

DEMOGRAPHY

Sardinia's Mysterious Male Methuselahs

More men live past 100 on this Italian island, proportionally, than anywhere else, it appears. Scientists are now trying to explain why

SASSARI, ITALY—When Antonio Todde celebrated his 112th birthday on 22 January in the tiny village of Tiana in the Sardinian mountains, among the hundreds of pilgrims who journeyed to the frail shepherd's house to wish him *akentannos*—congratulations for living to a century—were a half-dozen Italian scientists and a Belgian demographer. The hairpin turns on the narrow road into Tiana may have given the longevity researchers gray hairs, but the harrowing trip was worth it. Nowhere else in the world, they believe, does a larger proportion of men survive to the ripe old age of 100 than on this sunny Mediterranean island. Todde—the world's oldest living man, according to *The Guinness Book of World Records*—and his long-lived Sardinian brethren could hold clues to the secrets of long life.

In countries with reliable data, five women reach the century mark for every man who reaches this milestone. Two years ago, researchers here at the University of Sassari found that on Sardinia, the female-male centenarian ratio is only about two to one. And in Sardinia's mountainous interior, there are roughly equal numbers of 100-year-old men and women. "The proportion is remarkable," says Claudio Franceschi, Italy's leading centenarian researcher and an immunologist at the University of Bologna—which, as the world's oldest university, is a fitting venue for longevity research. Because relatively few people have moved to Sardinia's villages, he says, the island's genetic isolation makes it "a perfect place to pursue research into complex traits such as longevity."

Whereas many past claims of extreme male longevity around the world have withered under scrutiny, the Sardinia data appear to be holding up. That's why researchers are

now beating a path to Todde's door, and to many other homes in the Sardinian uplands, to take blood samples and quiz the centenarians about their genealogies and lifestyles. A clear explanation for what sets these men apart has not yet emerged, but there are hints that genetic factors—perhaps even inbreeding—may play a role. "Centenarians are the



Toast to long life. If it isn't the Sardinian wine that keeps Antonio Todde (above) and the Brundu brothers going, could it be something in their genes?

pioneers on the frontiers of survival," says James W. Vaupel, director of the Max Planck Institute for Demographic Research in Rostock, Germany. "By studying these pioneers, we can learn a lot about why they reach such extreme age and perhaps [about] what can be done to help others reach that age."

A checkered past

Although no one knows the exact number, as many as 100,000 centenarians worldwide may have witnessed the dawn of the new millennium. Vaupel and other demographers agree that the ranks of 100-year-olds are rising rapidly as nutrition and health care improve. Still, a minuscule fraction of the population attains supercentenarian status—

someone like Todde who has lived past the age of 110. While no biblical Methuselah, said to have lived more than 900 years, the oldest person today is a 114-year-old Frenchwoman. But the numbers of supercentenarians are on the rise, according to Jean-Marie Robine of INSERM's demography institute in Montpellier, who helped validate the world's most long-lived person according to verifiable records, Jeanne Calment, who died in 1997 at the age of 122.

At first, European demographers dismissed the Sardinian longevity numbers as too fantastic to be true. At a meeting in France in October 1999, Vaupel recalls, "I stood up and said: 'There's no need to present these results, because they are almost certainly false.'" He had good reason to be suspicious. Similar claims of extreme longevity—among populations in the Caucasus, the Andes, western China, and on the island of Okinawa—all fell apart because they could not be documented. Even past U.S. censuses have exaggerated the number of American centenarians. The 1980 census, for example, listed more than twice what demographers believe to be the true number due to errors such as elderly people misreporting their ages or 4-year-olds reported as 104 because their birth years were marked wrongly. Several projects are gathering more robust data on centenarians (see table), and top centenarian

researchers around the world have banded together to form the Alliance for Research on Longevity and Exceptional Survival.

In light of the research area's checkered past, Vaupel cut a deal with Franceschi to split the cost of sending a crack historical demographer, Michel Poulain of the Catholic University of Louvain in Louvain-la-Neuve, Belgium, to vet the Sardinian numbers. After shuttling to the island last year to examine birth, marriage, and other records of



about three dozen of Sardinia's known centenarians, Poulain announced last October that those age claims were, in fact, checking out. "This was a shock for people who were supposing that the data were false," says Poulain, who adds that Sardinia's excellent records have allowed him so far to verify the ages of more than 50 of Sardinia's centenarians, including most of the oldest men. "This is really striking," says Vaupel. "It's the first case I know of an alleged male-longevity region looking plausible to demographers."

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An Island of 'Genetic Parks'

NUORO, ITALY—When the Romans invaded the island of Sardinia in the second century B.C., they called its rugged interior, where the native nuragic tribes had taken refuge, the land of barbarians. Today's descendants of those warriors are welcoming, not fighting, the latest wave of invaders: scientists mining the genetic riches of these isolated communities.

The first major genetic campaign has been mounted in Talana, a remote village near the Gennargentu Mountains southeast of Nuoro. While longevity researchers have zeroed in on a few dozen unusual individuals scattered across the highlands (see main text), molecular geneticist Mario Pirastu has turned the entire village of Talana into a "genetic park." The goal is to probe for genes that might contribute to illnesses common in Sardinia, including diabetes, kidney stones, and asthma.

Focusing on such Sardinian towns makes sense, says David Schlessinger of the U.S. National Institute on Aging's gerontology research center in Baltimore, as they often were settled by a few individuals. The small genetic variability of so-called founder populations, says Schlessinger, who has teamed up with a rival group of Sardinian scientists to look at other mountain villages, "makes the work of geneticists simpler."

Sardinia's genetically isolated populations, adds Pirastu, offer a more precise target for gene hunters than larger surveys such as one now under way in Iceland by deCODE Genetics Inc. (*Science*, 1 January 1999, p. 13). "In Iceland, they are examining the whole island," says Pirastu. "But here in Sardinia, we think that we can get the same sorts of results using maybe 5000 people in a few villages. You don't have to start with a half-million people."

With the support of Talana's 1200 residents, Pirastu—who heads the Italian national research agency's Institute of Molecular Genetics in Alghero—has spent 6 years building a research clinic, tracing the entire town's family tree back 400 years, canvassing health information, and taking DNA samples from every adult inhabitant. Now collaborators are helping to map the frequency of



Genetic Club Med. Researchers are flocking to Sardinia to study its isolated populations. The first major project is in Talana.

genetic polymorphisms, or variations, that Pirastu hopes to link to diseases. "His project looks very promising," says molecular geneticist James L. Weber, whose group at the Marshfield Medical Research Foundation in Wisconsin is one of two contracted to do the gene mapping. "We'll learn a lot about the population genetics of the village and maybe about Sardinia in general," he says. Weber's group and a team in Edinburgh together will sequence 1600 markers for each adult Talana inhabitant.

Some Italian critics contend that the Sardinians may not fully understand the project's implications and therefore might be ex-

ploited. Pirastu, however, says that villagers sign privacy waivers, and that Talana and other towns enrolled in the study get new clinics, free medical testing, and jobs connected with the project. The villagers, he notes, are aware of the study's goals: Pirastu says he gets written permission from each resident "every time we collect a new blood sample or clinical test." The villagers, he claims, "are happy to collaborate, because they feel they are part of something important."

Pirastu's research has attracted the attention of Sardinia's wealthiest man—Internet entrepreneur Renato Soru—who

last year put up most of the \$5 million in capital for a start-up company, called Shar.DNA, that is now discussing how to commercialize any findings from the mountain villages. Italy's national research agency—which could use its share of any future profits to beef up the research—is a partner in the firm, as are Soru and Sardinia's biggest bank. Pirastu is Shar.DNA's scientific director, but he says he holds no shares in the company.

Pirastu already has expanded the "genetic park" experiment well beyond Talana. The town of Perdasdefogu has given him a \$1 million grant and lab space to start plumbing the genetics of its 2400 inhabitants, for example, and he has extended his studies to several other isolated villages with populations of between 1000 and 2000. His group will soon publish a paper linking a region of chromosome 10 with kidney stones. "Have we found a gene? Not yet," says Pirastu. "But we think that we are very close. Our fingers are crossed."

—R.K.

Not everyone is fully convinced. "I'm still a bit skeptical, even though Poulain has done a fine job so far," says Odense University's Bernard Jeune, who heads up Denmark's centenarian project. He, Poulain, Vaupel, and several Italian scientists are planning to join forces on a study to fully verify all the age claims and extend the demographic analysis to Sardinian men in their 80s and 90s. Whereas female mortality among Sardinian women older than 80 "is about the same as elsewhere," Vaupel says, male mortality after age 80 "appears to be substantially lower than elsewhere." If the numbers are correct, Jeune says, "the mortality figures for the men over age 80 are

amazing." Indeed, the exceptional survival of males over 80 accounts for the large number of male centenarians on Sardinia.

Programmed for long life?

Some experts assume that a healthy, low-stress, agrarian lifestyle is the main reason why Sardinian centenarians have outlived most of their peers. "I worry about focusing so much attention on centenarians," says molecular geneticist Mario Pirastu of the Institute of Molecular Genetics in Alghero, who is also conducting genetic research in central Sardinia (see sidebar). "I suspect that most of the reasons for their longevity may turn out to be the style of life." Several stud-

ies of elderly, noncentenarian twins in Scandinavia suggest that only about 25% of the variance in adult longevity is attributable to genetic differences, says Vaupel. The other 75% seems to be related to early life events and a person's current environment.

But no one knows for sure whether that holds for centenarians. "It is possible that genetic factors are more important for them," Vaupel says. And if lifestyle factors are the answer, it's not clear what they would be. After helping interview more than 100 Sardinian centenarians, Gianni Pes and Ciriaco Carru of the University of Sassari point out that some of the men—while leading otherwise low-stress, outdoor lifestyles—were

once heavy smokers and weren't exactly risk adverse in their youth, having fought in one or both of the 20th century's world wars.

Franceschi and his Sardinian colleagues suspect that genetics underlies the unusual male longevity, which appears to run in families. Take the case of the Brundu brothers—Pietro, 103, and Antonio, 101—who share a house in the village of Erula. Whereas Pietro's health has declined in recent years, the dapper Antonio—a confirmed bachelor—is a raconteur who regales visitors with stories about his exploits as a policeman. Asked the secret of his longevity, Antonio laughs: "I have no explanation, other than a perfectly normal life." Todde attributes his longevity to a carefree attitude, but as Vaupel points out, "there are a lot of people with a good attitude toward life who drop dead at 50."

The Italian researchers are hoping to get more provocative answers by interrogating centenarian genes. Geneticists Giovanna De Benedictis of the University of Calabria and L. Luca Cavalli-Sforza of Stanford University are examining gene variations, or polymorphisms, of the male Y chromosome in Sardinian centenarians to see if these differ markedly from those of younger Sardinian men. In the general population, some Y chromosome polymorphisms are associated with a reduced likelihood of fathering children, says De Benedictis. Given the known trade-off between fertility and longevity, variations in Y chromosome genes, she says, could "in principle play a role in the high number of male centenarians found on the island."

De Benedictis has also teamed up with human geneticist Giuseppe Attardi of the California Institute of Technology in Pasadena—an expert on aging and mitochondrial DNA (mtDNA), which is inherited from the mother—to examine the mtDNA of centenarians. They're testing whether mtDNA gene variations protect cells against the ravages of aging. After examining mtDNA from 212 Italian centenarians and a control group, the team has found that the frequency of the J haplogroup—one of the groups of mtDNA types that population geneticists use to reconstruct human evolution lineages—"was notably higher in centenarians than in younger individuals," De Benedictis says. In male centenarians, the frequen-

cy was about 20%, versus about 2% in younger Italian males. Researchers are now trying to explain how the J haplogroup could be linked to long life.

One surprising indication from the research so far is that inbreeding, long known to increase the chances of inheriting recessive genes that can be detrimental to health,

sponse, centered on T and B lymphocytes, tends to deteriorate with age, the body's innate immunity—in which macrophages gobble up foreign proteins and cells—appears to improve, like a fine wine. "This phenomenon starts at about 60 to 70 years of age," says Franceschi, who believes that "those who live longer ... are able to adapt

THE CENTURY CLUB: A SAMPLING OF MAJOR CENTENARIAN STUDIES

Country	Institution and lead researcher	No. of centenarians studied (approximate)
China	The Chinese National Research Center on Aging, Peking Univ., Duke Univ., NIH, Max Planck Institute for Demographic Research; Zeng Yi, James Vaupel	4900
Denmark	Odense Univ., Bernard Jeune	275
France	IPSEN Foundation, Jean-Marie Robine and Michel Allard	900
	Supercentenarians database, Montpellier, France, INSERM; Robine and Vaupel	35
Italy	National Research Council and Ministries, Claudio Franceschi	2000
U.S.	New England: Harvard Univ., Tom Perls	650
	Georgia: Univ. of Georgia, Leonard Poon	140



Intriguing clues. Claudio Franceschi's work suggests that in some old people, innate immunity kicks into overdrive.

may actually help the Sardinian men live longer. In Sardinia's remote mountain villages, most residents are descendants of a few founding families, and preliminary data suggest that they do, indeed, have less genetic diversity than the general population. "At first glance, the finding seems to be counterintuitive," says Franceschi. One possible explanation could lie in the fact that extreme longevity itself is not a logical consequence of evolution: If life's *raison d'être* is to reproduce, excessively old individuals—well past their prime reproductive years—offer

little if any biological benefit to a population. "Longevity is a trait with some peculiarities because of the unnecessary nature of aging," says Franceschi.

Another interesting concept from the Italian research is that, in certain individuals, the immune system may adapt to aging. According to Franceschi, this "complex reshaping of the immune system" in extremely old persons tends to follow this pattern: Whereas the most sophisticated immune re-

sponse, centered on T and B lymphocytes, tends to deteriorate with age, the body's innate immunity—in which macrophages gobble up foreign proteins and cells—appears to improve, like a fine wine.

Other immunologists, including Beatrix Grubeck-Loebenstein of the Institute for Biomedical Aging Research in Innsbruck, Austria, have noticed similar immune-system "shifts" in certain healthy patients over age 65. When such shifts occur, says Georg Wick, an Innsbruck colleague who edits the journal *Experimental Gerontology*, the body's innate immunity "is not only preserved, but even compensates for the deteriorating response of the specific immunity."

Years of work lie ahead to decipher any lessons encrypted in the centenarians' unusual genetics. "When I first started this work a dozen years ago, centenarians were considered a rare curiosity," says Franceschi. "Now they are important subjects of research." He and his colleagues know the clock is ticking. Besides drawing blood from the oldsters, a team led by Giovannella Baggio and Luca Deiana has been banking DNA samples and taking detailed medical, genealogical, and mental-health histories.

Is the Fountain of Youth hidden in the verdant Sardinian mountains? Probably not, unless that vital liquid is the local red wine, which all the old men seem to enjoy. Asked whether he had learned any secrets of longevity from his 103-year-old father, 75-year-old Italo Brundu smiled and said: "I touch him every day, and I hope it rubs off."

—ROBERT KOENIG

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