A GENE FOR MALENESS

TEXT: ANDREAS LORENZ-MEYER

Brown algae are outsiders – neither plant nor animal, neither fungus nor bacteria. Their unique position in the tree of life makes them very interesting to Susana Coelho and her team at the Max Planck Institute for Biology in Tübingen. The researchers want to find out whether evolution has taken two different paths to the same important innovation: the emergence of female and male individuals.

Brown algae rarely make the news, and when they do, lately the headlines have been negative. The reason for this is the "Great Atlantic Sargassum Belt," an ever-growing carpet of brown *Sargassum* algae that has formed in the Atlantic every spring since 2011. It has since spread to the coasts of Florida and Mexico, where it threatens tourism and fishing.

Although *Sargassum* has yet to reach Tübingen, the incubators at the city's Max Planck Institute for Biology host a close relative: the brown alga *Ectocarpus*, which forms small, brownish clumps in glass flasks filled with seawater. The shapeless structures are gametophytes – the sexual phase, or generation, that produces eggs and sperm. Like mosses, ferns, and seed plants, brown algae alternate between sexual and asexual reproduction. A mostly unremarkable sexual generation is followed by one in which the algae use spores to reproduce asexually. In the case of *Sargassum*, the asexual generation is the one that is highly visible in the sea, sometimes covering vast areas.

Brown algae split off from green and red algae a billion years ago. A green algal ancestor went on to evolve into land plants, while brown algae went their own way. Their name derives from the brown pigment fucoxanthin, which is produced in the chloroplasts along with chlorophyll and conceals the latter's green pigment. The asexual generation takes a variety of forms depending on the species, ranging from small, branched cell filaments to organisms measuring several meters. The latter form extensive kelp forests along coasts.

Susana Coelho has been researching brown algae for years – first at the Station Biologique de Roscoff in Brittany, where the organisms populate the Atlantic coast, and starting in 2021, at the Max Planck Institute. In the Tübingen labs, incubators provide *Ectocarpus* with steady temperatures and precisely regulated artificial light. "Brown algae are tremen-

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PHOTO: SUSANA COELHO/MPI FOR BIOLOGY TÜBINGEN

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The brown algae Halopteris congesta is found in tropical oceans. The fan-shaped cell filaments of the algae are easy to recognize under a microscope.

PHOTO: JEAN-CLAUDE WINKLER

Susana Coelho at her lab in Tübingen. This is where she hopes to learn whether evolution took the same path more than once in producing male and female individuals.

dously adaptable and inventive. They perform photosynthesis and have leaves, stems, and claw-like structures resembling roots. They look like plants, but they're not. And they don't belong to the animal or fungi kingdom either."

Nature's misfits

That makes brown algae evolutionary mavericks. They have blazed their own trail to multicellularity, developing an ability to use the Sun's energy much like plants, but independently of them. Long ago, a single-celled ancestor of brown algae absorbed a photosynthetic red alga, which then provided its symbiotic partner with energy from sunlight. This formerly independent red alga gradually became the chloroplast of brown algae. The chloroplasts of plants, by contrast, originally came from free-roaming cyanobacteria.

Their independent evolution is what makes brown algae such promising research subjects for Susana Coelho. "They help us understand how evolution works. In the case of brown algae, for example, if we compare the genes that were crucial for the formation of female and male individuals with those of organisms of other lineages, we can find out whether the algae used the same genetic toolbox.

Susana Coelho and her team at the Max Planck Institute in Tübingen are particularly interested in the sexual phase of the life cycle of *Ectocarpus*. They want to find the gene that determines whether a brown alga is male or female. A comparison with the gene that determines sex in other groups of organisms will help provide clarity.

Did brown algae inherit their type of sexual development from the last ancestor they share with other groups, or did they find a different form? "Hopefully one day we will find out if evolution determines sex across all lineages with the same master gene."

The researchers have already discovered a candidate for the maleness gene. It resembles the SRY gene, which determines maleness in mammals. SRY stands for sex-determining region Y gene. It seems that the same kind of master gene, or a similar molecular mechanism, determines the sex of an individual brown alga just like with other lineages. Recently, the group described that an asexual individual emerged when removing the male master candidate from the *Ectocarpus* genome using the Crispr/Cas9 system, providing evidence that it is indeed the sex-determining gene.

Male algae with eggs

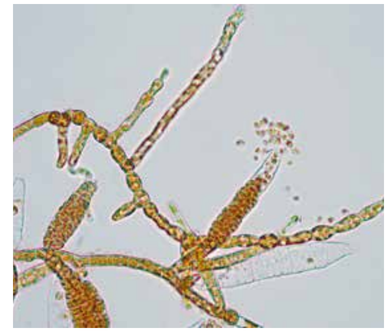
When it comes to sex, brown algae are flexible. For example, some male individuals are able to produce eggs as well as sperm, which might be an evolutionary step towards self-fertilization. This would enable them to reproduce even if they are unable to find partners in the vastness of the ocean. But how do males become such hermaphroditic creatures? That is what Daniel Liesner wants to find out. His approach is to study not *Ectocarpus*, but a brown algae called *Laminaria pallida*. Liesner brought presumably bisexual specimens of this species from southern Africa. He holds up a Petri dish containing filtered sea water. There is nothing to see at first, even though there are a few hundred male *Laminaria* floating in the dish. "The individuals of the sexual generation are tiny filaments of cells measuring 10 to 40 micrometers in size. Only later do they develop into sporophytes, that is, the asexual generation. These are the big brown algae we see on the beach," explains Liesner. The biologist places the Petri dish under a light microscope and focuses on one of the irregularly shaped brownish cell clusters. It is fringed here and there by small, whitish balls – the holders in which sperm are produced.

SUMMARY

Brown algae form their own lineage. They belong neither to the plant nor the animal kingdom.

They have a gene in their sex chromosome that determines whether an individual produces sperm or eggs. By comparing this gene with its counterpart in other groups of organisms, researchers hope to determine whether evolution followed the same path twice when determining sex.

Brown algae are of paramount importance for biodiversity and the global climate. One cause of their mass proliferation in some regions is the over-fertilization of the seas.

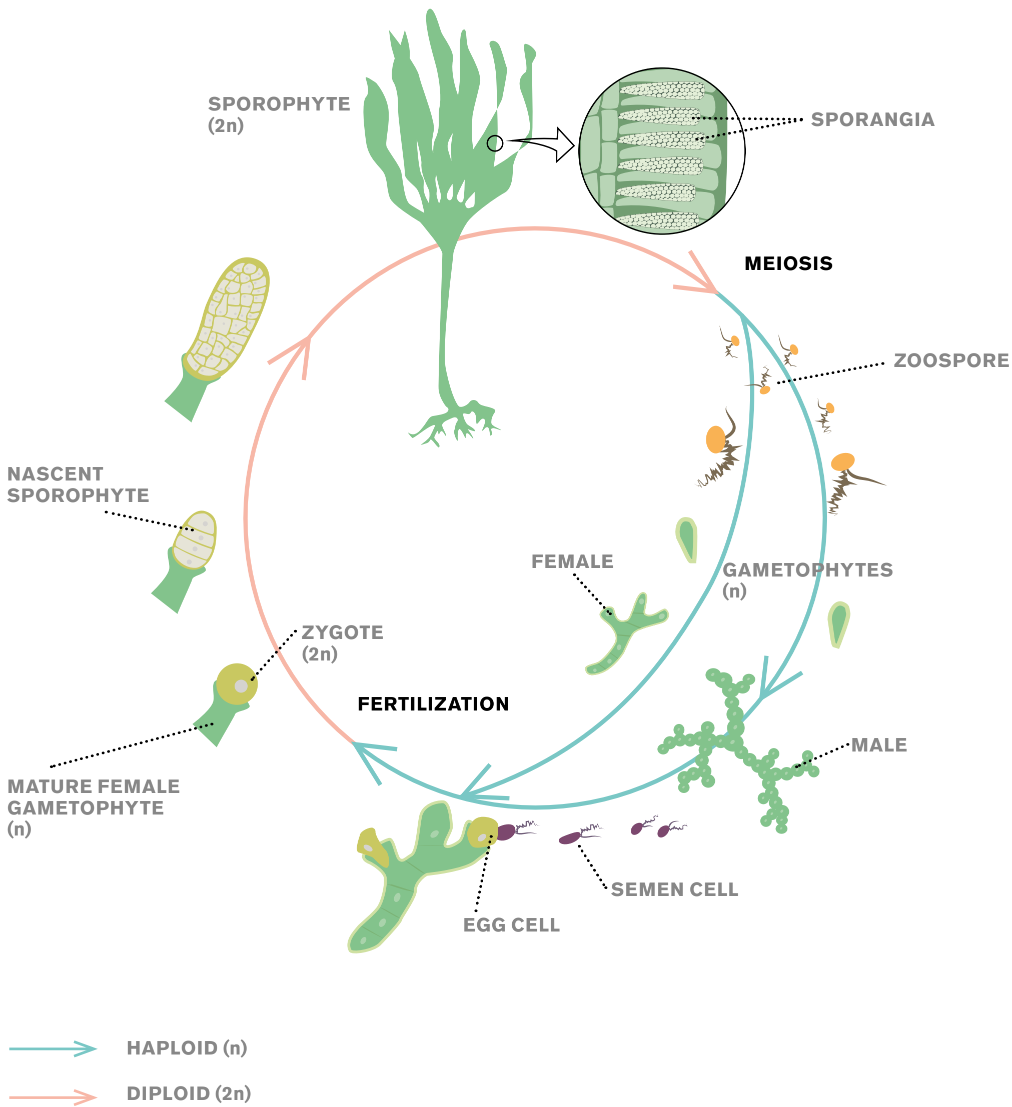


The sporophyte, or asexual generation, of an *Ectocarpus* alga releases spores from a spore holder. Whip-like appendages enable the spores to move.

PHOTO: DR. SUSANA COELHO/MPI FOR BIOLOGY TÜBINGEN

None of this is unusual for a male brown alga. However, the specimens in Liesner's dish have a special feature: a cell that is much larger and darker than the others. "If this is a functional egg, the male individuals would actually be hermaphrodites," says Liesner. The cell looks like an egg under the microscope, but tests have confirmed that only the male sex chromosome is present in the culture. It is yet unclear whether the egg (if it is one) is functional – in other words, whether the individual could fertilize itself.

In any case, brown algae have mastered additional forms of reproduction without the opposite sex. And these are being studied in Tübingen. Populations consisting exclusively of females have been discovered in the oceans. They reproduce clonally when unfertilized eggs divide inside them, giving rise to asexual sporophytes without the fusion of egg cells and sperm. This allows them to cir-



Reproductive cycle of the brown *Laminaria* alga, alternating between sexual and asexual reproduction.

The highly visible algal body of the *Laminaria* is the sporophyte, or the asexual generation (above). Spores produced in special holders undergo a unique form of cell division (meiosis) in which the genetic material is divided in a way that leaves each spore with only a single set of chromosomes (n). The spores grow into tiny little male and female gametophytes, which form the gametes. The zygote resulting from the fusion of semen and egg cells once again has a double set of chromosomes (2n) and goes on to develop into sporophytes.

GRAPHIC: GCO, ADAPTED FROM CAMPBELL BIOLOGY, 10TH EDITION, PEARSON

cumvent a problem faced by populations that reproduce sexually in the vastness of the oceans. How do sperm and eggs find each other? The egg cells help by releasing attractants into the water to attract sperm. Identifying these pheromones is another goal of the researchers. To this end, they study the air from the glass flasks where the algae grow in incubators at the Tübingen lab. The molecules of fragrance diffused from the algae water into the layer of air above it are filtered out and their chemical composition is analyzed using mass spectrometry. The pheromones even have a noticeable odor. "They smell like gin," says Susana Coelho.

Without a doubt, brown algae are fascinating organisms. And when they reproduce at normal levels, they are of paramount importance for the Earth's biodiversity and climate. Kelp forests provide a habitat for countless species and bind huge quantities of carbon dioxide, which is why they are called the rainforests of the oceans. The carpets of brown algae that swamp the coasts every year show how much brown algae ecosystems are already out of balance. This happens because large quantities of fertilizer used in agriculture ultimately end up in the sea, where they increase the concentration of nutrients. "This allows the *Sargassum* algae to multiply much more than before," explains Susana Coelho. Changes to ocean currents due to climate change could also have paved the way for *Sargassum* to spread to new regions of the world. "Ultimately, there is only one cure. We need to drastically reduce nutrient input to the oceans."