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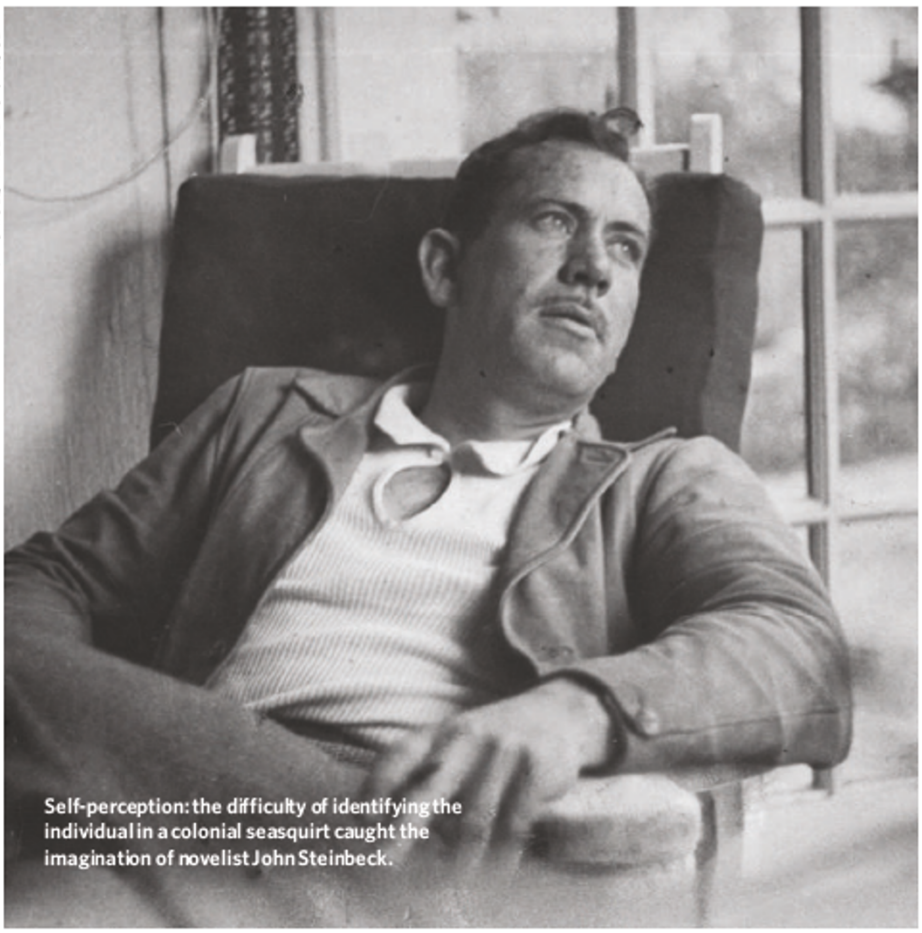
The story of *i*

Multicellular creatures can be battlegrounds for competing populations of cells. Claire Ainsworth learns how this way of looking at an individual is feeding into immunology and cancer biology.

"There are colonies of pelagic tunicates which have taken shape like the finger of a glove. Each member of the colony is an individual animal, but the colony is another individual animal, not at all like the sum of its individuals... So a man of individualistic reason, if he must ask, 'Which is the animal, the colony or the individual?' must abandon his particular kind of reason and say, 'Why, it's two animals and they aren't alike in any more than the cells of my body are like me. I am much more than the sum of my cells, and, for all I know, they are much more than the division of me.'"[1]

P. STACKPOLE/TIME LIFE PICTURES/GETTY



Self-perception: the difficulty of identifying the individual in a colonial seasquirt caught the imagination of novelist John Steinbeck.

There is something wonderful, whether for a child or a great man of letters, in the sight of a rock pool brimming with strange and secret creatures. The pelagic tunicates that so enraptured John Steinbeck when he sailed the seas off California in 1940 with his friend, marine biologist Edward Ricketts, were particularly worthy of such wonder. Today, similar tunicates are providing scientific insight into the nature of the individual, in two seemingly separate realms. Both immunologists, interested in how the self is distinguished from non-self, and evolutionary biologists, trying to establish the level of organization on which natural selection acts, have turned to the tunicate *Botryllus schlosseri* for answers.

Botryllus schlosseri is a beautiful, cosmopolitan and enigmatic creature. Its life cycle starts with a tadpole-like animal that possesses a rod of elastic tissue called a notochord — the evolutionary precursor to the backbone. This tadpole picks a suitable patch of rock or seaweed frond on which to settle and, in a bizarre and seemingly atavistic transformation, metamorphoses into a polyp-like creature. The adult then begins to bud, producing genetically identical offspring called zooids. Once mature, the zooids produce their own buds. In a coordinated weekly orgy of death and regeneration, the old zooids perish and shrink back to nothing while the new take over.

The entire colony of zooids is connected by a system of blood vessels and is sheathed in a jelly-like tunic. Underneath this mantle, the filter-feeding zooids cluster prettily like petals around communal siphons, through which they expel water. The question of where the individuality lies in such a creature is not easy to answer.

Irving Weissman, of Stanford University, California, has been studying the *Botryllus* colonies of nearby Monterey Bay for 30 years. Now an eminent stem-cell biologist, he began his career with a keen interest in immunology. His interest drew him to the strange interactions that take place place when the *Botryl-*

lus colonies infringe on each other's borders

When the blood vessels of two colonies make contact, one of two things can happen. The two systems can fuse, producing a new colony that contains cells of two different genetic make ups — a chimaera. Or an inflammatory reaction can take place in which the interacting blood vessels are destroyed and a scar is formed that prevents fusion. Whether the colonies fuse or reject each other is determined by the genetic make up of each colony. Together these responses look, to an immunologist's eyes, uncannily like the mechanisms by which a body accepts or rejects an organ transplant.

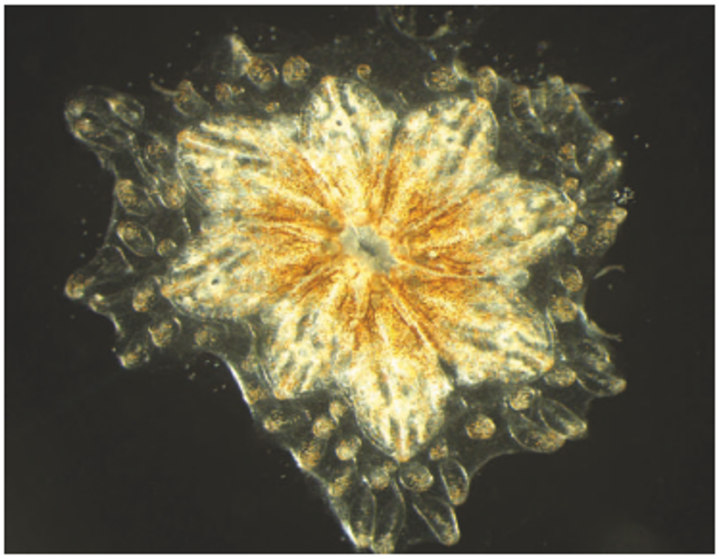
In the 1980s, Weissman's team found that the fusion–rejection decision depended on a genetically encoded system that behaved very much like the major histocompatibilty complex (MHC) in humans[2]. The MHC, which displays specific protein fragments on the surface of cells, acts a bit like an identification tag and determines whether the tissues of a human organ donor are accepted or rejected by a recipient. But why do the colonies try to fuse in the first place?

Skill sharing

One suggestion is that a combination of two (or more) tunicates is better than one. For example, in the lab, if a colony that thrives best at 15 °C merges with one that thrives at 25 °C, then the fused colony will do pretty well at both temperatures. "The chimaera is very flexible," says Baruch Rinkevich, a biologist at the National Institute of Oceanography in Haifa, Israel. Pooling talents in this way would make the colonies better able to deal with environmental change. It also means that a colony can spread itself over a larger area — handy if you get nibbled by hungry fish. But how can colonies merge their characteristics?

The answer lies in the tunicate's extraordinary ability to regenerate. The colonies can regrow themselves every seven days thanks to a particular sort of stem cell. In adult vertebrates, stem cells divide to produce both more stem cells and cells of various different types specific to a particular tissue; skin stem cells can produce various sorts of skin cell, for example. But take a tiny scrap of blood vessel from one of these tunicates, and it will regenerate the entire animal. In other words, some of the tunicate's adult stem cells behave like our embryonic stem cells; they have the power to make any cell type in the tunicate 'body'.

In creatures that develop once, such as humans, such powerful stem cells are needed only early on in development. But in a creature that regrows itself on a weekly basis, they must be on call all the time. "It's totally backwards," says Anthony De Tomaso, a former postdoc of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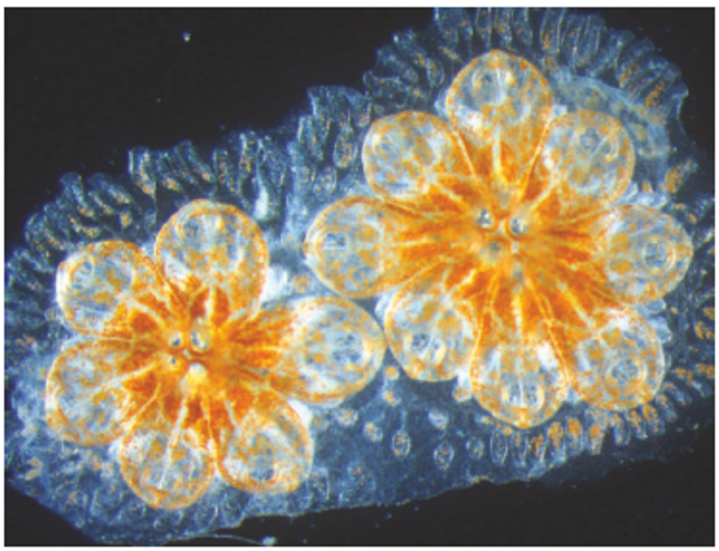
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Baruch Rinkevich (middle) sees parallels between cancer and the behaviour of stems cells of the tunicate *Botryllus schlosseri* (top and bottom), which run riot in the tissues of a closely related species.

Weissman's who has taken up the reins of the Stanford tunicate lab. "But it sets up this situation where you can have development occurring all the time."

When two colonies fuse, the stem cells of each are free to build tissues in what used to be a separate colony. This mixes up the different cells and gives the 'new' tunicate the benefit of a greater range of gene variants without all the fuss and bother of sex. But the fusion raises questions about what level of organization natural selection now acts on — the genetically distinct cell lines originating from the two colonies, or the merged colony as a whole?

It was another Weismann, differently spelled, who a century earlier argued that natural selection does not take place within the body. The nineteenth-century German biologist August Weismann divided the cells of multicellular creatures into two types: 'somatic cells', which make up almost all the parts of the body, and the cells of the 'germ line' — a small minority that produce just egg and sperm.

Crucially, he said, there is a wall — the Weismann barrier — between the soma and the germ line. This barrier ensures that somatic cells can never contribute to the next generation, and prevents any of the creature's acquired characteristics from being passed on to its descendants. Weismann's work was fundamental to the rediscovery of mendelian genetics and to twentieth-century understanding of darwinian evolution. This viewed natural selection as acting on the individual organism, with the quality of the creature's germ line tested by the fitness of its genetically identical somatic cells.

The idea of the individual as the only unit of selection has since been challenged by biologists advocating the role of higher and lower levels of organization. Group selectionists hold that evolution can select for attributes that benefit a group or species as a whole. Meanwhile, gene selectionists argue that evolution can be best understood in terms of choices between 'selfish' genes competing to copy themselves. The tale of the tunicates suggests another level, where selection acts directly on cell lines.

One champion of this idea is Leo Buss, a biologist at Yale University in New Haven, Connecticut, whose decades of work on the topic have only recently begun to be addressed by molecular biologists. Buss argues that in protists, fungi, plants and 19 of the 33 different animal phyla around today, the Weismann barrier can leak[3]. In these organisms, somatic cells can become germ cells, and thus genetic changes acquired during development within the soma can become heritable. Natural selection can thus act on competing cell lineages within the body, favouring those that get into the germ line.

One-way street

This competition began more than half a billion years ago, when a group of single-celled organisms made the great transition to forming a multicellular body. Understanding how natural selection resolved the competitive conflicts between cells, and between cells and the individual creature, says Buss, is key to understanding how multicellular organisms evolved into the forms we see today.

With Buss's work in mind, Weissman's team at Stanford traced the destiny of cells in fused tunicate colonies. The group found that, in some cases, cells from one colony seemed to completely replace the body tissues of another[4]. In others, cells from one colony sneaked into the gonads of the other, replacing the host's germ line[5,6]. In this fate worse than death, the tunicate is not just stopped from propagating its own genetic line, but effectively forced to churn out a competitor's offspring.

D. KRAFT

It is this dreadful spectre that gives rise to the tunicate's rejection reaction. To avoid being parasitized, *Botryllus* colonies have acquired a well-developed 'sense of self' — the genetically encoded tissue-matching system, now called the FuHC system, that Weissman's team found in the 1980s. One colony will only permit another to fuse with it if its FuHC tissue-matching genes are sufficiently similar. The FuHC gene, like the MHC genes, comes in thousands

THE BIGGEST PARASITE IN THE WORLD?

In 1876, a Russian vet called Nowinsky performed some rather disgusting experiments. He was interested in a strange cancer now called canine transmissible veneral sarcoma, which affects the faces and genitalia of dogs.

By transplanting tumours from the genitals of one dog to another, Nowinsky confirmed the initially surprising suggestion that this tumour was transmissible. Later, other scientists showed that the cancer was transmitted by its own cells, which have the ability to detach themselves from the tumour they start off in, in order to emigrate to a new host.

The disease probably originated from a single tumour in a single dog[9]. That dog is long dead, but the cell lineage it fostered is alive and well in the genitals of countless dogs and foxes all over the planet. It is common in strays in Japan, the United States, Europe, China, the Far East, the Middle East and parts of Africa. In some places it infects up to a third of the animals that might be susceptible. It may well be the biggest parasite in the world.

In most cases, the dog's immune system eradicates the cancer in months. But in puppies and dogs whose immune systems are depressed, the cancer spreads throughout the body — just like the *Botryllus* super-predator stem cells. Problems with the immune system might also underlie another transmissible cancer — one that is devastating populations of the Tasmanian devil in Australia. Devil facial-tumour disease spreads when the devils bite each other during fights or courtship. Like the dog tumour, it probably originated in the body of a single individual[10]; unlike the canine cancer it kills almost every devil it infects. This may be because the devil population is so inbred that catching the cancer is like getting a tissue transplant from a close relative; the cell lineage can escape the immune system's radar.

Humans, too, can occasionally catch a deadly cancer — if they receive cancerous organ transplants and their immune systems are subverted by immunosuppressive drugs. For this reason, organs are normally no longer harvested from the bodies of people who are suffering from or have died from cancer. **C.A.**

Irving Weissman (left) and Tony De Tomaso (right) have shown that a tissue-matching system analogous to that in humans operates in tunicates.

of different versions; two colonies are only likely to be a match if they are closely related. In this case, the costs of germline takeover are much diminished. If incoming stem cells do hijack the germ line, they will be closely enough related for it not to matter too much; most of the host's genes will effectively still get through.

De Tomaso and colleagues think that the FuHC operates in much the same way as the human MHC does in natural killer cells. Unlike T cells — white blood cells that actively look for foreign proteins associated with the MHC — natural killer cells look for 'missing self'; that is, for cells that lack the normal MHC. Although the molecular players appear to be different in the tunicate, the same logic seems to apply when one colony merges with another.

All creatures that can live as chimaeras, including fungi, flowering plants and primitive animals such as sponges, have some kind of ability to recognize foreign cells. Luis Cadavid, a biologist at the University of New Mexico in Albuquerque who has studied another colonial sea creature, a hydrozoan called *Hydractinia* finds that it, too, has a set of markers for self-hood. The commonality of this trait does not mean, however, that it has a shared origin. Although FuHC may function like the MHC, the sequence data of de Tomaso's team's shows that the two systems are not related[7].

"My impression is that there is a common theme to recognizing self versus non-self, but the mechanisms may have different origins," says Cadavid. Louis du Pasquier, a biologist at the University of Basel in Switzerland who works on the evolution of adaptive immunity, agrees. What the evidence points to, says Du Pasquier, is a common selection pressure — originally for a way to maintain the integrity of the self in the face of invasion, not by bacteria and viruses, but by members of your own species.

Self aware

This applies to humans too, argue De Tomaso and Weissman. Humans can play host to competing cell lines in a number of ways. For example, in an organ transplant or blood transfusion, blood stem cells from the donor can quickly colonize the recipient's body and may hang around indefinitely. But there are natural ways too. Although not an example of chimaerism, cancer involves a particular line of somatic cells declaring an independent identity from the body around it. In order to avoid alerting the adaptive immune system to their aberrant nature, some cancer cells stop expressing MHC genes.

Rinkevich and his team have studied, as an analogous process, cells from *Botryllus* running riot in the bodies of a closely related species of tunicate called *Botrylloides*. "It's like cancer," says Rinkevich. "It does have parallels, no doubt about it," agrees De Tomaso. "Cancer is somatic cell parasitism. It is selection inside a body for a variant."

Weissman thinks that *Botryllus* has a lot to teach us. Cancer researchers now think that our bodies' stem cells play a key role in both the origin and development of cancerous tumours[8], and Weissman sees intriguing parallels between such stem cells and the most highly successful of *Botryllus*'s 'winner' stem cells. He is keen to uncover the genes that give these cells their super-predatory powers. "I wouldn't be surprised if, when we finally isolate these genes, related genes are found in pathways that allow cancer cells to develop," he says.

> "Everything I see suggests that there is selection for you to be as rare as possible."
> — Anthony De Tomaso

Cancer is not generally a route to true immortality, because it is normally limited to a single body with a finite lifespan (although not always, see 'The biggest parasite in the world?'). To persist beyond this, stem cells need to get out of the soma and into a germ line — any germ line. In theory, the easiest way for this to happen would be through pregnancy. Scientists have known for years that in many mammals, stem cells can cross from fetus to fetus in multiple pregnancies. Stem cells can also break out from fetuses into the mother's blood. Most are hunted down and destroyed by the mother's immune system, but usually some remain — although no one knows why or how. A woman who has had several children could be a mixture of many different cell lineages.

Such embryonic stem cells could in principle colonize the germ lines of subsequent embryos, or even, conceivably, the mother's germ line. But germline chimaerism seems not to happen. And to Weissman, that implies that our MHC-based systems of selfhood, like those of the tunicates, know how to stop such takeovers. "The fact that we don't all walk around as germline chimaeras would imply that there is a protection mechanism" says Weissman.

De Tomaso suggests that the need to stop these cells from taking over could perhaps explain a long-standing mystery in immunology. The diversity seen in MHC genes is much greater than can be explained by the needs of the adaptive immune system. Perhaps the threat of stem-cell parasitism is to blame? So far, de Tomaso admits, the idea is just speculation, and there are a number of other theories that attempt to explain MHC diversity. But he's still intrigued by the idea that the need to distinguish ourselves might be a key driver of natural selection at the level of the organism, pushing people towards uniqueness. "Everything I see," he says, "suggests that there is selection for you to be as rare as possible."

And so the maintenance, even exaggeration, of individuality in humans and the circumstances under which other creatures will surrender themselves to greater groups may be underpinned by common mechanisms. The levels of the self, the cell and the gene all interact under rules that were laid down half a billion years ago, and yet are invigilated round the clock today. Understanding the drives of the self, the cell and the gene, and recognizing that cells are not just inert building blocks but entities with at least a capacity for selfdom, is, as Steinbeck wrote when pondering his tunicates, "the basis for a far deeper understanding of us and the world". ■

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1. Steinbeck, J. *The Log from the Sea of Cortez* (Penguin, 2000).
2. Scofield, V. L., Schlumpberger, J. M., West, L. A. & Weissman, I. L. *Nature* **295**, 499–502 (1982).
3. Buss L. W. *Proc. Natl Acad. Sci. USA* **80**, 1387–1391 (1983).
4. Stoner, D. S. & Weissman, I. L. *Proc. Natl. Acad. Sci. USA* **93**, 15254–15259 (1996).
5. Stoner, D. S., Rinkevich. B, Weissman, I. L. *Proc. Natl Acad. Sci. USA* **96**, 9148–9153 (1999).
6. Laird, D. J., De Tomaso, A. W. & Weissman, I. L. *Cell* **123**, 1351–1360 (2005).
6. De Tomaso, A. W. *et al. Nature* **438**, 454–459 (2005).
7. Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. *Nature* **414**, 105–111 (2001).
8. Katzir *et al. Oncogene* **1**, 445–448 (1987).
10. Pearse, A.-M. & Swift, K. *Nature* **439**, 549 (2006).