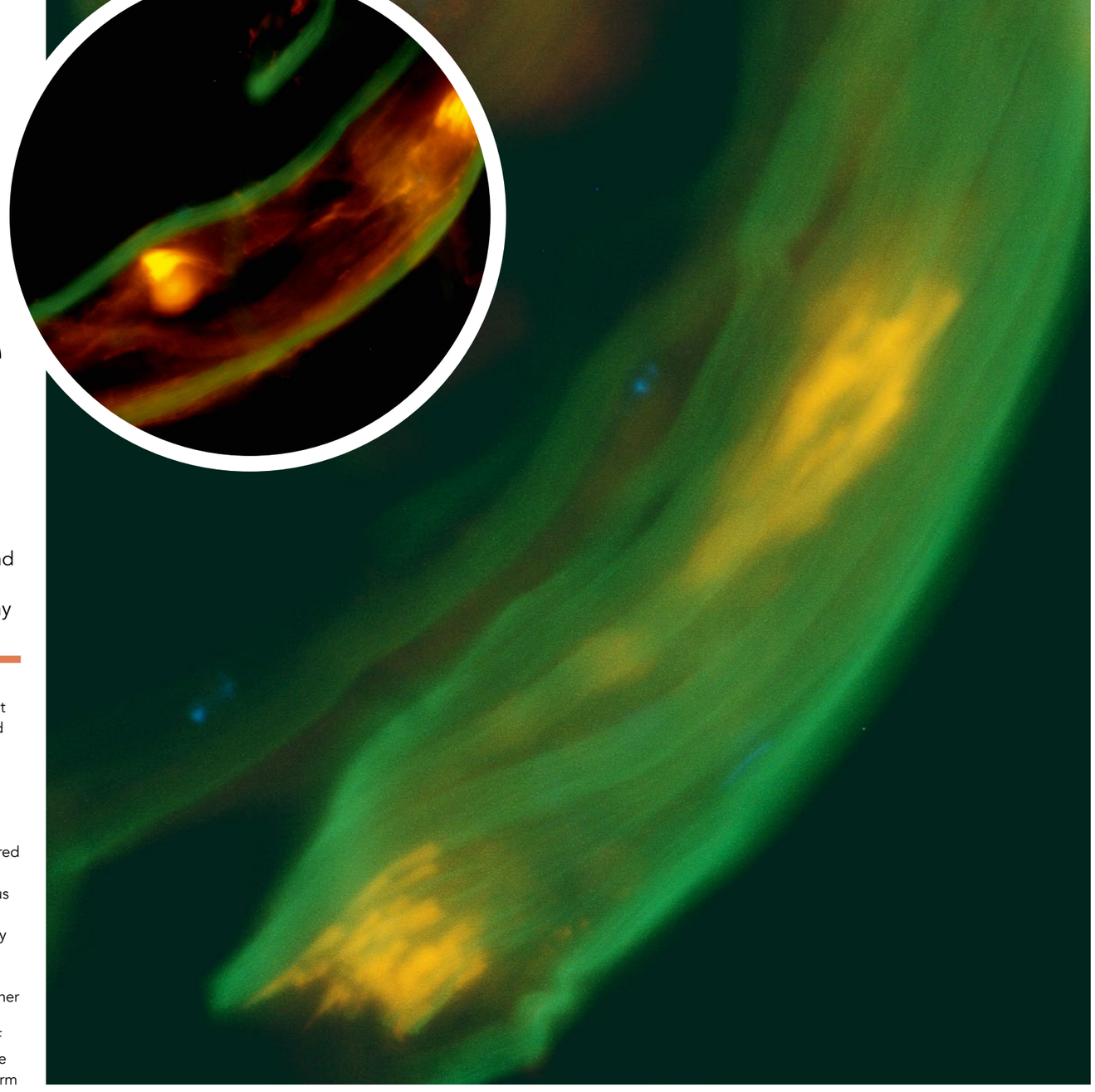
# Fruit flies help scientists seek treatment for male infertility

**Dr James Fabrizio** and his team of researchers from the College of Mount Saint Vincent have enlisted the humble fruit fly to help unravel the secrets of male infertility. The team are looking to better understand the molecular mechanisms behind sperm development. Exploring the genes involved in the late-stage maturation of sperm may pave the way toward new infertility treatments and contraceptives.



Many of us may associate the birds and the bees with reproduction however, the latest research from Dr James Fabrizio and his colleagues at the College of Mount Saint Vincent suggest another organism may be better suited to the role. The recent finding that the fruit fly (*Drosophila melanogaster*) shares important reproductive genetic similarities with mankind could help to tackle one of the leading causes of male infertility. Dr Fabrizio's research examines the consequences when things go wrong for genes involved in sperm development.

Dr Fabrizio works at a primarily undergraduate institution, and thus his approach is to identify and recruit the best undergraduate students he can find from his classes. These students are invited to fully participate in his cutting-edge research, from performing the actual experiments to collating the data and preparing posters for presentation at international research conferences. Dr Fabrizio has also found that encouraging collaboration with established scientists both enhances his work and exposes his students to a wider world of science that may ultimately inspire them.

**HOW SPERM ARE FORMED**

During spermatogenesis, sperm are clustered together in a ball of cytoplasm, known as a syncytium. Infertility can occur for numerous reasons but the leading cause in human males is failure of sperm cells to be properly resolved, or "carved out" of the syncytium in which they were matured. During sperm development, sperm cells are joined together in this syncytium by bridges of cytoplasm and remain connected until the very end of spermatogenesis, sperm development. The process of being separated from other sperm is known as individualisation. This process 'shrink wraps' each cell within a plasma membrane and removes the cytoplasmic bridges and other excess cytoplasm. The resulting sperm, each now free of cytoplasm and invested in their own plasma membranes, are now streamlined free swimming "guided missiles", each carrying a unique genetic payload. If individualisation fails, however, clumps of excess cytoplasm are left behind on the sperm tails, severely impeding their abilities to swim and fertilise an egg.

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In *Drosophila melanogaster*, 64 sperm cells are also clustered together within a syncytium known as a cyst. They are able to become distinct using an individualisation complex (IC). This individualisation complex consists of 64 cones, called investment cones, one of which assembles around the head of each of the sperm cells. Investment cones are made of actin filaments (or microfilaments), very fine structural components of the cytoskeleton. The complex travels from the head to the tail of each cell removing the cytoplasm bridges, creating 64 individually packaged sperm cells.

The individualisation process is controlled by many genes, ensuring that well-formed sperm cells can be consistently produced. Indeed, most mutations that disrupt spermatogenesis disrupt individualisation, indicating the complexity of the process and the large number of genes involved. However, mutation of these genes may lead to their function being corrupted. When this happens during the production of sperm cells the genetic material may not be packaged well enough to ensure successful fertilisation.

*Figure:* A disrupted individualisation complex, IC (orange), is associated with interflagellar microtubules (green) in a *mulet* mutant cyst. The persistence of these microtubules likely causes this disruption. Indeed, it almost appears as though the IC becomes "derailed" by attempting to follow these microtubules, which should have been removed by wild-type Tubulin-binding cofactor E-like (TBCEL), the protein product of the *mulet* gene.

*Inset:* These inter-flagellar microtubules are removed in cysts dissected from wild-type testes, so that intact wild-type ICs (orange) are always seen traveling along cysts lacking these microtubules (green).

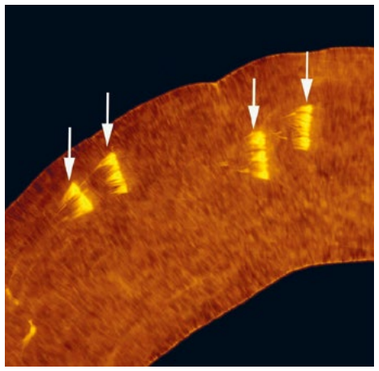
Though there are some differences in the method of sperm production between animals, the fundamentals remain the same. This research takes advantage of an important similarity between flies and humans to find out more about what happens when the individualisation process doesn't work.

**THE GENE WITH THE ANSWERS**

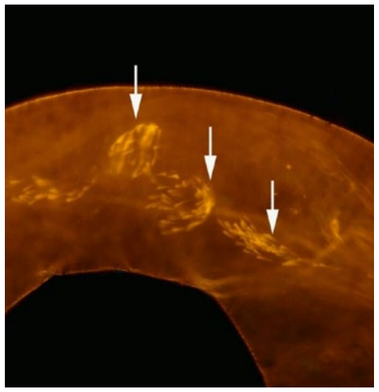
Using a red fluorescent dye, rhodamine-conjugated phalloidin, to mark the actin microfilaments within the investment cones allows the individualisation complex to be visualised. DNA-binding dyes (such as Hoechst 33258 or DAPI) highlight the sperm heads. Applying this dye combination to fly testes with different known mutations and studying the outcome of the attempted individualisation process in each mutant allowed Dr Fabrizio to recognise several genes required for individualisation. Flies with certain genes mutated displayed disruption of the individualisation complex, and thus could not produce functional sperm cells.

One of these genes is *mulet (mlt)*. While we know that *mlt* is required for sperm individualisation in *Drosophila*, and we know that there is a human homologue, we do not know for sure if *mlt* does the same thing in humans as it does in flies. But the fact that the human homologue is expressed in the testis suggests that the functions are conserved between the species. Individualisation complexes from *mulet* mutant testes become severely discoordinated and fail to individualise, indicating that *mulet* is required for individualisation.

In order to understand more about the role of *mlt* in spermatogenesis, the team conducted a series of tests to establish the DNA sequences and protein product of the gene. The group took advantage of the fact that there was a transposon, or "jumping gene" in the control region of the mutant versions of the gene. This tagged the gene and allowed the researchers to fish out the gene from all the other genes in the



Wild-type individualisation complexes (ICs, arrows) visualised with rhodamine-conjugated phalloidin. Note the ICs are intact and the investment cones are traveling down the cysts as a coordinated ensemble.



Rhodamine-phalloidin also stains the disrupted individualisation complexes (ICs, arrows) of *mulet* mutant testes..

genome. And since the transposon was in the control region of the mutated gene, the actual protein-coding part of the gene was left unaffected. The team was thus able to retrieve the normal gene sequence from these mutants.

The procedure included inverse polymerase chain reaction (PCR) and cycle sequencing which allowed the researchers to amplify the DNA next to the transposon insertion and thus map the gene. The team were then able to identify two genes that mutant *mlt* mapped onto, called *KCNQ* and *CG12214*. Interestingly, *CG12214* is a smaller gene that actually resides within *KCNQ*. So which is *mlt, CG12214* or *KCNQ*?

Further testing explored the expectation that mutated *mlt* and *CG12214*, if they were the same, would be unable to complement each other. When males carrying one copy of mutant *mlt* and one copy of *CG12214* were studied these results were confirmed; *mlt* is *CG12214*. Similar testing revealed that *mlt* and *KCNQ* did complement one another and were thus mutations in different genes.

The genome of the insect family *Drosophilidae* has been intensively studied leading to the creation of the genetic database 'FlyBase' in 1992. The species used in this investigation, *Drosophila melanogaster*, is the most intensively studied of the fruit fly family. The 'computed gene' *CG12214*, which proved so important in this research, was identified by 'FlyBase'.

This study also sought to understand how the function of the protein product of *mlt* was affected by mutation. The protein product is Tubilin-binding cofactor E-like (TBCEL) which helps to individualise sperm cells by removing microtubules between them. When *mlt* is mutated the function of TBCEL is also disrupted, causing these microtubules to remain, which appears to disrupt the ICs by derailing and discoordinating the investment cones. Dr Fabrizio has secured funding to explore this process in finer detail. He will focus on the mechanics of the IC process. In particular he will study how different molecular markers of the IC are affected by the *mlt* mutation, and if the *mlt* mutation can be rescued by driving expression of the *mlt* gene in the sperm cells.

Dr Fabrizio anticipates future studies "will contribute to our knowledge of reproductive biology by uncovering a previously undocumented role for microtubule dynamics in spermatid individualisation." Furthermore, increased understanding of the function and importance of TBCEL may "shift the focus" of reproductive science towards the individualisation process.

**Increased understanding of the function and importance of TBCEL may "shift the focus" of reproductive science towards the individualisation process.**

## Q&A

***You worked with undergraduate researchers for this project- do you enjoy this dynamic in your team?***
I love working with undergraduates. It allows me to combine my love of teaching with my love of research. The students working on this project were directly recruited from my Genetics class, where I look for students who are smart and talented but who also love what they are doing. And we have wonderful students. Indeed, there are usually no more than 40 students taking Genetics in a given semester, so they are usually friends before they even set foot in my lab! But it is true that the lab brings us all together in a special way and forges bonds that last a lifetime. And I am right in the lab working with them – I do not stay on the side-lines and tell them what to do. That is the beauty of working at a small school like the College of Mount Saint Vincent; I can get to know my students well and together we build a research cohort that does fun science together.

***How unexpected were the results of your research? Were there any surprises along the way?***
I suppose the biggest surprise was the location of our gene *CG12214* within the larger *KCNQ* gene. Indeed, in the very beginning we thought that *mulet* was *KCNQ*! And since *KCNQ* encodes a voltage-gated potassium channel, we imagined that a potassium influx might trigger the coordinated departure of investment cones from the nuclear bundle-sort of like a sperm action potential! A wonderfully incorrect idea!

***What was the biggest challenge you encountered?***
The major challenge at a primarily undergraduate teaching institution is time. As I said earlier, I love to teach and I pour my heart and soul into my classes. And my research students are often very busy with my class and/or other classes. So finding time for all of us to do experiments during the academic year is my biggest challenge. But the College of Mount Saint Vincent has been very supportive; they provided us with a wonderful lab space along with equipment and supplies. And the grant we have from the NIH was instrumental in all of our more recent successes in the lab.

***What excites you the most about the findings from this research?***
I am excited by the microtubular dynamics behind individualisation, specifically that there is a population of microtubules between the sperm tails that must be removed for individualisation to occur, and that the *mulet* gene product (TBCEL) is responsible for their removal. Indeed, the persistence of these microtubules in the *mlt* mutant appears to be responsible for the disruption of the IC. So *mlt* does not encode part of the IC at all, but it is responsible for creating an environment in which the IC can work. That is totally new, and was definitely the most exciting aspect of the work for me.

***Do you envisage fruit flies may hold the answers to other human problems in their genetics?***
Fruit flies have already secured their place in scientific history as a wonderful model organism with tremendous applications to human biology. The same genes that control eye, heart and limb development in the fly control their development in humans and other mammals. Genes that encode critical components of the major cell signalling pathways were discovered first in flies, and the same genes encoding the same signalling molecules were then found in mammals. Genes that control pattern formation in the embryo, such as the *Hox* genes, were found first in flies and later in mammals. Currently, many of the genetic mechanisms behind stem cell differentiation and self-renewal are being investigated using *Drosophila*, along with the mechanisms behind neural differentiation, the genetics of behaviour, addiction, courtship and even sexual orientation! Indeed, according to http://modencode.sciencemag.org, "approximately 60% of a group of readily identified genes that are mutated, amplified, or deleted in a diverse set of human diseases have a counterpart in *Drosophila*. Studying these genes in *Drosophila* lets scientists bypass some of the ethical issues of biomedical research involving human subjects." These diseases include neurodegenerative diseases, cleft palate, Fragile X syndrome and even cancer. In addition, every year at the International *Drosophila* Research Conference sponsored by the Genetics Society of America, new molecular techniques are revealed that make the *Drosophila* genome even more accessible and manipulable to basic researchers like myself; this can only increase the number of discoveries made with the fly. So yes, as long as we continue to ask the right questions, the fly will continue to reveal answers about human biology.

**THE HUMAN IMPACT**
Looking forward, the human impact of this research has the potential to be life-changing. Not only could understanding more about the process of sperm formation help to eradicate male infertility, it could also allow for the development of different male contraceptives. An estimated 7% of men are affected by fertility problems with the predominant cause being poor sperm quality. Treatment to ensure the consistent production of high quality sperm could help to reduce these numbers.

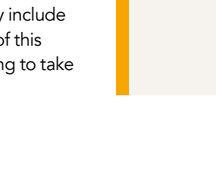
In addition, Dr Fabrizio's research may also show how to produce deliberately corrupted sperm. Further work in this area, including how to reverse the process if desired, could provide an alternative form of contraception. Current methods, such as physical barriers to sperm or hormone treatments for women can be inconvenient, expensive and may include unpleasant side effects. The future of this field has a lot to offer to those wishing to take greater control of their fertility.

## Detail

**RESEARCH OBJECTIVES**
Dr Fabrizio's lab seeks to uncover genes involved in the late-stage maturation of spermatozoa in the fruit fly. Given the large genetic similarity between Drosophila and humans, this research will shed light on the molecular mechanisms responsible for human male fertility.

**FUNDING**
National Institutes for Health (NIH)

**COLLABORATORS**
- Dr Stephen DiNardo, University of Pennsylvania
- Dr Christopher Bazinet, St. John's University



**BIO**
After graduating from Manhattan College with a BS in Biology in 1993, Dr Fabrizio was awarded his PhD in 1999 for work on sperm individualisation under Christopher Bazinet at St. John's University. Dr Fabrizio worked as post-doc under Stephen DiNardo at the University of Pennsylvania until 2002, when he was hired by the College of Mount Saint Vincent, and promoted to full professor in 2015.

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