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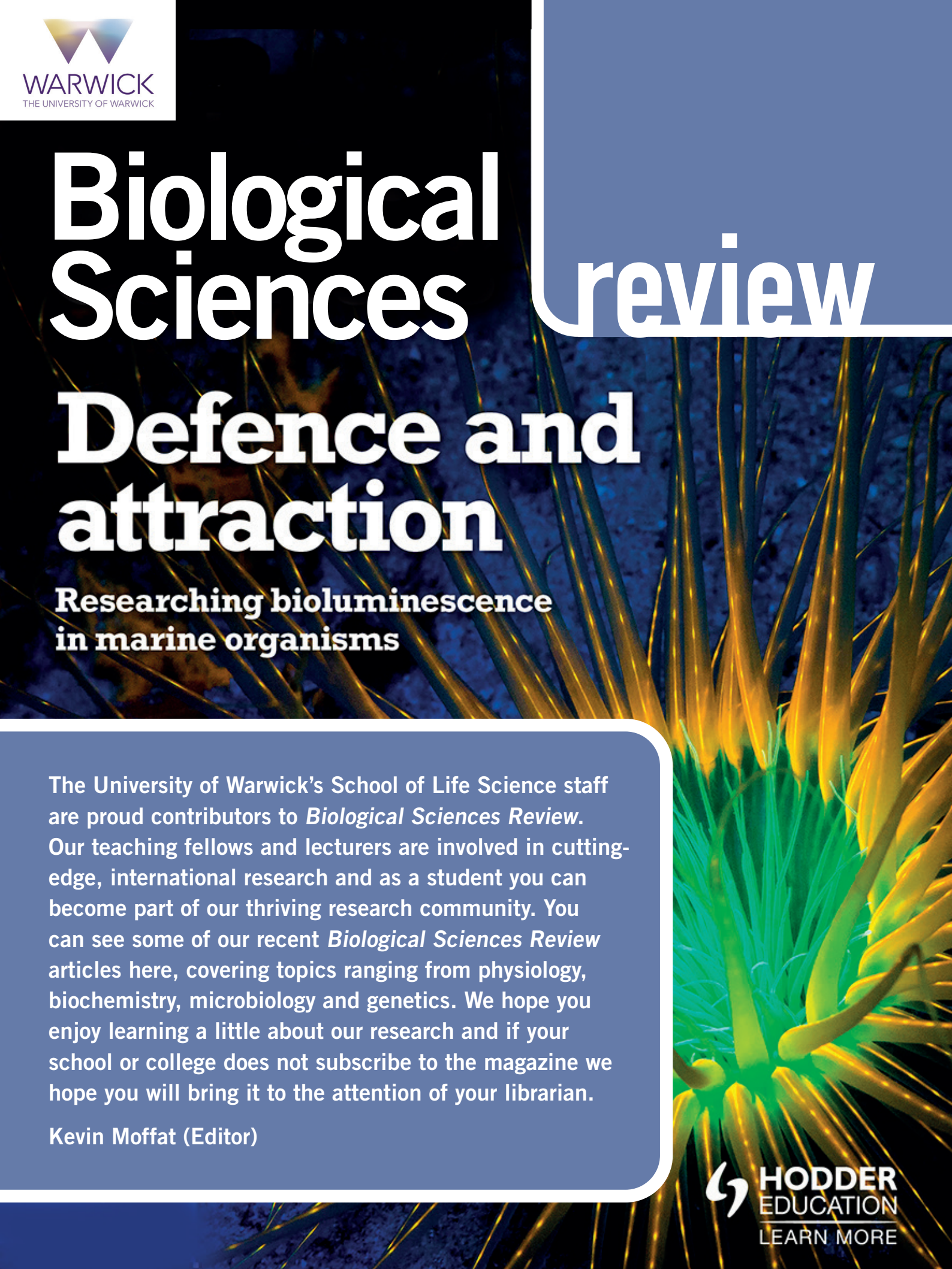
Biological Sciences review

Defence and attraction

Researching bioluminescence in marine organisms



The University of Warwick's School of Life Science staff are proud contributors to *Biological Sciences Review*. Our teaching fellows and lecturers are involved in cutting-edge, international research and as a student you can become part of our thriving research community. You can see some of our recent *Biological Sciences Review* articles here, covering topics ranging from physiology, biochemistry, microbiology and genetics. We hope you enjoy learning a little about our research and if your school or college does not subscribe to the magazine we hope you will bring it to the attention of your librarian.

Kevin Moffat (Editor)

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Marine bacteria and the plastisphere

Robyn Wright

It is estimated that over 8 million tonnes of plastics are being added to the oceans each year. PhD student Robyn Wright explores potential microbiological solutions to the problem

AQA: 3.5.4 Nutrient cycles
Edexcel A: 4.15 Replacement of oil-based plastics; 5 On the wild side
Edexcel B: 10.4 Human effects on ecosystems
OCR A: 4.2.1(i) Conservation agreements; 6.3.2 Populations and sustainability
OCR B: 4.3.2(b) Impact of the rise in human population on ecosystems
WJEC Eduqas: 2.1.6 Human impact on the environment

Key words
Microplastics
Biodegradation
Marine biology
Microbiology

Recently the Ellen MacArthur Foundation stated, 'By 2050 there will be, by weight, more plastics than fish in the oceans!' Plastic, from the Latin 'plasticus' or Greek 'plastikos', means 'able to be moulded' and is the term we use for the materials we derive from oil. Plastics are hydrocarbon **polymers**, predominantly made up of carbon and hydrogen, sometimes with other functional groups including esters. Many plastics are derivatives of very long chains of alkanes (see Figure 1).

We have been making plastics since the end of the nineteenth century and production is now hundreds of millions of tonnes annually. Unfortunately, the properties that make plastics so widely used underlie the reasons they are so hard to break down. This difficulty in breaking down plastics is heard in the phrase 'it will take hundreds of years for plastics to break down in the ocean'. In reality, hundreds of years have not passed since the mass production of plastics began in the 1950s, so we do not know how long they will remain in the oceans. Current research suggests that plastics

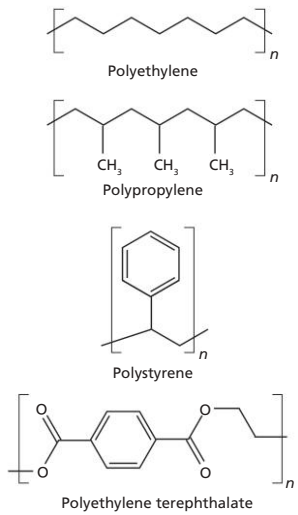

Figure 1 Chemical structures of some plastic polymers made predominantly from long chains of alkanes

simply fragment into smaller and smaller pieces but never truly disappear. The Marine Debris Working Group in the USA estimates that over 8 billion tonnes of plastics have been produced since the 1950s — and almost half of this in the last 13 years. About 80% of the waste ends up in landfill or the environment.

Where are the plastics in the oceans?

Wherever we have looked in our oceans, we have found plastics. From coastal areas to the deep sea. From the Arctic to other unpopulated areas, such as the Chagos Archipelago in the Indian Ocean. Some plastics, such as polyethylene (PE), polypropylene (PP) and expanded polystyrene (PS), are less dense than seawater, so they float and are carried by ocean currents. These have a tendency to accumulate in **gyres** in the middle of the oceans, often termed 'garbage patches'. Other plastics, including polyethylene terephthalate (PET) and polyvinyl chloride (PVC), are denser than seawater and so sink.

Floating plastics might also sink from the surface of the sea following colonisation by bacteria and the larvae of larger organisms. Scientists from the Woods Hole Oceanographic Institution in Massachusetts termed these colonising organisms the '**plastisphere**', a **microbiome** for plastics. These bacterial communities are distinct from the surrounding seawater and from those that colonise natural surfaces in the ocean.

Unfortunately, plastics are also removed from the surface seawater by animals that ingest them, and are found in the faeces of animals including zooplankton, fish, seals and whales, which also sink.

How do plastics get into the oceans?

Plastics end up in the ocean by a number of different routes, such as fibres that are released each time we wash our clothes, or through **microbeads** in cosmetics such as face washes. Microbeads have now been widely banned in the UK, but there are loopholes allowing them in 'leave on' products, including makeup and sun cream. Other routes for plastic pollution include lost fishing gear such as nets and traps, or items lost from ships. The largest quantity of plastic pollution comes from the mismanagement of waste originating from land. Much rubbish enters the oceans from developing coastal countries that lack adequate sewage treatment or waste collections.

Why does it matter that plastics are in the oceans?

The effects of plastics on marine organisms can be both direct and indirect. The direct effects are usually through entanglement or ingestion. For example, fish will often seek out plastics to eat because when they have been colonised by the plastisphere they smell like food. When plastics are either manufactured to be **microplastics** or break down into small pieces from

Box 1 A youth-driven challenge

'Bye Bye Plastic Bags' was founded in 2013 by Melati and Isabel Wijsen. Melati was 12 and Isabel just 10 years old. They had grown up on the Indonesian island of Bali, and seeing the pollution there inspired the sisters to act. Together, they collected over 77 000 signatures supporting a ban on plastic bags in Bali, which local government has enforced since January 2018. This was not the end of their journey, and they continue to campaign for 'a world free of plastic bags and where the young generation are empowered to take action'. Speaking in March 2018 at the International Marine Debris Conference in San Diego, Melati stated, 'youth are 25% of the population, but 100% of the future'. They continue to win awards and attend international conferences to publicise this youth-driven challenge.

Melati and Isabel Wijsen

Dead black-footed albatross — its stomach filled with plastic consumed accidentally

larger plastics, they become accessible to more organisms. Indeed, plastics bioaccumulate and thus have a greater effect on animals at the top of food chains. Often, ingested plastics pass through digestive systems, but many microplastics in the oceans are fibres and can clump together, becoming obstructive.

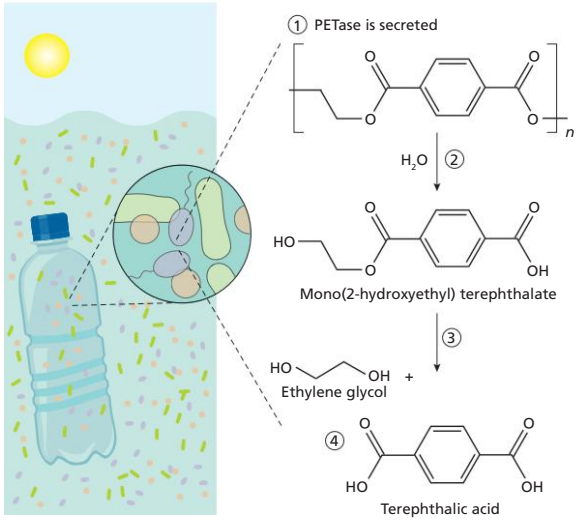

Figure 2 Degradation of PET by microbes. **1**, Extracellular enzymes secreted, **2**, enzymes attach to the surface and cleave the PET polymer, **3**, intermediates assimilated into cells, **4**, short degradation intermediates dissolved into the water

Terms explained

Gyres A large system of circular ocean currents formed by global wind patterns and forces created by the Earth's rotation. The largest ones are found in the middle of oceans.

Microbeads Microplastics found in cosmetics and toiletries.

Microbiome All of the microorganisms in a particular environment.

Microplastics Plastics below 5 mm in size.

Plastisphere The microorganisms colonising the outside of plastic marine debris. It is usually distinct from the surrounding seawater and includes bacteria that could degrade plastics as well as potential pathogens.

Polymers Materials with a molecular structure primarily made of a large number of the same units (monomers) bonded together.

Sequencing DNA sequencing is the process of determining the order of nucleotides in a DNA molecule. This is used for whole communities of microorganisms in order to identify what is there, even when we cannot see it.

Plastics are manufactured with chemical additives — many are toxic, and other poisonous hydrophobic chemicals have been found on plastics at concentrations a million times higher than the surrounding sea water. This can lead to indirect effects via transfer to animals either when they are eaten or through contact. Because plastics are able to travel large distances in the oceans, the biological accessibility of the associated toxin is increased. Such chemical effects can potentially include reproductive failure, hormonal defects, growth of tumours and even death.

What are we doing to clean up the oceans?

Many scientists are researching the main sources of plastics in the oceans, where they end up, and what the effects of these plastics are. This will help to advise policy makers on strategies for cutting down the plastic waste that reaches the ocean, which areas need to be targeted for clean-up, and how we can improve our efficiency in removing plastics before they reach the oceans.

Importantly, some bacteria and fungi in landfills and soils have been shown to be capable of degrading plastics. Could these organisms also be present in the ocean? The terrestrial microorganisms tend to be relatively slow at degrading plastics, generally taking months for a noticeable difference in plastic mass to be observed. Additionally, the process only takes place at relatively high temperatures (optimum temperature of these microbial enzymes is typically above 60°C). So, can we do anything to speed up these processes?

How do bacteria break down plastics?

A group of Japanese scientists discovered a bacterium (*Ideonella sakaiensis*) that can break down PET, the polymer from which most plastic bottles are made, and characterised the enzymes that the bacterium used (see Figure 2). The bacteria were found to attach to the plastic and then release an enzyme called PETase, which breaks the PET into monomers (mono(2-hydroxyethyl) terephthalate, ethylene glycol and terephthalic acid). These monomers are taken up through bacterial membranes. The bacteria are able to break down these monomers and use them as an energy source, producing carbon dioxide and water. In April 2018, a team of scientists at the University of Portsmouth, UK, managed to engineer this enzyme to increase its efficiency. While this is significant, it will realistically still take many months for there to be a noticeable change in plastics degraded by this enzyme.

The ocean is too cold...

The enzymes so far found to break down plastics in a terrestrial environment have relatively high optimum temperatures. At the surface of the ocean, the average global temperature is approximately 16°C, but the average depth of the ocean is 3500 metres. Sunlight only reaches down to approximately 200 metres and the majority of the oceans are therefore cold, about 0–3°C. It is difficult to find enzymes that are active at these temperatures. Further, plastics are challenging to degrade. Their breakdown requires several steps using several different enzymes. It has therefore been suggested that a community of microorganisms may be more able to degrade plastics than an individual species or a single enzyme.

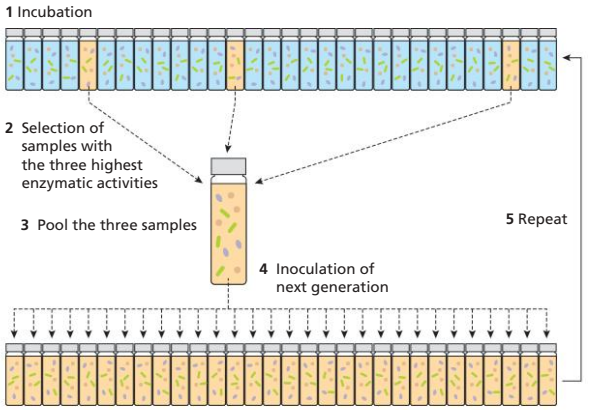

Figure 3 Workflow for the artificial selection of communities of microorganisms for polymer degradation

My research group at The University of Warwick, UK, is currently trying to select plastic degrading communities of microorganisms. In the laboratory we are testing microorganisms that colonise plastics from coastal waters around the UK. First, using an 'enrichment experiment': communities are left with the plastics as the only carbon source to see if any survive. Second, we take many replicate communities and test the activity of enzymes that could be involved with plastic degradation (see Figure 3). Subsequently, communities with the highest activities are mixed. This is carried out over 20 times to select communities that can become better at degrading plastics over time. This technique is reminiscent of selective breeding of animals and plants but is far more complex.

Currently the technique has been used to find microbial communities able to degrade chitin, the natural polymer present in the shells of crustaceans. We have demonstrated that, within 1 month of growth, a community could be selected to have increased chitinase activity up to 90 fold. Analysis of this community by DNA **sequencing** shows that thousands of different species are present. These include bacteria able to degrade chitin, bacteria able to use the products of chitin degradation, and eukaryotes, including protoctists, that feed on the bacteria. Now we know the technique works, investigations using PET are in progress. Microbial communities may help us in the future to finally degrade the plastics with which we pollute our oceans.

Further reading

Keep up to date and learn more about the group that Robyn is working with at The University of Warwick: **www.christieoleza-lab.com**

Read about the Ellen MacArthur Foundation proposals for sustainable 'circular economies': **https://tinyurl.com/y7sbsvxz**

'Our campaign to ban plastic bags in Bali', TED talk by Melati and Isabel Wijsen: **https://tinyurl.com/jgfgkhe**

'Bye Bye Plastic Bags', a movement powered by youth around the world to say 'no' to plastic bags: **www.byebyeplasticbags.org**

New plastic-munching bacteria could fuel a recycling revolution', a news article about *Ideonella sakaiensis*: **https://tinyurl.com/zjqp2yz**

'Recycling hope for plastic-hungry enzyme', a news article about engineered PETase: **https://tinyurl.com/ydgettak**

Robyn Wright is currently a PhD student at The University of Warwick, developing the community selection methodology to identify plastic-eating microbes that can survive in our oceans. She is an active blogger on marine biology: **robynw371.wixsite.com/mainlymarine**

Key points

- Plastics are key polluters of the marine environment.
- Plastics, and the molecules associated with them, are toxic to a wide range of organisms, both physically and chemically.
- Biodegradation of plastics by single species of microbes is at best slow.
- Microbial communities are likely to be more efficient at plastic removal than an individual species or a single enzyme.

Water

Transport and regulation in the body

Kevin Moffat

Biologist Kevin Moffat explains the link between your blood, your brain and your kidneys and how together they control the hydration of your body

AQA: 3.1.7 Water; 3.2.3 Transport across cell membranes; 3.6.4.3 Control of blood water potential

Edexcel A: 1.2 The importance of water; 2.3 Osmosis; 8.8 Brain structure

Edexcel B: 1.7 Water; 4.2 Cell transport mechanisms; 9.9 Osmoregulation

OCR A:2.1.2(a) Properties of water; 2.1.5(e) Osmosis; 5.1.1 Principles of homeostasis; 5.1.2(c)(i) Structure and function of the mammalian kidney; 5.1.2(d) Control of water potential of blood; 5.1.5(h) The human brain

OCR B: 2.1.2 Water and its importance; 5.3.3 Kidney functions and malfunctions

WJEC Eduqas: Core 1(b) The importance of water; Core 3(c) Osmosis and water potential; 2.3.4 Homeostasis and the kidney

Key words

Water potential
Osmosis
Antidiuretic hormone
Kidney
Pituitary gland
Brain

By weight, the average human female is about 50% water and the average male 60%. For a 70 kg male that's around 42 dm^3 of water. Differences are due to disparities in body fat and muscle composition. But where is this water, how is it regulated and what happens when this regulation goes wrong?

Kate Mori was running her fourth marathon. Fit, experienced and a sports scientist, she was 'ahead of her thirst', well hydrated before she began. It was April 2007 and one of the hottest London marathons on record, with temperatures over 23 °C. Amid frequent reminders from the race officials about dehydration, Kate made sure that she drank at every opportunity. However, as she passed the 18-mile mark, she felt ill. She knew she was in trouble. Determined to finish for her charity, and with support from other runners, she staggered to the finish line. Shortly afterwards she collapsed and was put on a saline drip in hospital. Her problem? Over-hydration, resulting in a potentially lethal low concentration of sodium in her blood and tissues.

This condition is termed **hyponatremia**. The combination of exercise, drinking too much and inappropriate release of **anti-diuretic hormone** (ADH) resulted in Kate suffering from exercise-associated hyponatremia (EAH). This is a well-documented condition and, although it is treatable with a saline drip to replace the sodium, it can be fatal. Victims of EAH include American footballers, on-duty soldiers and policemen, canoeists, ironmen, long distance swimmers and mountain bikers. Symptoms of hyponatremia have also been linked to cases of people involved in water drinking games or those with seemingly unquenchable thirst after taking drugs such as ecstasy. They all risk death.

The UK National Health Service advises adults to drink 2 dm^3 of water a day. If we don't drink fluids we will die of dehydration in a few days. We might be lucky and last a week. However, we may die far sooner if we drink too much water too quickly. The **median lethal dose** (LD50) of water for humans is just 6 dm^3 — dangerous if drunk over a period of a few hours. To understand this, we need to consider where our body stores water and how our physiology controls it.

Where is our water?

About two-thirds of the water in our body is inside cells. The rest is extracellular. The extracellular water is divided between the plasma in the blood vessels (intravascular) and the fluid surrounding the cells and organs (interstitial) (see Figure 1). Plasma volume is normally kept at around 3 dm^3 for men and 2.3 dm^3 for women. Plasma is the main fluid compartment that interacts directly with water inputs to the body — from food and drink — and water outputs including urine, sweat, breath and secretions from the gut. To understand how water in the body is controlled, we need to consider its movement between plasma, interstitial fluid and cells. Water moves by **osmosis**, owing to differences in **water potential**.

Why does water move?

Early in our biology education we learn about osmosis, particularly in the context of plant roots taking up water, or changes in the size of cells.

Terms explained

Anti-diuretic hormone (ADH) A short, nine amino acid polypeptide hormone.

Baroreceptors Receptors that sense pressure in arteries.

Blood brain barrier Cells that normally prevent the movement of substances between the blood and the brain.

Cytokines Molecules secreted by cells that stimulate other cells, frequently found in the immune system.

Homeostasis The maintenance of a physiological state.

Hyponatremia A concentration of sodium ions in body fluids that is below normal physiological concentration.

MDMA 3,4-Methylenedioxymethamphetamine, also known as ecstasy.

Median lethal dose (LD50) The dose of a substance that will kill 50% of a sample population.

Osmoreceptors Receptors that sense changes in the water potential of arterial blood.

Osmosis The movement of a solvent across a semipermeable membrane from a dilute to a concentrated solution.

TRPV (transient receptor potential vanilloid) channels Membrane ion channels that respond to mechanical movement, often as a result of osmotic changes to cells.

Water potential A quantitative measure of the tendency of water to move by osmosis.

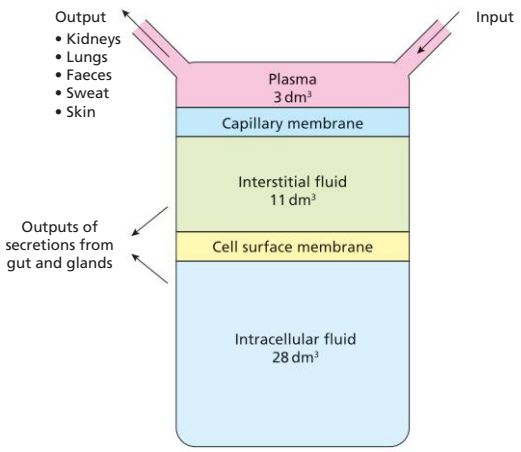

Figure 1 Fluid compartments. Our body fluids can be considered to be in three compartments separated by two partially permeable membranes. Blood plasma is responsible for the majority of exchange between the external environment and the fluid found inside our cells

Osmosis explains why water moves across a partially permeable membrane. The solvent (water) moves from the less concentrated solution to the more concentrated solution. Understanding water potential allows us to appreciate the forces underlying this movement (see Box 1).

In plant cells, the rigid cell walls contribute to the pressure component of water potential inside the cell. In animal cells, however, it is the concentrations of solutes that are the major determinants of water potential. A simple way to consider this is that the solute prevents water from moving freely by interacting with it, thus reducing the amount of free water and resulting in a lower water potential (see Box 1, Figure 1.1).

Sensing we are thirsty

When we sweat, urinate, defaecate and breathe, we lose water. The initial physiological response is the movement of water between compartments in the body owing to changes in water potential. Water loss increases solute concentration and hence decreases water potential in the plasma, resulting in the remaining water moving first from interstitial fluid and subsequently from cells (see Figure 1).

We normally maintain our water **homeostasis** by regulating thirst and urination. In severe situations, such as acute injury and blood loss, our arterioles undergo vasoconstriction in order to maintain blood pressure. This is mediated via **baroreceptors** located in the walls of blood vessels. An increase in concentration of solutes in blood plasma is sensed by **osmoreceptors**. Significant to thirst are osmoreceptors that are found in the brain, controlling the release of an important hormone, ADH.

Osmoreceptors are proteins called **TRPV channels** that are found in the cell surface membranes of neurones in brain regions where there is little or no **blood brain barrier**. Here they have close contact with blood vessels and

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Festival goers who take ecstasy risk developing EAH

therefore blood plasma (see Figure 2). These neurones shrink or expand as a result of changes in the water potential of blood, and they are exquisitely sensitive.

A decrease in water potential of the blood results in thirst and concentrates urine. This process is initiated by the mechanical movement of the TRPV channels as the neurone shrinks. The TRPV channels then directly alter the flow of ions across the neuronal membrane, generating action potentials that are sent to the brain's hypothalamus. The hypothalamus controls ADH secretion into the blood from the pituitary gland (see Figure 2). ADH then regulates water retention in our kidneys and, in the brain, gives rise to the sensation of thirst.

The kidneys

The major sites of fluid regulation are the kidneys' nephrons (see Figure 3). An ultrafiltrate is produced from the blood in the capillaries of the glomerulus. This then moves into the proximal convoluted tubules before entering the loop of Henle. The fluid moves through the distal convoluted tubule, enters the collecting ducts, and finally reaches the bladder for excretion from the body.

Human kidneys filter the blood around 20–25 times each day, producing about 1.5 dm^3 of urine, the majority of our daily water loss. The initial ultrafiltrate is similar to blood plasma, except for the proteins, which are not removed. In the proximal convoluted tubule, many ions, amino acids and vitamins are actively recovered. The descending loop of Henle is largely impermeable to ions, and water easily moves out by osmosis into the surrounding kidney medulla. The water potential in this part of the loop falls, becoming more negative.

Fluid movement through the ascending loop results in an increase in water potential, as this region is impermeable to water. Ions therefore move out, first by diffusion and then actively using energy from ATP. More water and ions are removed in the distal tubule, and the fluid entering the collecting duct has a high water potential.

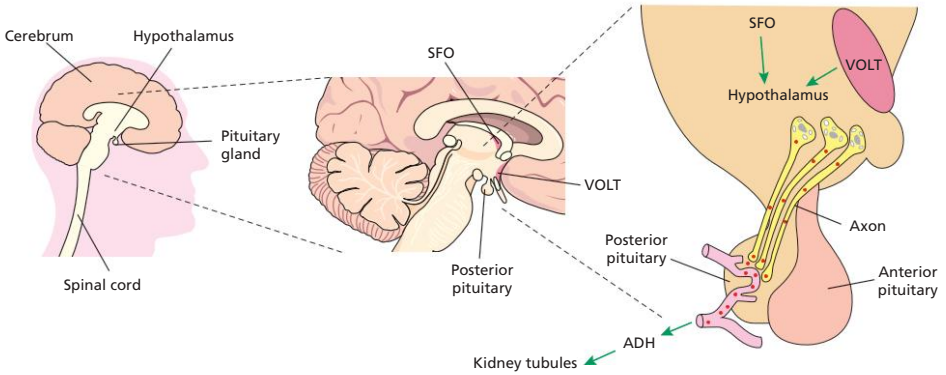


Figure 2 Osmoreception in the brain. Osmoreceptors are located in neurones in the vascular organ of lamina terminalis (VOLT) and the subfornical organ (SFO). These neurones send action potentials to hormone-producing neurosecretory cells in the hypothalamus, which regulate ADH secretion from the posterior pituitary gland

Box 1 Water potential

Water moves from a region of high water potential to a region of lower water potential (see Figure 1.1). Water potential is determined by two things — pressure and solute concentration. Water potential is represented by the Greek letter ψ (psi) with units of kilopascals (kPa). Water potential is effectively a measure of free water molecules in a solution. Pure water has the highest ψ of 0 kPa. The more solutes, the less free water. This is because solute interacts with water molecules so that they are not free to move. And the lower the water potential the more negative the kPa value. For example, a 0.15 M solution of sucrose has a ψ of −370 kPa.

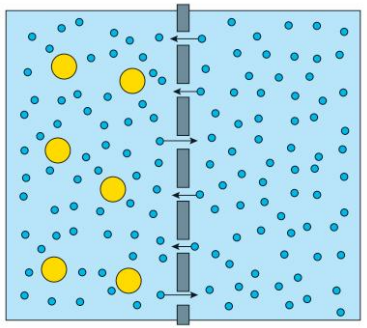


Figure 1.1 Osmosis and water potential. Yellow circles represent the solute (e.g. ions) and blue circles represent the solvent, water. The two sides are separated by a partially permeable membrane. With the solute only on the left, the higher concentration of free water is on the right, giving the right-hand side a higher water potential. The net flow of water is therefore to the left by osmosis

Further reading

Read Kate Mori's story as reported in the *Telegraph*, 26 March 2012: **https://tinyurl.com/z36gs3j**

Watch a 4-minute documentary on the death of Jennifer Strange, after trying to win a Wii in a water drinking competition on a US radio station: **www.youtube.com/watch?v=ioKDf-JvKDo**

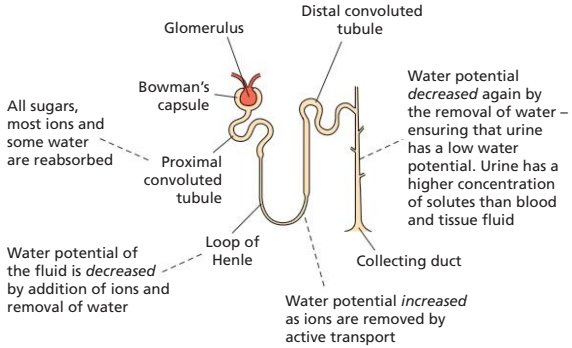


Figure 3 Water potential changes in a nephron of the kidney during production of urine

As it passes down the collecting duct, the high concentration of ions in the surrounding medulla results in a flow of water out of the duct. This results in urine that has a lower water potential — more concentrated solutes — than blood.

Importantly, the amount of water removed from the collecting duct is regulated by controlling its permeability to water. Dehydration lowers water potential in the blood, driving the thirst response via TRPV channels. The ADH subsequently released from the pituitary gland binds to receptors on the epithelial walls of the collecting ducts, stimulating an increase in the number of water channels — called aquaporins — in their cell surface membranes. This allows more water to leave the collecting duct by osmosis, resulting in further concentration of urine.

What can go wrong?

There are multiple causes of EAH: loss of sodium ions in sweat, internal production of water from metabolism, as well as a large intake of fluid through drinking. An Italian research team found that during glycogen metabolism and muscle injury — both common in intense exercise — a **cytokine** called IL-6 is released from muscle. This cytokine stimulates ADH release independently of osmoreceptors. Thus, during extreme exercise, even when well hydrated, ADH may continue to stimulate thirst and reduce urination, increasing water retention and therefore sodium dilution.

The causes of hyponatremia for users of ecstasy (**MDMA**) are different. Rather than IL-6, there is now evidence that a metabolite of MDMA can directly stimulate ADH secretion from the pituitary gland. The effect is the same as for EAH — to lower the concentration of sodium ions in body tissues. At best this is treatable with saline drips. At worst it results in death.

Kevin Moffat is a professorial teaching fellow in the School of Life Sciences at The University of Warwick. His research background is in physiology, genetics and neuroscience.

Key points

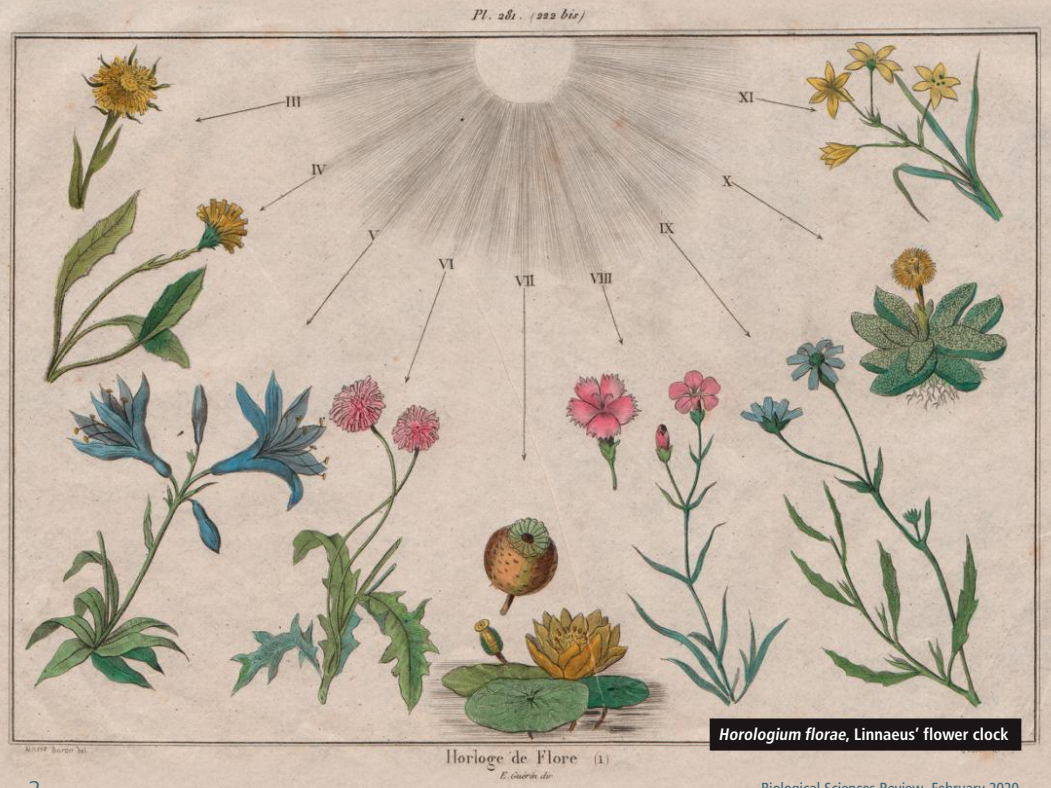
- Controlling hydration levels is important for health.
- Water moves between body compartments by osmosis owing to differences in water potential.
- The brain and pituitary hormones are critical for maintaining homeostasis.
- Water potential of blood plasma is controlled by the kidney.

Biological clocks in plants

Environmental responses regulating gene expression

Isabelle Carré
Amy Newman

Most organisms have a biological clock, and their components and the molecular mechanisms used are often similar across species. Here circadian biologists Isabelle Carré and Amy Newman review the plant clock and the adaptations it drives



***Horologium florae*, Linnaeus' flower clock**

Exam links

AQA Mass transport in plants; Survival and response; Control of gene expression
Edexcel A How genes can be switched on and off; Responses in plants
Edexcel B Transport in plants; Chemical control in plants
OCR A Plant and animal responses; The regulatory mechanisms that control gene expression
OCR B Stomatal opening; Control of flowering in plants
WJEC Eduqas Stomatal opening

As the Earth rotates around its axis, we experience changes in temperatures and light intensities throughout the day as our position on Earth moves to face towards and away from the Sun. Most plants cannot change their location to compensate for these changes in environmental conditions, but they have adaptations to deal with environmental stresses (Biological Sciences Review, Vol. 31, No. 3, pp. 2–6). The circadian clock is one of them — it allows plants to prepare themselves ahead of cold temperatures at night, or heat and water loss during the day.

A **circadian** clock is a network of genes regulating each other's activity, resulting in rhythmic changes in an organism's metabolism, physiology and behaviour over the course of the day. Our own circadian clocks are perhaps obvious to us. We sleep at certain times, eat at certain times and go to the toilet at certain times each day. Disruption of this clock is the reason we get jetlag when we travel between time zones. It takes about a week to readjust, and we are often unable to sleep at the right time and we feel unwell.

Daily rhythms in plants

Plants also synchronise with their environment. Their daily rhythms were described over 2000 years ago (see Box 1). The circadian clock influences many aspects of plant biology. Perhaps the most noticeable example is the opening and closing of flowers as the day goes on. In 1751, the influential biologist Carl Linnaeus proposed that knowledge of these rhythms could be used to plant a garden where you would be able to tell the time of day depending on which flowers were open.

We also see rhythms in the movement of leaves. Their opening during the day maximises light capture for photosynthesis, whereas their folding at night protects vital growth structures (such as the **meristems**) from damage due to cold temperatures. In addition, many flowers release their fragrances at specific times, coinciding with when their pollinators are most active. For example, honeysuckle flowers are particularly strongly perfumed in the evening, thus attracting the nocturnal moths that pollinate them.

The circadian clock controls the timing of scent production in these honeysuckle flowers, thus attracting the night-flying hawk moth that pollinates them

Properties of circadian clocks

All circadian clocks generate rhythms that have a period, or cycle duration, of approximately 24 hours. Under natural day–night conditions, these rhythms are synchronised to diurnal changes in light and temperature. Having a clock that does not keep time with its environment would be a serious disadvantage. However, circadian oscillations persist in the lab in the absence of day–night cycles, indicating that they are being generated from within the organism and not caused by any external influences. The period length under constant conditions is often a little longer or shorter than 24 hours and is determined by the organism's genes. These clocks also keep time independently of temperature, unlike other biochemical reactions which, broadly speaking, speed up two-fold with a 10°C increase in temperature.

Not only do circadian clocks keep track of the time of day, they also respond to photoperiods — the duration of light in each day. This allows **anticipation** of changes in season. In many plants this is important in order for flowering to take place at the appropriate time of the year, so that seed can be produced and the life cycle can be completed before the following cold season.

Box 1 The discovery of circadian rhythms

While molecular biology has uncovered much about how circadian clocks work, we have been aware of circadian rhythms for centuries. The earliest recorded observation is thought to be by Androsthenes of Thasos in ancient Greece, around the year 400 BCE. He noticed that the leaves of the tamarind tree opened and faced towards the Sun during the day and folded up at night. Over 2000 years later, in 1729, the French astronomer Jean-Jacques d'Ortous de Mairan was the first to demonstrate that sunlight was not needed to trigger such changes in leaf position, by placing plants in constant darkness and noting that the rhythms still occurred.

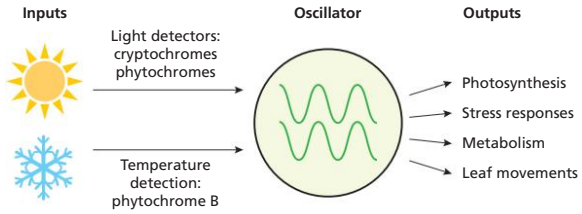


Figure 1 The circadian clock has three elements: input pathways, a core oscillator, and output pathways

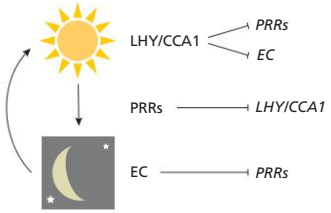


Figure 2 The molecular mechanism of the circadian oscillator in plants. In the morning, the LHY and CCA1 proteins switch off the expression of all the other clock genes. Later in the day, the *LHY* and *CCA1* genes are switched off by the PRR proteins, and in the evening the *PRR* genes are switched off by the proteins that make up the 'evening complex' (EC). This lifts the repression of the *LHY* and *CCA1* genes so that the cycle can begin again the next day. Arrows with flat ends indicate the repression of gene expression

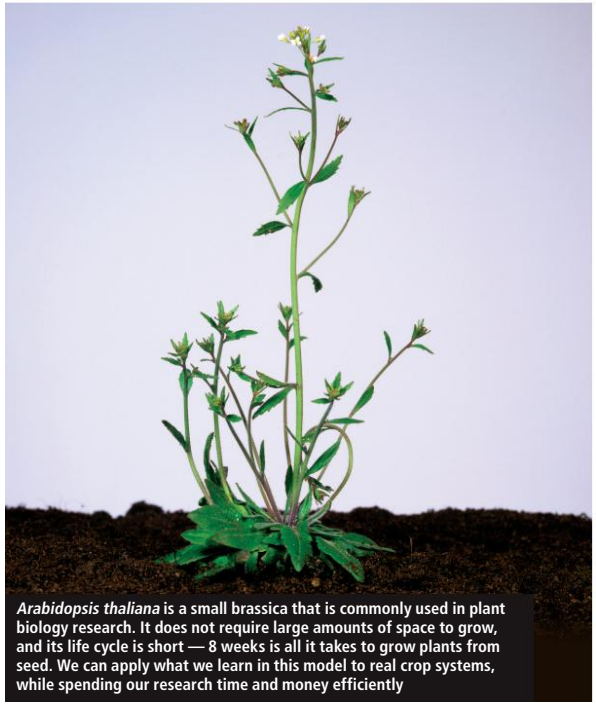
How the clock ticks

We can think of the circadian clock as having three key elements (see Figure 1):

- input pathways, which allow synchronisation of the oscillator to daily light–dark cycles
- a timing mechanism known as a central oscillator
- output pathways, which control various aspects of plant physiology and metabolism

Since the 1990s, scientists have used thale cress — *Arabidopsis thaliana*, a small member of the Brassicaceae — as a genetic model to identify the molecular components of the plant clock.

The oscillator itself is composed of about 20 genes and is found in every cell. Its oscillations are generated by molecular feedback loops based on three inhibitory steps (see Figure 2). Light influences expression of some of the clock genes, allowing synchronisation of the clock to light–dark cycles. Plants use light-sensing molecules called cryptochromes and phytochromes which, on stimulation, send signals inside cells, which then either increase or decrease expression of specific genes. The core components of the circadian oscillator control expression of many other genes that drive rhythms downstream of the clock. It is estimated that the circadian clock controls expression of around 15–30% of all the genes in plants.



Arabidopsis thaliana **is a small brassica that is commonly used in plant biology research. It does not require large amounts of space to grow, and its life cycle is short — 8 weeks is all it takes to grow plants from seed. We can apply what we learn in this model to real crop systems, while spending our research time and money efficiently**

Timing photosynthesis and starch metabolism

The circadian clock plays a key role in maximising the efficiency of photosynthesis while also minimising water loss. Gas exchange for photosynthesis comes at the cost of water loss due to transpiration, but the circadian clock acts to limit this by controlling the opening and closing of stomata on the surface of leaves. Most plants open their stomata during the day and close them at night. This allows carbon fixation and production of sugar through the Calvin cycle to take place when high concentrations of ATP and reduced NADP are produced from the light-dependent stage of photosynthesis.

However, many plants in dry environments have a different type of photosynthesis known as crassulacean acid metabolism (CAM) where carbon dioxide fixation takes place at night. This adaptation enables plants to keep their stomata closed when temperatures are the highest, during the day, and minimises water loss. Carbon dioxide is initially assimilated during the night, and it is then released during the day for synthesis of sugars.

The Calvin cycle only operates during the day, and many processes that are dependent on its products also show circadian rhythms. The best-studied example is the way in which the circadian clock controls the rates of

Terms explained

Anticipation In the context of circadian biology, these are the biochemical changes preparing the organism for the next phase of the day–night light cycle.

Calvin cycle The metabolic cycle used in most plants to fix carbon dioxide into sugar.

Circadian From the Latin *circa* for about, and *dies* for day.

Cryptochromes Molecules that sense blue light.

Expression mRNA synthesis from DNA encoding a gene.

Meristem Plant tissue found in the growing tips of roots and shoots, containing dividing cells.

Phytochromes Molecules that sense red light.

Examples of CAM plants include (a) orchids, (b) agave and (c and d) cacti including the Christmas cactus

Botrytis cinerea **is a mould that grows on grapes and many other fruits and vegetables. Plants are less vulnerable to this pathogen when infected at dawn, corresponding to the time when the fungus normally releases its spores**

carbohydrate use, thus maximising growth and avoiding starvation. During the day, plants store some of the sugars produced by photosynthesis as starch. Starch is then used during the night to sustain growth. The circadian clock allows plants to biologically 'anticipate' when dawn will arrive, adjusting their rate of starch consumption so that they don't run out before it is light again, when more can be made.

Coping with environmental challenges

Plants face daily challenges from other organisms, including pathogens and herbivores. Again, the circadian clock helps them put defence mechanisms in place at times when attacks are most likely to occur. Some plants produce a deterrent chemical called jasmonic acid at the time when caterpillars are most likely to feed, limiting the amount of damage caused by these insects. Similarly, the fungal pathogen *Botrytis cinerea* causes less severe disease in plants when it infects them in the morning, because plants can deploy their defence system faster at this time. Because stress tolerance mechanisms often come with an energy cost, we think that deploying them at critical times of the day could allow a compromise between stress tolerance and sustained growth.

The clock's influence on interactions with other organisms even extends to plant roots. The abundance of many different types of microbes living on the roots fluctuates over daily cycles, due to rhythmic changes in chemicals that are released by plant roots into the soil. Indeed, plants with dysfunctional clocks have increased numbers of pathogens on their roots, suggesting that circadian rhythms are important for plant health.

Better clocks for better crops?

A clock that is well adapted to its environment brings many advantages, optimising the timing of metabolic activities, growth and environmental responses. There is evidence that plants with a properly working clock outcompete plants with disrupted clocks, or with clocks poorly matched to the environmental light–dark cycle.

In the future, we may be able to apply such knowledge to develop more resilient crops. While we know a lot about the 'ticking' of the plant circadian clock and its wide range of influences, there is still much to learn about how we can apply this knowledge to the benefit of agriculture. Manipulating the clock to optimise the timing of one process may impact many other rhythms, and more work is required to understand fully the consequences of doing this — enough to keep scientists busy for many years.

Things to do

- Watch time-lapse videos of circadian rhythms of plant growth and leaf movements on the Plants in Motion website: **plantsinmotion.bio.indiana.edu**

Isabelle Carré is an associate professor at the University of Warwick. Her research aims to understand how the circadian clock contributes to environmental stress tolerance in plants. **Amy Newman** is a PhD student studying the impact of the plant circadian clock on microbial interactions with roots.

Further reading

Read the *Guardian* article about the research on circadian rhythms in humans and animals that received the 2017 Nobel prize for medicine:
https://tinyurl.com/ya4rorkv

Key points

- Plants respond to daily changes in their environment.
- The circadian clock is a network of genes regulating each other's expression.
- The clock provides survival advantages to plants.

Bird adaptations for running

Evolution of birds' feet

Currently, the most supported hypothesis is that birds evolved from a branch of **theropod** dinosaurs. When we compare the feet of theropods with those of early fossil relatives, such as *Archaeopteryx*, and of present day chickens, the similarity is remarkable (see Figure 1). The difference in the bird lineage is the presence of an opposable digit, the **hallux**. In 2015, scientists at the University of Chile reported that chemically preventing the movement of the hallux during chicken development resulted in limbs exactly like those of theropods.

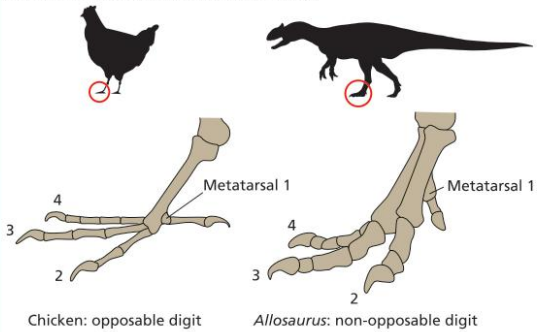


Figure 1 Comparison of the hind limb digits of chicken and theropod

The feet of two large ratites — emu (top) and ostrich (bottom)

Organisation of bird toes

Most birds' feet have four toes and they have a variety of forms (see Figure 2). Birds in the order Passeriformes (perching birds, such as the robin and the wheatear) show anisodactyly — an adaptation that facilitates perching and general walking. Woodpeckers, owls, cuckoos and roadrunners have a zygodactyl arrangement of toes, kingfishers a syndactyl arrangement, and swifts are an example of pamprodactyly.

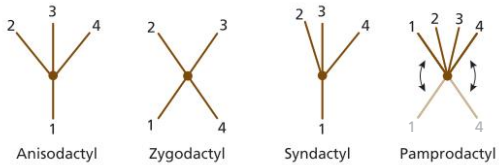


Figure 2 The organisation of birds' toes. Digit 1 is the hallux. In the pamprodactyl arrangement, digits 1 and 4 can move freely so help the feet to cling

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Flightless sprinters

From their theropod origins, birds have undergone remarkable radiations to provide the diversity we now see. After first evolving flight, some lineages reverted to flightlessness while others evolved aquatic adaptations. It's an extraordinary expansion. The emu, *Dromaius novaehollandiae*, is typical of many large flightless birds (ratites). Emus, along with other ratites such as cassowaries, have lost their hallux during evolution and have only three, forward-facing toes. Both species reach running speeds of around 50 km h^{-1}. The champion runner is of course the ostrich. The ostrich lost further toes during its evolution and now has only two. It reaches speeds over 70 km h^{-1}. The loss of toes contributes to a reduction in lower-leg mass and reduces ground contact. Such adaptations are found in many **cursorial** animals. For example, horses run on the toenail of their third digit.

Chasing the waves

Adaptations of birds' feet that facilitate running have evolved more than once. An example in the northern hemisphere is the sanderling. These waders breed on the Arctic tundra, migrating south in autumn and returning in summer. They are common on UK shores in spring, gathering in small flocks to probe the sand of wave-washed beaches for invertebrates. They can be seen sprinting in front of the receding waves to get their food, running back and forth. Like emus, sanderlings have three toes and no hallux.

Sanderling, showing a three-toed adaptation to running

Outrunning the coyote?

Other birds have different foot adaptations. As a child in the 1970s, I loved the Road Runner from the Warner Brothers' cartoon and his ability to outrun and outsmart Wylie Coyote. In real life, the roadrunner, *Geococcyx californicus*, lives in the deserts of southwestern USA and Central America, catching reptiles and small mammals and almost never flying. Its zygodactyl feet demonstrate relatedness to woodpeckers and cuckoos. This arrangement of toes is usually associated with climbing birds, but it is also suitable for running. However, while roadrunners can reach speeds of up to 30 km h^{-1}, this is not fast enough to outrun a hungry coyote!

A greater American roadrunner

Terms explained

Cursorial Animals adapted to running.

Hallux The first digit of the hind limb — big toe in humans.

Theropod A member from the dinosaur suborder theropoda. 'Thero', meaning beast, 'poda' meaning footed. They have hollow bones, a wishbone and three forward-facing toes.

Further viewing

How an ostrich outruns a top athlete:
www.youtube.com/watch?v=rJ0Ex4Xp3gM

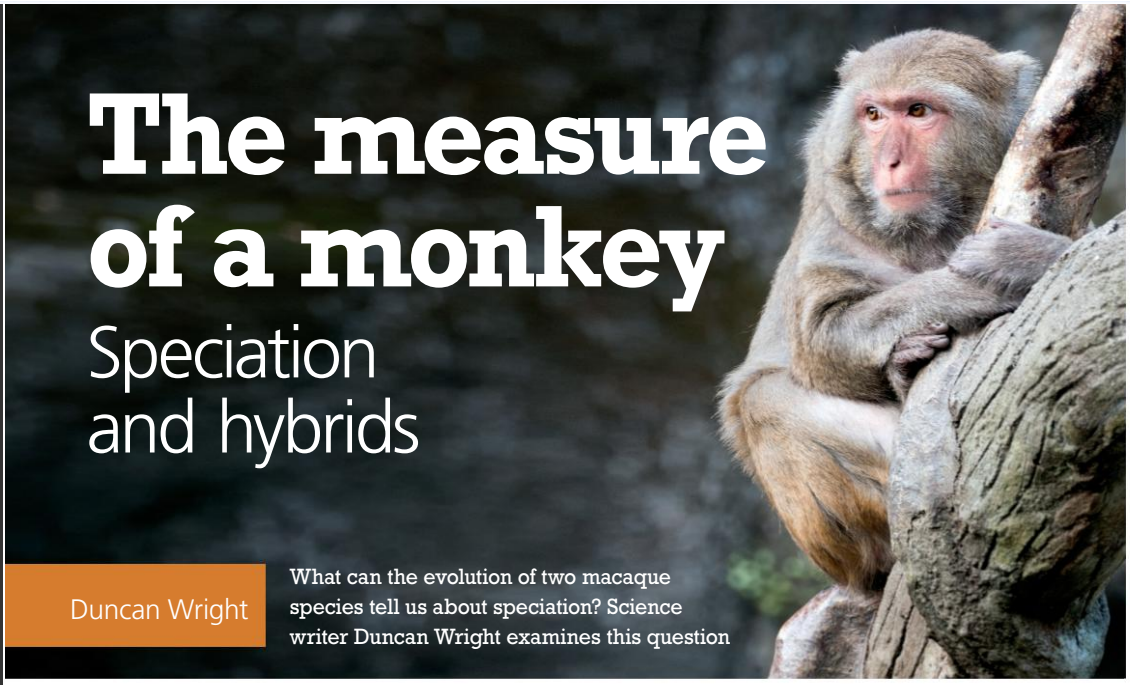
Sanderlings dodging the waves:
www.youtube.com/watch?v=HDyhPUkKiFY

A wheatear (*Oenanthe oenanthe*) showing anisodactyl toes

Professor **Kevin Moffat**, The University of Warwick

The measure of a monkey

Speciation and hybrids



Duncan Wright

What can the evolution of two macaque species tell us about speciation? Science writer Duncan Wright examines this question

AQA: 3.4.4 Genetic diversity and adaptation; 3.4.5 Species and taxonomy; 3.7.3 Evolution may lead to speciation

Edexcel A: 4.4 Natural selection can lead to adaptation; 4.6(i) Species concept; 5.19 Allopatric and sympatric speciation

Edexcel B: 3.1 Classification; 3.2 Natural selection

OCR A: 4.2.2 Classification and evolution; 6.1.2(g) Allopatric and sympatric speciation

OCR B: 3.1.3 The development of species; 5.1.2(e) Geographical and reproductive isolation

WJEC Eduqas: 2.2.1(f) The concept of species; 2.2.6(i) Isolation and speciation

Key words

Speciation
Vicariance
Hybridisation
Reproductive isolation

At last! After an hour's hike up the Tianmu Historic Trail in the unforgiving Taiwanese heat, I'd found them. To the left of the trail, on a raised patch of earth overlooking a sheer drop down the mountain, was a group of infant Formosan rock macaques. I crept forward to try to get a better shot with my camera, when suddenly — whump! The space between me and the infants was now occupied by an imposing male macaque, and the quiet mountain was suddenly filled with the sound of monkey chatter. Outmanoeuvred and out of my natural habitat, I began to think perhaps I had made a mistake. Eager to avoid a face full of macaque teeth, I broke eye contact and started to step slowly away, backwards. I didn't get my photo, but at least my face was still intact.

The Formosan rock macaque (*Macaca cyclopis*) is one of two primate species native to Taiwan, the other species being humans. Rock macaques primarily inhabit mountainous regions, where they forage for plant matter, insects and small vertebrates. Much like a hamster, the macaques store food in cheek pouches for later consumption. They are closely related to both rhesus macaques (*Macaca mulatta*), after which the Rhesus blood group is named, and Japanese macaques (*Macaca fuscata*).

Allopatric speciation

While rhesus macaques are broadly distributed across the Asian mainland, both Formosan rock macaques and Japanese macaques are **insular species**, restricted to Taiwan and the Japanese islands, respectively. How did these non-seafaring animals find themselves confined to these islands? The answer lies in their DNA and the Earth's history.

Taiwan and Japan have not always been islands: lower sea levels during **glacial periods** meant that Taiwan and Japan were connected to the mainland by **land bridges**. Comparison of **mitochondrial DNA** implies that an ancestral rhesus macaque population split between 380 000 and 440 000 years ago, and suggests that rhesus macaques migrated to Taiwan and Japan. A subsequent rise in sea levels cut off these islands from the mainland, causing the resident macaques to become isolated from the rest of the gene pool.

These island macaques evolved to become separate species with distinct physical characteristics. Japanese macaques have stumpy tails and distinctive red faces, while Formosan rock macaques have long tails and less colourful faces.

Terms explained

Allele A variant of a given gene.

Allopatric speciation The process whereby new species evolve from geographically isolated populations of a single species.

Courtship Behaviour and rituals used to attract a mate.

Gene flow The transfer of genes from one population to another.

Glacial period A time period associated with glacial advance.

Insular species A species with an island distribution.

Land bridge A connecting strip of land between two larger land areas.

Mitochondrial DNA The 16 569 bp of circular DNA found in mitochondria, encoding 37 genes.

Japanese macaques, famous for their enjoyment of hot springs, relaxing in Onsen Jigokudan Park, 130 km northwest of Tokyo

The emergence of these species is an example of **allopatric speciation**. The first step in this process is the geographical isolation (also known as vicariance) of populations of the same species. In our example, the macaques were isolated by rising sea levels. The isolated populations are unable to breed with one another, and there is thus no exchange of genetic material between them (i.e. there is no **gene flow**). The populations then slowly undergo genetic divergence, through various mechanisms (see Box 1). The gradual accumulation of genetic changes may ultimately result in reproductive isolation, meaning that, were the two populations to encounter one another again, they would be unable to exchange genes (see Figure 1). Reproductive isolation can be caused by several factors, as shown in Box 2.

Box 1 Genetic divergence

The gene pools of isolated populations can diverge through the following processes.

- Mutation: random genetic mutations occur in each population, resulting in distinct gene pools. Some of these mutations do not affect an organism's ability to survive and reproduce (they are 'neutral') but may still be passed on by chance (see 'genetic drift' below).
- Natural selection: other mutations may enhance an organism's ability to reproduce in a particular environment. Organisms with such mutations are thus more likely to pass their favourable **alleles** of genes to more offspring. For example, mutations that increase the chance of survival in Taiwan's subtropical heat would be more likely to be retained in Formosan rock macaques.
- Genetic drift: it is not always the fittest organisms that survive and pass on their genes — there is also an element of chance involved. If organisms fail to mate through bad luck (say, an encounter with a human hunter), their alleles may be lost from the gene pool. Such a random change in the frequencies of alleles is called genetic drift.

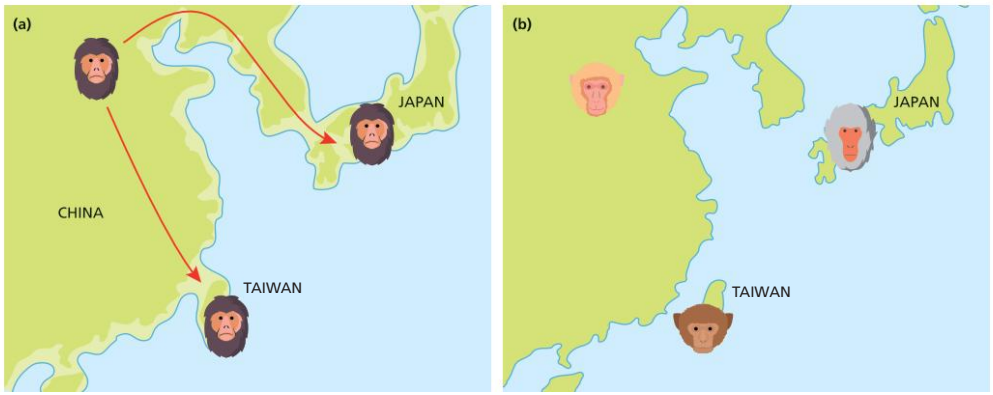
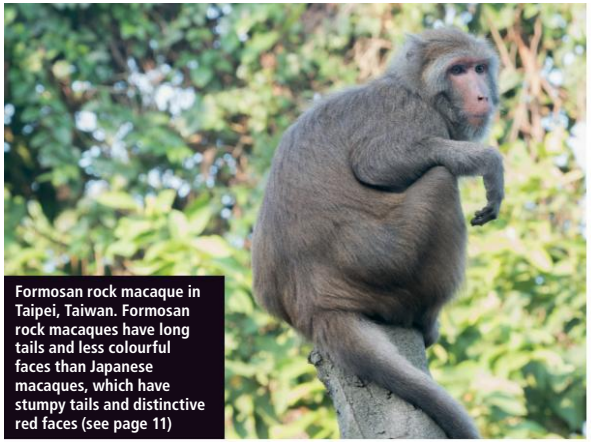


Figure 1 Speciation in macaques. **(a)** During earlier glacial periods, rhesus macaques migrated from the Asian mainland to Taiwan and Japan via land bridges. **(b)** Taiwan and Japan later became islands, isolating their resident macaque populations from the wider population. Subsequent genetic divergence caused distinct species to form in Taiwan, Japan and continental Asia



Formosan rock macaque in Taipei, Taiwan. Formosan rock macaques have long tails and less colourful faces than Japanese macaques, which have stumpy tails and distinctive red faces (see page 11)

Hybridisation

Although Formosan rock macaques can swim, it is unlikely they would have the stamina to swim over 1000 kilometres from Taiwan to Japan. Even if a macaque clung on to a piece of wood that was washed out to sea, and that wood just happened to float all the way to Japan, the macaque would be unlikely to find enough food to survive in the open ocean. The bottom line is that Formosan rock macaques and Japanese macaques would probably not have encountered one another had it not been for human intervention.

Formosan rock macaques were taken to zoos and research centres in Japan and some of them managed to escape. Indeed, enough macaques escaped from a private zoo in Wakayama City to set up their own breeding population (see Figure 2). This particular group has been known to mate with Japanese macaques, producing fertile hybrids between the two species.

But wait. We tend to think that different species cannot interbreed and even if they can, the offspring tend to be sterile. Matings between donkeys and horses produce (usually) sterile mules or hinnies. If Japanese macaques and Formosan rock macaques can produce fertile offspring, can they really be considered two different species?

Figure 2 Sites of *Macaca cyclopis* breeding populations in Japan

Box 2 Reproductive isolation

Reproductive isolation can occur at two main levels — pre-zygotic (before fertilisation or mating) and post-zygotic (after fertilisation).

Pre-zygotic barriers

- Behavioural isolation: two populations will not interbreed because of differences in courtship behaviour.
- Mechanical isolation: differences in the size, shape, and/or location of genitals prevent mating.
- Gametic isolation: the sperm and egg (or pollen and ovule) are incompatible for fertilisation.

Post-zygotic barriers

- Non-viable hybrid: the egg is fertilised but the embryo fails to develop normally.
- Hybrid sterility: the hybrid develops normally but has low fertility or is completely sterile.

Definition of species

This brings us to 'the species problem' — how does one define species? The biologist Ernst Mayr defined a species as:

> groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups

In other words, organisms can interbreed with members of the same species, but not with members of other species. However, there are several problems with this definition (see Box 3). One problem is that it does not account for hybridisation between closely related species, such as our macaques. Although Formosan rock macaques and Japanese macaques exhibit distinctive physical and genetic properties, they have not yet undergone complete reproductive isolation. This is also true for coyotes and wolves — canines that meet and mate where their territories overlap, creating hybrid zones in which 'coywolves' are common.

Hybrid success

Often, hybrids are less fertile and less adapted to their environment than their parents, meaning they are less likely to pass on their genes. In such cases, natural selection will favour organisms that mate with members of their own species, effectively reinforcing separation between the two species. Over time, complete reproductive isolation may develop, even in the absence of geographical isolation. Sometimes, however, hybrids may be so well adapted to their environment that they become a new species. One notable example is the European bison, which is believed to have arisen as a hybrid of two extinct species: the steppe bison and the aurochs (the former is also the ancestor of the American bison, and the latter is the ancestor of domesticated European cattle).

Which of these two fates awaits hybrids of Formosan rock macaques and Japanese macaques? We may never know, as natural selection is being supplanted by artificial selection. In 2003, the Wakayama Prefectural Government launched a campaign to exterminate hybrids of these two species, as part of a wider movement to limit damage to ecosystems caused by invasive alien species. Sadly, the greatest threat facing macaques is not hybridisation, but rather destruction of their environment by human encroachment. While a group of macaques may be able to scare off one human, they are defenceless against the destructive force that is human society.

Further reading

Read about the Formosan rock macaque here:
www.arkive.org/formosan-rock-macaque/macaca-cyclopis

And more about the Japanese macaque here:
www.arkive.org/japanese-macaque/macaca-fuscata

Read Richard Dawkins' book on evolution — *The Blind Watchmaker.*

Box 3 Defining species

The Mayr definition of species is challenged by the following situations.

- Asexual reproduction: members of asexually reproducing species (such as bacteria) cannot be defined based on their ability to interbreed.
- Ring species: sometimes it is not clear where one species ends and another begins. Ring species (such as the *Larus* gull, see p. 25, this issue) consist of a long chain of populations (say, 'ABCDEFGH') — neighbouring populations can interbreed (for example, members of A can breed with members of B), but the two ends are reproductively isolated from one another (members of A do not breed with H).
- Hybridisation: sometimes reproductive isolation between two related species is not complete, and members of distinct populations can interbreed to produce hybrids (e.g. macaques).

The European bison is an example of successful hybridisation

Dr Duncan Wright completed a PhD in molecular genetics at The University of Warwick and subsequently worked as a postdoctoral researcher in Taiwan. He currently works in scientific publishing.

Key points

- Rising sea levels caused the ancestors of Japanese macaques and Formosan rock macaques to become separated from each other.
- Geographical separation prevented interbreeding between the two groups, causing them to slowly undergo genetic divergence.
- Genetic divergence may eventually result in reproductive isolation, preventing organisms of two species from producing fertile offspring.
- Formosan rock macaques and Japanese macaques can still mate and produce fertile offspring, and so do not exhibit complete reproductive isolation.

Francis Crick Institute exhibition

Craft & Graft: Making Science Happen, Francis Crick Institute, 1 Midland Rd, London NW1 1ST (near Kings Cross Station) until 30 November 2019

A new exhibition from the Francis Crick Institute offers visitors the chance to go behind the scenes and see the work of five specialist departments which support the institute's ground-breaking research. They include:

- Glass Wash, which cleans 750 000 items of glassware a year
- Fly Facility, which nurtures over 1.5 million flies
- Cell Services (or 'Librarians of life-forms'), which grows around 100 billion cells a month

Biological Sciences Review has a link with the institute: its director Sir Paul Nurse is on our advisory panel and articles from Sir Paul and other authors from the institute regularly appear in the magazine (e.g. 'Cancer stem cells: the seeds of a tumour', Vol. 30, No. 4).

Opening hours:
Wednesday 10 a.m.–8 p.m.
Thursday, Friday, Saturday 10 a.m.–4 p.m
Admission is free

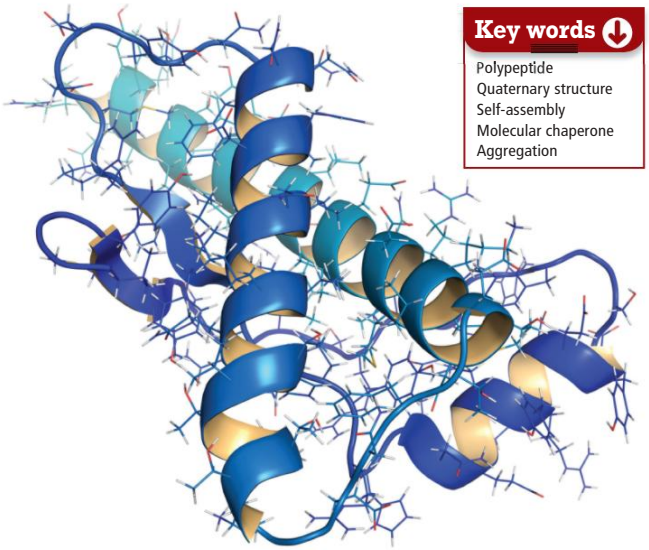
The Fly Facility

Protein folding

Principles and problems

John Ellis

Protein folding is one of the most important biological processes because it converts the linear information encoded in genes into the three-dimensional structures that give proteins their functional properties. Biochemist John Ellis explains how proteins fold, and introduces how problems with the process can lead to disease



Key words
Polypeptide
Quaternary structure
Self-assembly
Molecular chaperone
Aggregation

Proteins are the action molecules of all organisms. They are the molecules that carry out most of the processes necessary to sustain life. Genes are important because they contain the information to make these proteins. Proteins function by presenting binding sites on their surface for chemicals in their environment, including other proteins. These sites recognise and bind a wide range of chemicals in a highly specific fashion. 'Specific' means that each site binds just one type of chemical. Most proteins have more than one binding site, each specific for a different type of chemical.

Enzymes

Some proteins function as enzymes. Enzymes are defined as specific catalysts, that is, each type of enzyme promotes one type of chemical reaction, which would proceed very slowly, or not at all, in its absence. Each enzyme has its own name, which indicates the type of reaction that it catalyses. For example, hexokinase catalyses the transfer of a phosphate group from a donor molecule called ATP to a carbon atom at position six in glucose to give glucose-6-phosphate.

Figure 1 shows an atomic model of one molecule of the enzyme hexokinase. Each sphere in this model represents the size and position of an atom of carbon, oxygen or nitrogen; hydrogen atoms, of which there are many, are omitted for clarity. The shape of hexokinase is unique to that enzyme, and every molecule of hexokinase is identical. Hexokinase has on its surface a site that specifically binds a glucose molecule.

The way in which a chemical fits into a binding site is similar to the way a key fits into a lock. However, because most proteins change their shape as they bind their substrates, the term '**induced fit**' is a more helpful description. Proteins can change their shape because the bonds that hold the shape in place are weak **non-covalent bonds**, such as hydrogen bonds, electrostatic bonds and hydrophobic interactions. Such weak bonds are easily broken and reformed under the conditions found inside organisms. Figure 1 illustrates the change in shape of hexokinase following the binding of one glucose molecule.

Protein folding

Because each organism carries out several thousand different kinds of chemical reaction, it follows that each organism contains thousands of different kinds of enzyme. When we add to their roles as catalysts the many other functions of proteins — such as acting as signalling molecules, transport systems, structural components and antibodies — it is clear that proteins are a remarkable group of chemicals. What determines the specific properties of all these different proteins?

Proteins are linear polymers of different kinds of amino acid strung together like beads on a chain. Amino acids have the same basic structure but vary in their side chains, of which there are at least 20 different types found in proteins (see Box 1). Each chain is called a polypeptide and has a unique sequence of amino acids encoded in the base sequence of its gene. This amino acid sequence is called the primary structure.

Terms explained

Dialysis A method of separating small molecules from larger ones.
Induced fit The idea that enzymes are flexible structures with binding sites that alter shape as substrates bind to them.
Non-covalent bonds Bonds that differ from covalent bonds in that they do not involve the sharing of electrons but involve weaker more dispersive electromagnetic interactions.

A polypeptide does not exist as a straight chain for long after it is synthesised, but folds into helices and sheets, forming the secondary structure. This intermediate then folds further into a more compact stable shape, called the tertiary structure or monomer.

Protein folding is very rapid, taking only a few seconds to minutes. Most proteins consist of more than one monomer, which may be identical to, or different from, one another. These monomers assemble together into oligomers, which are said to have quaternary structure. For example, hexokinase is an oligomer of two dissimilar subunits. Figure 2 illustrates these processes, with different secondary structures depicted as blue and red ribbons.

What determines the specific binding properties of a given protein? The answer is that it is the sequence of amino acids that determines exactly how each chain folds into the specific three-dimensional shape, called its conformation, which displays binding sites on its surface. The conformation unique to hexokinase is formed by specific interactions between the side chains of the different amino acids along the polypeptide chain. What interactions are possible, and hence the shape of the folded molecule, is determined solely by the sequence of amino acids. Once that sequence is synthesised, the chain folds into its functional conformation. The amino acid sequence of each polypeptide is encoded by the base sequence of its gene.

Genes in action

The biochemical process by which a gene determines the amino acid sequence of a polypeptide chain is extraordinarily complicated. This complexity is required because one chemical language — the base sequence of DNA — has to be translated into another chemical language — the amino acid sequence of polypeptide chains. It is important to realise that this process does not involve a conversion of the bases themselves into amino acids. What flows from bases to amino acids is sequence information, not material.

The mechanism of gene expression is outlined in Figure 3. DNA acts as a template that allows the

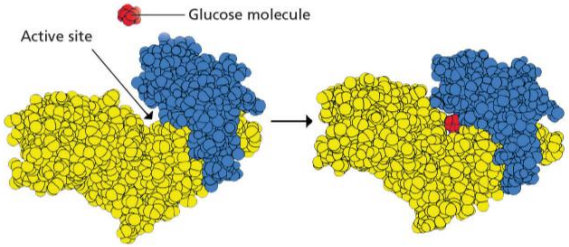


Figure 1 The enzyme hexokinase is made of two different subunits, here coloured blue and yellow. One molecule of glucose binds to its active site, and the enzyme then changes shape to enclose the glucose

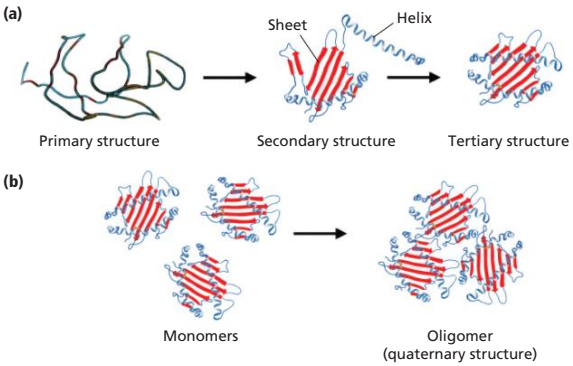
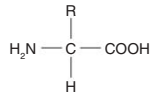


Figure 2 **(a)** Protein folding involves the rapid collapse of an extended polypeptide chain into a stable compact structure called a monomer. **(b)** Monomers may be functional on their own or assemble with identical or different monomers to form oligomers

Box 1 Amino acid structure

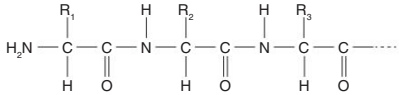
The basic structure of an amino acid is:



where R represents an amino acid side chain. For example:

- in glycine, R = H
- in alanine, R = CH_3
- in lysine, R = $CH_2CH_2CH_2CH_2NH_3^+$
- in aspartic acid, R = CH_2COO^-

Amino acids are linked together via peptide bonds to form a polypeptide.



The type and sequence of amino acid side chains (R_1, R_2, R_3 etc.) give the polypeptide chain its unique properties.

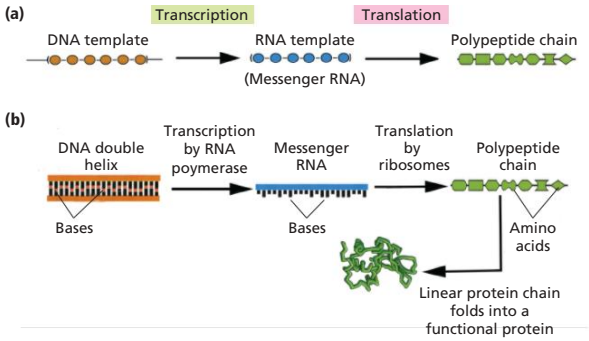


Figure 3 **(a)** Sequence information flows from DNA to protein. **(b)** Linear DNA information produces a folded polypeptide

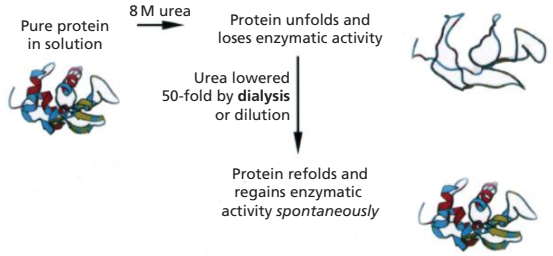


Figure 4 The classic refolding experiment of Anfinsen demonstrates the principle of protein self-assembly

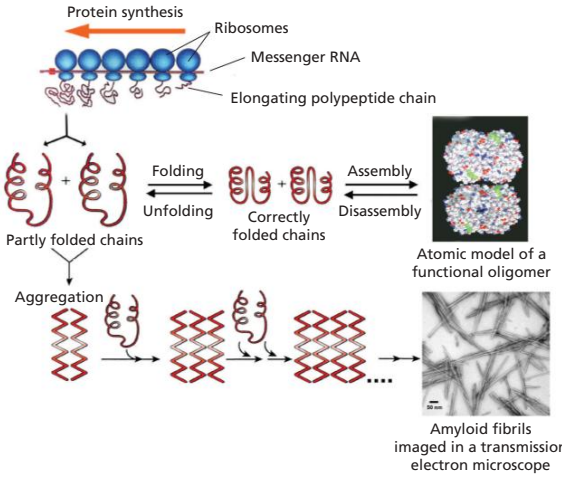


Figure 5 Newly synthesised chains can either fold correctly to produce functional proteins or aggregate together into non-functional fibrillar complexes. These complexes are called amyloid fibrils because when first discovered they were thought (incorrectly) to be made of carbohydrate

sequence of bases in one strand of the double helix of each gene to be copied in the form of RNA. This process is called transcription, and it produces from each gene many copies of an RNA molecule called messenger RNA (mRNA). Each molecule of mRNA is about the same length as the gene that acts as a template for its synthesis, and the enzyme that carries out this synthesis is called RNA polymerase. The order of bases in mRNA is the same as the order of bases in one strand of the double helix of DNA from which it is copied.

The mRNA in turn acts as a template for a second process called translation, in which amino acids are joined together by ribosomes in a linear order determined by the order of bases in the mRNA. Each molecule of mRNA can programme the synthesis of many molecules of the protein chain that it encodes.

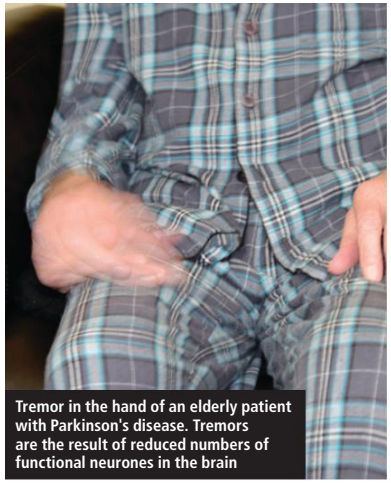
The principle of self-assembly

Experiments with isolated proteins show that protein folding is spontaneous — no other source of information or energy is required for folding to occur once the sequence has been made. This fact is sometimes called the 'principle of self-assembly'.

This principle was established by experiments carried out by Christian Anfinsen, which earned him the Nobel prize in chemistry in 1972. He purified the enzyme ribonuclease, which hydrolyses RNA, until no other proteins were present. He then incubated the pure enzyme in aqueous solution with a high concentration of urea. This treatment breaks the weak non-covalent bonds holding the tertiary structure together. The protein thus unfolds and loses its enzymatic activity because it no longer has binding sites for its RNA substrate. Such an unfolded protein is said to be denatured. If the urea concentration is diluted by a large factor so that it is no longer effective as a denaturing agent, the protein then refolds spontaneously into an active enzyme (see Figure 4). This type of refolding experiment has been carried out successfully with many isolated proteins. But inside organisms the situation is not so simple.

Problems with folding

Mutations that change the primary structure of a polypeptide may result in the failure of protein folding to produce a functional protein (see Biological Sciences Review, Vol. 26, No. 3, pp. 22–25). However, mutations are rare — every time a human cell divides, only approximately three base pairs in the entire genome of three billion base pairs are changed. But there is another folding problem that potentially affects every polypeptide chain, whether mutated or not. This is the problem of protein aggregation.



Tremor in the hand of an elderly patient with Parkinson's disease. Tremors are the result of reduced numbers of functional neurones in the brain

Protein folding is rapid, but not fast enough to completely prevent partly folded chains from interacting prematurely with one another to form incorrectly folded structures called aggregates (see Figure 5). Aggregation is highly specific — only chains that are identical or nearly identical in sequence will form aggregates, because the structures that aggregate have some secondary and tertiary structure. It is also a progressive process — more and more partly folded chains are added together to form growing fibrils.

Fibrils that are large enough to become insoluble are called amyloid plaques. Such amyloid plaques are characteristic of human neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease, where they accumulate in the brain (see Figure 6). It is thought that it is the smaller, soluble aggregates that cause neurones to become damaged and die.

Chaperones

Protein aggregation is a universal cellular problem, so it is not surprising that all cells have evolved means to combat this problem. A major mechanism is the existence of proteins that recognise partly folded polypeptides and bind to them transiently. This binding prevents the partly folded chains from aggregating with one another. The binding is then reversed under conditions that favour correct folding. These protective proteins are called molecular chaperones. The term 'molecular chaperone' was coined because their properties are a precise molecular analogy of the role of human chaperones. The traditional role of human chaperones is to prevent incorrect interactions between pairs of humans.

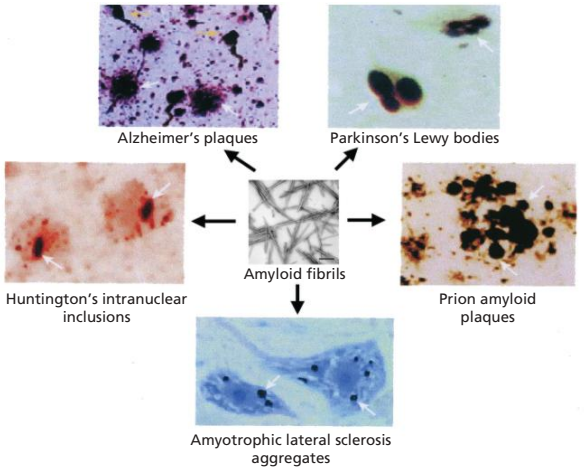


Figure 6 Amyloid plaques in the brain are characteristic of most human neurodegenerative diseases

Unlike other proteins, most chaperones have broad specificity and bind to a wide range of different partly folded polypeptides. Some chaperones bind to monomers released by the disassembly of oligomers, and prevent them from aggregating. Most chaperones use ATP as an energy source to perform their function.

The discovery of chaperones in the 1980s resulted in the replacement of the original principle of spontaneous self-assembly by the current principle of assisted self-assembly. Note that the basic concept of self-assembly is retained, but modified to include assistance by other proteins (molecular chaperones) that minimise incorrect folding. This discovery has enabled researchers to devise novel strategies to develop chemicals that can mimic molecular chaperones, thereby providing treatments for diseases caused by protein aggregation.

John Ellis is an emeritus professor in life sciences at the University of Warwick. Visit **http://en.wikipedia.org/wiki/R._John_Ellis** for downloadable lectures and a book called *How Science Works: Evolution*

Key points

- Proteins are the action molecules of life, and they function by binding biochemicals in a highly specific manner.
- Proteins are made as linear chains of amino acids but then fold rapidly into three-dimensional shapes with specific binding sites on their surfaces.
- Partly folded chains may aggregate together to form non-functional structures that can be toxic.
- Aggregation is prevented by some proteins acting as molecular chaperones that bind transiently to partly folded chains.

what is…?

A ring species

A species can be defined as a group of individuals that can interbreed in the wild. What mechanisms might cause speciation — the generation of new species from an ancestral species? Biologists Robert Spooner and Raksha Gohel examine the concept of a ring species

Speciation, the generation of new species from an ancestral species, requires reproductive isolation. If the geographical area that a species occupies is larger than the average life-time travel of any individual, then although the population is continuous, gene flow will be restricted to geographic neighbours. Could collections of genetic changes at the extremes of a geographic range of a population result in speciation?

Salamanders

This has been observed in ring species, where an ancestral species encountered a geographical barrier, migrated around it in two directions and the populations then met again.

The classic example is that of multi-coloured *Ensatina eschscholtzii* salamanders in the Central Valley of California. This dry grassy valley acts as a geographical barrier to gene flow for salamanders, which require moist habitats. A population at the north of the valley migrated south down both the east and west flanks of the valley, evolving on the way into 19 different subspecies. A subspecies can be considered somewhat physically and genetically different from the rest of the species, but still similar enough to interbreed. Indeed, individual salamanders in adjacent subspecies appear to mate freely. However, where the eastern and western populations meet at the southern end of the Valley (where the 'ring' closes), the two populations look distinctly different and do not mate with each other, so they have become two different species.

Gulls

You don't need to travel to California to see an example of a ring species. If you are in the northern hemisphere, just look up (although the closer you are to a coast, the more likely you are to see it).

There is a circumpolar ring of *Larus* gull subspecies (see Figure 1), with the Arctic ice acting as a physical gene flow barrier. Where the ring closes above Britain and Scandinavia, the physical differences between the gulls are extreme, as in the salamanders mentioned above. The herring gull (*Larus argentatus*) has grey feathers on the backs of its wings, pink legs, pink feet and golden/orange eye-rims. The lesser black-backed gull (*Larus fuscus*) has dark charcoal/black feathers on the backs of its wings, yellow legs, yellow feet and red eye-rims. There is no interbreeding between these gulls, so they have become different species.

Observational studies from Walney Island in northwest Lancashire, UK, showed that no hybridisation between herring gulls and lesser black-backed gulls happened in the wild. However, in captive conditions, herring gulls and lesser black-backed gulls will mate and produce fully fertile hybrids. So, why don't they mate in the wild? Why do we consider them different species? Are there other factors that favour speciation? In part it appears to depend on the female's choice of mate. She likely reacts to specific cues: differences in call-notes, the colour of the back, the colour of the eye-ring, and the colour of the legs have all been suggested. Perhaps such behavioural barriers will subdivide the ring further, ultimately generating multiple species. Recent DNA studies on *Larus* gulls measuring gene flow indicate that this process is already underway and these subspecies are indeed becoming separate species.

Figure 1 The ring species of *Larus* gulls. The numbers around the Arctic Circle are placed at the approximate geographical centre of the range of each subspecies. **1**, *Larus fuscus* (lesser black-backed gull); **2**, *Larus heuglini* (Heuglin's gull); **3**, *Larus argentatus birulai* (Birula's gull); **4**, *Larus vegae* (east Siberian herring gull); **5**, *Larus smithsonianus* (American herring gull); **6**, *Larus argentatus* (herring gull). The double-headed arrows show gene flow. Each subspecies can breed with its neighbour subspecies except over northern Europe where the herring gull and the lesser black-backed gull do not interbreed, and where speciation is complete

Robert Spooner is a senior teaching fellow. **Raksha Gohel** is an undergraduate student and a curriculum advisor to the Royal Society of Biology. Both are in the School of Life Sciences, The University of Warwick.

Transcription factors

How transcription factors work

Proteins involved in the regulation of gene expression by controlling the transcription of DNA are called transcription factors. Transcription factors bind to DNA at specific sequences — for example, a gene's **promoter region** — and it is only then that the **RNA polymerase** can bind to make mRNA (see Figure 1). The combination of transcription factors and RNA polymerase is known as the transcription initiation complex. This protein complex starts the transcription of DNA into mRNA, which subsequently acts as the template for protein translation. Transcription factors that are activators enhance gene expression. Reciprocally, transcription factors that are repressors decrease or block gene expression (see Figure 1). Together, these control the identity of cell types by ensuring each type of cell expresses a specific subset of genes.

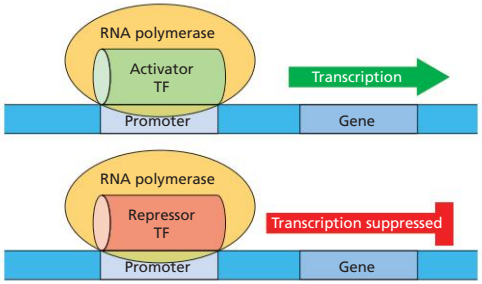


Figure 1 Transcription factors can stimulate or suppress transcription of a target gene

Figure 2 Coloured scanning electron micrograph of Pax6 mutant *Drosophila melanogaster* on the left opposite wild-type on the right. The mutant fly has not developed eyes ×100

Flies with legs on their heads

All the cells in the body have the same genome and it is transcription factors that determine when, where and which specific genes are expressed. In the **model organism** *Drosophila melanogaster*, the transcription factor known as 'Tinman' is responsible for the development of the insect's heart. If Tinman is missing, a heart cannot form. Pax6 is a transcription factor involved in eye development. In *Drosophila*, mutant flies lacking Pax6 are noticeably different from **wild-type** flies — they have no eyes (see Figure 2). The process of the formation of antennae in *Drosophila* is dependent on the transcription factor Antennapedia. It has been observed that if, due to a mutation, this transcription factor is itself expressed in the wrong place during development, the flies grow legs on their heads where the antennae should be (see Figure 3).

Terms explained

Model organism An organism suitable for studying a specific trait or disease that has a characterised genome.

Multipotent Cells that have the ability to self-renew by dividing and develop into many specialised cell types.

Promoter region An area of DNA that defines where transcription of a gene by RNA polymerase begins.

RNA polymerase The enzyme that synthesises RNA from a DNA template during transcription.

Wild-type The normal, non-mutated version of a gene common in nature.

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Stem cells

In humans, one role of transcription factors is to maintain the cellular function of stem cells. Stem cells are undifferentiated cells that can develop into either specialised cell types in the human body or simply divide to produce more stem cells. There is more than one type of stem cell, depending on the stage of human life. Embryonic stem cells — stem cells derived from the undifferentiated inner mass cells of a human embryo — are pluripotent, meaning they can form any cell type in the human body. Adult stem cells are **multipotent** and are vital for renewing damaged cells and tissues. Four transcription factors — Oct4, Sox2, Klf4 and c-Myc — play important roles in stem cell control. Oct4 forms a complex with Sox2 and controls the expression of genes responsible for pluripotency in embryonic stem cells. Klf4 can act as both activator and repressor of itself and genes involved in cell death (see pp. 10–13 this issue), and c-Myc activates the transcription of growth-related genes.

Transcription factors are essential components in all organisms for the correct control of gene expression. Without these important factors our bodies would not be able function correctly.

Figure 3 Antennapedia mutant *Drosophila*. Legs have grown where antennae should be ×100

Rachel Clewes, University of Warwick

Uncoupling mitochondria turns up the heat

Katrine Wallis

In eukaryotes, including animals and plants, the production of adenosine triphosphate (ATP) largely reflects the actions of mitochondria and chloroplasts. The energy usually transferred into the phosphate bonds of ATP can, alternatively, lead to the production of heat. This is accomplished through the actions of special proteins called uncouplers. Senior teaching fellow Katrine Wallis explains this remarkable process

Exam links

AQA Photosynthesis; Respiration
Edexcel A Light-dependent reactions of photosynthesis; Respiration
Edexcel B Respiration; Photosynthesis
OCR A Photosynthesis; Respiration
OCR B Cellular respiration; Photosynthesis
WJEC Eduqas The importance of ATP; Photosynthesis; Respiration

Key words

Mitochondria
Chloroplast
Electron transport
Uncoupling
Hibernation

In early spring, from Nova Scotia to the foothills of Minnesota, USA, winter is still apparent. Nonetheless, the eastern skunk cabbage (*Symplocarpus foetidus*) is melting the snow. Despite the temperature being only just above freezing, parts of the plant can maintain a temperature of around 15°C. This elevated temperature allows the plant to emerge and flower early (see Figure 1). It also allows the flowers to release chemicals that attract pollinators. These smelly chemicals are what gives the plant its specific name — *foetidus* (from the Latin for foul-smelling).

In northern America, groundhogs and other small mammals are still hibernating in their burrows. Their body temperature can be as low as 3°C, and while it regularly spikes to normal mammalian levels (37°C) it cools down again. These physiological events are the result of mitochondrial function (see Figure 2), inside both the skunk cabbage and in the hibernating mammals.

Mitochondria are found in cells of all eukaryotes. Their main function is not to release heat but to produce ATP. Producing ATP and releasing heat are biochemically related processes. Unlike animals, plants have another organelle that can produce ATP. In chloroplasts (see Figure 2), the **light-dependent reactions** of photosynthesis trap energy from light and couple it to ATP production. Here we will compare the heat release and ATP production from these organelles.

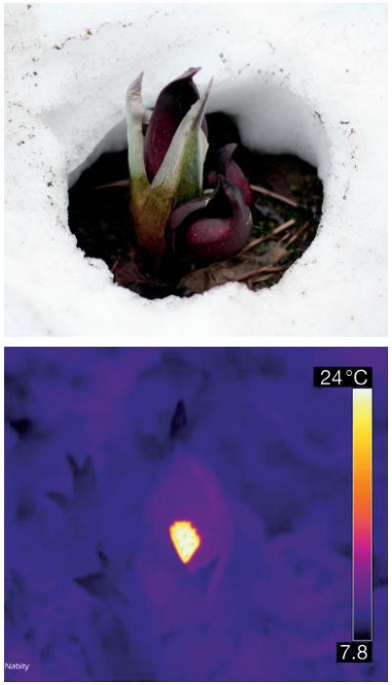


Figure 1 (Top) Skunk cabbage melting the snow. (Bottom) Thermal imaging of the plant shows that the **spadix** is some 16°C warmer than other plant tissues

Terms explained

Aerobic respiration The process of transferring cellular energy into ATP by involving oxygen.
Electron carrier A molecule capable of accepting an electron(s) before donating to another molecule.
Light-dependent reactions In photosynthesis, the creation of ATP and reduced NADP.
Light-independent reactions In plants, the use of carbon dioxide by the Calvin cycle to produce triose phosphate, some of which is then used to make glucose and other compounds.
Redox A change in reduction/oxidation state of a molecule.
Spadix A spike of small flowers packed tightly together on a small stem.
Thylakoid A membrane-bound compartment inside chloroplasts.

Mitochondria and respiration

The energy needed to make ATP or to release heat comes from a series of reactions that make up **aerobic respiration**. The energy released from this process is linked to adding phosphate groups (P_i) to ADP molecules, creating ATP:

glucose + oxygen + P_i + ADP → carbon dioxide + water + ATP

As part of this process, glucose is oxidised to carbon dioxide and some of its electrons are transferred to oxygen, which is reduced to water. This transfer of electrons is the key energy-transfer step, resulting in production of ATP. The electrons are not transferred directly from glucose to oxygen, but instead are transferred via a series of **electron carriers**, resulting in a gradual, controlled release of energy. Much of this process takes place in mitochondria (see Box 1). The energy released in this process is used to move protons (H^+) from the matrix of the mitochondrion to the inter-membrane space (IMS) resulting in a proton gradient building up, with a high concentration of protons in the IMS and a low concentration in the matrix (see Box 1).

Glucose has now been fully oxidised to carbon dioxide and the electrons have been transferred to their final destination, which is oxygen, but not much ATP has been made yet. Instead there is a proton gradient across the inner mitochondrial membrane (see Box 2). Just like a dam prevents water from flowing down the river, the membrane prevents the protons flowing back into the matrix. In the same way that dammed water can be used to release energy by passing the water through a turbine, the protons can flow through a membrane protein called ATP synthase. The ATP synthase acts like a turbine, as the flow of protons is used to spin part of the protein inside the membrane. This spinning results in the production of ATP (see Box 2).

Uncoupling proteins allow hibernating groundhogs to warm up

The generation of a proton gradient used to drive the formation of ATP was termed the chemiosmosis theory by Peter Mitchell from the University of Oxford in 1969. We call this process **oxidative phosphorylation** because the oxidative process of moving electrons and building a proton gradient is coupled to addition of inorganic phosphate to ADP forming ATP.

Uncoupling

The coupling of the proton gradient with ATP production creates the vast majority of ATP that cells need. Sometimes the two are uncoupled to allow release of heat. This is equivalent to releasing some water from a dammed river without flowing it through the turbine. It is carried out by uncoupling proteins (UCP) in the inner mitochondrial membrane (see Box 2). In this case the energy from the proton gradient is released as heat.

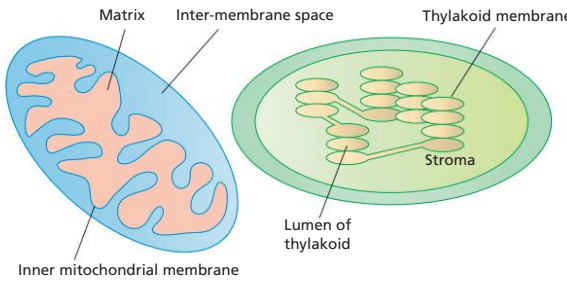


Figure 2 Structure of mitochondrion (left) and chloroplast (right)

First World War munitions workers lost weight as a consequence of working with DNP

In mammals this heat generating process mainly happens in specialised fat tissue known as brown fat owing to its large number of mitochondria and rich blood supply (which give it a brown shade). It is this uncoupling process that allows hibernating animals, such as groundhogs, to warm up their bodies to normal temperature. Other mammals can also generate heat through uncoupling. The process is particularly common in young animals that can struggle to keep warm. Uncoupling proteins are found in all known mammals that have been investigated except the pig.

Photosynthesis

Just like animals, plants use aerobic respiration to produce ATP. Unlike animals they can make it by photosynthesis. Photosynthesis happens in two stages. First, in the light-dependent reactions, light is harvested to make ATP. Then, in the **light-independent reactions**, the ATP is used to convert carbon dioxide into organic compounds. If we look at the energy-harvesting parts — the light-dependent reactions — they are similar to oxidative phosphorylation, used to generate ATP during aerobic respiration. Electrons are transported along a series of carriers and the energy released in these **redox** processes results in a proton gradient (see Box 1). Just like in respiration, this proton gradient is used to make ATP, except that this process happens in chloroplasts rather than mitochondria.

Box 1 Electron transport

Mitochondrial electron transport

The electrons taken from glucose during its oxidation in glycolysis and the Krebs cycle are carried by reduced NAD (NADH). Electrons from NADH are transported along a series of electron carriers until they reach oxygen (see Figure 1.1, red arrows). The oxygen is then reduced to water. The carriers of electrons consist of three big protein complexes (I, III and IV) embedded in the membrane, and smaller carriers that ferry the electrons from one complex to the next. A carrier is in the reduced state when it is carrying electrons and in its oxidised state when it gives up the electrons. This process of transporting electrons releases energy, a little in each step, which is linked to transporting protons from the matrix, across the inner mitochondrial membrane to the IMS (green arrows). This results in a high concentration of protons in the IMS.

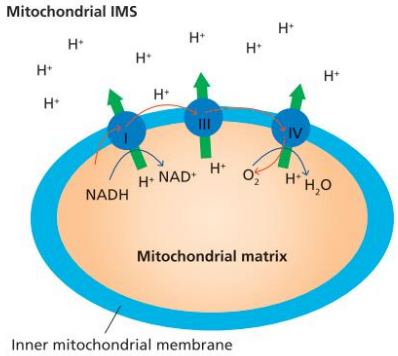


Figure 1.1

Light-dependent reactions of photosynthesis

The electron transport process is similar to the mitochondrial electron transport. Again, there are three large protein complexes in the membrane — photosystem 2 (PS2), cytochrome bf and photosystem 1 (PS1) (see Figure 1.2) and smaller carriers that ferry the electrons from one to the next (red arrows). As the electrons are transported from one carrier to the next, energy release is linked to transporting protons across the thylakoid membrane from the stroma into the lumen of the **thylakoid** (green arrows). However, unlike respiration, regular inputs of energy are needed during this process of electron transport. They are provided by light, which is harvested by photosynthetic pigments associated with PS2 and PS1. The final destination is oxidised NADP (NADP+), which becomes reduced NADP (NADPH). PS2 replaces the electrons it lost by taking electrons from water in a process called photolysis, resulting in the release of oxygen.

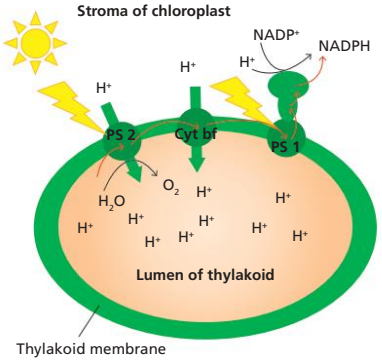


Figure 1.2

Further reading

'Toxic chemical DNP in weight loss products causes several deaths in Australia: NSW Health': **https://tinyurl.com/yyfnweee**

Uncoupling in plants

Most plants are less able than animals to move away if their environment becomes unfavourable. Most cannot move into the shade if there is too much sunlight. Plants have several mechanisms for dealing with too much light, including ways to uncouple the electron transport chain from the production of ATP.

Excess sunlight results in production of too much reduced NADP (see Box 1). This reduced NADP can be removed from the chloroplasts and converted into NADH, which is then transferred to the mitochondria. Plant mitochondria have mechanisms for releasing energy from NADH without creating ATP. They can bypass the electron transport chain by oxidising NADH without generating a proton gradient, simply releasing the energy as heat. They can also use uncoupling proteins, like those in mammalian mitochondria, to get rid of the proton gradient, releasing heat. Unlike animals, only a few plants, like the skunk cabbage, are adapted to use these mechanisms in physiological processes.

Pharmacological uncoupling

During the First World War it was noticed that some munitions workers were inexplicably losing weight. The cause was dinitrophenol (DNP) — a chemical used in the production of ammunition. Shortly thereafter, DNP was on the market as a weight loss drug. DNP can move protons through the inner mitochondrial membrane, hence it is an uncoupler. However, it quickly became apparent that far from being a wonder drug, its use can lead to liver damage and in many cases death, due to dangerous increases in body temperature. The drug was withdrawn from the market, but it is still sold illegally via the internet to people desperate to lose weight, often with tragic consequences.

Despite these poor results, it remains a hope that in the future we can make drugs that can uncouple electron transport and ATP production sufficiently to cause weight loss but not so much that it becomes life threatening. It is hoped that such drugs could help combat obesity.

In summary, mitochondria and chloroplasts function as power stations of the cells by using electron transport chains to generate a proton gradient across key membranes. This proton gradient is then coupled to ATP production, exploiting the energy released by allowing protons to cross the membranes. Disrupting this process through uncoupling can serve important physiological processes, such as generating heat or, in plants, helping to deal with excess sunlight. However, if this is not tightly regulated it can have disastrous consequences for the organism, as has been seen when drugs have been used to cause uncoupling.

Box 2 How can protons cross back over the membrane?

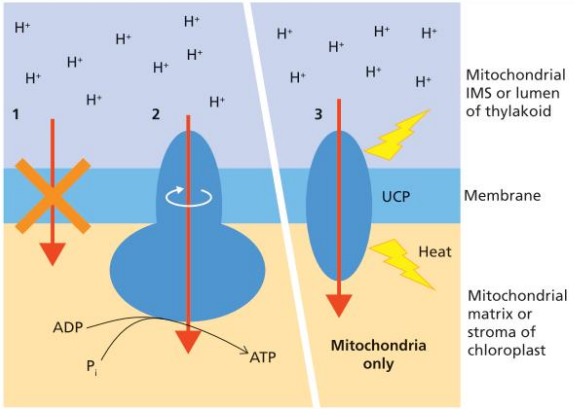


Figure 2.1

Electron transport, whether in the chloroplast or mitochondria, results in a high concentration of protons on one side of a membrane and a low concentration on the other side. Such a concentration gradient has potential energy, which can be released by letting the protons back to the side of the membrane where there are fewer protons. The protons, however, cannot pass freely through either the inner mitochondrial membrane or the thylakoid membrane (1) so must pass through the ATP synthase (2). The ATP synthase uses the energy released to spin part of its structure. The energy obtained from the spinning allows the production of ATP. In the mitochondria, uncoupling proteins (UCP) allow protons to go back into the matrix bypassing the ATP synthase under controlled conditions (3).

Dr Katrine Wallis has spent her research career as a biochemist studying the molecular mechanisms of protein disulfide isomerase. She is currently a senior teaching fellow in the School of Life Sciences at The University of Warwick.

Key points

- Mitochondria can uncouple oxidative phosphorylation in both plants and animals.
- Uncoupling of oxidative phosphorylation in mitochondria transfers energy into heat as an alternative to creating ATP.
- Ecological adaptations can be achieved by some animals and plants by using biochemical uncoupling.

Speciation and the Tube mosquito

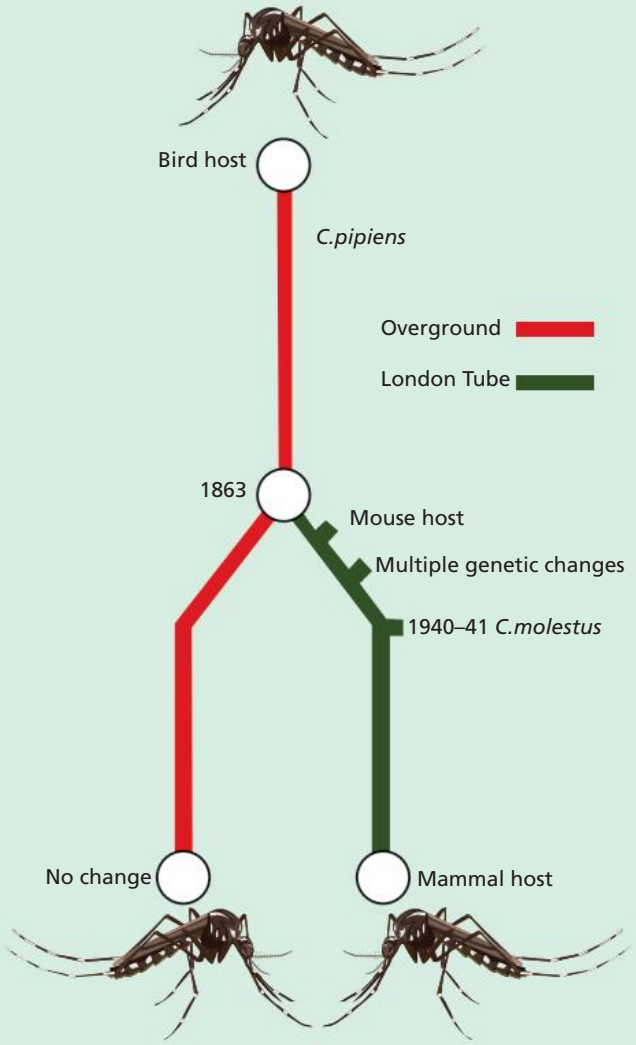
Charles Darwin suggested that speciation takes timescales of tens to hundreds of thousands of generations. This example appears to have occurred more quickly.

Culex pipiens is a mosquito. It mates seasonally, in large swarms in open spaces. The brood size is 150–350 eggs and their development requires a high-energy food source. Mated females obtain this energy from a blood meal, typically taken from birds. They then lay eggs in open-air pools of stagnant water. Gravid females (females carrying eggs) can hibernate over winter.

The London Underground (otherwise known as 'the Tube') opened in 1863 and it is thought that some *C. pipiens* were blown into the tunnels by trains, or ventured in looking for puddles of water in which to lay their eggs. Tube tunnels are largely sealed from the surface, and some of the mosquitoes became trapped underground. This physical barrier was responsible for the evolution of a new mosquito. There were no birds in these tunnels but there were mammals (for example, mice and people). A few *C. pipiens* females took blood meals from rodents, adapting to mammalian hosts. During 1940 and 1941, the tunnels were used as overnight bomb shelters throughout the Blitz of the Second World War, and Londoners complained bitterly about the misery inflicted by the bites of these mosquitoes, which were re-named *C. molestus*.

In the late 1990s, Katherine Byrne and Richard Nicholls, from Queen Mary University of London, sampled mosquitoes from seven underground sites in the London Tube network and compared these mosquitoes with overground mosquitoes. They showed that the subterranean mosquitoes had become reproductively isolated from those above ground, and had multiple adaptations to life underground — the females take blood meals from mammals, lay eggs without a prior blood meal and in enclosed spaces, breed continuously rather than seasonally, they do not swarm and remain active during the winter. *C. pipiens* and *C. molestus* are now so distinct they no longer interbreed, even in artificial laboratory conditions — in other words, we have a historical record of speciation that has occurred within approximately 150 years of the construction of the London Tube system. It seems to have taken only a few hundred generations.

Mosquito larvae under the water surface



Dr Robert Spooner, School of Life Sciences, The University of Warwick

Tetrachromat chat

The male bluethroat (*Luscinia svecica*) is one of the UK's most beautiful migrants. These birds are called chats, and they visit the UK in spring and autumn. They fly from their winter homes in Africa and India, up to the Arctic Circle. Bluethroats are 'Old World flycatchers'. They are Passerines, perching and songbirds, in the family Muscicapidae, which includes robins, flycatchers and other chats.

You are lucky if you see a bluethroat in the UK. Data recorded through the 1960s recorded only around 600 birds. They are seen mainly around the southern and eastern coasts of the UK, from Cornwall anticlockwise to the north of Scotland. Only one breeding pair in the UK has been reported, in Scotland in 1968. They construct nests of moss, twigs and grass, lining them with material such as soft animal hair. They can produce two broods each year, laying up to seven eggs. They typically prefer thick vegetation, flitting quickly in and out of bushes. During the mating season the males find open perches, fan their tails and sing. They can mimic more than 50 other bird calls.

But all is not what we see. In 1998, the University of Oslo's museum zoologists reported that, as shown for zebra finches, in bluethroats, ultraviolet signalling influenced female mate selection. Such species of birds have tetrachromatic vision. We humans have three cone-cell types in our retinas, containing different opsins that detect red, green and blue wavelengths of light. Tetrachromats have a fourth cone type, sensitive to light wavelengths between 300 and 400 nm. These ultraviolet wavelengths below 400 nm are not detectable by our eyes.

Studies of zebra finch behaviour and the striking blue breast plate of the bluethroat had led Norwegian scientists to perform cage studies, before doing field experiments. They coated the throat plumage of males with an ultraviolet-absorbing fat smear and compared the attractiveness of these males with that of control males. Females associated less with the 'ultraviolet-reduced' males. Controls were crucial. The researchers physically ensured an even brightness, precisely measuring the ultraviolet-reflectivity of the birds with spectrophotometry. This was first performed on museum specimens to ensure the smears would work. The images here show a classic 'skin mount', illuminated with both natural and ultraviolet light. The skin of the animal was removed and chemically preserved before being placed on a support structure and sculpted into as close to a natural posture as possible.

For research, multiple 'study skins' can be used. These skins are stuffed with cotton and stored unposed, and can yield DNA for phylogenetic studies, obviating the need to disturb or capture live animals. This shows the continued relevance of taxidermy collections. When prepared well in curated archives, they are not just an amazing record of the biodiversity of our world but have modern research relevance.

Professor Kevin Moffat and **Paolo Juan**, University of Warwick

Further reading

Duncan, O. (2018), 'Not just a pretty face: why museums need study skins': **https://tinyurl.com/yyr4efv9**

'Molecular and phenotypic divergence in the bluethroat (*Luscinia svecica*) subspecies complex': **https://tinyurl.com/yxmshl94**

ISBN 978-1-5104-7032-3

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