Synopses of Research Articles

Space Matters: Estimating Species Diversity in the Fossil Record

To get a sense of the challenges in measuring biodiversity, consider this: estimates for the number of living species on earth range from 3.5 million to over 30 million. Only 1.9 million species have been classified and described. (Of these, 15,589 currently face extinction.) Now imagine trying to calculate patterns of biodiversity through the paleontological record. One tool ecologists rely on to identify patterns of biological diversity is a long-established rule of thumb called the species–area effect: the tendency for species number, or richness, to increase in a regular way with area.

Paleontologists typically have been unable to apply the species–area rule in estimating paleodiversity and instead use species counts for a given time interval to calculate historical biodiversity, with the assumption that other sampling considerations override the species–area effect. In a new study, Anthony Barnosky, Marc Carrasco, and Edward Davis test this assumption and discover that the golden rule of ecology holds for the rock record as well. Just as geographic sampling influences diversity counts in the modern landscape, the species–area effect influences counts in the fossil record.

To get a true picture of geographic conditions, Barnosky et al. used mapping and imaging systems that generate direct measures of the geography for a given set of fossil species. To get a sense of diversity across time and space, the authors used a recently completed archival database (which they also built) that integrates the geographic data

with fossil datasets, called the Miocene Mammal Mapping Project (MIOMAP). MIOMAP includes all western North American mammals from 5–30 million years ago—3,100 localities and 14,000 occurrences of species in all.

The authors first tested the fossil data for species–area effects with species from a time period with robust geographic data (the Early Barstovian, about 14.8–15.9 million years ago). They plotted species richness against geographic area, using what's known as nested sets of fauna (species that occur in a smaller area represent a subset of species in a larger area) by starting with one biogeographic region and successively adding species from others. They also used unnested sets of fauna to plot species richness within a given time period for nine different geographic regions against the geographic sampling area. After correcting for possible biases in sample size that might influence the number of species, Barnosky et al. found a strong species–area effect in both analyses.

These results, they argue, suggest that many fluctuations in diversity seen in fossil analyses actually arise from the species–area effect. Given the lack of uniform geographic sampling in paleontological data, the impact of this effect may be significant—and likely applies to other taxa as well. Once the effect is factored in, one might expect significant adjustments in accepted patterns of global and regional paleodiversity. And because an important metric for understanding current extinctions relies on descriptions

A new, comprehensive database compiles mammalian fossils including this upper jaw of the *Sthenicitis campestris*, a weasel from about 12 million years ago (Photo: Alan B. Shabel)

of past extinction events, controlling for a paleodiversity–area effect may provide a better frame of reference for understanding the current biodiversity crisis. Estimates of paleodiversity also have important evolutionary implications for understanding how and when new species emerge. Thanks to the innovative text-mining tools and approach presented here, future studies can more easily correct for area effects and explore these issues. And given the parallels between species–area relationships in paleontology and ecology, collaborations across disciplines may offer valuable insights into ecological dynamics through time.

Barnosky AD, Carrasco MA, Davis EB (2005) The impact of the species–area relationship on estimates of paleodiversity. DOI: 10.1371/journal.pbio.0030266

## Mitochondrial DNA Provides a Link between Polynesians and Indigenous Taiwanese

According to folklore, Polynesians originated from a mythical homeland called Hawaiki. The existence of such a place, however, as well as its location, has been the subject of much speculation. Significant research efforts have attempted to elucidate these claims through archeological, linguistic, and, more recently, biological evidence. Jean Trejaut and colleagues are among the research groups attempting to determine the link between Polynesians and other Southeast Asian populations. In their recent study, they provide evidence that indigenous Taiwanese and Polynesians share a common ancestral link.

Two main theories have previously emerged to explain the origins of modern day Polynesians: the express train model and the slow boat model. The express train model proposes that early ancestors migrated from mainland China and Southeast Asia,

colonizing Taiwan first and then spreading rapidly to the other Pacific Islands. The slow boat model assumes that Polynesian culture was influenced by gradual, complex interactions with neighboring islands before reaching Polynesia.

Recently, genetic techniques involving mitochondrial DNA (mtDNA) have been used to compare the genetic profiles of Polynesians with people from mainland China, Southeast Asia, and Taiwan. Mitochondria reside in the cell cytoplasm and contain separate DNA that is inherited only from the mother. This means that while a person's nuclear DNA comes from a large number of ancestors, mtDNA can be traced back to a single ancestor. In theory, every person should have a copy of mtDNA identical to this original ancestor. In practice, this is not the case because random errors occur in the replication
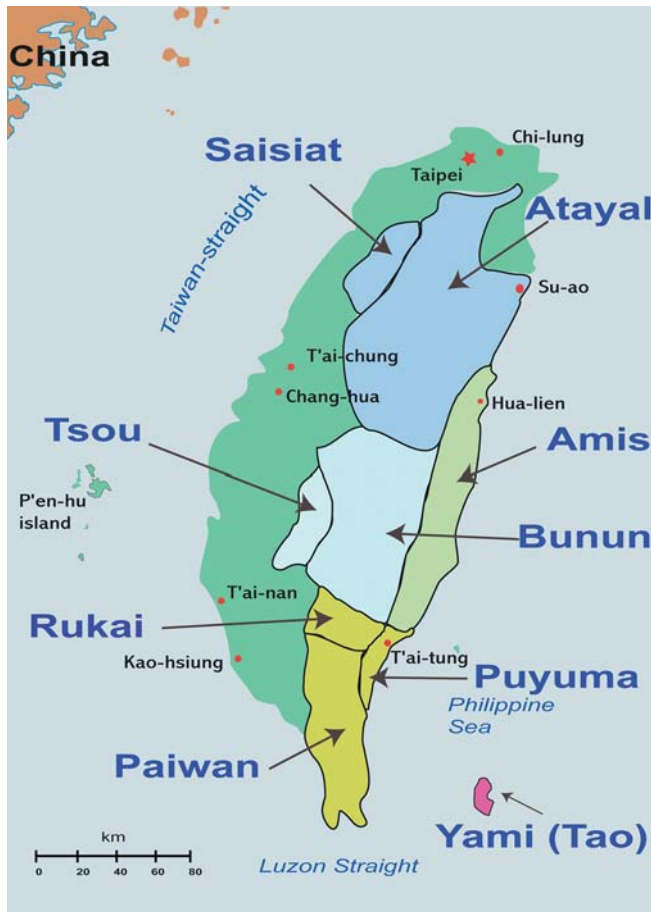
Ancient mitochondrial DNA from these nine indigenous Taiwanese tribes provided clues about their movement throughout Polynesia

process. Different populations will experience mutations at different locations in their mtDNA, and these will be passed on to future generations. The result is that some groups of people will end up with mtDNA that is very different from another group. By comparing how much mtDNA different populations have in common, an ancestral relationship can be determined and dated.

Early results from studies using mtDNA to explore this question were conflicting or inconclusive; however, recent research by Trejaut et al. has shed more light on the subject. Trejaut et al. analyzed DNA from people in China, Southeast Asia, Polynesia, and Taiwan. The authors focused specifically on the aboriginal populations of Taiwan, thought to be ancestors of today's Polynesians, and looked for unique genetic markers that occurred in the aboriginal people. They then compared these markers to those found in mainland Chinese, Taiwanese, and other Southeast Asian peoples, and asked: do the aboriginal people of Taiwan have a common ancestor in mainland China, and if yes, how long ago? And do the aboriginal Taiwanese share a common ancestry with the Polynesians?

Although Taiwan is currently inhabited mainly by migrants of recent Chinese origin, this has not always been the case. Today, roughly 2% of the inhabitants are direct descendents of the island's indigenous people and have a unique culture, language, and genetic makeup. And while the Chinese immigrants colonized Taiwan a mere 400 years ago, archeological records show that Taiwan may have been inhabited for the last 15,000 years.

Trejaut et al. found that the indigenous Taiwanese, Melanesian, and Polynesian populations share three specific mutations in their mtDNA that do not occur in mainland east Asian populations. Furthermore, they showed that there were enough different mtDNA mutations between the mainland Chinese population and the aboriginal Taiwanese to support the archeological findings suggesting a long period of habitation.

Taken together, these results suggest that Taiwanese aboriginal populations have genetically been isolated from mainland Chinese for 10,000 to 20,000 years, though the whereabouts of their origin in the Asian region is still unclear. Additionally, these results demonstrate that Polynesian migration most likely originated from people identical to the aboriginal Taiwanese. These findings provide the first direct evidence for the common ancestry of Polynesians and indigenous Taiwanese, and suggest that Taiwan genetically belongs to that region of insular Southeast Asia that might have been the point from where Polynesians started their migration across the Pacific, followed by later cultures that developed from their descendents in east Indonesia and Melanesia. Further research will be necessary to accurately determine the origins of the aboriginal Taiwanese; however, these results are a step towards clarifying the origins of Polynesians.

Trejaut JA, Kivisild T, Loo JH, Lee CL, He CL, et al. (2005) Traces of archaic mitochondrial lineages persist in Austronesian-speaking Formosan populations. DOI: 10.1371/journal.pbio.0030247

New World Pleistocene Horses: Pruning the Equid Tree

Darwin's evolutionary theories got a big boost when Othniel Charles Marsh assembled his North American horse fossils in the 1870s. They showed the gradual (presumed linear) evolution of a small, multi-toed *Hyracotherium* (then known as *Eohippus*) into the larger, hoofed modern *Equus*. As new fossils appeared, it became clear that the equid tree—which includes modern horses, zebras, burros, and the like—shows a complex evolutionary pattern with many branches. By the early 1900s, over 50 equid species were named for the Pleistocene (1.8 million years [Ma] to 10,000 years ago) alone.

Since then, the validity, origin, and relationships of these classifications have been revised, especially for the species of the Pliocene (5 Ma to 1.8 Ma ago) and Pleistocene epochs. In a new paper, Jaco Weinstock, Alan Cooper, and their colleagues use ancient DNA to upset conventional models describing the origins of two extinct forms: the North American "stilt-legged" horses and the stocky South American genus, *Hippidion*.

New World "stilt-legged" horses often appear in fossil deposits along with a second equid form that shares morphological characteristics with Eurasian caballines—a group that includes the domestic horse and the nearly extinct Przewalskii horse of Mongolia. Because stilt-legged horses had builds similar to the onager and kiang, rare wild asses that live in Asia (Asian hemionids), it's been suggested

that stilt-leggeds migrated from Asia across the Bering Strait. *Hippidion* shows up in South American fossils about 2.5 Ma ago. It has been considered a descendant of a primitive Miocene (23.8 Ma to 5.3 Ma ago) horse that diverged from the ancestral *Equus* lineage about 10 Ma ago. But a recent genetic analysis of Patagonian *Hippidion* specimens placed the fossils within an extinct *Equus* lineage that migrated to South America more recently—suggesting that the specimen had been misidentified as *Hippidion*.

To analyze the horse fossils, Weinstock et al. used a well-established genetic technique based on mitochondrial DNA (mtDNA). Mitochondria provide most of a cell's energy needs, but it is their genome that interests evolutionary biologists—specifically, a stretch of mtDNA sequence called the control region. This region mutates at a high rate, but the patterns of mutations remain stable over thousands of generations, providing a tool for inferring evolutionary relationships.

The authors first extracted mtDNA from horse bones (mostly toes) from Eurasia and North and South America dating back to 53,000 years ago. Their genetic analysis showed that *Hippidion*, stilt-leggeds, and caballines all arose from a common lineage. Even though stilt-leggeds share morphological traits with the Asian hemionids, they are genetically distinct, suggesting a convergence of form rather than a common ancestry. And because

DOI: 10.1371/journal.pbio.0030275.g001

Like this wild horse from North Carolina, free-ranging American horses descended from European stock. Horses native to the New World became extinct about 10,000 years ago

none of the Old World specimens had genetic sequences similar to sequences extracted from the stilt-legged horses, it's likely that the stilt-legged horses were North American endemics. The genetic analysis also showed that stilt-legged specimens from north and south of the ice sheets that bisected North America during long stretches of the Pleistocene belong to the same taxon, suggesting a wide-ranging species.

Hippidion and stilt-leggeds cluster as sister taxa—in direct contrast to paleontological models of *Hippidion*'s ancient form. The sequences are truly from *Hippidion* fossils (rather than the *Equus* lineage proposed in a recent

report), the authors argue, because all the fossils came from Late Pleistocene cave deposits in southern Patagonia known to contain only *Hippidion saldiasi* specimens—and all but one specimen came from these deposits. Along with telling morphological and size characteristics of the bones, these results suggest that *Hippidion* emerged closer to 3 Ma ago.

A final analysis of horse specimens from the Pleistocene, historic, and recent caballines—which have been grouped as separate species based on their diverse size—suggests that all North American caballines may belong to the same species. Altogether, the results suggest that just two horse lineages—caballine and stilt-legged—may have lived in North America during the Late Pleistocene. Both lineages showed regional and temporal variations in size and form. Though these variations have been taken to represent many different species, the authors propose that the two lineages are more likely just two species whose variations reflect adaptations to different environments. If true, this model could provide a tool for exploring how environmental adaptations give rise to morphological variation.

Weinstock J, Willerslev E, Sher A, Tong W, Ho SYW, et al. (2005) Evolution, systematics, and phylogeography of Pleistocene horses in the new world: A molecular perspective. DOI: 10.1371/journal.pbio.0030241

## Improved Statistical Tools Reveal Many Linked Loci

Using traditional statistical tools to analyze the modern wealth of biological data is a bit like trying to move a muscle car with a buggy whip—you're not likely to get anywhere very fast. The problem is perhaps most acute in the quest to understand how genes interact to regulate one another's expression. The amount of RNA made by any one gene is likely influenced by DNA at dozens of loci, or locations around the genome. Such loci are often situated within genes that participate in the same pathway as the gene being influenced, and a central goal is to understand this network of mutually influential genes and loci. Consider piecing together this puzzle for each of the many thousands of genes and many thousands of potentially influential loci, and the old analytical tools simply can't keep up. In this issue of *PLoS Biology*, John Storey and

colleagues tackle the challenge with a new approach.

The authors began by mating two strains of yeast that have minor differences in their DNA at more than 3,000 loci—creating over 3,000 markers—and then tracking the inheritance of these markers in the yeast offspring. Because the two genomes randomly reshuffle upon mating, any single offspring will contain some random combination of marker outcomes from each parent. The authors also examined the amount of RNA produced by over 6,000 individual genes in each offspring. The next step was to determine how these two large sets of data—variations at specific loci and variations in expression of specific genes—were correlated.

Straightforward statistical tests performed on each gene's expression
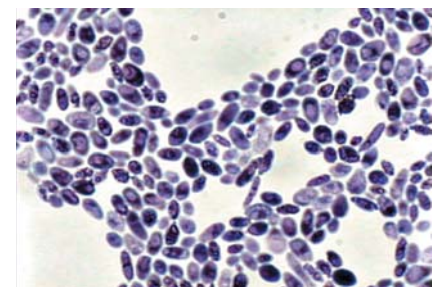
DOI: 10.1371/journal.pbio.0030294.g001

To unravel the tangled web of interactions between yeast genes, researchers bred two distinct yeast strains and statistically analyzed how gene expression varied between their offspring (Photo: David Byres)

revealed the single most influential location in the DNA. But such tests don't reveal the more complicated

reality that any single gene is likely to be influenced by more than one locus. Linking expression of a single gene (or "expression trait," in genetic parlance) to more than one locus has been stymied by the inability of conventional statistical approaches to cope with the mountains of data involved. Not only is an exhaustive pair-by-pair testing of all possible interactions computationally demanding, but it can also be very difficult to distinguish whether elevated expression is due to one or both of the loci being tested. The problem becomes exponentially harder as more potentially linked loci are tested.

To overcome the limitations of standard approaches, Storey et al. used a novel statistical approach that exploited what they had the most of—data. They began by determining the single most significant locus for each expression trait. They then moved on to the next most significant locus for that trait, but tested its linkage (that is, its influence on expression) with the assumption that the first locus was also linked. The ability to assign a "probability of linkage" to the first locus greatly simplified the calculations for the subsequent locus, reducing by almost a thousand-fold the number of possibilities that needed to be tested.

As opposed to standard methods, the authors show that their approach is able to assess true *joint* linkage of two loci to an expression trait, while requiring substantially less computation. In addition, they found that about one in seven expression traits is controlled by "epistatic," or hierarchical, relationships among the two loci, while the standard method revealed none. This method can be adapted to search for even larger numbers of linked loci, to provide insights into the many interlocking pathways that make up the gene regulatory network, and ultimately result in the organism itself.

Storey JD, Akey JM, Kruglyak L (2005) Multiple locus linkage analysis of genomewide expression in yeast. DOI: 10.1371/journal.pbio.0030267
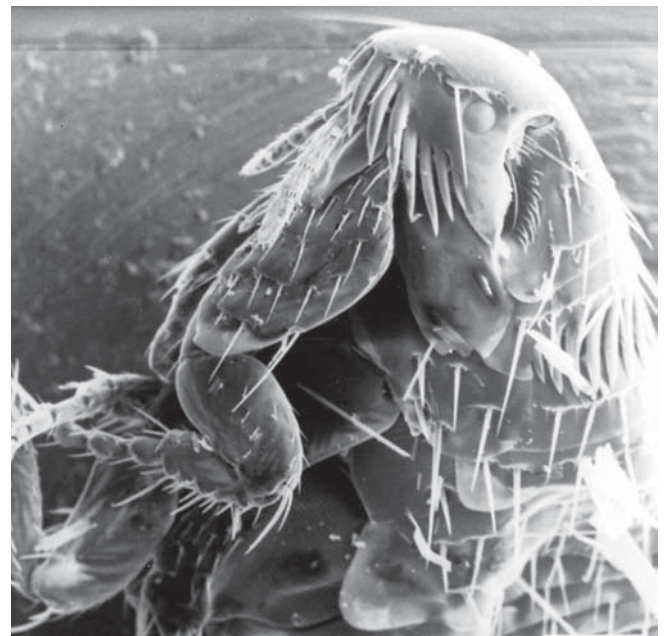
Using the Genomic Shortcut to Predict Bacterial Behavior

While many infectious bacteria remain outside human cells as they do their damage, others, including the various species of *Rickettsia*, take up residence inside. This makes them especially hard to study in vivo. In this issue, Hiroyuki Ogata and colleagues show that important clues to bacterial phenotype and pathogenicity can be learned about such an intracellular pathogen by sequencing and analyzing its genome.

The bacterium they studied was *R. felis*, which is carried by fleas and infects cats, dogs, and even humans. Its close relatives include species that cause the deadly human diseases of typhus and Rocky Mountain spotted fever. The researchers found that the *R. felis* genome includes not only the expected large circular chromosome, but also two small circular plasmids, bits of DNA carrying relatively few genes that are often transferred from bacterium to bacterium. This is the first species of *Rickettsia* in which plasmids have been found. While Ogata et al. have not yet proved that *R. felis* plasmids are exchanged between bacterial cells, the larger of the two plasmids contains several genes known to facilitate this type of exchange, called conjugation.

The bacterial chromosome itself appears to code for about 1,500 proteins, the largest so far of the sequenced *Rickettsia* genomes. More than 500 of these appear to be unique to *R. felis*. Among these are a large number of transposases, enzymes that cut and paste chromosomal DNA, whose existence correlates with the large amount of repeated DNA in the genome (about 5% of the total), and the finding that the *R. felis* chromosome has been rearranged many times. Indeed, the chromosome bears traces of multiple types of mobile gene elements, along with gene transfers back and forth between the chromosome and the plasmids, and acquisition of genes from other, non-rickettsial, bacteria.

The authors also scanned the genome for clues to the behavior of the bacterial cell. They found genes that in other bacteria code for pili, hair-like protrusions from the cell membrane. This clue prompted them to look for pili with electron microscopy, and they found them—two types, in fact, which appear to play roles in conjugation and cell adhesion. They also found a gene that induces polymerization of actin filaments in the host cell, suggesting that *R. felis* uses

The common flea can carry *Rickettsia felis* bacteria in its cells

the host cytoskeleton to get around inside the cell, as do other *Rickettsia* species.

The genomic shortcut to predicting bacterial behavior may have applications in other intracellular species both in *Rickettsia* and beyond. Perhaps even more importantly, the discovery of plasmids in *R. felis* may provide a key tool for study of other *Rickettsia* species. Plasmids can be modified to carry other genes, and as such may offer a route for examining the biology of the more pathogenic species, including those that cause typhus, a widespread and often deadly disease.

Ogata H, Renesto P, Audic S, Robert C, Blanc G, et al. (2005) The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. DOI: 10.1371/journal.pbio.0030248

Light-Sensing Protein Illuminates Sun-Loving Ocean Bacteria

Biologists have a knack for finding life in the harshest or loneliest regions of the Earth. Microbes, in particular, have adapted to wild variations in environment, and different strains of bacteria thrive under extremely acidic, sweltering, low-nutrient, and even oxygen-free conditions. Unfortunately, humans lack such flexibility, so they cannot always create lab conditions cozy enough for both biologists and their bacterial cultures. Until recently, no bacterial culture meant no genomic information.

Enter the polymerase chain reaction, a technique to amplify genes, and the environmental shotgun sequencing approach, a strategy to order vast numbers of genes. With the advent of these techniques, harvesting precious bacterial cultures was no longer the sole strategy for understanding bacterial genetics. Like the earlier shotgun approach, which compiles sequences of randomly fractured human genes, the environmental shotgun approach compiles sequences of bacterial genes skimmed from the top layer of the ocean. These bacteria are known as oligotrophs; given the dearth of nutrients in their environment, they must make use of a variety of energy sources. Findings from meta-genetic studies of Sargasso Sea bacteria corresponded with earlier discoveries of proteorhodopsin, a membrane protein that harnesses sunlight's energy. Scientists needed to do further research to understand how many and which marine bacteria had these light-sensitive proton-pump proteins.

In a new study by scientists from Israel, Austria, Korea, and the United States, Oded Béjà and colleagues sought answers to these questions by isolating large segments of DNA from the top, or photic layer, of the Mediterranean and Red Seas. Then, they inserted large segments of this DNA into host bacteria. This creates what is known as a large-insert bacterial artificial chromosome library, an amplified collection of the genome of interest. Like shotgun sequencing, this technique helped solve the human genome and now is helping to solve more exotic genomes.

By analyzing the library, the scientists located specific genes in relation to the entire genome. This analysis led to several insights. By estimating the average size of bacterial genomes, Béjà et. al were able to calculate that 13% of bacteria near the ocean's surface contain proteorhodopsin. While their bacterial artificial chromosome library revealed diversity among proteorhodopsin genes, it also revealed that proteorhodopsin is uniquely suited to make use of the high-radiation sunlight that illuminates the sea. Some evidence suggests that many of the bacteria with proteorhodopsin might also be able to metabolize sulfur, a common energy source for deep sea life. Additionally, Béjà and colleagues found some potential evidence that the marine bacteria are able to manufacture retinal, a molecule typically associated with vision.

If we can believe that austere, solitary stretches of open ocean are in reality rife with diverse bacteria, it's no great stretch to imagine that these bacteria make use of light energy. What else

Ocean bacteria with the light-sensitive proteorhodopsin enzyme live several meters above this coral reef (Photo: Boaz Harel)

would they feed on in the nutrient-poor upper layers of foam and froth? Many other organisms use proteins resembling proteorhodopsin for different functions. Humans, for instance, use rhodopsin to sense light in the eyeball. The presence of rhodopsin-like proteins in a wide range of life may eventually provide hints to the protein's evolutionary age. That this large class of transmembrane proteins was so well-conserved over a long evolutionary time scale provides evidence for complex ancient proteins. Another question that remains is whether the proteorhodopsin has any sensory function as does rhodopsin in humans, or whether the bacteria use the protein purely for energy transduction.

Sabehi G, Loy A, Jung KH, Partha R, Spudich J, et al. (2005) New insights into metabolic properties of marine bacteria encoding proteorhodopsins. DOI: 10.1371/journal.pbio.0030273

Prevalence of Infection in a Population Can Shape Parasite Virulence

If necessity is the mother of invention, the coevolutionary arms race is the mother of adaptation. For parasites and hosts engaged in an ongoing battle to gain advantage, those adaptations take many forms. A host continually tinkers with resistance mechanisms while the parasite adjusts its means of infection. But if infection causes too much damage to its host, the parasite could destroy its chances of transmission and, thus, survival. Thus, from the parasite's point of view, natural selection must create an optimal balance between the costs of parasitic infection (or virulence)—host death—and the benefits—eluding host defenses and establishing an infection.

Stephanie Bedhomme, Yannis Michalakis, and their colleagues study the coevolution of parasite virulence and host life history traits. In a new study, the researchers modify the standard

approach to studying the costs of infection (comparing infected and uninfected groups) by introducing another variable: intraspecific competition between hosts. Specifically, they ask, does infection affect competitive interactions between individuals? As expected, the authors find that infected individuals pay a cost compared to their healthy counterparts. But surprisingly, both infected and uninfected individuals do better when their competitor is infected: parasite costs depend on the infection status of the competitors.

To study the interplay between parasitism and intraspecific competition, Bedhomme et al. worked with the yellow fever mosquito *Aedes aegypti* and its natural enemy, the single-celled parasite *Vavraia culicis*. In the wild, mosquitoes get *V. culicis* infections when larvae ingest spores of the parasite, which enter

By accounting for competition between infected and uninfected mosquitoes, researchers discovered a link between parasitic virulence and the prevalence of infection in a population

larval gut cells, where they develop and reproduce. Because the spores need water to survive, it's thought that successful transmission depends on larvae, which live in water, and on two host life history traits: larval developmental time and probability of emergence of the host.

To study this process in the lab, Bedhomme et al. divided recently hatched mosquito larvae into groups of 60 larvae, and exposed half of the groups to the parasite. Larvae were then placed two by two into vials. Vials contained either two uninfected larvae, two infected larvae, or one infected and one uninfected individual.

Larvae were treated with high- and low-food diets. Competitive performance was measured by the probability of reaching adulthood and the time to develop. On the high-food diet, infected and uninfected larvae mostly grew normally and reached adulthood. On a low-food diet, however, infected larvae grew

slowly and were less likely to reach adulthood—probably because the parasite had more time to kill them. Being paired with an infected versus uninfected partner did not influence this outcome.

As for time to develop, infected pairs took longer to develop than uninfected pairs, as expected. But with infected and uninfected pairs, infected larvae took longer to develop than their healthy partners, meaning they should be more likely to succumb to the parasite. Competing against a healthy partner increased virulence by increasing development time. Interestingly, infected mosquitoes also fared better when paired with an infected competitor.

These results suggest that a high incidence, or prevalence, of parasitic infection in the population means that healthy larvae face less competition and do better than they would if they had to compete with healthy individuals. Infected individuals will also do better if there's a high prevalence of infection because they are more likely to compete against equally poor competitors. Thus, by ignoring the effects of competition, standard models underestimate the full costs of virulence—and, more important, miss a significant link between a parasite's prevalence in a population and its virulence.

Because the virulence of the parasite varies with its distribution in the population, this phenomenon may affect the population dynamics of parasite and host: infected hosts can't compete as well and take longer to develop, increasing the parasite's chances of transmission. The evolutionary implications of this relationship may be a selective pressure for host resistance to parasites. In traditional models, in low-virulence conditions, resistance is selected against because the cost of resistance reduces the fitness of the uninfected individual. But when low prevalence is associated with high virulence, the benefits of resistance would increase the individual's fitness. For an infected mosquito, at least, you stand a better chance of getting your wings and leaving the natal lagoon if more of your larval neighbors are infected too.

Bedhomme S, Agnew P, Vital Y, Sidobre C, Michalakis Y (2005) Prevalence-dependent costs of parasite virulence. DOI: 10.1371/journal.pbio.0030262

Sniffing Out the Structure of a Pheromone Receptor-Associated MHC Molecule

Although humans are thought to use their sense of smell to some extent when choosing a mate—or to a great extent, if perfume advertisements are to be believed—for many animals odor is centrally important in their mating decisions. Male moths, for example, are attracted over great distances to receptive mates by powerful species- and gender-specific cues known as pheromones.

Mammals, too, produce pheromones, although few mammalian pheromones have been unambiguously identified. These compounds have both long-lasting effects on the hormonal state of the animal receiving the signal and short-term effects on its social behavior. In rodents, pheromones are processed by a special part of the olfactory system called

the vomeronasal organ (VNO), which lies between the nasal cavity and the top of the mouth.

In rats and mice, the VNO expresses two large families of genes encoding putative pheromone receptors—the V1Rs and the V2Rs. In 2003, it was discovered that for V2R receptors to be functional, they have to associate with members of the M10 and M1 families of non-classical major histocompatibility complex (MHC) class Ib molecules. Classical MHC class Ia molecules are a huge family of closely related, immunologically important molecules that present small pieces of foreign proteins (peptides) to T lymphocytes to help them recognize invading pathogens. By contrast, the smaller group of non-classical MHC molecules

have both immune and non-immune functions. Intriguingly, peptides that bind to classical MHC class Ia molecules have been reported to activate V2R-expressing neurons. Furthermore, mice and rats mate preferentially with animals expressing MHC molecules different from their own.

Given all these pieces of information implicating both classical and non-classical MHC molecules in the social behavior of rodents, Pamela Bjorkman and her colleagues wanted to discover more about the relationship between these two classes of MHC molecules. To do this, they undertook a structural study of M10.5, one of the nine VNO-specific MHC class Ib proteins. They crystallized molecules of M10.5 expressed in insect cells and then used X ray crystallography

The structure of a pheromone receptor-associated molecule, shown here as a ribbon model, suggests that the molecule binds to an unknown compound

to investigate how the M10.5 structure compares to that of classical MHC molecules.

Overall, the structure turned out to be very similar but there was one big surprise. MHC class Ia molecules contain a characteristic open groove, which has thus far always been occupied by a peptide in crystal structures. The analogous open groove in the M10.5 was unexpectedly empty. However, experiments showed that the empty M10.5 molecule was thermally unstable, suggesting that the groove is normally occupied.

The researchers tried several approaches to identify the mysterious M10.5 ligand(s). First, they expressed M10.5 in mammalian cells rather than insect cells—insect cells lack the cellular machinery that normally loads peptides into MHC molecules—but still no peptides bound in the M10.5 groove. Then, they provided the M10.5 molecule with a mixture of peptides known to bind to MHC class I molecules. Again, no sign of peptide binding. Finally, the researchers used computer modeling to predict potential M10.5 groove occupants. From this analysis, they concluded that M10.5 and other M10s could bind a more restricted but longer set of peptides than MHC class Ia molecules. One possibility is that the M10.5 groove provides a binding site for V2Rs, but it might also bind pheromones.

Further experiments are now needed to identify the true binding partners of M10.5 and the other MHC class Ib molecules that are expressed in the VNO. Their eventual identification should provide insights into pheromone detection and facilitate the understanding of mating preferences in rodents. As for the mating preferences of humans, researchers will have to look elsewhere to solve that mystery since we do not appear to have M10 proteins, or a VNO!

Olson RA, Huey-Tubman KE, Dulac C, Bjorkman PJ (2005) Structure of a pheromone receptor-associated MHC molecule with an open and empty groove. DOI: 10.1371/journal.pbio.0030257
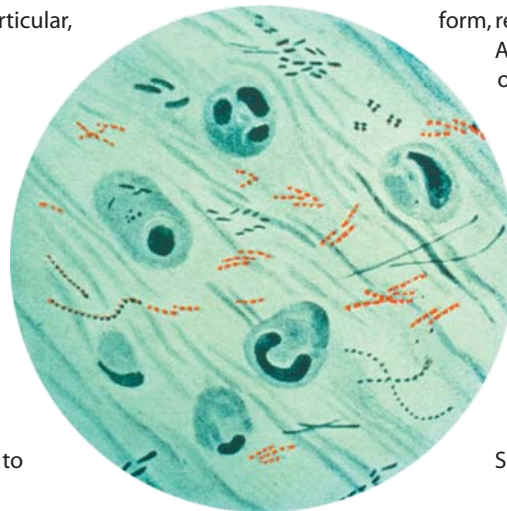
Reducing the Mysteries of Sulfur Metabolism

Sulfur is one of life's essential atoms. It is found in two amino acids—methionine and cysteine—and in several vitamins. Outside of living organisms, sulfur is mostly found in its fully oxidized form, the sulfate ion (SO_4^{2-}). To incorporate it into biomolecules, enzymes must reduce it, stripping it of oxygens. As is the case with so many other metabolic pathways, microorganisms possess a richer and more varied sulfur metabolism than humans do. In particular, bacteria, including the human pathogen *Mycobacterium tuberculosis*, use enzymes called sulfonucleotide reductases to begin the reduction process, creating reduced sulfite (SO_3^{2-}). In this issue of *PLoS Biology*, Carolyn Bertozzi and colleagues elucidate the novel molecular mechanism at the heart of this reaction, and show that it is conserved among a wide variety of organisms, from bacteria to plants.

The process begins when sulfate links up with an ATP molecule to form a species, abbreviated APS. The authors used a variety of biochemical techniques to discover what happens next. When APS was incubated with one kind of sulfonucleotide reductase, called APS reductase, they found that the weight of the enzyme increased by 80 Daltons, exactly the weight of a covalently bound sulfite, suggesting that the sulfur of the APS had been

reduced and linked to the enzyme. By mutating the enzyme, they showed that the sulfite links to a critical cysteine amino acid, which itself contains a sulfur (sulfur–sulfur covalent bonds are common in biochemical molecules). To regenerate the enzyme and liberate the sulfite, yet another sulfur-containing compound, thioredoxin, joins the action, the result of which is that sulfite is released and the enzyme is restored to its original form, ready to react again.

Analysis of the same pathway in two other types of bacteria confirmed that this same mechanism occurs in each. Combined with previous observations of a similar mechanism in the model plant *Arabidopsis*, these results demonstrate a remarkable bit of functional conservation over many millions of years of evolution. As well as elucidating an important piece of sulfur biochemistry, the deeper understanding of this critical biochemical process provided by this study may have practical implications. Since humans do not possess this pathway, drugs that target it might make an effective antibiotic with a low risk for side effects.

Researchers deduced the mechanism of the highly conserved sulfonucleotide reductase enzyme in *Mycobacterium tuberculosis* pictured here (Image: US Centers for Disease Control and Prevention)

Carroll KS, Gao H, Chen H, Stout CD, Leary JA, et al. (2005) A conserved mechanism for sulfonucleotide reduction. DOI: 10.1371/journal.pbio.0030250

Fish and Chips: A Fast Track to Understanding Blood Development

Each day, the bone marrow of an adult makes upwards of 200 billion new red blood cells, along with lesser numbers of white blood cells and platelets. This process, called hematopoiesis, depends on hematopoietic stem cells (HSCs), which divide to make both more stem cells and progenitor cells that differentiate into all the cell types of the blood. The genetic controls on this process are poorly understood. In this issue, Catherine Verfaillie and colleagues show how a two-stage analysis, generation of transcript microarrays followed by functional validation in zebrafish, can identify key regulators of the hematopoietic process.

The study of human hematopoiesis has been hampered in part because it's not possible to use surface markers to identify and isolate HSCs, a technique used to purify other cell types. The authors used a strategy they had previously developed to isolate HSCs from bone marrow and umbilical cord blood that produces a yield up to 10-fold greater than standard protocols for purifying human HSC. Gene expression in this HSC-enriched cell population was then compared with that in an HSC-depleted population using transcript microarrays ("RNA chips") to identify those genes whose expression was most
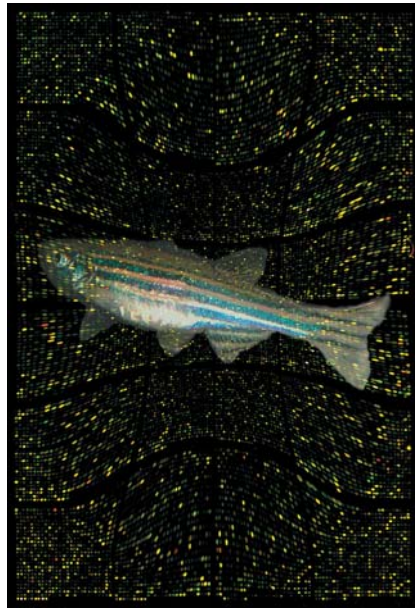
Using a zebrafish microarray, researchers discovered which human genes are active during hematopoiesis, the formation of blood cells

different between the two groups of cells. They identified 277 genes whose expression in both marrow and cord blood significantly different between the HSC-enriched and -depleted populations.

Of these 277 genes, Verfaillie and colleagues identified 61 whose functions were not already known and which had close matches in the zebrafish, a small fish in which hematopoiesis follows essentially the same path as in humans. To prevent expression of these 61 genes, they designed complementary antisense molecules against them, and injected them into zebrafish embryos. In 14 of the 61 genes, knocking down expression led to observable defects in hematopoiesis.

The authors note that three of these 14 genes are involved in signaling of fibroblast growth factor, a powerful regulator of development, suggesting that fibroblast growth factor may play a central role in hematopoiesis. More generally, they believe that the combination of using gene transcript microarrays to identify candidates and producing antisense molecules in zebrafish for functional screening of these candidates offers a way to quickly identify genes with central roles in vertebrate development.

Eckfeldt CE, Mendenhall EM, Flynn CM, Wang TF, Pickart MA, et al. (2005) Functional analysis of human hematopoietic stem cell gene expression using zebrafish. DOI: 10.1371/journal.pbio.0030254

A Mouse Model for Parkinson Disease

The debilitating effects of Parkinson disease are well known: muscle rigidity, impaired movement, and the uncontrollable shaking that makes even the most mundane activity a challenge. The symptoms result from a progressive deterioration of the nervous system and degeneration of neurons that control motor function and coordinated movement. These neurons, found in the midbrain, produce dopamine, a chemical that transmits brain signals (called a neurotransmitter) that regulate motor control, energy levels, and motivation to achieve goals. In Parkinson disease, dopamine levels drop as neurons degenerate, producing the characteristic symptoms. With no cure on the horizon, the most common treatment involves administration of the dopamine precursor, L-DOPA, usually in pill form. Though symptoms subside at first, this treatment is rendered ineffective over time.

To develop better therapies, scientists use animal models of human diseases to test the efficacy and toxicity of potential treatments before moving on to human trials. To be effective, animal models must recapitulate the disease symptoms as closely as possible. However, while previous models of Parkinson with incomplete dopamine depletion captured some of the manifestations of this condition, the dominance of the dopamine

To model Parkinson disease, researchers bred mice with severe dopamine deficiencies that displayed rigidity, inhibited motion, and, as seen here, freezing behavior

system on the control of locomotion has made it difficult to elucidate the contribution of other neurotransmitter systems. In a new study, Tatyana Sotnikova and colleagues from Duke University created such a model that recapitulates many of the symptoms of Parkinson. By eliminating the dopamine transporter—the protein responsible for recycling the chemical into neurons—in mice, the authors reduced dopamine levels in the midbrain by 20-fold. In addition, chemically inhibiting dopamine production in these mice resulted in essentially unmeasurable levels of the neurotransmitter, since it could now neither be produced at normal levels nor be recycled. Because these mice exhibited the symptoms of Parkinson disease remarkably well, the authors could test how well drugs that act independently of dopamine ameliorated symptoms of the disease. This approach allows the identification of drugs that may serve to improve treatment at later stages of the disease, when dopamine-producing neurons have been severely reduced in number and L-DOPA efficacy has been reduced.

The authors tested a number of drugs at various doses and found that in addition to L-DOPA-related treatments, drugs related to amphetamine were effective in ameliorating muscle rigidity, tremor, and impaired movement. Most effective was methylenedioxymethamphetamine HCl (MDMA), commonly known as ecstasy. It has been shown that amphetamines can trigger release of neurotransmitters such as dopamine, serotonin, and norepinephrine and cause sudden bursts in neurotransmission, leading to a feeling of alertness, increased muscular activity, and reduced fatigue. This study, however, shows that treating mice with MDMA does not increase dopamine levels; furthermore, treating the mice with drugs related to serotonin or norepinephrine did not ameliorate

the disease's symptoms. These results suggest that MDMA likely acts through a pathway unrelated to these common neurotransmitters.

The authors tested the possibility that MDMA may be increasing transmission via receptors that respond to compounds that are normally present at very low levels, called trace amines. Activation of these receptors reduced rigidity and akinesia, as with MDMA, though to a much lower level. Thus, while it is possible that MDMA is acting through trace amine receptors, this may not be the only pathway used. Future studies will be required to elucidate how MDMA ameliorates Parkinson symptoms.

The largest effects of MDMA on reducing symptoms was seen at levels that produce neurotoxic effects in wild-type mice, though administering non-neurotoxic doses of MDMA along with tiny amounts of L-DOPA that are normally ineffective proved as effective as higher doses of MDMA alone. Interestingly, the authors report an absence of significant side effects of even very high doses of MDMA administration on mice lacking the dopamine transporter; however, since patients with Parkinson disease do not necessarily lack the dopamine transporter, toxicity of MDMA and related compounds will need to be studied in greater detail in the future. This study opens the door to a search for compounds related to ecstasy, which may provide a more effective treatment for symptoms of Parkinson in the later stages of the disease—and hopefully allow patients to perform the simple functions of everyday life independently again.

How Do Embryos Know Left from Right?

On the outside, humans and other vertebrates seem to be bilaterally symmetrical. Draw a line down your body from the top of your head to your feet, and what is to the left of the line is pretty much the mirror image of what is to the right. But think about your internal organs. Your heart is on the left of your body, while your liver is over to the right. This left–right asymmetry, like the other asymmetries of vertebrate bodies, is established early in embryonic life. The symmetrical ball of cells formed after fertilization of the egg quickly develops a head and tail end (an anteroposterior axis) and a front and back (a dorsoventral axis). Once these identities have been established, the embryo then specifies its left and right sides—an event called left–right symmetry breaking.

Work in early mouse embryos suggests that left–right symmetry breaking arises from the leftward flow of extra-embryonic fluid. This flow is somehow generated by beating cilia, rod-like structures that originally propelled
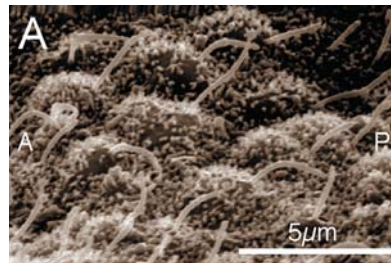
DOI: 10.1371/journal.pbio.0030291.g001

A scanning electron micrograph of mouse embryo node cilia helped researchers determine the cilia's tilt direction, a factor that contributes to embryo asymmetry

unicellular organisms through water. During evolution, however, multicellular organisms retained cilia on cells that move extracellular fluid; for example, respiratory tract cells use cilia to flush away bacteria and other debris. And in the embryo, cilia on cells in a region called the node produce the symmetry-breaking leftward flow, or "nodal flow." As a result, in mouse embryos with mutant cilia that fail to beat, nodal flow is not

established, and some mice develop with their internal organs on the wrong side.

But it's not clear how beating nodal cilia actually produce a leftward fluid flow. Unlike most cilia, which beat in a whip-like back-and-forth motion, nodal cilia beat by twirling in a circle; cilia twirling in fluid should act like a propeller and create a vortex (a circular flow of water). But nodal cilia don't produce a vortex; they produce a leftward flow. Models of fluid dynamics suggest that the nodal cilia might be able to do this if they are tilted. And as Hiroshi Hamada and colleagues now report, this is indeed the case; nodal cell cilia are tilted toward the embryo's tail end, rather than sticking straight up out of the plane of the node.

To investigate whether the cilia in mouse embryonic nodes are tilted, the researchers first traced their trajectories using a high-speed camera. Tracing the path of the tip of a straight-up cilium as it beats should yield a circular trace, but the researchers actually recorded elliptical and D-shaped traces. The authors explain that elliptical traces

are representative of beating cilia tips viewed at an angle, while the D-shaped traces result when the cilia are tilted so much that they slam into the "floor"— the embryonic cell surface—during their circuit. Scanning electron microscopy then revealed that all the cilia in the node tilted toward the posterior of the embryo and that each cilium was located toward the back of its node cell. Finally, the researchers built a 1,000× scale model of an embryonic node using wires to represent cilia and thick silicone

fluid to represent the extra-embryonic fluid. As predicted by theoretical fluid dynamics, the liquid in this model always flowed leftward when the tilted wires were rotated clockwise.

Hamada and colleagues propose that the position of the cilia and their tilt is determined by the pre-existing axis asymmetries (anteroposterior and dorsoventral asymmetries) in the embryo. The tendency of the cilia to rotate clockwise then produces the leftward fluid flow across the node that

breaks embryonic left–right symmetry. Whether a similar mechanism occurs in vertebrates other than mice, and how exactly leftward flow contributes to left–right axis establishment remains to be determined, but at least the mystery of how rotating cilia can produce a linear flow has now been solved.

Nonaka S, Yoshiba S, Watanabe D, Ikeuchi S, Goto T, et al. (2005) De novo formation of left–right asymmetry by posterior tilt of nodal cilia. DOI: 10.1371/journal. pbio.0030268

Creating a Window into the Developing Brain: Observing Axon Growth in Live Mice

Soon after a human baby emerges from the womb, its brain churns out new cells at an unbelievable rate of 250,000 neurons per minute. Each neuron typically acquires multiple dendrites to pick up electrical signals from other cells, and just one axon to transmit those signals to other cells. An axon can grow several feet in the service of relaying signals to its target tissue, but how it manages this growth has been an open question. Now Carlos Portera-Cailliau and colleagues use the real-time fidelity offered by two-photon microscopy to peer inside the brain of a living neonatal mouse and observe axons growing and reorganizing. By creating a window into the developing mouse brain, the authors could watch and record time-lapse images of living axons as they migrated through the neural landscape.

Researchers have long been aware that a complex process of axon growth and retraction occurs during the first few weeks of life, but until now have not been able to observe it for themselves in real time, at least not in mammals. Axon growth and guidance have been studied extensively in cultured neurons, and, along with time-lapse imaging of the brains of live frogs and fish, have provided insights into brain development in lower vertebrates. But because studies of mammalian axon growth have relied on measurements taken from fixed brain tissue samples, very little is known about the details of the growth and refinement of axonal projections in mammals.

Portera-Cailliau et al. solved this problem by using two-photon microscopy, a state-of-the-art imaging technique that allowed them to watch the same developing axons in the cerebral cortex of live mice during the first two weeks of life. The group used a line of transgenic mice that expressed green fluorescent protein in small subsets of neurons. The procedure involved removing a portion of the skull and replacing it with a glass coverslip, thus creating a window for viewing live brain tissue at different developmental stages. Although traditional confocal microscopy can also be used to view fluorescently labeled cells, two-photon microscopy can image deeper into tissue without damaging
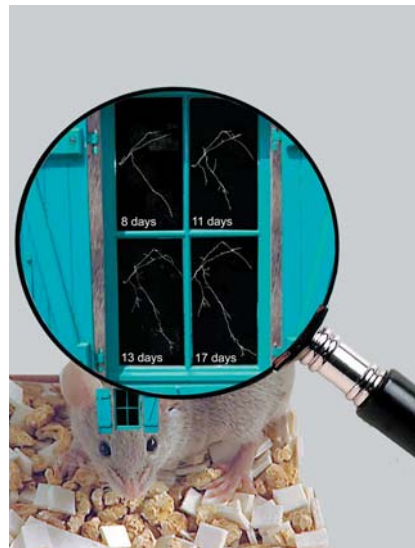
DOI: 10.1371/journal.pbio.0030301.g001

Implanting "windows" into the skulls of newborn mice allowed researchers to observe axon development

the brain cells, which allows imaging repeatedly over long periods of time.

Time-lapse images were generated from the mice over a period of several minutes to as long as three weeks, allowing the researchers to observe changes in neuronal structure throughout the critical period of cortical development. Portera-Cailliau et al. observed changes in two very different types of neurons: thalamocortical (TC) neurons and Cajal-Retzius (CR) neurons. Both of these neurons send axons to the outermost layer of the cortex, but CR neurons project only locally and TC neurons project over long distances. The structure and dynamics of axons from these neurons, it turns out, are also very different, indicating that axonal development is not homogeneous across cell types. For example, TC axons grew quickly in long, straight paths and added new branches frequently. By contrast, CR axons grew much more slowly, along tortuous paths. Additionally, TC axons exhibited both short branch retraction (tens of microns) and elimination of larger branches (hundreds of microns or more), while CR axons only used branch tip retraction for pruning. Thus, the structure and dynamics of axon elaboration are dependent on neuronal cell type, even when they grow side by side in the same environment. This suggests that different neurons may exhibit different axon elaboration programs and/or interpret differently cues from their surroundings.

This study provides insight into the process of refinement and optimization of neuronal circuitry that occurs in mammals in the early stages of life, and begins to solve the mystery about how axons develop in the cortex. By observing axon growth in real time, scientists have taken the first step in understanding the cues that control each twist and turn of every axon in the cortex. Such efforts may one day suggest ways to prevent or treat the many types of cognitive disabilities that arise from abnormalities in brain development.

Portera-Cailliau C, Weimer RM, De Paola V, Caroni P, Svoboda K (2005) Diverse modes of axon elaboration in the developing neocortex. DOI: 10.1371/journal.pbio.0030272

Now You Don't See It, Now You Do: Filling In Creates the Illusion of Motion

The parade of lights flashing on a theater marquee provides an important lesson in how the brain creates the illusion of motion. While we know each bulb remains stationary, the lighting and dimming of each in succession makes it appear that light is moving across the marquee. Even when successive bulbs are separated by a large space, our brains fill in the missing data to create the illusion that the motion has occurred smoothly from one point to the next. But where in the brain does the illusion occur? In this issue of *PLoS Biology*, Lars Muckli and colleagues demonstrate that the first cortical area in the visual processing stream, the primary visual cortex, participates in sustaining the illusion, probably under the influence of higher visual centers in the brain that likely create it.

In this study, human subjects observed a simple white square on a computer screen, flashed first in one spot, and then again several centimeters away. A functional magnetic resonance image of their brain activity was recorded, and the most active areas were determined. In the primary visual cortex, or V1, activity was found in one area that corresponded to the location in the visual field of the first flash, and in another area corresponding to the location of the second flash. Remarkably, there was also activity in between these two locations, corresponding to a region of the visual field that, while not itself illuminated, was on the path between the two flashes. This same area was active when the subjects viewed the white square moving smoothly between the two spots, and was absent when the square was flashed at only the initial or only the final spot, or when squares in both spots flickered simultaneously. The subjects' brains, it seems, filled in the missing information when a pattern of activity suggesting motion was detected. Further evidence that such "fill-in" activity in V1 corresponds to the conscious perception of motion came from an additional experiment, in which the flashing pattern of a quartet of white squares suggested alternating, and mutually exclusive, horizontal or vertical motion. When the observers reported the quartet as moving vertically, there was more activity in the V1 region corresponding to the vertical apparent motion.

The source of this filling in is not likely to be in V1 itself, the authors argue, because the middle area is too far from either
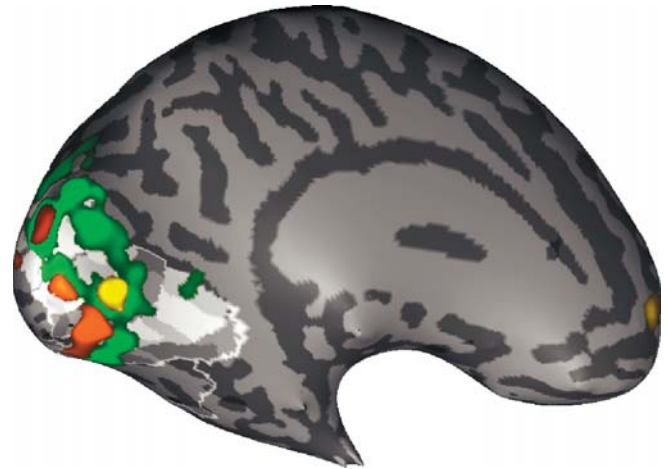
The brain regions highlighted above allow us to see a series of flashing images as smooth motion. This activity occurs in the primary visual cortex.

of the ends to be stimulated by them directly. Instead, they propose the most likely source is activity in a visual area much higher in the processing chain, called hMT/V5+. Projections from here are known to influence V1 activity, and cover large enough areas to encompass the entire region of V1 activation in these experiments.

These results provide further demonstration that the brain masterfully creates a continuous, yet sometimes imagined, whole from individual, and often incomplete, parts. Most surprisingly, even the brain areas originally thought to be "literalist" in their representation of the environment are actually accomplices in the construction of illusory views. While such synthetic activity occasionally leaves us prey to optical illusions, it's a small price to pay for what we get in return: a seamless understanding of often fractured perspectives.

Muckli L, Kohler A, Kriegeskorte N, Singer W (2005) Primary visual cortex activity along the apparent-motion trace reflects illusory perception. DOI: 10.1371/journal.pbio.0030265

# Cancer-Causing Genes Can Convert Even the Most Committed Cells

Healthy, normal cells follow the rules: don't crowd the neighbors, stick to your own tissue, and die when it's time. When cells no longer observe these regulations, they become cancerous, dividing uncontrollably, pushing out their healthy counterparts, and eventually invading other tissues. All cells appear to have the capacity to become cancerous, but most don't. Just what turns a healthy cell into an outlaw remains uncertain, but cancer researchers Terence Rabbitts and colleagues at the MRC Laboratory of Molecular Biology in Cambridge, United Kingdom, think the genetic changes regularly observed in cancer cells can provide some clues.

Tumor cells commonly exhibit chromosomal abnormalities. One type of aberration occurs when DNA strands break and segments between two different chromosomes are swapped, a process called chromosomal translocation. Depending on where

these chromosomal breaks occur, the newly fused DNA can produce novel genes called fusion genes. Fusion genes are the result of explicit chromosomal changes associated with different cell types and result in distinct types of cancers. In human connective tissue cancers (called sarcomas), genetic exchange between Chromosomes 21 and 22 produces the *EWS-ERG* fusion gene; this translocation is thought to initiate tumor formation in undifferentiated "progenitor" cells called mesenchymal cells.

Progenitor mesenchymal cells are long-lived and self-renewing, and can give rise to many specialized cells, including muscle cells, bone cells, and connective tissue cells. Because the *EWS-ERG* fusion gene in humans was observed only in sarcomas derived from progenitor lineages, researchers thought it initiated a differentiation program that transformed uncommitted non-cancer cells into committed cancer cells.
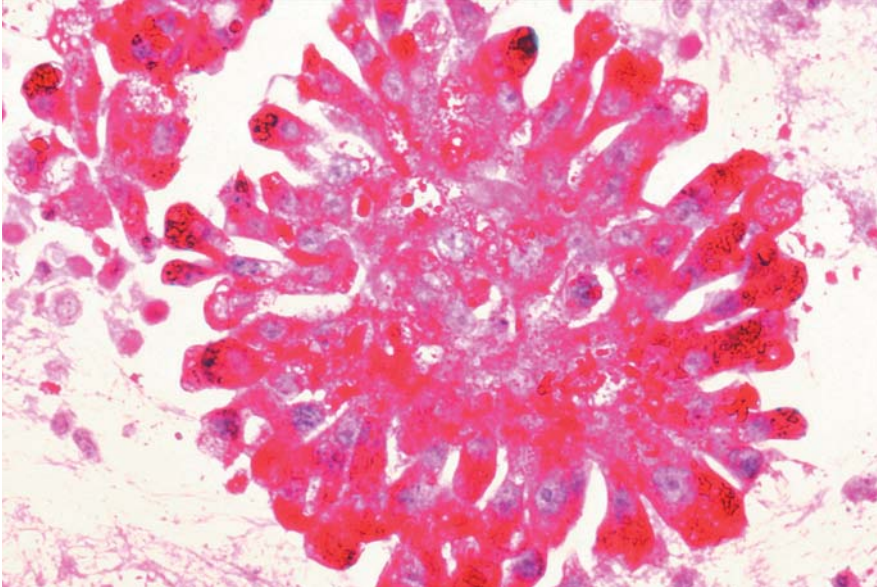
DOI: 10.1371/journal.pbio.0030276.g001

Genetic exchange between chromosomes can cause cells to become cancerous, like these cells from metastasized Ewing's sarcoma (Image: Lance Liotta Laboratory)

To determine whether the Ews-ERG fusion protein could initiate tumorigenesis in other lineages, particularly lineages of committed cells not typical of human cancers, Rabbitts and colleagues genetically engineered mice to express an Ews-ERG fusion protein exclusively in committed B and T immune cells. Mice that expressed the fusion protein developed T cell tumors, demonstrating for the first time that Ews-ERG could cause blood-borne cancers from committed cells, but B cell tumors were not observed. Since the Ews-ERG fusion protein was expressed in both B and T cells in the mutant mice, these results suggest that other factors influence the fusion protein's potential to cause cancer.

These results reveal important information about the way tumors are generated. The cancer-causing effects of the Ews-ERG fusion protein are neither specific to a given tissue type, nor do they exclusively activate tumor cell differentiation in progenitor cells, as originally thought. Rather, *EWS-ERG* may be able to act as a universal cancer-causing gene, inappropriately activating signaling pathways responsible for regulating the cellular lifecycle. These observations, if shown to extend to other known cancer fusion genes, may indicate that the apparent tissue specificity of Ews-ERG and other similar fusion proteins stems from a particular propensity for chromosomal translocations in a given cell type, rather than from the specificity of the resulting fusion proteins.

Codrington R, Pannell R, Forster A, Drynan LF, Daser A, et al. (2005) The Ews-ERG fusion protein can initiate neoplasia from lineage-committed haematopoietic cells. DOI: 10.1371/journal.pbio.0030242