right orange spheres tumble over each other, RACING THROUGH CLEAR, BRANCHING CHANNELS. SUDDENLY, THEY STOP, PAUSE, AND REVERSE DIRECTION. THE PARTICIPANTS IN THIS CONFUSED DANCE ARE THE BLOOD CELLS OF A MARINE SEA SQUIRT, CALLED *BOTRYLLUS SCHLOSSERI*. THE REVERSIBLE HEART THAT PUMPS THEM IS JUST ONE OF THE MANY ODDITIES OF THIS ORGANISM. CANNIBALISM, PROGRAMMED SELF-DESTRUCTION, AND GENETIC HIJACKING ARE ALSO STANDARD CONDUCT.

Botryllus, a beautiful creature, resembles a flower in which each petal is an individual organism. And, on the surface, its life is peaceful enough: The existing petals produce more petals until the flower grows large enough to split, with external, wandering blood vessels still connecting the two resulting flowers.

This idyllic cycle is interrupted when two unrelated colonies of *Botryllus* grow too close to each other. "In their natural state, *Botrylli* are in a massive competition for space," explains Irving Weissman, MD, professor of pathology. "Whoever gets the space gets the food that drifts over it." *Botryllus* can dominate more space by fusing with its neighbors, but only if they are close relatives. As genetic competitors, non-relatives are frozen out — rejected in a flurry of cellular death and destruction that is reminiscent of organ rejection in humans. It is that connection to humans that caught Weissman's interest.

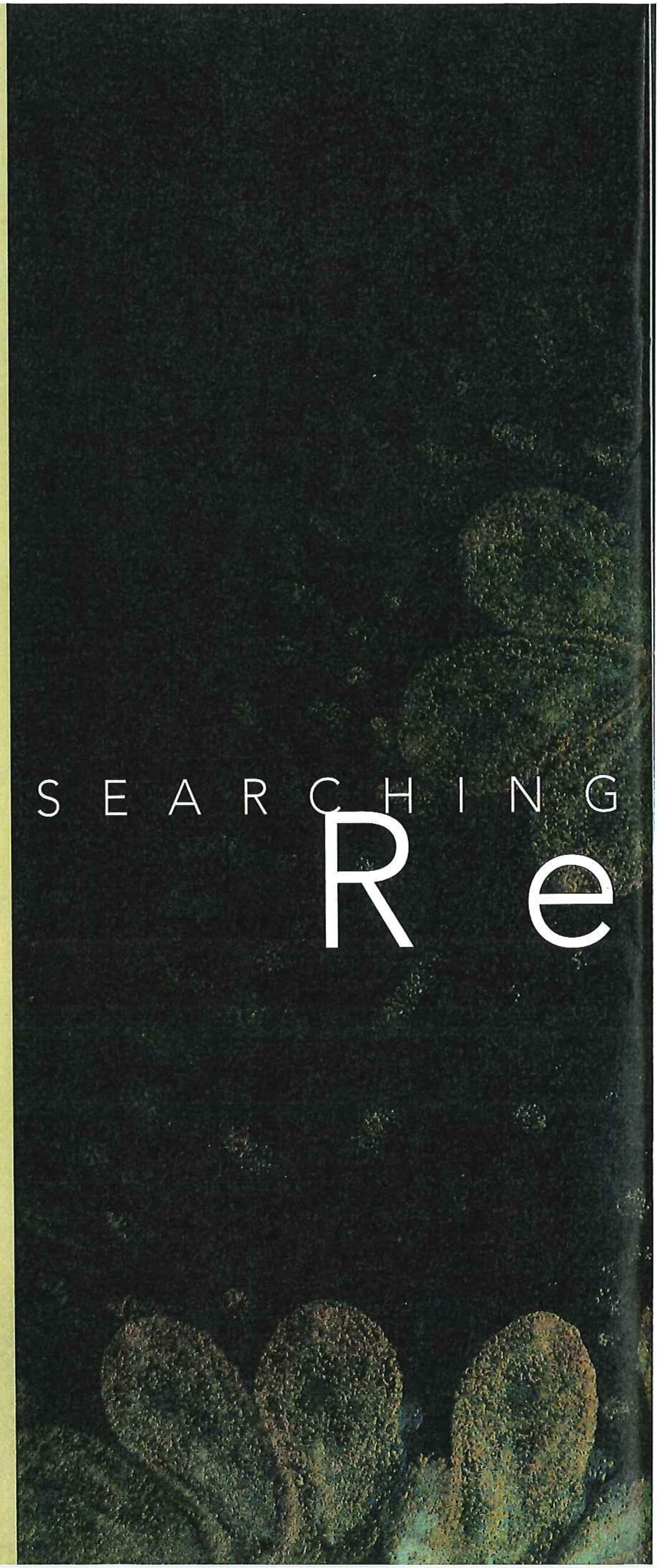
Weissman's primary research focuses on immune cell development, how special "stem" cells generate all the different cells in the immune system. But he was prompted to take a turn into left field — the study of sea squirts — by what he saw as a misguided approach to the study of the human immune system.

"Our immune systems were not designed to 'see' a transplant," explains Tony De Tomaso, PhD, a postdoctoral fellow in Weissman's outpost laboratory at Hopkins Marine Station in Monterey. And yet many researchers aim to understand the immune system based on these unnatural situations. *Botryllus* seems a perfect alternative. "For this animal," says De Tomaso, "transplantation is part of its lifestyle."

The problem with human transplantation comes about because certain molecules vary wildly between individuals. These highly variable molecules, called the major histocompatibility complex (or MHC) proteins, grasp onto foreign proteins and display them to the immune system. With the help of the MHC proteins, the immune system can then decipher "self" proteins from "non-self" or foreign proteins.

MHC variability helps individuals (who have several different copies of the MHC proteins) pick up a wider variety of proteins and, as a result, fend off a wider variety of viruses and bacteria. But it also means that a transplanted organ will have the donor's strange MHC proteins on its surface. The recipient treats the graft like infected tissue: It kills it off.

Weissman wants to know whether *Botryllus* self/non-self recognition (or allorecognition) works the same way. In 1982 he published a report in the journal *Nature* showing that the allorecognition was controlled by a single stretch of DNA that was variable like the MHC genes. This genetic site has been dubbed the fusibility and histocompatibility locus, or Fu/HC for short. Probably around 300 different versions of it are found in the *Botryllus* in Monterey Bay alone. Although the researchers now know that the Fu/HC locus exists, they don't know exactly where it is in the DNA or what it looks like. "The big thing now," says De Tomaso, "is to find



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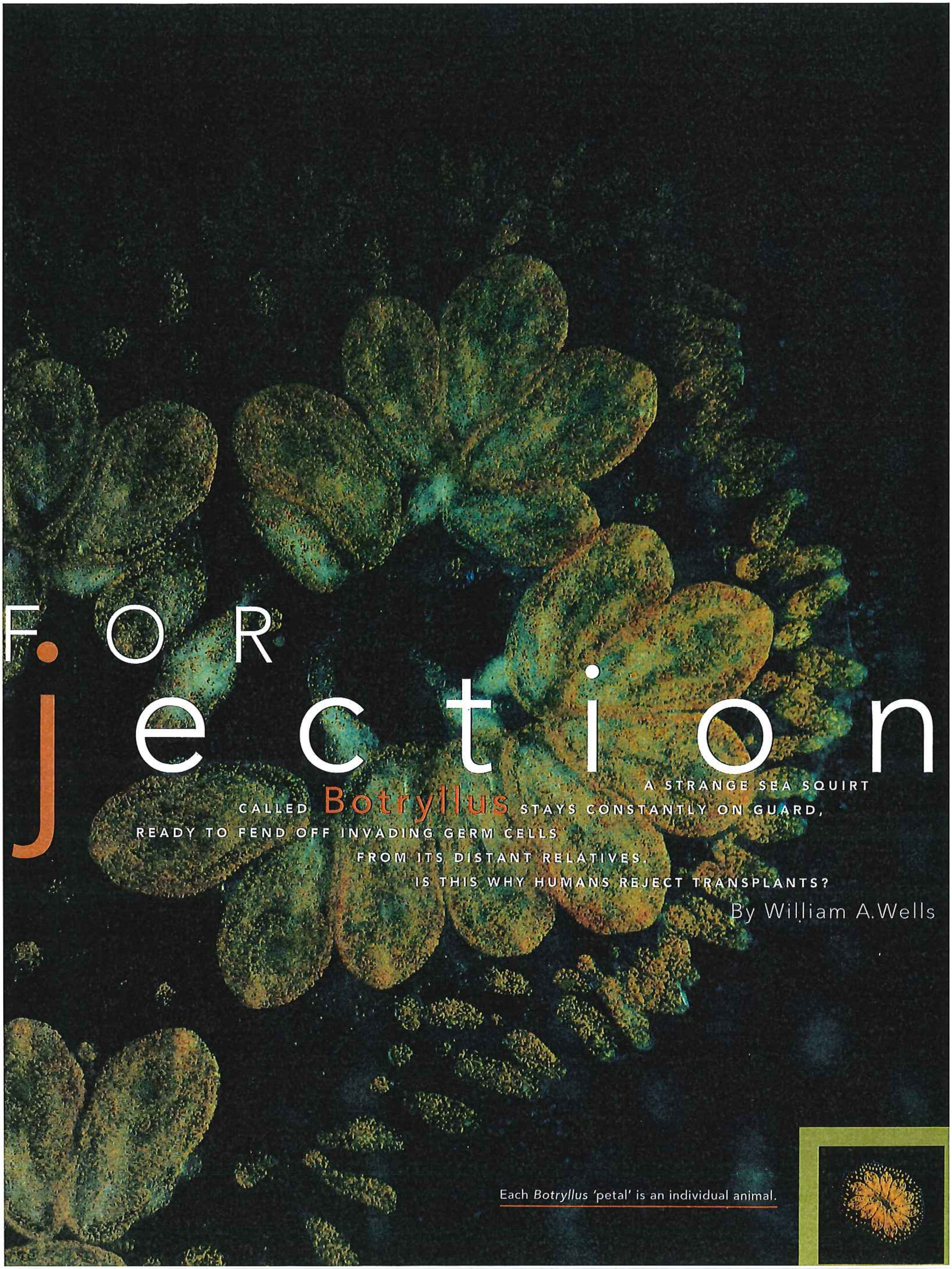


FOR jection

A STRANGE SEA SQUIRT CALLED **Botryllus** STAYS CONSTANTLY ON GUARD, READY TO FEND OFF INVADING GERM CELLS FROM ITS DISTANT RELATIVES. IS THIS WHY HUMANS REJECT TRANSPLANTS?

By William A. Wells

Each *Botryllus* 'petal' is an individual animal.

Fu/HC and determine if it's related to the immune response elements in higher animals."

Perhaps because of the evolutionary chasm between *Botryllus* and man, Weissman has not been able to isolate *Botryllus* versions of human immune system genes based on their similarity, so, he has resorted to an old-fashioned and lengthy way of tracking down Fu/HC. That hunt is ongoing, but the past decade has not been without its discoveries. While searching for Fu/HC, the team at Hopkins marine lab has uncovered the strange story of how *Botryllus* lives and works.

Life for *Botryllus* begins with a fertilized egg, whose early growth is somewhat like that of a frog. The *Botryllus* tadpole, the size of a pencil tip and complete with segmented muscles and a primitive spinal cord, then swims off to settle new territories. It lands head first and then metamorphoses like a butterfly. But this metamorphosis involves a reversal of the evolutionary clock, a step back towards primeval sea sludge. The frog-like muscles and spinal cord disappear, the tail withers away, and life as a petal-shaped filter-feeder begins.

As it munches plankton, the new *Botryllus* petal reproduces asexually, putting out buds that grow to become the other petals of the flower. The petals, each just a few millimeters long, are linked by a common blood supply and a single, central channel for disposing of filtered sea water. The petals suck in, blow out, and produce new buds, all in synchrony with each other.

The organisms don't last long. As each set of buds reaches maturity, the generation of petals that produced it simultaneously self-destructs, with a wave of cellular suicide that rolls from the front to the back of each organism. The stomachs are the first to go, and the hearts follow. The hearts of the new generation, which are already connected to the same blood system, take over the pumping duties, and the young move into the barely vacated territory, trampling on their dying parents in the process.

Every week, this cycle repeats itself. Meanwhile eggs are developing, eggs that need several weeks before they are ready for fertilization. To escape the weekly destruction, the eggs must know when to leave, then migrate through the blood vessels to take up residence in the ovaries of next week's generation. Eventually, all of the colony's ovaries open simultaneously to allow fertilization, and a week later the new tadpoles swim away.

After several cycles of producing tadpoles, the entire colony abruptly dies off. The synchrony of death remains even if parts of the colony were separated for long periods and grown under different environmental conditions. And when Weissman fused two colonies of a different age, the different parts of the colony died off at their different, pre-programmed times. All this suggests to Weissman that the lifespan of *Botryllus* is controlled genetically. A search for the genes involved may not be far off.

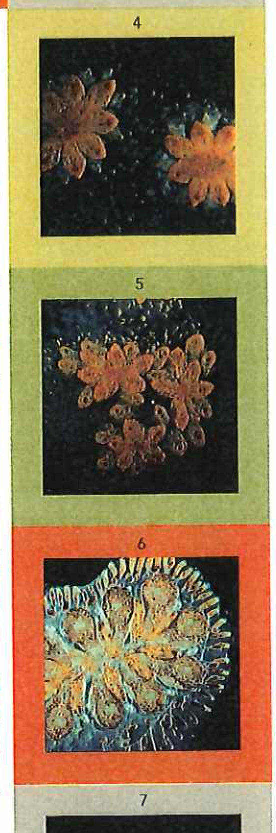
That search would be aided by the work of De Tomaso. He is constructing a genetic roadmap of *Botryllus* in an attempt to track down Fu/HC. "We can work on *Botryllus* now only because of new genomic techniques," he says. "We're going about it the old-fashioned way — by mating animals and making a map — but using these new techniques we can do 50 years' worth of 1940s work in one or two years."

The new techniques include a revised method for finding the tiny differences, or polymorphisms, that can be used as signposts to track the inheritance of different segments of DNA. Previously, polymorphisms were identified one by one, but with the new technique De Tomaso can define and look at up to 200 in one day.

Each *Botryllus* individual has two copies of Fu/HC, one from the sperm DNA and one from the egg DNA. Each parent passes only one of its copies on to its progeny, and with it goes one version of each of the polymorphisms. If the polymorphism is close to Fu/HC, the same version will always be co-inherited with the same version of Fu/HC; if it is farther away, the inheritance pattern will be random.

De Tomaso looks for polymorphisms that are more and more often co-inherited with Fu/HC and therefore closer and closer to Fu/HC, until he closes in on a small enough region to feasibly determine all the letters of the DNA that include Fu/HC. De Tomaso is now down to perhaps a few million DNA letters, or bases. Ron Davis, PhD, professor of biochemistry and director of the Stanford DNA Sequencing and Technology Center, has promised rapid-fire decoding of hundreds of thousands of bases, so the end is in sight. And although all the data have not been analyzed, De Tomaso's experiments contain the information needed to make a map with signposts for the entire genetic complement of *Botryllus*, which would enable others to find different genes such as those controlling lifespan.

Brad Magor, PhD, also a postdoctoral fellow at Hopkins Marine Station, is using an alternate approach to the hunt for Fu/HC. Going with the theory that Fu/HC is related to the vertebrate MHC, Magor and doctoral student Melinda Fagan have been cloning the *Botryllus* versions of a number of genes that, in organisms from frogs to chickens to man, are



LIFE AND DEATH among the sea squirts

1. A single system of *Botryllus*. If you think of it as a flower, each petal is an individual animal with its own heart, gut, gonads, etc. The petals, called zooids, are connected by a common blood supply that runs through a colony, which can consist of more than 100 systems connected together. The orange color is from pigmented cells in the blood. The orange dots ringing the colony are called ampullae — blood-vessel "fingers" which surround the colony and serve as the site of allorecognition reactions.

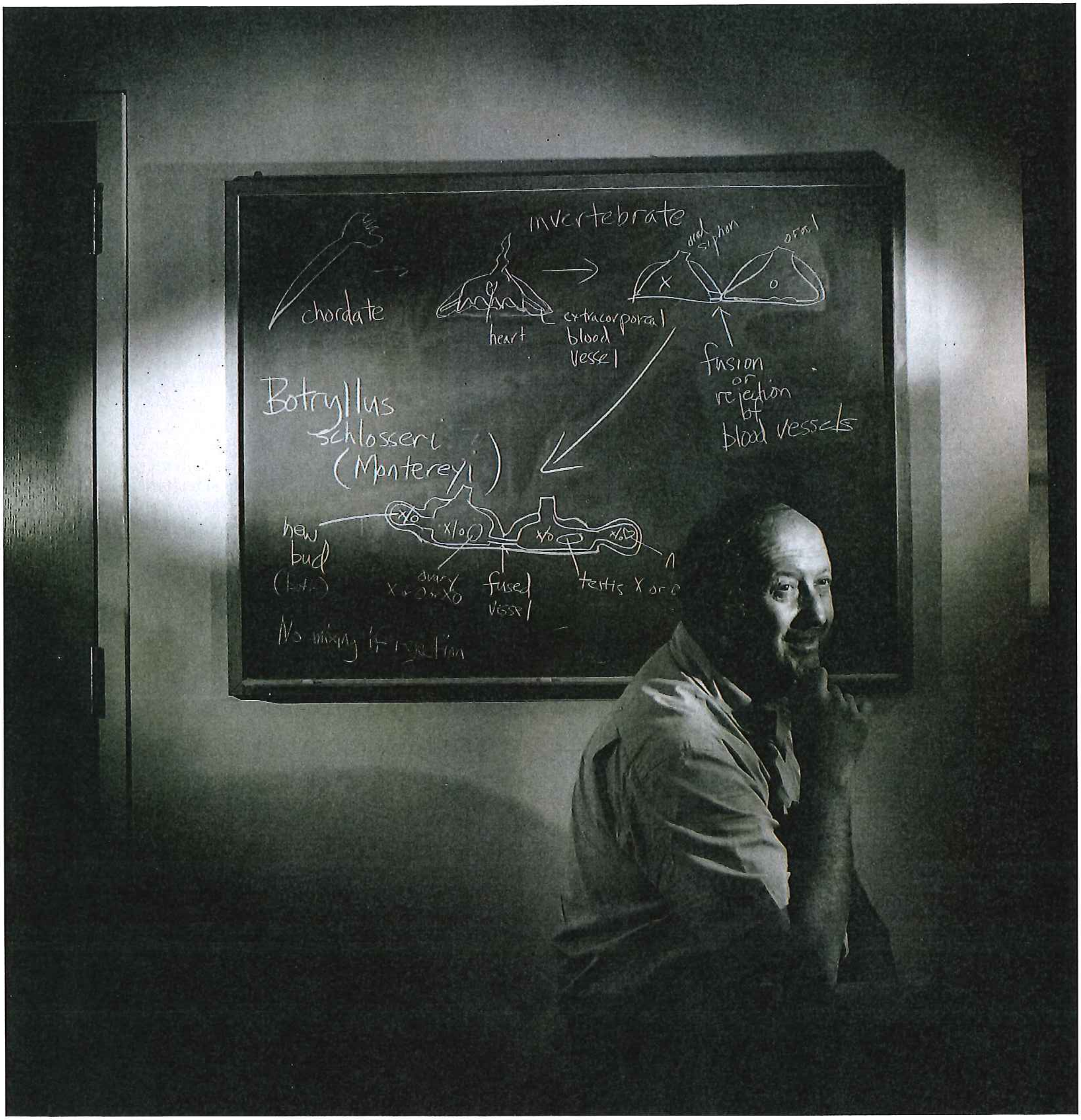
2, 3 and 4. Two systems, each showing signs of initiating the weekly cycle of asexual reproduction. The small white bulbs at the back end of each zooid are buds that will mature into adults. The buds get larger and larger.

5. The pigmented cells enter the buds, which are now about 50 to 60 percent of the size of the zooid.

6. The beginning of "takeover," a roughly day-long process in which all adults throughout the colony commit suicide. The adults have shrunk and the buds have grown much larger.

7. The very end of takeover. The buds are now adults and you can see just the rudiments of the previous generation of adults. The new adults will move and arrange themselves into a system and start budding again.

KATHI ISHIZUKA



Irving Weissman, MD

SAUL BROMBERGER AND SANDRA HOOVER

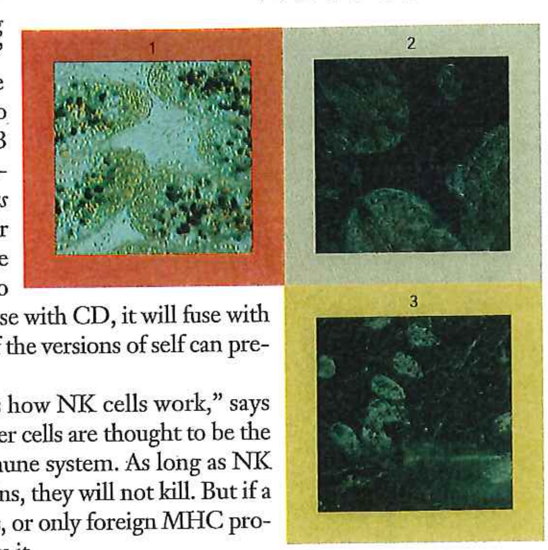
found interspersed in the genes of the MHC. Unlike the variable genes of the MHC, these genes are very important for the functioning of all cells, and therefore always look very similar, no matter which organism you look in.

Magor and Fagan are hoping that the *Botryllus* genes will be sprinkled among the genes of the *Botryllus* MHC, the Fu/HC. Unfortunately their first gene, called hsp70, is not close to Fu/HC, emphasizing the advantage of what De Tomaso calls "the no-brainer approach." According to Weissman, "you can guess and guess and guess and spend a life doing that [isolating genes that may be near Fu/HC], but with genomics you know that you can clone a gene eventually. Unless something weird comes up, it's just a matter of time and money and will."

Magor is pressing on, however, driven by another possibility. "Even if these genes aren't close to Fu/HC, " he says, "if they are close to each other, that would be interesting." That would define an MHC-like region in *Botryllus*, with a mysterious, non-Fu/HC function.

Once Fu/HC is identified, Weissman believes that uncovering the mechanics of the rejection system in *Botryllus* — the identity of the cells that do the rejecting, the way they differentiate self and non-self — will be relatively easy.

AFTER CONTACT: FUSION OR ruin?



1. Two ampullae making contact.

2. Fusion. If conditions are right, the ampullae will fuse, connecting their blood vessels.

3. Rejection. An inflammation reaction takes place at the tips of the ampullae. Blood cells pour out and begin killing each other.

Already there are clues from the genetics of rejection. "It's more forgiving than the vertebrate MHC," says Magor. The vertebrate immune system reacts to any difference, so only AB tissue is accepted by AB tissue. But when two *Botryllus* meet, if any one of their two versions of Fu/HC are the same, they will fuse. So while strain AB will not fuse with CD, it will fuse with BC. Recognition of any of the versions of self can prevent rejection.

"In vertebrates that is how NK cells work," says Magor. NK or natural killer cells are thought to be the primitive part of our immune system. As long as NK cells see self-MHC proteins, they will not kill. But if a cell has no MHC proteins, or only foreign MHC proteins, the NK cell destroys it.

The targets for NK cells are usually MHC-deficient tumor or virus-infected cells. For example, some viruses stop the transport of the MHC (and the associated viral protein fragments) to the outer coat of the cell, in an effort to hide their proteins from the T-cell surveillance system.

Recognizing self, the task of NK cells and Fu/HC, is simpler than the boundless recognition of non-self achieved by the T and B cells of the "adaptive" vertebrate immune system. And only the adaptive system has a memory mechanism that enables a larger response when the foreigner is detected a second time. So perhaps the Fu/HC system will turn out to be the predecessor of the NK system, which may in turn be the predecessor of the MHC.

A relationship between Fu/HC and NK cells would make the Hopkins team very happy. NK cells are potential tumor killers and are important in the rejection of certain transplants, yet little is known about how their proteins detect self.

Weissman is hoping that Fu/HC will supply some clues. Whatever the relation to NK cells, there must be a reason for Fu/HC's existence. The MHC and, in particular, its variability are maintained by the selective pressure of infections. But why does *Botryllus* keep the complex Fu/HC system?

Leo Buss, PhD, a biology professor at Yale University, first suggested a solution: *Botryllus* may be protecting itself from a genetic takeover of its eggs and sperm (its germ cells).

Douglas Stoner, PhD, a postdoctoral fellow in Weissman's laboratory, set out to test Buss' theory. After two *Botryllus* colonies fused, he tested to see whose DNA (as defined by the version of Fu/HC) was in their eggs and sperm. Remarkably, he found that *Botryllus* number one was often nurturing the progeny of *Botryllus* number two. The continuation of the genetic heritage of *Botryllus* number one — the whole reason for its existence and the focus of all its food-gathering efforts — had been subverted by *Botryllus* number two.

This deception occurred even after *Botryllus* number two had died. Although two *Botryllus* colonies that share only one version of Fu/HC can fuse by joining their blood systems, one of them is usually destroyed several weeks later in a process called resorption, leaving the victor to move into the open territory. But, says Weissman, "the one you see could be the genetic loser," pumping out the germ cells of its vanquished relative. "To a vertebrate biologist," he says, "that is amazing."

"We wrote, when we first described resorption, that it was a great strategy," says Weissman. After dominating more space by fusing with its neighbor, an individual *Botryllus* then uses resorption to dispose of the neighbor's genetic input. "But if you get germ-cell parasitism," says Weissman, "all bets are off."

That may be why *Botryllus* fuses only with its close relatives, so that the genetic losers will at least be supporting the generation of progeny that are very similar to themselves. This "selfish-DNA" strategy also keeps different populations distinct and so maintains the genetic diversity of the population, helping protect the animals from disease. Making big colonies by fusion minimizes the effect of predators, says Magor, but "fusing without prejudice dilutes the genetic fitness. They have to strike a balance."

Stoner found that more than germ cells were migrating to new partners: Complex organ systems with the fusion partner's DNA were appearing in the *Botryllus* colonies, suggesting that stem cells capable of making these organs are circulating in the blood of *Botryllus*. In asexual growth, such stem cells probably migrate from the dying petal to the new generation. They grow up to yield an organism that is identical to that produced by sexual reproduction, but the developmental path is completely different. "By studying asexual reproduction we are probably studying something that is akin to regeneration of tissues like the liver, or of the blood by hematopoetic stem cells," says Weissman.

Some genetic takeovers can be seen by the naked eye. Kathi Ishizuka and Karla Palmeri, the technicians in the Hopkins laboratory, noticed that one orange *Botryllus* was turning purple, and genetic testing from tissue isolated before and after the event confirmed that an unusual parasitic event had occurred. The offspring of a *Botryllus* colony often settle next to their parent and then fuse with it; inevitably the offspring lose out in the resorption process. But the chance color-change observation confirmed that, before the resorption is complete, the Oedipal offspring can foist their stem and germ cells onto their parent. "They're going at it with each

A COMMON sense

IN SOME WAYS WE ARE NOT SO DIFFERENT FROM *BOTRYLLUS*, says pathology professor Irving Weissman, MD. And he has examples to back up his assertion.

- One feature that has been documented in both mammals and tunicates is the ability to sense MHC (in rodents) or Fu/HC (in *Botryllus*) at a distance. Rick Grosberg, PhD, a professor at the University of California, Davis, found that *Botryllus* tadpoles, in an effort to maximize later fusion events, preferentially settle near other *Botryllus* with at least one shared version of Fu/HC. Presumably they detect products of Fu/HC through the water.
- At Sloan Kettering Cancer Institute, New York, researchers Ted Boyse, PhD, and Lewis Thomas, MD, found that mice take the opposite approach. In a maze test in which they cannot see their prospective partners, mice will head toward a mate with an MHC that is different from their own. This should maximize the diversity of their progeny's MHC and, thus, their disease resistance (see main story). If a female is already pregnant after mating with an MHC-identical male, more drastic measures are called for. When a male with a dissimilar MHC, or even just a cloth soaked with the urine of that male, is placed into such a female's cage, she spontaneously aborts the existing fetus so she can accept the new male's advances. As Weissman says, "It's the cruelest thing on earth."

KATHI ISHIZUKA

Douglas Stoner, left; Tony De Tomaso, center; and Brad Magor in *Botryllus* habitat at Hopkins Marine Station

SAUL BROMBERGER AND SANDRA HOOVER

other, even within their own families," says De Tomaso.

All this hijacking makes computing evolutionary effects very complicated. "Now selection is not on the basis of individuals, but on the mechanism of germ-cell parasitism," says Weissman. "The unit of inheritance is not the individual."

As Stoner and Weissman politely stated at the end of the paper announcing these findings, "We are presently at a loss to formulate mathematically the likely outcomes of such an inheritance mode." Or, as Weissman puts it, "Somebody else can handle the math."

Germ-cell parasitism also makes keeping track of Fu/HC inheritance a real challenge. Unless they separate out and track the organism from the fertilized egg stage, the researchers can never be sure whether the *Botryllus* is an individual or a mixture of individuals.

"If you like cell biology or genetics, there are a lot easier organisms to work on," says De Tomaso. "But the implications are so broad — where adaptive immunity came from, what remnants from *Botryllus* might still exist in vertebrates." Besides, from the window he can see the seals lolling on the rocks, the birds scattering as waves crash to shore, and the sun setting into the Pacific Ocean. And the *Botrylli*, he says, "are a whole lot prettier than lab mice." SM