DEVELOPMENTAL BIOLOGY

Long Search for Sea Urchin Sperm Receptor Pays Off

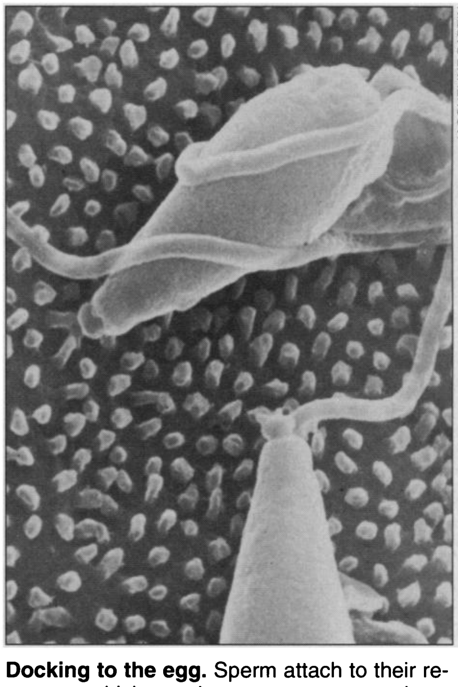
Like most marine invertebrates, sea urchins have a problem when it comes to the mating game. They dump their eggs into the open sea to be fertilized externally, which means that they need very specific recognition mechanisms to ensure that the eggs are fertilized only by sperm of the same species. Biologists have been trying to figure out the components of this system for years, but the search has been frustrating. They thought they were close 15 years ago, when a protein that sea urchin sperm use to recognize and bind to eggs was identified. But its partner in this molecular mating dance, the receptor on the egg surface that this protein binds to, remained elusive—until now, that is. On page 1421, a team led by William Lennarz of the State University of New York at Stony Brook reports the first characterization of a sea urchin sperm receptor.

The discovery should enable researchers to identify the precise differences between the sperm receptors from closely related sea urchin species that prevent cross-species fertilization. "Now the basis for the species-specificity of fertilization can be explored in a real structural sense," says Victor Vacquier of the Scripps Institution of Oceanography, who in the 1970s identified the egg-recognition molecule on sperm, a protein called bindin. A better understanding of those differences could bring new insights into how species arise during evolution. The chance occurrence of both a mutant sperm receptor and of a mutant bindin that could recognize it may have been the event that gave rise to sea urchins that could no longer breed with others of their ilk and have since diverged to become distinct species.

Hopes are also high that the advance will lead researchers to the internal signaling pathway that brings about the rapid transformation of a fertilized egg from a slumbering giant into a metabolically active cell, ready to take on board the sperm's cargo of DNA and begin the rapid series of cell divisions that forms the embryo. If so—and if similar pathways occur in the eggs of higher organisms such as mammals—then the discovery may also lead eventually to new methods of birth control that work by interfering with the pathways and preventing egg activation.

Lennarz's discovery of the sperm receptor didn't happen overnight. He began studying the molecular biology of egg fertilization in the mid-1970s, expecting that his previous expertise in bacteriology would lead to rapid progress. "It struck me that we knew all about how a bacteriophage enters a bacterium," he recalls, and that might well serve as a guide to understanding how sperm "infect" eggs. Lennarz became even more optimistic in 1977 when Vacquier discovered bindin. Since that protein presumably bound to a receptor on the surface of the egg, it could be used like a template to identify its partner. In the event, however, repeated attempts to extract the receptor from the mass of tangled extracellular material that surrounds sea urchin eggs all met with failure. "It's been the hardest project of my 30-year career," Lennarz observes.

The problem, explains cell biologist Kathleen Foltz, who worked on the project as a postdoc, is that the protease enzymes used to snip off the extracellular part of the bindin receptor protein were only too effective: They completely destroyed the molecule. It wasn't until a new generation of proteases that clip much more specifically became available a couple of years ago, she says, that it became possible to extract appreciable quantities of the pure protein. Once that was achieved, the researchers were able to raise antibodies against it that they could use to screen a library of cDNA clones from the sea urchin *Strongylocentrotus purpuratus* for the gene region that makes the extracellular part of the sperm receptor molecule. With that in hand, the rest of the task—finding overlapping chunks of DNA that code for the remainder of the receptor and then working out the whole receptor structure—was a formality.



Docking to the egg. Sperm attach to their receptors, which may be more concentrated on the small projections called microvilli.

To Foltz and Lennarz's surprise, the structure they came up with bears little resemblance to any other known cell membrane receptors. Indeed, the only notable similarity with any known protein they can detect is between the region of their receptor that interacts with bindin and members of a family of heat shock proteins that are involved in the transport of peptides around the cell and in protein folding. Just what that similarity means is still uncertain, but the cloning of the *S. purpuratus* sperm receptor now opens the door to searching for similar receptors in higher organisms. Indeed, Foltz, who is now at the University of California, Santa Barbara, has already started searching through mammalian cDNA libraries for sequences that might encode a look-alike receptor. "My guess is that we are going to find similar molecules in other species," says Scripps' Vacquier.

If a mammalian receptor is found, it probably won't play a role in ensuring that eggs only bind to sperm of the same species, as two proteins found outside the egg in a jelly-like layer called the zona pellucida seem to do this job. But there's another, potentially even more intriguing, possibility. Unlike these mammalian zona pellucida proteins, the sea urchin receptor is embedded in the egg membrane with part projecting through into the cytoplasm. That suggests it's responsible for triggering the signal transduction pathway that activates the egg following fertilization. If so, similar molecules in other species might play a comparable role.

For the time being, Foltz and Lennarz aren't willing to stick their necks out and claim that their receptor will prove to be such a signal transducer. Bindin and its receptor might merely serve as the "molecular glue" that brings the sperm and egg together, Lennarz says, with other molecules actually transmitting the activation signal to the egg interior. But other researchers aren't so cautious. "I don't think there is any other molecule ...that you could blame this on," says developmental biologist Eric Davidson of the California Institute of Technology.

Lennarz's group has already found one promising sign that Davidson is right. The cDNA encoding the cytoplasmic part of the *S. purpuratus* receptor molecule hybridizes with DNA from two other sea urchin species, whereas the cDNA encoding the domain that extends outside the plasma membrane does not. This is exactly what would be expected for a molecule whose external portion determines the species specificity of fertilization, but whose internal part causes an identical signal transduction event in each species.

Unfortunately, the fact that the structure of the cytoplasmic domain of the sea urchin bindin receptor doesn't resemble other known receptors means there are few clues as to exactly what the hypothetical signal transduction pathway might be. But it should be possible to prove that the receptor is transmitting a signal by expressing it in eggs from related species, such as starfish, to see if the modified eggs can be activated by *S. purpuratus* sperm or bindin. And if this activation could then be prevented by mutating the receptor's cytoplasmic domain, the case would be closed. Foltz and Lennarz have already begun these experiments, collaborating independently with developmental biologist Laurinda Jaffe of the University of Connecticut. So far their efforts haven't met with success. But if 15 years spent struggling to get this far have taught Lennarz anything, it's the value of persistence. "We haven't given up," he promises.

–Peter Aldhous

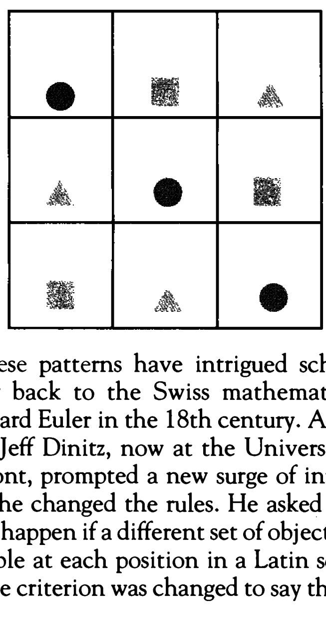
MATHEMATICS

If You're Stumped, Try Something Harder

In mathematics, it's often hard to tell the difference between an "easy" problem and a "hard" one. Some of the simplest-sounding questions turn out to be the most challenging to solve. And to get the answers, researchers may have to work through a thicket of seemingly much harder theoretical problems.

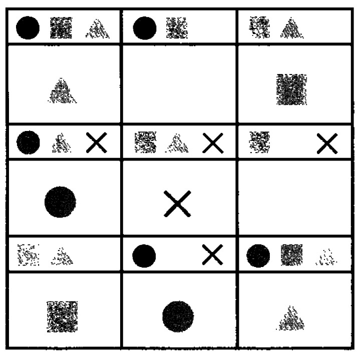
Something like that happened recently in the mathematical subspecialty known as combinatorial design—an area with implications for computer algorithms, scheduling, and experimental design. Jeannette Janssen, a graduate student at Lehigh University, has solved a variation of a "simple" combinatorial problem that has stumped mathematicians since it was first posed in the late 1970s. To do so, she had to turn to more abstruse-sounding theoretical developments that arose from earlier attacks on the problem. While her new result doesn't completely solve the original problem, notes Herb Wilf, a combinatorialist at the University of Pennsylvania, "it moves the problem much closer to a resolution than anyone had expected."

The problem Janssen tackled has to do with what mathematicians call Latin squares. A Latin square is an n-by-n arrangement of n objects in which each object appears exactly once in each row and each column (see below). Even though the definition sounds very restrictive, it's easy to produce a Latin square of any size.



These patterns have intrigued scholars dating back to the Swiss mathematician Leonhard Euler in the 18th century. And in 1977, Jeff Dinitz, now at the University of Vermont, prompted a new surge of interest when he changed the rules. He asked what would happen if a different set of objects was available at each position in a Latin square and the criterion was changed to say that no object can appear more than once in any row or column. In particular, Dinitz wondered whether it would always be possible to form such a "generalized Latin square" having n rows and columns as long as each entry had n objects to choose from. Although that sounds like an even simpler puzzle than the classical version, it may not be: If the sets of choices overlap, there's no obvious rule for avoiding repetition (see below).

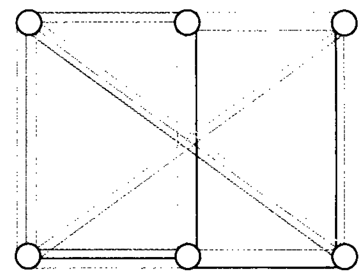
Dinitz's problem is just one case of a broader meta-problem: Given a bunch of constraints on what you're allowed to do (such as



not scheduling the same person or piece of machinery to be in two places at the same time), how can you tell if what you're trying to accomplish is even feasible? And the deceptive simplicity of Dinitz's problem has inspired a lot of related work. "A good question will motivate people to work in an area," says Dinitz, who describes his problem as "easy to state and easy to see, but very hard to prove."

Hard enough that mathematicians had been unable, until recently, to solve the problem for squares larger than $n=3$. Then, in 1991, Noga Alon and Michael Tarsi at Tel Aviv University in Israel proved that you can always find a generalized Latin square for $n=4$ and $n=6$ also. They did so in a time-honored way: by proving a much deeper, more abstract theorem first.

Alon and Tarsi's main theorem is about "coloring" the edges of graphs so that no two edges with the same color meet at a vertex (the edges of a graph are the lines or curves that connect pairs of vertices). Alon and Tarsi were able to find a condition under which arbitrary graphs, with an arbitrary list of allowed colors assigned to each edge, are guaranteed to have a "legal" coloring. That result



had immediate implications for Dinitz's problem, because the combinatorics of n-by-n Latin squares translate readily into graph-coloring terms: Think of n vertices, representing the rows in a square, connected to another n "column" vertices by n^2 edges corresponding to the entries in the square (see above).

Alon and Tarsi showed that the condition of their theorem is satisfied by the Dinitz-problem graph when $n=4$ and $n=6$, thereby solving Dinitz's problem in those two cases. However, the condition isn't satisfied for $n=5$—nor for any other odd value of n. Alon and Tarsi's theorem, it seemed, could only apply to Dinitz's problem for even values of n.

Enter Janssen. His inspired idea was to apply Alon and Tarsi's theorem to Latin *rectangles*. She proved that for generalized Latin rectangles of any size, with n rows and fewer than n columns, it's enough to have n choices for each entry. That's a big advance over what had been proved before, according to Jeff Kahn at Rutgers University. The previous best result had been that n choices sufficed for rectangles having no more than two-sevenths as many columns as rows.

Moreover, Janssen's rectangle result comes extremely close to solving the original Dinitz problem: It implies that you can always build a generalized n-by-n square if you have $n+1$ choices for each entry. The trick is to think of the n-by-n square as part of an $n+1$-by-n rectangle; since $n+1$ objects per entry suffice for the rectangle, they'll do for the smaller square as well.

Whether Janssen's breakthrough presages a complete solution to Dinitz's problem is impossible to predict. However, Kahn thinks it may well lead to the solution of other long-standing problems in graph coloring—and that bodes well for the simple-seeming problem that started it all. Not only can a good question motivate work in an area, but so can a good solution.

–Barry Cipra