and take over the discipline. If we want to make sure that the biology of the future preserves our hard-won biological perspectives, knowledge and insights, we need to be able to do the analyses and deal with all these data ourselves.
For young scientists embarking on a PhD, make sure your PhD topic is something you love, and that your question is one whose answer you care deeply about. Don't settle for less than this, or you'll lack the drive needed to work to your own full potential. And if there's some body of knowledge or theory that is important to your question, whatever the discipline, just roll up your sleeves and learn it. For post-docs, one word: 'publish!' And don't spend months trying to get a paper perfect in every detail before submitting: your reviewers will find flaws no matter what. Spend your time getting the experiments and analysis right, not perfecting the writing.

Your website says you play music and paint: does science influence your creative work? While in college I seriously considered a career in the arts, and many of my closest friends and band-mates went on to become professional musicians. I've played in rock and salsa bands and an African drumming ensemble, and I still play a lot of guitar and write and record songs. And recently, we've been studying the biology and evolution of music, and I've really been enjoying the opportunity to combine music with scientific research.
Regarding the influence of science, I draw the figures for a lot of my publications (ink drawings or watercolors, reworked with Adobe Illustrator). I've also written some biological songs, including "*I Don't Believe in Evolution*", which pokes fun at creationists and has been live-broadcast on Italian radio (and even served as a ring tone on some of my students' phones!).
But frankly, I'm happy to have science as my 'day job' and music and painting as hobbies: I think the pressure to make money with art would take the fun out of it.

So you're glad you became a scientist? Absolutely. I feel incredibly fortunate to be a scientist. Sure, scientists' salaries are not usually commensurate to their education and ability. But how many people are lucky enough to be paid to follow their interests and satisfy their own curiosity every day?

What are the most exciting topics you are researching right now? At the moment I'm very excited about our new research program in empirical aesthetics, trying to understand the biological roots of the visual arts, and in particular of the human love for symmetry and order. Humans around the planet surround themselves with decorative patterns, with no obvious function, such as weaving, quilting, decorated pottery, clothes, tattoos and architectural ornament. Oddly, art historians have largely focused on representational art by great geniuses, and neglected this much more widespread, popular and presumably ancient form of art (often relegated to 'craft'). We've been bringing ordinary people into the lab and studying the kinds of patterns they make using computer interfaces (as well as what they like, and what kind of rules they can perceive). It looks like there is a deep biological drive in our species — what the art historian Ernst Gombrich called our 'sense of order' — that hasn't received enough attention.
I'm also very excited about our work in bioacoustics, trying to understand how animals produce their sounds. This is a truly inter-disciplinary bridging area, spanning an amazing breadth of disciplines from physics to physiology to behavior, cognition, and evolution. It also relies on comparative anatomy, so you get to dig out old anatomical papers documenting weird and wonderful adaptations for sound production that were forgotten long ago, and then try to understand them from the viewpoint of modern acoustics and nonlinear dynamics. We're studying vocal production in alligators, deer, primates, ravens, parrots, and lots of other species, but it is amazing how the same physical phenomena and principles (mostly originally discovered in human speech) seem to underlie all this diversity. It's a comparative biologist's dream come true.

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Quick guide

Aquaporins

A.S. Verkman

What are aquaporins? Aquaporins (often called aquaporin water channels) are a family of small, integral membrane proteins that are expressed broadly throughout the animal and plant kingdoms. They have a similar basic structure, with aquaporin monomers consisting of six transmembrane helical segments and two short helical segments that surround cytoplasmic and extracellular vestibules connected by a narrow aqueous pore (Figure 1A). They contain several conserved motifs, including NPA sequences in their short helical segments. Aquaporin monomers assemble as tetramers in membranes, with each monomer functioning independently. Some aquaporins, such as mammalian AQP4, can further aggregate in cell membranes to form supramolecular crystalline assemblies called orthogonal arrays of particles.

What do aquaporins do at the molecular level? The primary function of most aquaporins is to transport water across cell membranes in response to osmotic gradients created by active solute transport. Because the water transport capacity of aquaporin monomers is low, membranes often contain a high density of aquaporins, up to 10,000 per square micron, to increase water permeability substantially above that in the absence of aquaporins. Molecular dynamics simulations suggest that steric factors and electrostatic interactions in the aqueous pore are responsible for the selectivity of aquaporins for water. A subset of aquaporins, called aquaglyceroporins also transport glycerol. The pore diameter of the aquaglyceroporins is slightly greater than that of the water-selective aquaporins, and the pore is lined by relatively hydrophobic residues compared with the pore of a water-selective aquaporin. In addition to water and glycerol, there is evidence, some of which is controversial, that some aquaporins pass gases (CO_2, NH_3, NO, O_2), various small solutes such as H_2O_2 and arsenite, and

even ions (K+, Cl-). Non-transporting functions for some aquaporins have also been suggested, such as cell–cell adhesion, membrane polarization and regulation of interacting proteins, such as ion channels.

Why do some membranes need high water or glycerol permeability? Most cells do not express aquaporins. Virtually all biological membranes are reasonably water permeable as a consequence of water diffusion though membrane lipids, such that cell volume equilibrates in minutes or less in response to an osmotic gradient. So then why should high membrane water permeability be needed in some cells? One example is fluid secretion and absorption across epithelial cell layers, such as in kidney tubules and exocrine glands, where normal kidney function and secretion of bodily fluids, such as saliva, require high, aquaporin-facilitated transepithelial water permeability. High water permeability also facilitates water movement across fluid–tissue barriers, such as between blood and brain parenchyma across the blood–brain barrier. Other cellular and tissue functions of aquaporin water transport, and of aquaglyceroporin-facilitated glycerol transport, are not obvious *a priori*.

Where are aquaporins expressed? The mammalian aquaporins, which number about a dozen, are expressed in many cell types involved in fluid transport, including epithelia and endothelia in kidney, lung, exocrine glands, eye and gastrointestinal organs. However, aquaporins are also expressed in cells that do not have an obvious role in fluid transport, such as erythrocytes and some leukocytes, adipocytes and skeletal muscle. Aquaporins are also expressed in astrocytes throughout the central nervous system, and in supportive cells (but not electrically excitable cells) in sensory organs such as retinal Müller cells. In the eye, aquaporins are expressed in cornea, lens and ciliary epithelium. The tissue distribution of mammalian aquaporins provided initial clues about their cell and organ functions, though many of the initial guesses have not been confirmed in phenotype studies on aquaporin knockout mice.

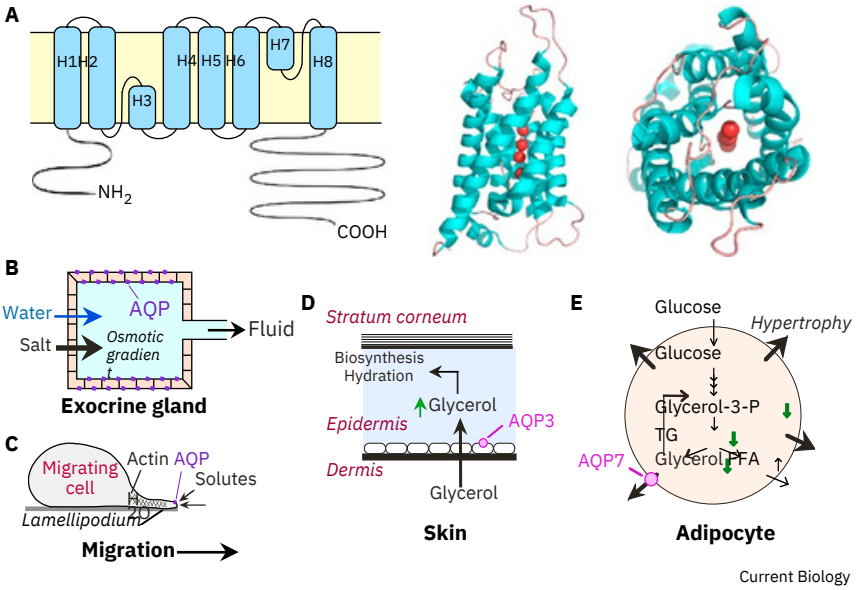

Figure 1. Structure and cellular functions of aquaporins.
(A) Membrane topography of the aquaporin monomer (left) and crystal structure (AQP1, PDB 1j4n) (side view, left; top view, right) with four water molcules (red balls) shown in aqueous pore region. Helices are labeled H1–H8. (B) Epithelial fluid secretion. High transepithelial water per- meability facilitates active, near-isosmolar fluid secretion in exocrine glands. (C) Cell migration. AQP-facilitated cell migration involves water entry into protruding lamellipodia in migrating cells. (D) Skin hydration. AQP3 maintains high levels of glycerol in the stratum corneum; glyc- erol acts as a humectant to retain water. (E) Adipocyte metabolism. AQP7 facilitates glycerol exit from adipocytes, preventing intracellular glycerol and triglyceride accumulation. AQP, aq- uaporin; TG, triglyceride; FFA, free fatty acid. (Panels B–E adapted from Verkman, A.S. (2011). Aquaporins at a glance. J. Cell Sci. *124*, 2107–2112.)

What about aquaporins in plants and lower organisms? Aquaporins are expressed in various plants and microbes, including bacteria and yeast. A large number of plant aquaporins have been identified, called PIPs (plasma membrane intrinsic proteins) and TIPs (tonoplast intrinsic proteins) for their cellular expression patterns. Deletion studies have elucidated a variety of interesting roles for aquaporins in plants, including transpiration, metabolism and reproduction, which are important in plant adaptation to various environmental stresses. Though aquaporins are also expressed in some microbes, deletion studies have not revealed any clear phenotype, though there is suggestive evidence that aquaporins may be protective in freeze–thaw stress, perhaps to maintain the water permeability of cell membranes at low temperature. The high surface-to-volume ratio of microbes predicts rapid osmotic equilibration, making aquaporins unnecessary for most functions, such as osmoregulation.

What anticipated roles of aquaporins have been confirmed from knockout mice? Phenotypic analysis of aquaporin knockout mice has confirmed the anticipated role of aquaporins in transepithelial fluid transport (Figure 1B). Deletion of various aquaporins in kidney tubules causes excessive urine output and impaired urinary concentrating ability. High, aquaporin-dependent water permeability is needed in the kidney proximal tubule, for near-isosmolar fluid absorption, in the thin descending limb of Henlé, for countercurrent multiplication, and in the collecting duct, for water absorption. Aquaporins also facilitate epithelial fluid secretion in salivary and airway submucosal glands, and in the choroid plexus (from which cerebrospinal fluid is secreted) and ciliary epithelium (from which ocular aqueous fluid is secreted). There is an important caveat, however: aquaporins are required for transepithelial fluid transport when fluid transport rate (per epithelial surface area) is very high, as in proximal tubule and salivary gland. Though aquaporins are expressed and

functional in lung, intestinal and sweat gland epithelia, they do not appear to have a significant physiological role.

What are some less obvious roles of aquaporin-facilitated water transport? An unanticipated role of aquaporins is in cell migration. AQP1 deletion in mice impairs angiogenesis as a consequence of a reduction in the migration speed of microvascular endothelial cells. Aquaporin-dependent migration has been found in a wide variety of cell types. Biophysical studies suggest that aquaporin polarization to the leading edge of migrating cells facilitates water influx during lamellipodial extension (Figure 1C). In contrast to transepithelial fluid transport, where aquaporins facilitate water movement across cell layers, aquaporins in migrating cells facilitate local, transient water transport. Another unanticipated role of an aquaporin is in neuroexcitation: deletion of AQP4 impairs neurosensory signaling and alters seizure and cortical spreading depression dynamics. Although the mechanism has not been fully resolved, it appears that AQP4-facilitated water transport is needed for rapid changes in extracellular space volume and K+ concentration, and that loss of AQP4 leads to delayed K+ reuptake following neuroexcitation. Other unexpected roles of water-selective aquaporins include their involvement in corneal and lens transparency, brain and spinal cord swelling, neuroinflammation and pain.

What about glycerol transport by aquaglyceroporins? The mammalian aquaglyceroporins include AQP3, AQP7 and AQP9. AQP3 is expressed in the basal layer of proliferating keratinocytes in the epidermis (Figure 1D). Mice lacking AQP3 have reduced skin hydration and elasticity, which is due to impaired glycerol transport from the blood to the epidermis and consequent reduced glycerol content in the epidermis and stratum corneum. Reduced glycerol impairs skin hydration and elasticity, as glycerol is a major humectant (water-retaining osmolyte). Reduced glycerol also impairs epidermal proliferation, as glycerol is an important epidermal cell metabolite involved in ATP generation and membrane lipid biosynthesis. There has been considerable interest in AQP3 in the cosmetic industry, with marketed products claiming to increase AQP3 expression. Recent studies have suggested an important role of AQP3 in macrophage and T-lymphocyte function, perhaps involving AQP3-facilitated H2O2 transport. Another intriguing observation, made in mice lacking AQP7, is progressive obesity with adipocyte hypertrophy, which appears to be a consequence of impaired glycerol efflux from adipocytes. Reduced plasma membrane glycerol permeability resulting from AQP7 deficiency leads to intracellular accumulation of glycerol and triglycerides (Figure 1E), suggesting the possibility of AQP7 upregulation to reduce fat mass in obesity. Finally, AQP9 expression in hepatocytes has been proposed to facilitate hepatic glycerol uptake, though the significance of hepatic glycerol uptake in human metabolism is unclear.

What about aquaporins in cancer? Provocative data implicate aquaporins in some cancers. A large descriptive literature reports strong expression of aquaporins in brain, skin, gastrointestinal, lung and other cancers, often with correlations between aquaporin expression and tumor grade. Tumors often overexpress aquaporin(s) found in the cell type of origin, such as AQP4 in astrocyte-derived glioblastomas, although sometimes tumor cells express aquaporins that are not seen in the cells of origin. Several mechanisms have been proposed that link aquaporin expression with cancer. AQP1 is strongly expressed in tumor microvessels, and its deletion in mice reduces tumor angiogenesis. Expression of various aquaporins in tumor cells increases their migration and metastatic potential in cell culture and animal models. AQP3 increases the proliferation of various tumor cells, and mice lacking AQP3 are highly resistant to skin cancer. Though many questions remain to be addressed, aquaporin inhibition for cancer therapy is an intriguing idea.

Are any human diseases caused by aquaporin abnormalities? One rare hereditary disease is caused by loss-of-function mutations in AQP2, a water channel expressed in the kidney collecting duct. AQP2 facilitates transepithelial water absorption in the kidney collecting duct in response to antidiuretic hormone, and this process involves the exocytic insertion of AQP2-containing vesicles into the apical membrane of the cell. Mutations in AQP2 that interfere with its cellular trafficking or water transport function result in the disease nephrogenic diabetes insipidus, in which large volumes of dilute urine, sometimes exceeding 30 liters per day, are excreted. Mutations in AQP0 (originally called ‘major intrinsic protein of lens fiber’) are associated with congenital cataracts; however, the mechanism of cataractogenesis is not known as it is unclear whether the main function of AQP0 is in lens structure or the water permeability of lens fiber cells. Loss-of-function mutations in several other aquaporins have been identified in a handful of individuals, although none appears to be associated with significant disease. An interesting autoimmune neurological disease, neuromyelitis optica, is caused by autoantibodies directed against extracellular epitopes on AQP4. Autoantibody binding causes complement- and cell-mediated astrocyte cytotoxicity, which leads to inflammation, with cytokine release, leukocyte infiltration and blood–brain barrier disruption. The inflammatory demyelinating lesions in the spinal cord and optic nerve lead to paralysis and blindness. So far other aquaporin-based autoimmune diseases have not been discovered.

What about aquaporin-based therapy for human diseases? There is considerable interest though little progress in aquaporin-based therapeutics. Data from cell and animal models provide a compelling rationale for the development of aquaporin modulators as therapeutics: AQP1 inhibitors are predicted to have utility in diuretic-refractory edema, tumor angiogenesis and glaucoma; AQP4 inhibitors in some forms of brain edema and injury; and AQP3 inhibitors in some cancers. Upregulators of the expression of some aquaporins have potential therapeutic utility for obesity, wound healing and spinal cord injury. Certain heavy metal (mercury, gold) containing compounds that react non-specifically with protein sulfhydryls inhibit the water permeability of some aquaporins. However, the identification of non-toxic, small-molecule aquaporin

inhibitors has not been possible, perhaps for a combination of reasons, including challenges in function-based screening assays and the intrinsic refractoriness of aquaporins to drug discovery (termed non-druggability). One area of recent progress is in AQP4-based therapeutics for neuromyelitis optica. The idea is to block the binding of pathogenic autoantibodies to astrocyte AQP4, which may provide a targeted, non-immunosuppressive approach to block the disease-initiating event in neuromyelitis optica. One approach has been the engineering of a non-pathogenic, tight-binding anti-AQP4 antibody ('aquaporumab') that competes with pathogenic autoantibodies; another approach has been the identification, by high-throughput screening, of small-molecule blockers of the autoantibody–AQP4 interaction.

What's next? There remain significant gaps in our knowledge about the cellular mechanisms of certain aquaporin functions, such as aquaporin-facilitated cell migration, cell proliferation, and neuroexcitatory phenomena. Though much of the low-hanging fruit in the aquaporin field has been picked and digested, as in areas of aquaporin protein structure and phenotypic analysis of knockout mice, new discoveries and ideas continue to emerge. Compelling recent data warrant further investigation of aquaporins in cancer, immune cell function and obesity. Lastly, notwithstanding the challenges and limited progress in the identification of aquaporin inhibitors, broad opportunities remain in aquaporin-based therapeutics.

Where can I find out more?

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In vivo male fertility is affected by naturally occurring mitochondrial haplotypes

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We harness an experimental design that enables us to completely disentangle mitochondrial from nuclear genetic effects in the fruit fly ***Drosophila melanogaster***. Using this design, we directly link male fertility outcomes to the mitochondrial haplotype. Specifically, we show that competitive male fertility, measured ***in vivo***, differs across naturally occurring mitochondrial haplotypes. We discuss this result in the context of recent studies that support the evolutionary hypothesis according to which maternal inheritance of mitochondria will facilitate the accumulation of male-harming mutations in the mitochondrial genome, when these same mutations are benign, beneficial or slightly deleterious in their effects on females [1–3]. We predict that at least some of the mitochondrial allelic variance affecting competitive male fertility across the sampled haplotypes will have accumulated under this evolutionary process and be male-specific in its effect on the phenotype. We suggest that the existence of male-harming mitochondrial mutations for male fertility would place strong selection on the interacting nuclear genome to evolve compensatory counter-adaptations that offset the negative effects, and we present support for this idea.

The mitochondrial genome encodes products that are crucial for achieving uncompromised metabolic function in both sexes. Yet, its maternal inheritance suggests that adaptation within the mitochondrial genome will proceed chiefly via natural selection on females. In theory, this could render mitochondrial genomes prone to the accumulation of alleles that — while optimised for female function — are potentially maladaptive for aspects of male function [1–3], particularly those traits exhibiting sexually dimorphic or sex limited expression [2,4]. Candidate traits likely to be prone to the effects of male-specific maladaptive mitochondrial alleles are those integral to male reproductive function, such as the testes and sperm. This hypothesis has received recent experimental support from studies reporting large male biases in the magnitude of mitochondrial genetic effects on patterns of gene and phenotypic expression in ***Drosophila melanogaster*** [2,5]. Such male-biases are indicative of the existence of mutations in the mitochondrial DNA (mtDNA) that exert male-biased effects. In one study, Innocenti ***et al***. [2] found that genetic variance across mitochondrial haplotypes, while having a negligible effect on the female transcriptome, mediated the expression patterns of more than 1000 nuclear genes in males, with manifold effects on gene expression in the male reproductive tissues. Here, we show that these effects on the male transcriptome have ultimate downstream consequences on the outcomes of male fertility, measured under ***in vivo*** conditions in standard-genotype tester females.

We used six naturally occurring mitochondrial haplotypes, derived from different worldwide ***D. melanogaster*** populations (Brownsville (Texas, USA), Dahomey (Benin), Israel, Madang (Papua New Guinea), Puerto Montt (Chile), and Zimbabwe), which had been placed alongside a standard nuclear background in ***D. melanogaster*** [5,6] (Supplemental information), and then assessed the fertility of males harboring each haplotype under competitive (each male competing for fertilizations within a once-mated female) and non-competitive (each male mated once to a virgin female) scenarios. Significant effects of the mitochondrial haplotype were found for competitive male fertility ($F_{5, 677} = 24.14$; $p < 0.0001$; Figure 1A; Supplemental information), but not for non-competitive fertility ($F_{5,7.48} = 1.42$; $p = 0.318$; Figure 1B; Supplemental information). Although the patterns for competitive fertility appeared to be driven by a large effect of the Brownsville mtDNA haplotype (Figure 1A), they remained significant when analysed without this haplotype