natural History Museum, Florence, Italy (photo by Balázs Gerics) and its leg posture (B). (C,D) Two possible corrections. Erroneously, stepping by the right hind leg is followed by raising the left foreleg, which does not occur in quadruped walking. Instead, it should be followed by raising right foreleg (C), or raising left foreleg should be preceded by the step of left hind leg (D).