9

The impact of gravity on life

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ABSTRACT

See Plate 14

Gravity is a well-known, but little understood, physical force. Its intensity and direction have been constant throughout evolutionary history on Earth, making it difficult to understand what role, if any, this vector force may have on life as we know it. Only since the launch of Sputnik in October of 1957 has life left the planet Earth and ventured into space, so that we could begin to investigate what happens to life with minimal gravity. To date, we only have fascinating snapshots of life in space. Completion of the International Space Station should allow long-duration studies over multiple generations in multiple species.

This chapter explores four questions: What is gravity? What happens to life when gravity changes? Is gravity necessary for life as we know it? Did gravity play a role in evolution of life on Earth? Life from the cellular level through adult humans exposed to spaceflight is briefly examined and examples from spaceflight and ground-based experiments are discussed. The conclusion from these studies suggests that gravity is necessary for life as we know it, and that 'gravity shapes life'.

9.1 Introduction

Gravity has been constant throughout the history of Earth. This simple fact masks the complexity of gravity as an evolutionary force. Gravity is a vector, i.e. a force that has magnitude and direction at each point in space. Gravitational loading is directional toward the center of the Earth. Gravitational loading acts on all masses at the Earth's surface and defines the weight of each object. Weight is the product of the object's mass times the force of gravity, which on Earth is equal to 1 G. Weight drives many chemical, biological, and ecological processes on Earth. Altering weight changes these processes. Given these facts, one should not be surprised that changes in gravity could alter life, as we know it. If gravity causes changes to biology, then gravity, *per se*, must be a major physical environmental force shaping life on Earth.

Life evolved from the sea. Neutrally buoyant aquatic species still have gravity acting upon them, but the uniform pressure around them and internal organs, such as the swim bladder, tend to counterbalance the intensity of the gravitational signal. However, some aquatic species appear to use gravity as a directional cue. When life evolved from the sea, it likely experienced gravitational loading for the first time. Land species changing their orientation with respect to the gravity vector or increasing in height probably began to develop adaptive mechanisms for coping with directional changes and for moving fluids and structures against this load. Due to gravity, the force necessary to lift an object above the surface of the Earth increases with the distance that an object is lifted. Birds had to solve the lift/drag problem related to air density and gravity before they could fly and had to evolve a musculoskeletal system that could provide adequate thrust. As species on land increased in

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size, they required support structures appropriate for the loads imposed. Species that crawled along the ground didn't need the same mechanisms for countering gravity's effects as those species alternating between horizontal and vertical positions. The latter species required more complex systems for balance or gravity sensing, fluid regulation, and locomotion. So, gravity, though constant, may have played a major role in evolution as species crawled from the sea and began to populate the land (see also Rayner, this volume, Chapter 10).

By altering gravity, we are able to investigate those biological systems that were developed to detect or oppose this unique force. Decreasing gravity on Earth for more than several seconds is impossible with existing technology. Until Sputnik was launched in October 1957, we had little opportunity to study how lowering this physical force influenced life. By decreasing gravity through spaceflight, we are beginning to understand that not only gravity, but also the physical changes that occur in the absence of gravity, may have profound effects on evolution of species and their ecologies. By going into space, we can gain a better understanding of how gravity shaped life on Earth. This chapter attempts to provide answers to four questions:

- What is gravity?
- What happens to life when gravity changes?
- Is gravity necessary for life as we know it?
- Does gravity play a role in evolution? If so, what role might it play?

I am privileged to share recent research results from investigators whom I personally thank for allowing me to present their data, suggesting that gravity has been and continues to be a major player in the evolution of species.

9.2 What is gravity?

In 1665/1666, Sir Isaac Newton first developed the universal law of gravitation and the laws of motion, which form the basis for our understanding of planetary motion and spaceflight (Guillen, 1995). The universal law of gravitation states that the attractive force between any two bodies is given by:

$$F_g = G_u \frac{Mm}{d^2} \tag{9.1}$$

where M (of Earth) and m (of any object) are the masses of the two attracting bodies, d is the distance between their centers of mass and G_u is the universal gravitational constant ($6.67 \times 10^{-8}\,\text{cm}^3/\text{g}\cdot\text{s}^2$) (Pace, 1977). In other words, the force of gravity is directly proportional to the product of the masses and inversely proportional to the square of the distance between them. Thus, each time the distance between the center of two masses doubles, the force is cut to one quarter of the previous value. Microgravity (10^{-6} G) requires a significant distance between the two masses (~ 1000 earth radii or 6.37×10^6 km). Low Earth orbit is only about 300 km above Earth. How, then, can we state that microgravity is found in low Earth orbit? The next paragraph suggests an answer to this apparent discrepancy.

A force is defined as equal to the mass of an object times its acceleration (i.e. $F = ma$). Equation (9.1) can be rewritten as:

$$a = G_u \frac{M}{d^2} \tag{9.2}$$

Thus, an object of any mass at the surface of the Earth accelerates toward the center of the Earth at approximately $9.8\,\text{m/sec}^2$. This gravitational acceleration is 1 G. A spacecraft in orbit above Earth moves at a constant velocity in a straight trajectory (Figure 9.1). Earth's gravitational acceleration at that vehicle's center of mass alters the direction of the spacecraft from a straight path into a circular orbit normal to the gravitational vector via centripetal acceleration. Centrifugal force, the apparent force in a rotating system, deflects masses radially outward from the axis of rotation and is equal and opposite centrifugal force per unit mass. Thus, a spacecraft in a circular orbit above Earth is in 'free' fall around Earth. Centrifugal force counterbalances centripetal acceleration causing momentary resultant gravitational forces that range between 10^{-3} and 10^{-6} G even though gravity *per se* is

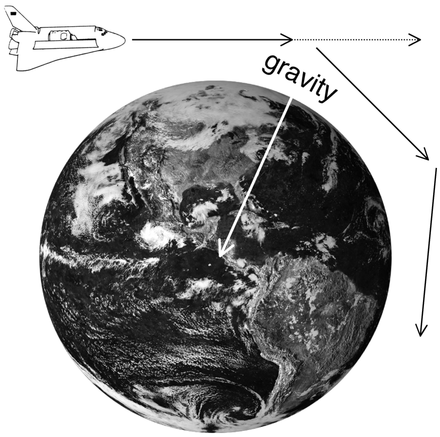


Figure 9.1 Spacecraft move in a straight line at a given acceleration rate. Rather than flying into space in a straight trajectory, forces acting on the craft cause it to 'free fall' and circle the Earth. The force of gravity in low Earth orbit is only reduced about 10% from that on Earth.

reduced only about 10% at the altitude of low Earth orbit (Klaus, 2001).

Gravity is one of the four fundamental physical forces of nature. The other three are the nuclear strong and weak forces, and electromagnetic forces. Given the intensity of the forces (adapted from http://learn.lincoln.ac.nz/ phsc103 / lectures / intro / 4_forces_ of_ physics.htm)

Nuclear strong force	10^{40}
Electromagnetic force	10^{38}
Nuclear weak force	10^{26}
Gravitational force	10^{1}

one quickly sees that gravitational force is far weaker than other forces. How could such a weak force affect all living systems? A brief description of the various forces may help in understanding this apparent discrepancy. The strength of a force depends on the distance over which it is acting. The strong force holds together protons and neutrons in the nucleus of an atom and is effective over a relatively short distance. Electromagnetic force (emf) is the force between charged particles; whether the force is attractive or repulsive is determined by the charges between interacting particles. The strength of the force drops with the inverse of the distance between charges. The weak force is effective over an incredibly small distance and can be pictured as the force that causes the decaying processes of unstable nuclear particles through time. Gravitational force is the weakest of the four fundamental physical forces of nature. Similar to emf, this force gets smaller as the objects get further apart. Yet, you feel the force of gravity and not emf, because an object at rest on Earth is pressed against Earth's surface by the force of gravity so that continuous loading is imposed upon the object. In orbit around Earth, objects have mass but almost no weight because the acceleration due to gravity is balanced by the centrifugal acceleration that keeps the object in orbit.

9.3 What happens to life when gravity changes?

Gravitational acceleration has been constant throughout the ~4 billion years of biological evolution on Earth. Gravity interacts with other environmental factors to produce today's Earth; for example, gravity is responsible for giving weight to objects on Earth so gravity is necessary for rain to fall, for water to drain, for heat to dissipate (i.e. convective force), for air and water to separate, etc. In addition to gravity's influence on the environment, it is probably a major contributor to biological changes as species evolve from water to land. To counteract gravity, new land species would need to develop systems for fluid flow and regulation, postural stability, structural support and locomotion to function and thrive in a 1 G terrestrial environment. How will terrestrial biota transported beyond Earth evolve in different gravity regimes? How much gravity is required to maintain life, as we know it? Is the Moon's gravity (1/6 G) sufficient for stimulating gravity thresholds while the lower gravity levels in space (10^{-6} G) are not? Could life evolving in space successfully return to Earth? Could Earth-based life readily evolve on planets larger than Earth with a higher gravity field? The ability to evolve under increased gravity appears related to size. Single cells and nematodes withstand 10^5 G for brief periods, young plants easily cope for 10 minutes at 30–40 G without noticeable structural changes, rats withstand 15 G for 10 minutes while 20 G is lethal, and humans are capable of tolerating only 4–5 G for 10 minutes. Gravitational levels, like other physical environmental factors, appear to determine the boundaries for life.

Microbes are less gravity sensitive than larger species and should have less difficulty transiting between planets and different gravity levels than humans. Complex spacecraft are required to transport and maintain humans off Earth while microbes survive outside spacecraft with minimal protection. Microbes fit into many ecological niches and began to evolve as soon as an environment is hospitable to their life form. Complex life forms require complex ecosystems for survival and evolution, suggesting that prototypes of Earth's ecology may have to be included, at least initially, to allow survival and evolution of these complex forms on other planets. Thus, the ability to thrive beyond Earth may be determined, at least initially, by the size of organisms and their environmental requirements.

To fully appreciate the effects of altered gravity on biological species, multiple generations must be studied at that gravity level. Subtle biological changes due to altered gravity are difficult to define over a single generation. Acute changes can be studied in less than one generation; the duration of most altered gravity experiments. Spaceflight studies in vertebrates suggest that gravity plays an incredibly important role in the development of these organisms even though studies have been limited to a small portion of the life cycle of the animal. Most spacecraft return specimens to Earth after several days to several weeks in space and most samples are collected following, rather than during, flight. Postflight data are confounded by a recovery period superimposed upon the spaceflight. Minimal spacecraft flights have had the time and the facilities for collecting inflight samples. Thus, predicting evolutionary changes from these meager, yet extremely important, data is a monumental challenge. The time required to initiate a second generation for species that NASA is considering for the International Space Station is listed in Table 9.1.

We seldom are exposed to gravity levels other than 1 G for any length of time on Earth making it very difficult to grasp the subtleties of altered gravity. Thus, we have developed an evolutionary '1 G mentality'. '1 G mentality' means that we use gravity in our daily life without even thinking about it and have difficulty comprehending life without gravity. In fact, we subconsciously design hardware and habitats for a 1 G, rather than an altered G, environment.

According to NASA, approximately 40% of equipment flown in space for the first time does not work, often due to heat build-up from lack of convection, air bubbles impeding fluid flow, or habitats based on designs more appropriate for Earth. In space, animals can use all sides of their cages and aren't limited to the floor as on Earth suggesting that housing standards appropriate for specimens on Earth may not be as appropriate for specimens in space. Understanding and appreciating the differences between Earth's physical environment and the spaceflight environment is critical if one is to provide a habitat that will keep organisms healthy and happy in altered gravity. To answer the question 'Can terrestrial life be sustained and thrive beyond Earth?' we need to understand the importance of gravity to living systems and to appreciate the role of gravity during evolution on Earth. When we can readily transition our thought processes between multiple gravity levels, we finally will be able to design space hardware and habitats that take advantage of, rather than depend on, the ambient gravity level.

Dr Maurice Averner, NASA Program Manager for Fundamental Biology, has likened gravity levels to light levels; that is, microgravity and 1 G can be compared to no light vs light. Profound differences occur in dark vs light environments and subtle changes occur as the light level increases above ambient. Gravity levels may be similar, i.e. more striking changes occur when

Table 9.1 Species doubling times vary greatly between species. Invertebrates ranging from bacteria through insects can go through multiple generations during the 90d crew rotation planned for the International Space Station, while vertebrates have a minimal doubling time of approximately two months.

SPECIES	*APPROXIMATE DOUBLING TIME*
Escherichia coli (Bacteria):	0.01d (16 min)
Yeast:	0.07d (100 min)
Protozoa (Euglena in the dark):	0.5d
Paramecium:	0.75d
Eukaryotic cells in culture:	1d
C. Elegans:	4d (on plates) or 8d (in suspension culture)
Arabidopsis (Plant):	25d (light dependent)
Drosophila:	13d (at 25C)
Rodent:	63d (2 mo)
Zebrafish:	90d (3 mo)
Quail/chicken:	90d (3 mo)
Xenopus (Frog):	152d (diploid, 5 mo) or 730d (pseudo tetraploid, 2 yr)
Human:	5380d (15 yr)

gravity is turned on or off with more subtle difference as the gravity level increases above 1 G.

The science of gravitational biology took a giant step forward with the advent of the space program. It provided the first opportunity to examine living organisms in gravity environments lower than could be sustained on Earth. Organisms ranging in complexity from single cells through humans are responsive to Earth's gravity; thus, these organisms most likely would be affected by a lack of gravity. Our knowledge of the biological consequences of decreased gravity (i.e. spaceflight) has increased significantly since 1957, yet we only have snapshots of biological changes in multiple species. This chapter will focus primarily on altered gravity responses of cells in culture, ecosystems, vertebrate development, and adult humans.

9.3.1 Cells

Physics predicts that altered gravity will not cause any changes in cells because gravity is extremely weak compared with other physical forces acting on or within cells (Brown, 1991). Yet, cellular changes have been reported. Are the physical scientists wrong or are there other previously unconsidered factors at work on cells when gravity changes? Purely physical mechanisms for gravitational responses probably can be eliminated (Hemmersbach *et al.*, 1999). Yet, cells appear to respond to changes in the environment (Klaus *et al.*, 1997) and to have evolved structures that interact directly with the outside environment to sense the environmental loads placed upon them (Hemmersbach *et al.*, 1999; Ingber, 1998).

The bacterium *E. coli* has flown experimentally in culture seven times aboard the space shuttle (Klaus *et al*., 1997). During spaceflight, *E. coli* exhibited a shortened lag phase, an increased duration of exponential growth, and an approximate doubling of final cell population density compared to ground controls. These differences may be related to the lack of convective fluid mixing and sedimentation, processes that require gravity. During exponential growth in minimal gravity, the more uniform distribution of suspended cells may initially increase nutrient availability compared to the 1 G-sedimenting cells that concentrate on the container bottom away from available nutrients remaining in solution. Also, local toxic by-products could become concentrated on the bottom of the 1 G container with cells in increased proximity to each other. Such a process could limit cell growth. Thus, changes in *E. coli* and possibly other cells during spaceflight may be related to alterations in the microenvironment surrounding non-motile cells. If true, then the extracellular environment plays a critical role in evolution of single cells through controlling nutrients and waste. This response to the extracellular environment suggests that intracellular gravity sensors are not essential for cells to elicit a gravitational response. Earlier predictions that microgravity could not affect cells were focused on the physical inability of gravity, an extremely weak intracellular force, to elicit an immediate or 'direct' response from organisms of such small mass. Rather than a 'direct' response, reduced gravity more likely initiates a cascade of events – the altered physical force leads to an altered chemical environment, which in turn gives rise to an altered physiological response. Modeling cell behavior predicts how cells evolve in different physical environments, including Earth, by including gravity as an integral part of the equations; hence, changes in sedimentation, convection, nutrient availability, and waste removal with altered gravity can be predicted.

Hammond, Kaysen, and colleagues (Kaysen *et al*., 1999) cultured renal cells under different conditions. They concluded that differentiation of renal cells in culture most likely requires three simultaneous conditions: low shear and low turbulence, three-dimensional configuration of the cell mass (i.e. free-floating), and co-spatial arrangement of different cell types and substrates. They have cultured human renal cells in rotating-wall vessels and in centrifuged bags on Earth, and in stationary bags flown aboard the shuttle (Hammond *et al*., 1999). Controls for all experiments were simultaneous, ground-based, bag cultures. All cultures contained liquid medium and the bags were made of material that was non-adherent for cells. A plethora of changes in steady-state level of mRNA expression occurred in space-flown human cells (1632 of 10 000 genes or 16.3%) compared to the Earth-based bag cultures. These patterns were unrelated to the changes in gene expression found in rotating-wall vessel experiments. Shear stress response elements and genes for heat shock proteins showed no change in steady-state gene expression in the flight culture. Specific transcription factors underwent large changes during flight (full data set at http://www.tmc.tulane.edu/astrobiology/microarray). In the rotating-wall vessel, 914 genes or 9% changed expression. In the centrifuge, increasing gravity to 3 G caused only four genes to change expression greater than threefold. In addition to the unique changes in gene expression noted during flight, structural changes in the cultured rat kidney cells also occurred. Far more microvilli were formed in renal cells grown in space or in the rotating-wall vessel than in the 1 G static bag culture or during centrifugation (Hammond *et al*., 2000). These studies suggest that renal cells flown in space have unique patterns of gene expression unrelated to the best Earth-based model of spaceflight (i.e. rotating-wall vessel), and that the ability to form a three-dimensional, free-floating structure in culture appears critical to induce tissue-specific, differentiated features in renal cells.

The data from bacterial and renal cells suggest that spaceflight may affect cells via their external environment and that differentiation of renal tissue may be enhanced during spaceflight. Such studies are demonstrating how physical factors, specifically gravity, regulate expression of specific genes, creating an organism specific for that environment. Thus, some cells and tissues may show greater differentiation of specific features while others may show the reverse. In fact, the timing of gene expression may be beneficial or detrimental to downstream effects and, hence, alter the final protein product and, ultimately, the organism. Evolution is more likely to cause changes through altered gene expression rather than through genomic modifications as the latter are more likely spontaneous mutations. Data are indicating that gravity may actually be a critical environmental factor in determining the differentiation and maturation of cells on Earth.

Early results with cultured cells from the musculoskeletal system suggest that spaceflight induces a variety of responses. Delayed differentiation and changes in the cytoskeleton, nuclear morphology, and gene expression have been reported for bone cells (Hughes-Fulford and Lewis, 1996; Landis, 1999). Dr Herman Vandenburgh has flown fused myoblasts (i.e. muscle fibers) to investigate the effects of microgravity on cultured muscle fibers. He found that flight muscle organoids were 10–20% thinner (i.e. atrophied) compared with ground controls due to decreases in protein synthesis rather than increases in protein degradation (Vandenburgh *et al*., 1999). Interestingly, atrophy of the isolated muscle fibers in culture was very similar to the amount of muscle atrophy reported in flight animals. These preliminary data from bone and muscle cells suggest that spaceflight affects adherent cells and tissues even when isolated from systemic factors and that the physical environment might direct the ultimate development of cells, organs, and tissues.

Changes in the physical environment surrounding cells, *in vivo* or *in vitro*, can lead indirectly to changes within the cell. Little is known about if or how individual cells sense mechanical signals or how they transduce those signals into a biochemical response. A cellular mechanosensing system might initiate changes in numerous signaling pathways. Such a system has been found in cells that attach to an extracellular matrix (i.e. the cell substratum) and the cellular components are beginning to be defined. These cellular interactions probably suppress or amplify signals generated by gravitational loading. We now know that the extracellular matrix to which cells attach contains adhesive proteins that bind to regulatory proteins that traverse the cell membrane. These transmembrane regulatory proteins (e.g. integrins), in turn, connect to the cytoskeleton and the cytoskeleton ultimately connects to the cell nucleus. Given these connections, activation of the regulatory proteins in the cell membrane can lead directly to regulation of gene expression, thereby eliminating the need for a solely intracellular gravity sensor. Living cells may be hardwired to respond immediately to external mechanical stresses. Exciting research on the interaction of the cell cytoskeleton with membrane components and the extracellular matrix is shedding light on possible 'force sensors' at the cellular level that might be essential for the differentiation process (Ingber, 1997, 1998; Globus *et al*., 1998; Schwuchow and Sack, 1994; Wayne *et al*., 1992). Ingber has applied to cells the concept of 'tensegrity' (i.e. tensional integrity), a tension-dependent form of cellular architecture that organizes the cytoskeleton and stabilizes cellular form (Ingber, 1999). This architecture may be the cellular system that initiates a response to mechanical loading as a result of stress-dependent changes in structure and may have been a key factor in the origin of cellular life (Ingber, 2000).

Definition of the cellular connections that might sense and transduce mechanical signals into a biochemical response may also shed light on the events initiating cell maturation. As a cell matures, it stops dividing and begins to express characteristics of a mature cell type. If a cell does not mature, it will continue to divide – the definition of a cancer cell. The maturation process may be triggered by multiple factors, including loads placed on the extracellular matrix during different phases of development.

With exciting new molecular tools in hand and the development of facilities for increasing gravity on Earth (i.e. centrifugation) or decreasing gravity on space platforms, great strides will be made in understanding the influence of gravity in living systems at the cellular level within the next decade.

In summary, the local environment around cells may be altered in space. Such changes may affect cellular metabolism and steady-state gene expression may change. Potential adaptive systems in eukaryotic cells include force coupling through the cellular skeleton, ion channels, and other load-sensitive cellular structures that might alter cellular signaling. Further investigation into cellular changes at multiple gravity levels is required. Research may show that cellular architecture in eukaryotic cells evolved to oppose loading or amplify directional cues. Thus, physical changes in the aqueous medium surrounding cells in culture and cellular structures that oppose or respond to mechanical loads may provide cells with the ability to respond to gravity.

9.3.2 Ecosystems

Algal mats and protists are fascinating! If orientation and stratification are weight-dependent, then the microgravity of space could significantly alter interactions between organisms. How these organisms would fare without gravity is unknown, but changes from Earth-based mats would be predicted. For example, microbes migrating during the day using gravity as an environmental cue to minimize exposure to solar radiation would not be able to migrate and could have greater radiation damage. Such damage would tend to select species with radiation resistance. Protists appear to detect gravity at about 0.1 G. Thus, the general principle of mechanoreceptors in metazoa is represented in unicellular organisms (Hemmersbach *et al.*, 1999) suggesting that the ability to detect and use Earth's gravity must have occurred very early in the evolutionary history. In fact, the Hemmersbach *et al.* (1999) review article suggests that these organisms have evolved structures, such as mechano- or stretch-sensitive ion channels, cytoskeletal elements and second messengers, to amplify the gravity signal rather than evolving intracellular gravity sensors. Interestingly, the genes for light sensing and gravity sensing occur very early in evolution. Some organisms seem to be able to use either or both as directional cues. Space, with very low gravity levels, provides a unique laboratory to sort out the importance of light in a relatively gravity-free environment. Or, perhaps these physical environmental factors have created redundant biological sensing systems.

Decreased gravity causes very complex changes in the environment. For example, gaseous boundary layers build up due to lack of convective mixing in the atmosphere and these boundary layers expose plants and simple ecosystems to stratified environments not present on Earth. Soil substrates in space have a different shape, do not pack like Earth soils, and wet in a very different way than on Earth. In space, water does not drain through soil, as that process requires gravity. Such changes make the management of simple ecosystems in space rather difficult. Plants may be the most difficult species to evolve efficiently in space, as they must adapt simultaneously to two environments (above and below the ground) that change during spaceflight (Musgrave *et al.*, 1997). Atmospheric issues include pollination and mixing of gases. During spaceflight, insects, gravity, and wind may be missing in the plant habitat. In addition, the gaseous environment above the ground may stratify due to lack of convective mixing and expose plant shoots to boundary layers that are new and novel. Such stratification may be responsible for the uneven ripening of seedpods noted during flight, i.e. ripening begins at the tip and is not uniform. Levels of potentially dwarfing compounds (e.g. ethylene and CO_2) might cling to the plants creating shorter plants rather than the taller ones that one might expect with lower gravity levels. Not only will plant shoots have to adapt to novel environments in flight, but also water management for the roots may be problematic.

Soil issues during spaceflight focus on root-zone management. The team from Utah State University has grappled with water-management issues while studying evolution of plant systems in space by attempting to grow multiple generations of wheat on the Russian space station MIR (Jones and Or, 1999). Their results show that we have a lot to learn before we can achieve successful and repeatable plant growth under reduced gravity. Root media wet differently in space. On Earth, water drains vertically through a soil column where each particle is held in contact by gravity. In space, the wetting front is free to move in all directions. The wetting front bulges out to reach a neighbor particle that may be floating a small distance away, 'gulps' the particle, and then opens an air space on the other side of it. Where particles are touching, water wicks along the particle surfaces partially filling the channels around the soil particulates. This tends to trap air between the particles rather than forming the saturated slurries that one finds on Earth. In a partially hydrated system, water wicks between particles as it does on Earth, but in all directions rather than just flowing 'down'. This wicking, due to capillary attraction, inhibits exchange of nutrients and, by bridging between the particle contact points, can suffocate plants at lower water contents than would be observed on Earth. Soil-based systems in space can be managed to resemble hydroponic (i.e. water-based) systems on Earth. It is impossible to simulate space-substrate/water-content conditions using the same soil substrate on Earth. In space, water forced into a substrate does not drain and can fill up to 90% of the soil matrix. Until the water is wicked out of the substrate, the flooded volume will stay in the substrate, creating an oxygen-free zone that is not usable by most plants. Proper root-zone management in space is an active process that requires sensors for continuous monitoring of both water and oxygen content in the soil matrix during spaceflight. Such monitoring is essential for effectively managing plant systems and for learning how the decreased gravity experienced during spaceflight alters the environment. Understanding and controlling the environment is often a prelude to survival and adaptation in unique environmental niches.

Many challenges remain for plant growth and crop management in space, including understanding of boundary layers above the ground and water and oxygen management in the root media. All physical environmental factors must be considered when predicting the evolutionary fate of a species in a unique environment.

9.3.3 Vertebrate development

Studies on Earth and in space suggest that gravity has shaped life. Studies with tadpoles, birds, and rats on Earth and in space are shedding light on the importance of gravity to animal systems. Unlike plants, no vertebrate has completed a life cycle in space. In fact, humans have spent about 1% of their life cycle in space, and rats have spent about 2% of a single generation. Building habits for multiple generations of complex species is a challenge not only in space, but also on Earth.

9.3.3.1 *Amphibian development*

The most elegant and definitive developmental biology experiment in space used the amphibian as a model (Souza *et al*., 1995, Figure 9.2). This experiment had an on-board 1 G centrifuge control. On Earth, the fertilized frog egg rotates upon sperm penetration, and this rotation is thought to be essential for normal development. Upon fertilization, the egg begins to divide and form the embryo that, after an appropriate time, emerges from the jelly-like egg as a tadpole.

Female frogs were sent into space and induced to shed eggs that were artificially inseminated. The eggs did not rotate and yet, surprisingly, the tadpoles emerged and appeared normal. After return to Earth within 2–3 days of hatching, the tadpoles metamorphosed and matured into normal frogs.

Development appeared normal during spaceflight, yet some morphological changes in embryos and tadpoles occurred. The embryo had a thicker blastocoel roof that should have created abnormalities in the tadpole, but no deformations appeared, suggesting plasticity of the embryo. The flight tadpoles did not inflate their lungs until they returned to Earth. The lungs appeared normal by the time the tadpoles were 10 days old. If the lungs didn't inflate and the animals remained in space, then would the gills remain as the tadpoles metamorphosed into frogs? If the gills resorbed without inflated lungs, would the defect be lethal? Why didn't the lungs inflate? We don't know the answers to these

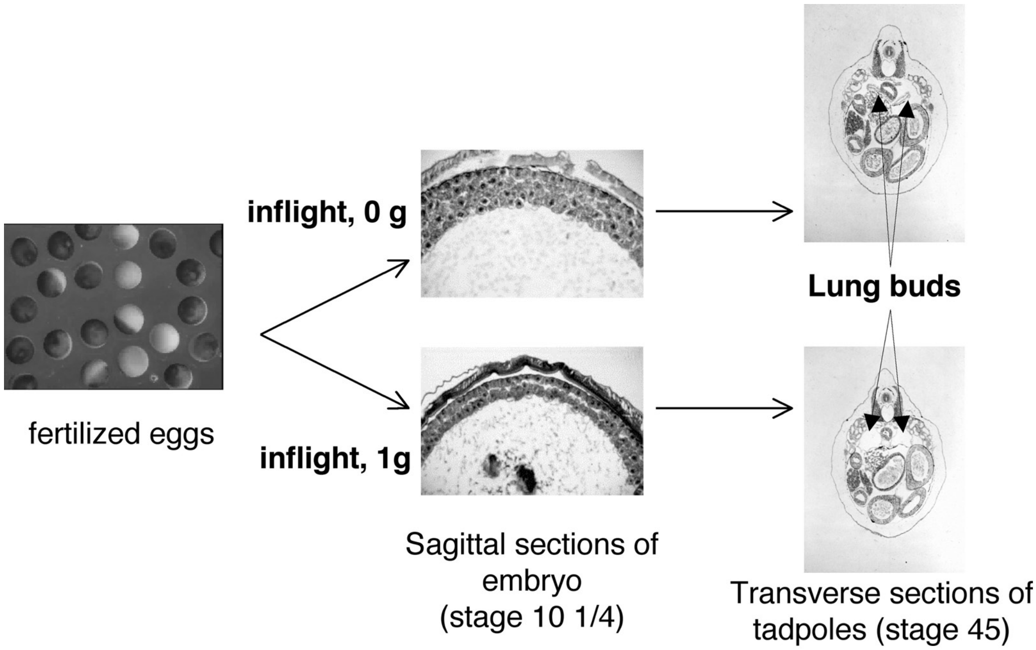


Figure 9.2 Fertilized frog eggs on Earth rotate upon fertilization and this rotation was hypothesized to be required for normal development. In space, the eggs appeared to develop normally whether or not they rotated. Several interesting abnormalities were noted in histological sections taken from the embryos and tadpoles. In the embryos, an extra cell layer was noted in the embryo and yet no gross abnormalities were found in the tadpoles. In the tadpole, the lung buds did not appear to inflate. The lack of inflation of the lungs might be cause for concern for frogs, but the tadpoles were returned to Earth when they were less than four days of age, i.e. before they metamorphosed to frogs. Pictures courtesy of Kenneth Souza, NASA Ames Research Center.

questions, but we do know that air bubbles were present in the tadpole aquatic habitat on orbit. Possibly, lack of directional cues or increased surface tension between the air/water interface interfering with penetration of the air bubbles may be involved in this interesting observation.

This developmental study produced multiple important findings. It showed that vertebrates can be induced to ovulate in space and that rotation of fertilized eggs is not required for normal development in space. The flight-induced changes, including a thicker blastocoel roof with more cell layers and uninflated lungs, appeared correctable in this experimental paradigm. In conclusion, the vertebrate embryo is very adaptive and the system is plastic, yet the long-term fate of the animal throughout its life in space remains unknown.

9.3.3.2 Quail

Adult quail on MIR adapted quickly to the space environment. They learned to soar with minimal wing flapping and held onto their perch for stability when eating rather than being propelled backwards when they pecked their food (i.e. for every action there is an equal and opposite reaction). Fertilized quail eggs appeared to undergo normal embryogenesis in space, but serious problems occurred after hatching (Jones, 1992). When a cosmonaut took a hatchling from its habitat, the chick appeared content as long as it was held. But once released, the bird first flapped its wings for orientation and began to spin like a ballerina, then kicked its legs causing it to tumble – it became a spinning ball. The cosmonaut noted that the chick would fix its eyes on the cosmonaut while trying to orient in space. When placed in their habitat, the chicks had difficulty flying to their perch to eat, and, unlike the adults, had difficulty grasping the perch for stability when eating. The hatchlings ate normally only when fed by the crew and, thus, did not survive.

9.3.3.3 Rat development

The force of gravity may influence events underlying the postnatal development of motor function in rats, similar to those noted in hatchling quail. Such effects most likely depend on the age of the animal, duration of the altered gravitational loading, and the specific motor function.

Walton (1998) reported differences in righting-reflex and locomotion in neonatal rats when the musculoskeletal system did not bear weight. Walton's data suggest that there are critical development periods during which biomechanical loading of limbs is essential to give cues to nerves. Without the cues, brain development and limb innervation may not occur normally and animals may develop an abnormal walking behavior. At the Final Results Symposium for the 17-day Neurolab Shuttle Mission, Dr Walton suggested that neonatal rats flown in space exhibited altered locomotor behavioral development that persisted for the 1-month recovery period and that righting-reflex strategies were still abnormal 5 months after return to Earth. Dr Danny Riley showed delayed development of certain nerve connections to muscles in these neonates. The connections returned to normal after return to Earth, yet fibers in hindlimb muscles did not reach normal size even after a month back on Earth. The Riley team found similar results in neonates that were not allowed to bear weight on their hindlimbs on Earth (Huckstorf *et al.*, 2000). The data suggest that biomechanical loading of limbs during early development may be essential for innervation of muscles and development of normal muscle fiber size.

These vertebrate studies suggest that embryonic development in frogs and birds proceeds normally in space, although unexplained changes occur during embryogenesis and early development. In birds and rats, biomechanical loading may be required for Earth-like development and innervation of certain structures. We are learning that habitats in which early development occurs on orbit may have to be very different from Earth cages. Without gravity, rat and bird neonates float freely. Without a surface to crawl against, the animals thrash about and their health may degrade if the housing provided is too large. In space the animals can use all three dimensions of their habitat rather than the two dimensions available in Earth habitats. Perhaps space habitats should be sized to the individuals, suggesting that more confining habitats may be appropriate for neonates until they are able to grasp and walk. Only after development of appropriate motor function should cage size be expanded. Cages that accommodate all stages in the life of vertebrates are critical if we are to understand the influence of gravity on development of vertebrate systems in a free-fall environment. Interestingly, evolutionary development with increased gravity may also require special habitats so that the pups are not crushed beneath the dam and can still obtain essential nutrients. If the habitat for a particular species is not compatible with survival throughout life, then evolution of that species will not occur in that environment.

9.3.4 Adult humans

Early predictions of the response of humans to spaceflight assumed that space adaptation would be analogous to human disease processes rather than to normal physiology. Through studies of bed-rested healthy adults and medical examinations of crews returning from space, we now recognize the adaptive nature of the human responses to spaceflight or its ground-based models. We are also aware of the necessity to minimize the flight-induced changes so that crews maintain their Earth-readiness and avoid injury on landing. Lack of gravitational loading affects multiple physiological systems, especially fluid flow, balance, and support structures that are particularly vulnerable to change or injury during re-entry and renewed exposure to gravitational forces. To minimize these changes, most crew members exercise extensively during long-duration flight. Although many physiological systems appear to be affected by spaceflight, only the cardiovascular, vestibular, and musculoskeletal systems are covered in this chapter.

9.3.4.1 Cardiovascular system

To understand how the human cardiovascular system adapts to gravitational loading, it is helpful to think about the system as the body's 'plumbing', which consists of the 'pump' (heart), 'pipes' (blood vessels), and 'control system' (nerves, hormones, and local factors). The cardiovascular system is designed for a 1 G environment. When crews go into space, strange things happen. Spaceflight causes a fluid shift from the legs toward the head, producing a puffy face and bird-like legs. The fluid shift increases the amount of blood in the chest region, causing the heart and fluid-volume sensors in the neck to detect an increase in fluid volume. The increased chest fluid initially increases heart size (i.e. amount of blood), but regulatory mechanisms quickly kick in and return the fluid to an appropriate, lower level. The loss of fluid results in a reduced plasma or blood volume. To keep blood thin, the decrease in plasma volume triggers a destruction of newly synthesized, immature, red blood cells, probably by a mechanism of programmed cell death or apoptosis (Alfrey *et al.*, 1996). The shift of fluids to the upper body and the distended facial veins noted in astronauts suggest that central venous pressure should increase. Surprisingly, it decreases, suggesting that our concepts of pressure and volume regulation need revision (Buckey *et al.*, 1996). These changes are appropriate for the spaceflight environment. However, upon return to Earth, many crew members have difficulty standing, usually due to the rush of blood to the feet that can cause fainting (Buckey *et al.*, 1996). This readaptation to Earth's gravitational force following spaceflight could pose a problem if crews are expected to stand and function normally immediately after landing on any planetary body.

9.3.4.2 The vestibular system

The vestibular system is our guidance system that controls eye movements, posture, and balance. Its main purpose is to create a stable platform for the eyes so that we can orient to the vertical – up is up and down is down. Deep within our inner ear is the vestibular organ with thousands of tiny hair cells. Resting atop these hair cells are microscopic crystals that move and bend the hair cells, sending information to the central nervous system for the reflex control of eye movements, posture, and balance. In space, the eyes send signals that confuse the brain because the visual references that we rely on for stability are missing (Merfeld, 1996; Merfeld *et al.*, 1996; Oman *et al.*, 1996). These mismatched sensory inputs may be one cause of 'Space Adaptation Syndrome' (SAS), an adaptive process that often involves nausea and can lead to vomiting. Another possible cause of SAS is sensor adaptation to a novel gravitational environment to increase the gain of sensory cells, possibly by increasing the number of synapses (Ross and Tomko, 1998). After several days in space, crews begin to function effortlessly, signaling that adaptation is complete. Crews initially rely on touch, sight, and muscle sensors for orientation (Young *et al.*, 1996). As soon as they switch to an internal alignment and use the feet to signal down, they are able to function normally. Upon return to Earth, the brain is confused once again as gravity is now available for orientation. This confusion creates postural instability that is compounded with the cardiovascular difficulty in standing. Also, reflexes associated with posture and balance are slowed even on short-duration missions. With long-duration flights, changes in reflexes, visual perception, and eye/hand coordination may become major issues for re-entry and readaptation to Earth.

9.3.4.3 The musculoskeletal system

The musculoskeletal system provides the magic of movement. This system is very responsive to changes in load. In fact, exercise is necessary to maintain muscle and bone mass on Earth. Without gravitational load, muscles and bones associated with posture and weight-bearing become weaker. The intensive exercises performed by crews are not able to counteract the loss of bone/muscle mass and strength because exercising in space without gravity does not produce the same level of mechanical loading possible on Earth. With the fluid shifts and decreased bone loading, calcium is lost from bone and calcium excretion increases. The higher calcium load presented to the kidneys is of concern for potential kidney-stone formation. Our bodies tend to conserve calcium; during spaceflight, the amount of mineral in some bones, including the head, may increase to offset losses from other sites. Bone and muscle are lost *only* in the legs, back, and neck indicating that the musculoskeletal changes are site-specific – loss does not occur throughout the entire body. Bone loss primarily occurs at sites in weight-bearing bones where muscles (that are also losing mass) attach to that bone. The muscles that help maintain posture are most severely affected and change phenotype. This new phenotype resembles skeletal muscle that fatigues more readily. Muscles in the jaw may change function during spaceflight because on Earth the jaw opens with gravity and people have to work to keep their mouth shut. Upon return to Earth, reduced muscle strength and power, and even pain, occur. Following extended spaceflight missions, certain muscles and bones might be weaker and fracture more easily. Thus, re-entry from space is similar to returning from a long boat trip on a rough sea, but space adds the additional complexity of fluid redistribution and muscle weakness in addition to the dizziness.

In summary, the changes in humans are appropriate adaptations to the space environment. They are not life threatening for at least 1 year, which is the longest that humans have been in space. The adaptations are functional (see Fregly and Blatteis (1996) and Sulzman (1996) – Results of SLS1 and SLS2). That's the good news. The bad news is that adaptation to space creates problems upon returning to Earth. Difficulty standing, dizziness, and muscle weakness present problems after landing. Appropriate countermeasures must be developed. Crew members exercise in space to minimize the difficulties of re-entry. We ethically cannot request that they stop exercising. To learn about adaptation of mammals that do not exercise in space, we use appropriate animal models.

9.4 Is gravity necessary for life as we know it?

Life most likely will look and, perhaps, move quite differently after many generations in space. We have learned that life is 'plastic' and changes with the environment; it adapts at least transiently to changes in gravity. The microenvironments of spaceflight require more study so that we will understand how to use them effectively. We certainly have a lot to learn about the complexity of biological responses to altered gravity. Data to date suggest that certain biological structures have evolved to sense and oppose biomechanical loads, and those structures occur at the cellular as well as at the organismal level. Certainly, the Earth-tuned physiological systems of vertebrates change following acute exposure to space; what will happen over multiple generations is speculative. The 'functional hypothesis' theory suggests 'use it or lose it'. If this theory holds over multiple generations in space, then gravity-dependent structures may ultimately disappear or assume a very different appearance. Based on the studies described in this chapter, gravity most likely is essential for life, as we know it.

9.5 Does gravity play a role in evolution?

Gravity affects the environment. Its attractive force gives weight to mass and weight is required for many ecological processes on Earth. Sprinkled throughout this chapter are examples and suggestions of the importance of gravity to life, as we know it. Particularly important is the apparent evolutionary development of unique biological structures that amplify the force of gravity and specific gravity sensors that are required for orientation, balance, and movement in a gravity environment. Will these structures and sensors change with gravity levels less than 1 G? Only extended time in space with multiple generations will begin to answer this question.

One might predict that plants would grow taller without gravity. Yet, the boundary layers produced by a lack of gravity might concentrate growth-inhibitory or ageing factors around the plants, thereby causing them to dwarf; increased gravity might facilitate the dispersal of such factors and actually lead to taller plants. If plants on Earth are fine-tuned to a 1 G environment, then they might not function as well at either increased or decreased gravity levels.

Ecologies, such as algal mats, that stratify by weight on Earth might tend to form as three-dimensional communities without gravity. If the hierarchical structure achieved by stratification is essential for survival or fitness, then the communities would either become extinct or change their fitness leading to evolved characteristics appropriate for the new environment.

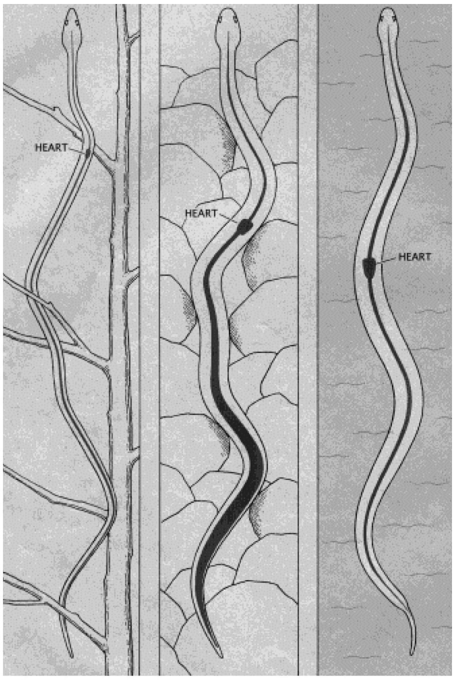
Gravity level is important in development of load-bearing structures. The scaling effect of gravity is well known: the percentage of body mass relegated to structural support is proportional to the size of a land animal (e.g. 20 g mouse = ~5%, 70 kg human = ~14%, and 7000 kg elephant = ~27%). The scaling effect in land animals would likely change in space and could result in a static scale comparable to marine mammals on Earth (~15% of mass as supporting tissues over a wide range of weights). However, increasing gravity would require altered support structures as scaling up existing structures without any modification in geometry would ultimately lead to failure.

Load-bearing limbs, so important on Earth, are less necessary in space. Human legs not only get in the way during spaceflight but also are involved in the fluid shifts that occur early in flight. Whether legs would disappear over time without gravity (perhaps similar to the extraterrestrial ET) or become more like grasping talons is unknown. Unlike evolution in a decreased gravity environment, higher gravity levels may lead to a different posture and a bipedal stance might become unusual with most species possibly existing as quadrupeds or even hexipeds. Larger species might become extinct at higher gravity levels unless these animals quickly adjust for brain-blood flow and placement of internal organs.

To ‘fall down’ probably requires a certain gravity level and the reflexes related to posture and equilibrium at 1 G are sluggish following spaceflight. Would such reflexes be innervated in species evolving in lower gravity fields? If the reflexes that keep us from falling down in a gravity environment do not develop, then would species evolving in a lower gravity field be able to move when placed in a higher gravity field?

Would the evolutionary response of biological systems be linear, logarithmic, or degraded at gravity levels other than 1 G? Some biological systems (e.g. metabolic rate which is proportional to body weight) increase with increased size on Earth. On the other hand, some life systems may have adapted to be maximally efficient at 1 G and degrade with changes in gravity (e.g. body temperature). Life as we know it is extremely adaptable and usually fits form to function during evolution in a hospitable environment. Humans readily adapt to a lower gravity regime aboard spacecraft yet require an extensive ‘recovery’ period when returning to Earth from space voyages suggesting that initial adaptation to a lower gravity environment might be easier than adapting to a higher gravity environment.

A fascinating suggestion that gravity might play a role in evolution comes from snakes (Figure 9.3). On Earth, snakes have evolved in different environments. For example, tree snakes spend their days crawling up and down trees and exist in an environment where they must cope with gravity. Land snakes spend most of their life in a horizontal position. Sea snakes are neutrally buoyant and spend their life swimming within their habitats. In other words, the orientation of the snake to the direction of the gravity force differs depending upon habitat, without a concomitant alteration in magnitude of gravity.



Tree Land Sea

Figure 9.3 The location of the heart in different species of snakes indicates a potential effect of gravity on organ position. The tree snake continuously changes its orientation with respect to the direction of gravity as it climbs up and down trees. Its heart is located closer to the head compared to either the land snake or the sea snake. The heart in the sea snake is approximately in the middle of its body. Pictures courtesy of Dr Harvey Lillywhite, University of FL, Gainesville, and published with the permission of Nelson Prentiss (Lillywhite, H.B. (1988) Snakes, blood circulation and gravity. *Scientific American*, **256**: 92–98).

Lillywhite (1988) noticed that the heart of the tree snake was closest to the brain, suggesting that it might be more gravity tolerant than the other snakes as it did not have to carry blood over as great a distance from the heart to the brain. He centrifuged the animals and found that the sea snake had the least gravity tolerance (i.e. fainting with increased gravity), the tree snake had the most, and the land snake was intermediate (Lillywhite *et al.*, 1997). Changes in heart position, likely related to gravity, most certainly happened over evolutionary, rather than single-generation, timescales. These studies suggest that changes in orientation of a species with respect to the direction of a gravitational force, without an alteration in the magnitude of gravity, may play a role in the evolution of that species on Earth. Gravity may determine the location and size of internal organs such as the heart.

So, what might evolving species at a higher or lower gravity field look like? Form follows function and as function changes, so will form. How much change and what form organisms and ecologies will assume over time in altered gravity is currently unknown. Increasing gravity within a survivable range will probably not cause dramatic differences in evolving Earth-like species while a gravity-free environment will likely produce significant changes in ecologies and species. ET may be a good example of a species evolving at a lower gravity field with the rotund body, duck-like flappers for feet, minimal legs, long thin arms and fingers, and a large head, large eyes, and minimal hair.

To quote ET, 'Love your planet', meaning that you are a product of your physical environment.We will begin to understand the influence of gravity on evolution of species only after prolonged exposure to different gravity levels. Today, the role of gravity in evolution remains speculative. But one certainly can say that *gravity shapes life*!

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Web addresses

E. coli: http://www.colorado.edu/ASEN/asen5016/
http://www.colorado.edu/engineering/
Fundamental forces of nature: http://learn.lincoln.ac.nz/phsc103/lectures/intro/4_forces_of_physics.htm
Human kidney cells: http://www.tmc.tulane.edu/astrobiology/microarray
Astrobiology/Life Sciences: http://space.arc.nasa.gov/
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