

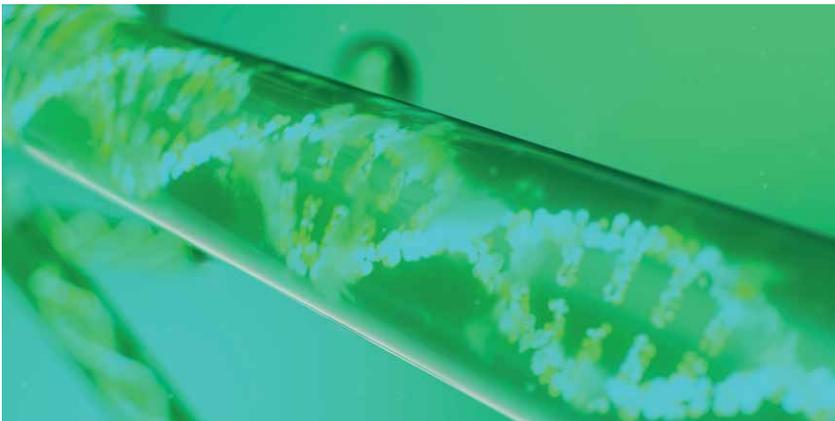


**Fern Genomics: Unfurling
the Mystery of Plant
Chromosome Numbers**

Dr Paul G. Wolf

FERN GENOMICS: UNFURLING THE MYSTERY OF PLANT CHROMOSOME NUMBERS

Plants are more tolerant of changes in their chromosome number than animals. Even dramatic changes, such as doubling of the entire genome, sometimes leads to beneficial outcomes. Though a history of genome doubling is common in most plants, the chromosome number in many plants does not reflect this. Complex genome downsizing processes help these plants shed extra genetic information, but are poorly understood. Through comparisons with ferns – a group with high chromosome numbers – **Dr Paul Wolf** from the University of Alabama in Huntsville aims to shed light on genome downsizing.



Plant Reproduction and Evolution

From mountains to the sea floor, plants dominate most of the planet's ecosystems. Over millennia, plants have evolved with a vast array of sizes, shapes, physiologies and reproductive strategies.

Ferns and mosses represent ancient groups of plants, with many characteristics distinct from those that evolved later, such as flowering plants. In particular, the evolution of the seed, which includes a protective layer for the embryo, has allowed more recently

evolved plants to thrive in drier habitats. Ferns, by contrast, do not produce seeds, instead relying on two separate and distinct life history phases to achieve reproduction.

In most ferns, large fronds produce spores which disperse to new areas, carried by wind currents. A smaller, but independent, life phase develops from these spores to produce eggs and sperm. In contrast, the egg and sperm-producing life stage has been dramatically reduced to only a few cells within flowering plants.

The spores that different plant groups produce also differ, depending on their evolutionary history. All seed-producing plants and some ferns are 'heterosporous'. In these plants, two types of spores are produced, though they are largely hidden from view. In contrast, the remaining plants, including most ferns, are 'homosporous', meaning that they produce only one type of spore.

Chromosome Number

More than 50 years ago, scientists noted that homosporous plants possess more chromosomes than heterosporous plants. One homosporous fern, *Ophioglossum reticulatum*, has more than 1400 chromosomes – the highest number for any plant, animal, or fungus. For comparison, humans have just 46 chromosomes, grouped into 23 pairs.

Most animals' chromosomes exist in pairs, with each pair comprising two of the same type of chromosome – one from each parent. However, plants are far more tolerant of deviations from this pattern. The number of sets of



chromosomes usually increases through genome doubling during reproduction, where complex processes lead to a phenomenon known as ‘polyploidy’ in the offspring. For example, this may lead to an organism with a set of four of each type of chromosome – or a ‘tetraploid’.

Such ‘Whole Genome Duplications’ are not uncommon, and are part of the evolutionary history of all plants and probably most animals. Many modern crop species, such as wheat, cotton and potatoes, are polyploid, and polyploidy can be induced artificially to aid the development of new crop varieties. However, polyploidy may also lead to genetic instability and infertility.

Heterosporous plants begin to lose this additional genetic content within a few generations through ‘genome downsizing’ processes, partially explaining why there is no correlation between chromosome number and genome size in most heterosporous lineages. It is thought that genome downsizing helps to streamline the organism’s genetic system by removing chromosomal material that is now redundant. In homosporous ferns, chromosome number and genome size are positively correlated, suggesting that they have fundamentally different, or absent, genome downsizing mechanisms.

Building on three decades of fern research, Dr Paul Wolf from the Department of Biological Sciences at the University of Alabama in Huntsville aims to shed light on these mechanisms through an extensive new research project. Given that homosporous is the ancestral condition, Dr Wolf is tackling these problems from a new perspective, by focusing on the question ‘Why do heterosporous plants have so few chromosomes?’

Genome Studies of Ferns

Initially, Dr Wolf’s research focused on creating a robust framework describing the evolutionary relationships among fern species. To achieve this, he sequenced the DNA contained in the plant cells’ chloroplasts, where photosynthesis happens.



However, this genetic information could only provide part of the picture. To understand the full evolutionary complexity of any plant species, researchers must also examine the plant’s major genome, which is packaged into chromosomes in the cell nucleus.

Technological advancements in genetic sequencing tools over the last decade have ushered in a new era of fern study. In 2011, Dr Wolf sequenced the more complex genome of a fern-like species for the first time, and in 2018 he published the first genetic sequences for two heterosporous fern species. Currently, Dr Wolf and his collaborators are assembling the first full genome sequence for the homosporous fern *Ceratopteris richardii*.

Polyploidy and Genome Downsizing

Ancient and recent polyploidy has played a large role in the evolution of species. However, its effects are complex. Polyploid lineages include evolutionary dead ends, as well as lineages where the physical effects of polyploidy have led to enhanced survival. Since the extra genetic material is typically unnecessary in polyploids, there is no pressure for the plants to retain it.

In addition to whole genome sequencing of *Ceratopteris richardii*, Dr Wolf and a PhD student in his lab, Sylvia Kinoshian, are also investigating genetic and chromosome variation within the whole *Ceratopteris* fern genus. Their hope is that unravelling the evolutionary relationships within this group of species will allow them to identify occurrences of gene duplications and evidence of ancient polyploidy. The researchers have also gathered spores from *Ceratopteris thalictroides*, a natural tetraploid, and are also generating hybrid tetraploid crosses between *Ceratopteris richardii* and *Ceratopteris pteridoides* in their lab.

‘Expanding the examination of heterospory and its correlated traits, and studying the effects of genome downsizing, can deepen our understanding of methods of crop improvement.’

CREDIT: Sylvia Kinoshian



One advantage of using *Ceratopteris* ferns as the model homosporous plant genus is their rapid lifecycle. Within their lab, Dr Wolf and his team can grow plants through an entire life cycle in as little as 145 days. This gives the researchers an opportunity to conduct multiple experiments as part of their research project in a relatively short period.

Within the next few months, they plan to examine how the chromosomes behave in these ferns during the cellular division that produces spores. This technique allows researchers to see the specially stained chromosomes through a microscope, and investigate how chromosomes are partitioned within the dividing cells. ‘This series of experiments will begin to address the question of whether ferns are unable to downsize genomes as rapidly as flowering plants,’ says Dr Wolf. However, he notes that additional work on other homosporous plants will be necessary to generalise their findings across the group.

Other Characteristics Associated with Spore Type

Although spore type correlates with chromosome number, other life history characteristics are also associated with homosporous versus heterospory. Thus, it remains plausible that other functional traits have a greater influence on the ability to downsize the genome following polyploidy. ‘So far, the chromosomal differences between homosporous and heterosporous plants have focused, naturally, on the direct aspects of spore production pattern, especially as this relates to mating systems. However, it is also possible that the correlation is not a function of direct causation,’ explains Dr Wolf.

To resolve this, Dr Wolf is examining the life history characteristics of these large groups of plants, and mapping functional traits to spore type. For example, unlike the functionally similar structures in heterosporous plants, the life

stage that produces eggs and sperm in homosporous plants is photosynthetic.

Dr Wolf envisages that this work will then feed back into other aspects of the research project, for a more detailed examination of the genes correlated with these traits, and an investigation into gene presence and expression. Previous evidence demonstrates that at least some homosporous plants turn off, or ‘silence’, extra genes instead of removing them. As such, they may be functionally negating the potentially negative effects of polyploidy.

Implications of this Research

This research represents a novel approach that could address broader biological concepts. ‘To date, research on this topic has focused on one or a few plant traits, or by examining genetic attributes of homosporous plants only,’ says Dr Wolf. ‘This synthesis will bring together data from multiple sources and view the problem from a broader perspective.’

Because many of our crop species are polyploid, a better understanding of the behaviour of chromosomes during reproductive cell division could have general implications for plant breeding. For example, exploitation of natural polyploidy or artificially induced polyploidy is an effective method to introduce beneficial characteristics into crop plant lineages. However, these lineages can be genetically unstable because of the effects of polyploidy on reproductive cell division.

‘Thus, expanding the examination of heterospory and its correlated traits, and studying the effects of genome downsizing, can deepen our understanding of methods of crop improvement,’ concludes Dr Wolf.



Meet the researcher

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Dr Paul G. Wolf earned his PhD in Botany from Washington State University, before continuing his postdoctoral research at the University of California, Irvine. He then climbed the ranks from Assistant Professor to full Professor at Utah State University, before taking his current position as Professor and Chair of the Department of Biological Sciences at the University of Alabama in Huntsville. Research in his lab focuses on plant evolutionary biology, spanning population genetics to deep phylogeny. During his career, Dr Wolf has published numerous papers in peer-reviewed journals, contributed book chapters, and presented his research at conferences and meetings across the world. In addition to his research activities, he also devotes his time to teaching multiple undergraduate and postgraduate courses, and supervising research students in his lab. Dr Wolf's research predominantly focuses on ferns, however other members of his research team are also researching flowering plants.

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FUNDING

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