and FWE3 are loser cells and those that express FWE2 and FWE4 are winner cells. Loser cells undergo a type of cell death called apoptosis, and the initiation of cell death requires direct contact between winner and loser cells.

The authors examined the expression of winner and loser versions of FWE in samples of human cancers. FWE^{Win} expression was higher in malignant tumours than in benign tumours. Madan and colleagues found that expression of FWE^{Lose} in normal cells adjacent to the tumour is higher than in normal cells farther away from it. Moreover, the level of FWE^{Lose} was higher in normal tissues adjacent to malignant tumours than in normal tissues that surrounded a benign tumour.

When the authors transplanted human breast cancer cells that express FWE^{Win} into mice, the mouse cells adjacent to the transplanted tumour cells increased their expression of mouse FWE^{Lose} compared with the levels in animals that had not received a tumour transplant. All these results suggest that FWE^{Win} expression in tumour cells induces FWE^{Lose} expression in neighbouring normal cells (Fig. 1). The mechanism responsible for such induction is unknown.

The authors report that, when human breast cancer cells expressing FWE^{Win} were transplanted into the breast region of mice engineered to express human FWE^{Lose}, the transplanted cells generated aggressive tumours. By contrast, less aggressive tumours were generated if FWE^{Lose}-expressing human breast cancer cells were transplanted into mouse breast tissue that expressed human FWE^{Win}. This indicates that it is the combination of high expression of FWE^{Win} in tumours and high expression of FWE^{Lose} in the tissue that surrounds them that aids cancer growth.

When the authors engineered human cancer cells to block expression of FWE and transplanted these cells into mouse legs, the cancer cells showed diminished growth and reduced capacity for migration (termed metastasis) to a secondary site compared with transplants of human cancer cells in which FWE expression was not blocked. When chemotherapy was also administered, growth of the engineered human cancer cells in the mouse legs was substantially inhibited.

Madan and colleagues suggest that FWE should be investigated as a possible therapeutic target in human tumours and in the tissues that surround them. However, whether human FWE can be selectively targeted using antibodies or chemical compounds should be examined before a clinical approach can be considered.

The authors have demonstrated convincingly that, in addition to its known role in *D. melanogaster*, FWE also functions in cell competition in mammals. In both mammals and flies, the expression of FWE^{Lose} is induced in loser cells; cells expressing FWE^{Lose} die only if they encounter cells that express FWE^{Win}; and it is the relative rather than the absolute levels of FWE^{Lose} and FWE^{Win} that trigger cell competition.

Several issues remain to be addressed. For example, the regulatory proteins that act upstream or downstream of FWE have not been identified. What controls the alternative splicing that generates different forms of FWE is unknown, and understanding this process might reveal other therapeutic targets. Previous work[4] suggests that membrane proteins of unknown identity can distinguish between winner and loser versions of FWE expressed on neighbouring cells. If such proteins exist, their identification will be necessary to understand how FWE-mediated cell competition functions.

Another key question is whether cancer-promoting mutations trigger FWE-mediated cell competition in mammals, and, if so, which mutations are responsible. There are reports that abnormal expression of the tumour-promoting proteins Myc or Wnt is involved in FWE-related cell competition in *D. melanogaster*[4–6]. Analyses of tumour cells from patients might shed light on whether this also occurs in humans.

Madan and colleagues' work should motivate researchers to analyse human-tumour samples to determine the involvement of FWE in cell competition and cancer development. If antibodies could be developed to specifically recognize human FWE^{Lose} proteins, this would greatly aid such studies. However, generating such antibodies is not straightforward, and the authors discuss the technical hurdles that would need to be overcome.

In *D. melanogaster*, other proteins in addition to FWE can regulate cell competition[7,8], and further studies in human cancer cells will be needed to gain a more complete picture of mammalian cell competition. Such work might offer new perspectives for improving cancer treatments. ■

Yasuyuki Fujita *is at the Institute for Genetic Medicine, Hokkaido University, Hokkaido 060-0815, Japan.*
e-mail: yasu@igm.hokudai.ac.jp

1 Morata, G. & Ripoll, P. *Dev. Biol.* **42**, 211–221 (1975).
2 Maruyama, T. & Fujita, Y. *Curr. Opin. Cell Biol.* **48**, 106–112 (2017).
3. Madan, E. *et al. Nature* **572**, 260–264 (2019).
4. Rhiner, C. *et al. Dev. Cell* **18**, 985–998 (2010).
5. Merino, M. M. *et al. Cell* **160**, 461–476 (2015).
6. Levayer, R., Hauert, B. & Moreno, E. *Nature* **524**, 476–480 (2015).
7. Yamamoto, M., Ohsawa, S., Kunimasa, K. & Igaki, T. *Nature* **542**, 246–250 (2017).
8. Meyer, S. N. *et al. Science* **346**, 1258236 (2014).

This article was published online on 24 July 2019.

GENOMICS

Evolution of flight loss caught in the act

The ability to fly has been lost in many groups of birds. A comparison of the wing structures and genomes of flighted and non-flighted species of steamer duck highlights a possible mechanism for the loss of flight.

JULIA A. CLARKE

We can gain insights into evolution by studying the sequence in which new features are acquired. But studying loss of features has its benefits, too. When a certain trait is lost multiple times in distinct groups of organisms, powerful statistical approaches can identify its genomic underpinnings. A study by Campagna *et al.* in *Evolution*[1] sheds light on the genetic changes associated with a loss of flight in birds. They compare the whole genomes of 59 individual steamer ducks (of the genus *Tachyeres*) to examine loss of flight as it is evolving.

Steamer ducks occupy coastal habitats and lakes in southern Chile, southern Argentina and the Falkland Islands[2]. They show a distinctive escape behaviour called steaming — rapid, synchronized paddling of their wings and feet across water that mimics the action of their namesake, paddle-steaming boats (Fig. 1). Of the four recognized species, three (*T. brachypterus*, *T. pteneres* and *T. leucocephalus*) are characterized by their inability to fly[2]. Some heavier, male ducks of the usually flighted species, *T. patachonicus*, are also unable to fly, because their wing loading (the ratio of body weight to wing surface area) is higher than that of their lighter counterparts.

All steamer ducks also walk proficiently on land, and dive to feed and to escape predators. Unlike puffins and penguins, which use wing movements in foraging and feeding, they do not steam to acquire food. However, they do use their wings when diving underwater, and the flight muscles in flightless species are only slightly proportionally smaller relative to body mass than in steamer ducks that can fly[2].

It has been debated whether the flightless species of steamer duck each independently

BILL COSTER/FLPA

Figure 1 | Steaming behaviour in a steamer duck. Flighted and flightless steamer ducks in the genus *Tachyeres* show a distinctive escape behaviour called steaming, in which they paddle their feet and short wings rapidly across the water. Campagna *et al.*[1] sequenced the genomes of individual steamer ducks from each of the *Tachyeres* species (including the flightless *Tachyeres brachypterus* pictured here), and analysed them together with the birds' wing measurements to propose changes in gene expression that might underpin the evolution of flightlessness.

lost the ability to fly or are all descended from a single flightless branch of ducks[2]. Resolving this debate would provide insight into some of the environmental or ecological factors that might promote flight loss.

Steamer ducks are an evolutionarily young group — estimated to be only about 2 million years old. Through their genome comparison, Campagna *et al.* show that the evolution of flightlessness in the two continental species, *T. pteneres* and *T. leucocephalus,* occurred early in the clade's history and within a relatively short time frame. By contrast, *T. patachonicus* and the coastal *T. brachypterus* are more closely related (they diverged only recently), and indeed might still interbreed. Overall, the authors' genome comparison suggests that flightlessness might have evolved independently on as many as three occasions, although there are alternative interpretations.

Campagna *et al.* also identified the parts of the genome that contain the highest number of differences in DNA sequence between flighted and flightless individuals, by mining the genomes for single nucleotide polymorphisms (SNPs): substitutions of single nucleotides at specific points in the DNA sequence. The authors correlated measurements of wing bones and bone proportions in the sequenced birds with the genome data, so that they could distinguish wing-shape-related genetic differences between individuals from those that were not relevant to wing shape or that had occurred by chance. Notably, some *T. patachonicus* and *T. brachypterus* ducks exhibited a mixture of both flight- and flightlessness-related versions of the genetic sequences linked to wing length. Thus, the evolution of flight loss seems to be caught in the act in steamer ducks.

Most of the SNPs that Campagna *et al.* found to be associated with differences in limb measurements occurred in or near a gene called *DYRK1A*. Thus, the authors suggest that changes in *DYRK1A* expression and function might contribute to the reduction in limb length relative to body weight that is observed in flightless individuals. They also note that mice that carry more copies of *DYRK1A* than normal show limb-skeleton differences[3]. Moreover, increases in the number of copies of *DYRK1A* have been implicated in certain symptoms of Down syndrome in humans, including differences in body size and the length of long bones, particularly those in the forelimbs[4]. Although Campagna *et al.* were unable to examine the number of copies of *DYRK1A* in *Tachyeres,* future work could examine the effects of observed genetic differences in bird development experimentally.

Flightless species are highly diverse, and flight loss has evolved in very different contexts. It has occurred after the acquisition or elaboration of an aquatic mode of locomotion, such as diving or steaming, and in largely terrestrial contexts in which there are few predators. As an example of the latter scenario, rails, which are relatives of cranes, have lost the ability to fly on nearly every oceanic island on which they have landed (and sometimes repeatedly on the same island[5]).

Regardless of the different contexts that might promote the loss of flight, in all cases of flight loss a reduction in the length of the wings relative to the rest of the body results in the wing loading becoming too high to allow flight. However, other changes in the wing musculature, skin and feathers, as well as the sensory systems and the rest of the skeleton, vary considerably among different flightless species, and it not always clear whether these changes are related to loss of flight or to other factors. For example, it is worth noting that the genetic and wing-shape changes associated with flight loss in steamer ducks are proposed to have occurred at the same time that these birds acquired steaming behaviour. Wings are typically relatively short in birds that use them to move through water. Thus, whether the genetic changes that affect wing shape are associated with the acquisition of steaming, or with the loss of flight, is difficult to determine.

The past few years have seen other substantial developments in research into the genetics of flight loss[6,7]. One study[6] identified differences between the genomes of three flying species of cormorant and their flightless relative, *Phalacrocorax harrisi*. Many of these variations were in or around genes involved in the function of cell protrusions called cilia, which mediate cell signals required for skeletal development. However, the flight muscles and associated parts of the sternal bones of *P. harrisi* are much smaller than those of its flighted relatives (differences not observed between the flightless and flighted steamer ducks[2]).

Another study[7] investigated a different basis for flight loss in ratites — a group of birds that includes the cassowary, ostrich and kiwi, and in which flight was lost multiple times in the deep past. Differences between flighted and flightless species were identified in regions of DNA that regulate the expression of genes involved in laying down the structure of the forelimb (but were distinct from the changes seen in the steamer ducks). Changes in the expression of several of these genes during

development result in short forelimbs[7].

The diverse mechanisms underlying flightlessness that have been identified in these genomic studies are not necessarily incompatible with each other. Indeed, an emerging perspective is that the genetic mechanisms that lead to changes in wing shape and length might be as diverse as the ecological contexts in which flight loss has occurred. Perhaps this is not surprising. Studies of digit reduction in mammals have shown similarly diverse mechanisms[8,9], and different genetic mechanisms underlie adaptations to high altitude in closely related hummingbird species[10]. More work with museum collections[11], and in developmental biology and anatomy, is needed to advance our understanding of the genetic changes that underpin traits such as flightlessness. ■

Julia A. Clarke *is at the Jackson School of Geosciences, The University of Texas at Austin, Austin, Texas 78712, USA.*
e-mail: julia_clarke@jsg.utexas.edu

1. Campagna, L., McCracken, K. G. & Lovette, I. J. *Evolution* https://doi.org/10.1111/evo.13758 (2019).
2. Livezey, B. C. & Humphrey, P. S. *Evolution* **40**, 540–558 (1986).
3. Blazek, J. D., Abeysekera, I., Li, J. & Roper, R. J. *Hum. Mol. Genet.* **24**, 5687–5696 (2015).
4. Bernstein, S. N., Saller, D. N., Catov, J. M. & Canavan, T. P. *Int. J. Gynecol. Obstet.* **133**, 287–290 (2016).
5. Hume, J. P. & Martill, D. *Zool. J. Linn. Soc.* **186**, 666–672 (2019).
6. Burga, A. *et al. Science* **356**, eaal3345 (2017).
7. Sackton, T. B. *et al. Science* **364**, 74–78 (2019).
8. Cooper, K. L. *et al. Nature* **511**, 41–45 (2014).
9. Lopez-Rios, J. *et al. Nature* **511**, 46–51 (2014).
10. Lim, M. C. W., Witt, C. C., Graham, C. H. & Dávalos, L. M. *Genome Biol. Evol.* **11**, 1573–1585 (2019).
11. Lamichhaney, S. *et al. Phil. Trans. R. Soc. B* **374**, 20180248 (2019).

This article was published online on 31 July 2019.

METABOLISM

A stress-coping strategy for yeast cells

Stressed yeast cells take up the amino acid lysine and reprogram their metabolism to free up supplies of a stress-relieving molecule. Lysine uptake therefore increases the tolerance of yeast cells to stress. SEE LETTER P.249

JENS NIELSEN

Metabolism is crucial for all living cells: it provides energy as well as the molecular building blocks required for growth. Some metabolic pathways protect cells against different types of stress, including the oxidative stress caused by other metabolic processes in the cell or by external factors. On page 249, Olin-Sandoval *et al.*[1] describe how yeast cells (*Saccharomyces cerevisiae*) can reprogram their metabolism so that they are better equipped to handle the oxidative stress that is caused by the accumulation of chemically reactive molecules known as reactive oxygen species (ROS).

Understanding how the many different metabolic pathways in a cell interact and ensure its proper functioning under varying environmental conditions is necessary for designing cell 'factories' — genetically engineered cells that can be cultured to produce fuels, chemicals, foods or pharmaceuticals. It is also important for gaining insight into the molecular mechanisms that underlie various human diseases, because metabolic changes are associated not only with disorders such as diabetes and cardiovascular disease that have conventionally been considered to be metabolic disorders, but also with conditions such as cancer and Alzheimer's disease[2].

Cell factories and human cells undergoing pronounced metabolic changes (such as cancer cells) experience different types of stress, including oxidative stress, which can be caused by the accumulation of ROS. These molecules disrupt many cellular processes: for example, they cause DNA damage and problems with protein folding. Cells have therefore evolved various defence mechanisms to cope with ROS accumulation.

The dominant pathway that cells use to combat accumulated ROS is to chemically reduce them with the thiol group (SH) of the antioxidant peptide glutathione; this reaction results in the formation of a sulfur bridge between two glutathione molecules (Fig. 1). To replenish the levels of glutathione, certain enzymes break the sulfur bridge apart, using the cofactor molecule NADPH as an electron acceptor to promote the reaction. Thus, when cells experience oxidative stress and must deplete accumulated ROS, they have a higher demand for NADPH than do non-stressed cells. However, NADPH is sometimes required for rapid cell growth; therefore, in growing cells, there might be less NADPH available for handling accumulated ROS than in non-growing cells. Olin-Sandoval *et al.*[1] demonstrate that, in the presence of the amino acid lysine, yeast cells can reprogram their metabolism such that they can allocate more NADPH for dealing with accumulated ROS.

The authors found this mechanism while studying a previously reported, yet largely unexplained, phenomenon: that yeast cells lacking Tpo1, an exporter protein that removes a group of chemicals called polyamines from the cell, are more sensitive to oxidative stress than wild-type cells[3]. Olin-Sandoval *et al.* used protein-expression analyses to demonstrate that, compared with wild-type yeast cells, yeast

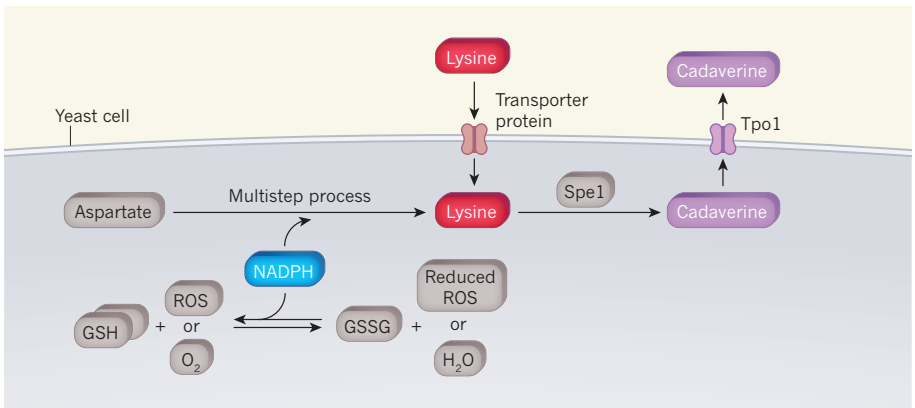

Figure 1 | Yeast cells reprogram their metabolism to reduce stress. In yeast cells, reactive oxygen species (ROS) and molecular oxygen (O_2) are chemically reduced by reaction with pairs of glutathione (GSH) peptides, which become linked by a sulfur bridge to form glutathione disulfide (GSSG). The enzyme-cofactor molecule NADPH is needed to replenish the levels of glutathione in the cell, and also for the multistep production of the amino acid lysine from another amino acid, aspartate. Olin-Sandoval *et al.*[1] found that yeast cells can harvest large amounts of lysine from outside the cell. The enzyme Spe1 converts lysine to the polyamine cadaverine, which is removed from the cell by the exporter protein Tpo1. Lysine harvesting results in an inhibition of lysine production (not shown), probably through a feedback mechanism. Thus, lysine harvesting reduces the use of NADPH for lysine synthesis, freeing it up for its role in handling accumulated ROS.