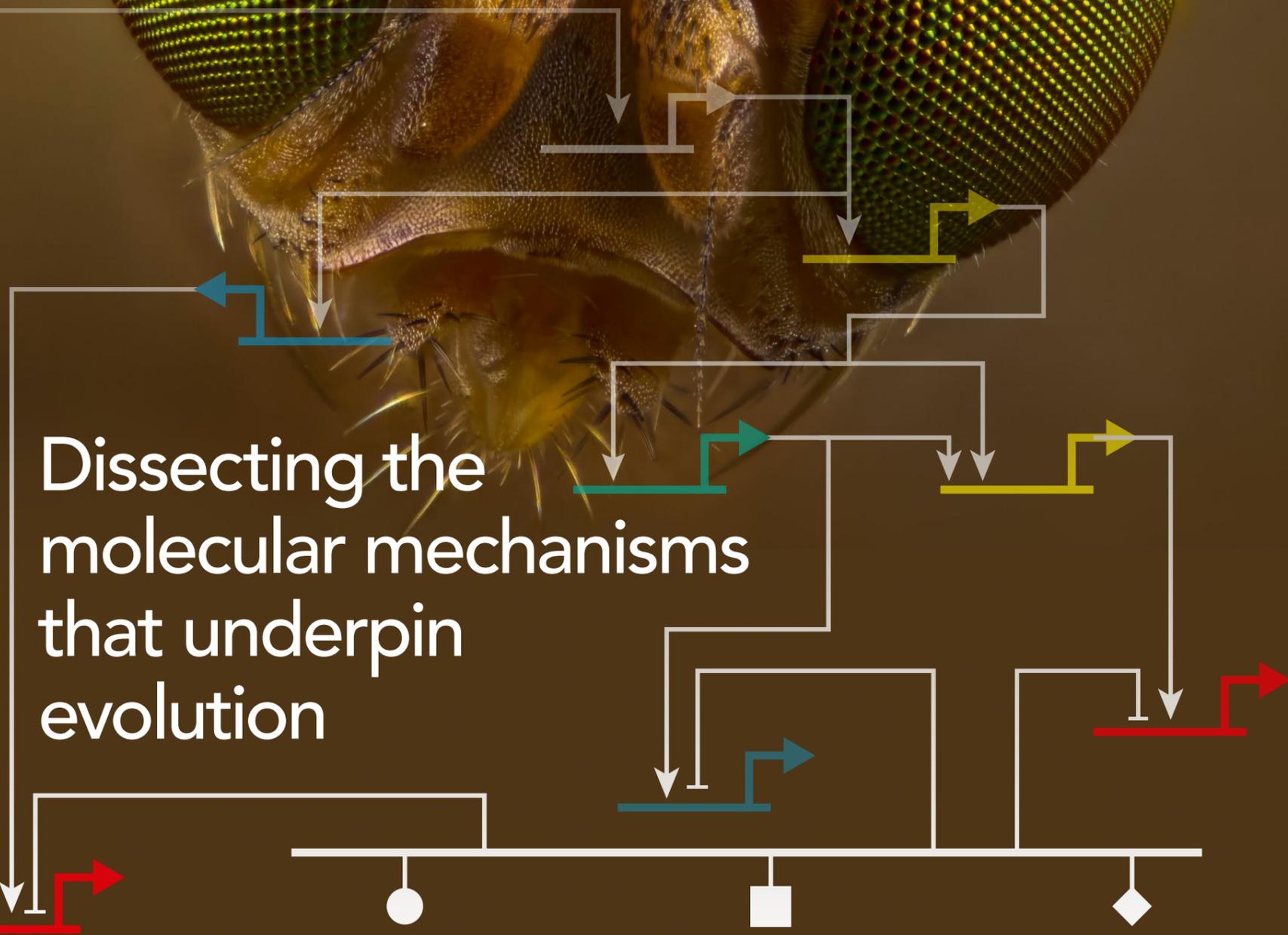


Dissecting the molecular mechanisms that underpin evolution



The question of how gene variation underlies the generation of biodiversity among organisms has long remained unanswered. **Dr J P Masly** is leading research to uncover the genetic and developmental mechanisms involved in the rapidly evolving morphology of *Drosophila*. His work is focused on how genetic differences impact the development of traits and how they ultimately result in the distinctive features that are characteristic among closely related species. In doing so, Dr Masly's research is yielding new insights into the key molecular processes that underpin the generation of biodiversity within the natural world.

At the University of Oklahoma, USA, Dr J P Masly is working to deepen our understanding of the mechanisms that generate biodiversity. He is using closely related fruit fly species with rapidly evolving morphology to dissect the genetic and developmental factors that underpin this process. One of the major goals of his research is to identify the forces that drive divergence in morphology among species, investigate how these forces shape the molecular variation that causes these differences, and understand the evolutionary consequences of these differences.

mechanisms underlying structural development have blossomed over the past few decades. This has enabled researchers such as Dr Masly to begin answering questions in evolutionary biology on an increasingly precise genetic level. His work is focused on the size and shape of reproductive traits, as they provide a particularly remarkable example of rapidly evolving biodiversity with the degree of morphological variation that exists between closely related species.

MAPPING THE GENOMES OF GENETIC HYBRIDS

The variation in reproductive structures of closely related species is usually the result of sexual selection or sexual conflict. Although this has been known for some time, how this variation develops throughout evolution at a molecular level had remained a mystery. The discovery of recently evolved distinct male genital structures in related species of fruit fly of the *Drosophila* genus have provided researchers with a powerful model for studying these processes. *Drosophila* have become one of the most widely used model organisms in the study of genetics due to their short life cycle, ease of culture in the laboratory, and amenability for generating interspecies crosses and transgenic lines. The species favoured by researchers is *Drosophila melanogaster*, which is a member of a large complex of closely related but distinct species.

To carry out their work, Dr Masly and his colleagues have developed several new resources to illuminate the genes that underpin morphological variation in rapidly evolving traits of *Drosophila*. His work is focused on two posterior lobes of the genital arch that have been found to be present in only four of these species. The lobes are composed of small cuticular projections that surround the external genitalia of males. There is an especially striking degree of variation between the species that have evolved this structure, with one species possessing a 'finger-shaped' lobe, and another featuring a 'goose-head-shaped' lobe that is around three times the size of the 'finger-shaped' trait.

To identify the mechanisms that underlie the development of these structures, Dr Masly and his team generated a novel collection of inter-species genetically-crossed lines between two of these posterior lobe-possessing species. Their

The technological tools available for unravelling the genetic and molecular

Dr Masly uses closely related fruit fly species with rapidly evolving morphology to dissect the genetic and developmental factors that underpin biodiversity

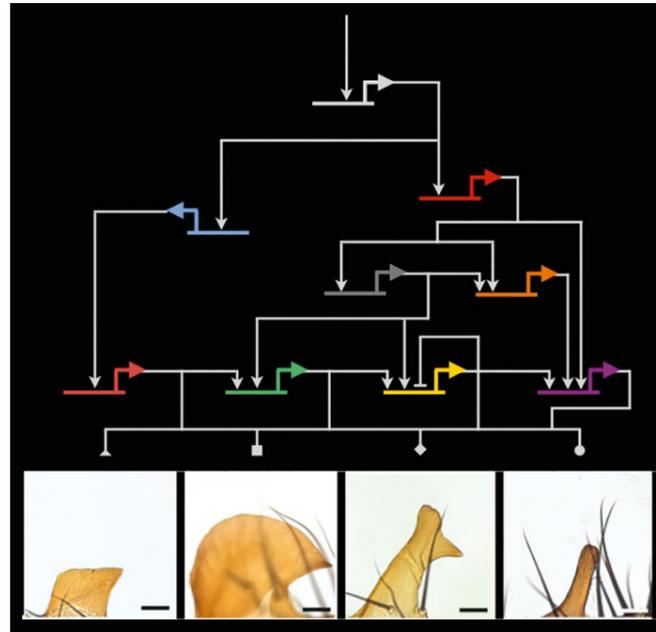
selected species are endemic to oceanic islands and last shared a common ancestor relatively recently (on an evolutionary timescale) – 240,000 years ago. They tested their extensive range of genome-wide genetic crosses that covered around 50% of the entire genome, looking for the morphological changes that resulted from placing genes from one species in the genetic background of another. This revealed several lines of flies that possessed large differences in the morphology of their posterior lobe size and shape.

Using these distinct genetic hybrids, Dr Masly and his team were able to map several regions of their genomes that were responsible for their species-specific traits. The researchers carried out live-cell imaging to analyse the process of the development of these structures in real time. This allowed them to characterise the structural development and investigate the differences in their formation. Their imaging analysis also revealed that the primary cause of variation in posterior lobe size is related to the duration of posterior lobe growth.

MOLECULAR REGULATORS OF MORPHOLOGY

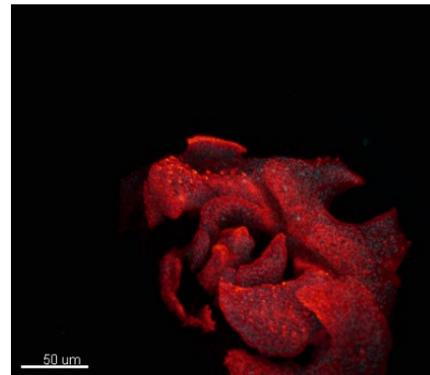
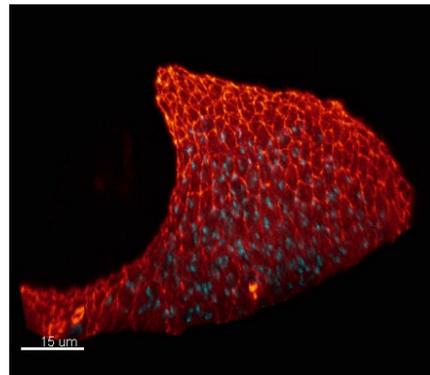
Dr Masly and his team had suspected that the morphological changes, which occur as the characteristics diverge during evolution, were due to alterations in the gene regulatory networks (GRNs). These are groups of molecular regulators that work together to control gene and, therefore, protein expression. It was thought that this typically occurs by the co-option or loss of entire GRNs. However, Dr Masly's team hypothesises that changes occur in the structure of the GRN itself, and that these may contribute to the incredibly fast evolution witnessed in morphologically complex structures between species.

Using next generation sequencing technology, Dr Masly and his team performed whole transcriptome gene expression analyses and were able to produce genetic maps with an abundance of data regarding specific gene locations



Top: Artistic rendering of a hypothetical gene regulatory network that illustrates how genes activate or repress other genes during development. Images below the network structure show the posterior lobe morphologies for adult males in the four species of the *Drosophila melanogaster* species group

Bottom: Developing reproductive structures of *D. melanogaster*. (Left) High resolution image of the developing posterior lobe. (Right) External male reproductive structures as they appear approximately 50 hours into pupal development



on the chromosomes. From these, they identified a novel gene that is responsible for specifying differences in posterior lobe size. Surprisingly, the gene they discovered is not a major regulator of DNA transcription into protein. Instead, the gene appears to code for a signalling protein whose level of expression precisely regulates the size of the structure during development. Dr Masly and his team are now creating a powerful new method, combining their live-cell imaging approach with functional genetic tests, to characterise exactly how variation at this locus is directing the developmental

differences. In addition to this, he and his team have identified numerous other genes that show promise in the divergence of posterior lobe morphology.

INVESTIGATING THE DRIVING FORCES OF SEXUAL SELECTION

It has been known for a long time that sexual selection affects changes in genital morphology. However, the specifics of this have been difficult to test and many questions have remained open, such as whether multiple selection pressures work in combination to drive this rapid evolution.

Dr Masly and his colleagues have tested the hypothesis that it is both pre- and post-copulatory sexual selection that drives this process. To do this, they genetically modified the structure of the posterior lobes of male *Drosophila* and investigated the effect this had on a selection of reproductive measures. This revealed that

Dr Masly's comprehensive research is helping to finally answer some of the fundamental questions that have fascinated evolutionary biologists



Q&A

What first motivated you to research the genetic basis of sexual selection?

Before I began my postdoctoral training, I became more interested in understanding how genetic variation shaped variation in developmental processes to give rise to species differences. Male reproductive structures seemed like an ideal model to study this, because if you are going to study species variation in genetics and development, you should select traits that possess robust differences. I also grew more interested in understanding how the genetics of divergence of reproductive traits might contribute to the genetics of reproductive isolation, and ultimately speciation, between diverging populations.

Did you face any difficulties when working with *Drosophila* in the laboratory?

No, *Drosophila* are incredibly easy to work with in the lab. I have a much greater appreciation for this luxury after my lab began working with another insect group, damselflies, which are much more difficult to rear and maintain in the lab.

Which aspect of your study would you say was the most technically challenging to obtain results for?

The live-cell imaging is particularly difficult, because *Drosophila* male genitalia develop well inside the body of the pupa. We are challenged by the law of physics – light refracts both going into and coming out of the developing pupa making it difficult to obtain clear, high-resolution images of structures such

as cell membranes. Also, because the individuals being imaged need to survive and develop normally, we cannot use high intensity light, otherwise we risk “cooking” our samples. Another challenge we have faced comes from working in “non-*D. melanogaster*” species, as we had to develop some molecular tools that work in these species to do our research.

What have been the most enjoyable aspects for you in carrying out this research?

Two things. First, there is no greater feeling for a scientist than discovering something new and exciting. Second, I relish the challenge of working on difficult problems. The opportunity to understand the evolutionary and molecular bases of complex traits that can potentially tell us something broadly about the generation of biodiversity satisfies both of these aspects of research that I really enjoy. In fact, these aspects of doing science represent some of the motivating factors for me having pursued a career in research in the first place.

What are the plans for your future research?

My lab has focused extensively on addressing questions at the interface of evolution, genetics, and development for male reproductive traits. Our results have given rise to an interesting set of questions that we will now pursue in females to understand how male and female reproductive traits co-evolve and how variation in those traits is patterned at the molecular level.

the recently evolved posterior lobes on some species are necessary for coupling and are also important for multiple post-copulatory factors, including sperm transfer amount. They also found that females which mated with males possessing smaller and narrower posterior lobes laid fewer eggs, and therefore produced fewer offspring. When combined, these factors influence morphological variation through natural selection and therefore act as a driving force for evolution.

Dr Masly's findings demonstrate the intricate subtleties involved in the sexual selection of traits within species, as these male structures do not make any direct contact with the female genitalia or reproductive organs when the flies mate. His comprehensive research is helping to finally answer some of the fundamental questions that have fascinated evolutionary biologists, including how unique characteristics, such as the posterior lobe, evolve in closely related species.

Detail

RESEARCH OBJECTIVES

Dr Masly's research focuses on understanding the mechanisms that generate biodiversity. To do this, he uses fruit flies to pinpoint the genes involved in speciation and phenotypic evolution.

FUNDING

National Science Foundation (NSF)

BIO

Dr Masly received his BS from Penn State University in 1998, spending the following year at the University of Crete as a Fulbright Fellow. He received his PhD in Evolutionary Genetics from the University of Rochester, and was a NIH/NRSA Postdoctoral Fellow at the University of Southern California until 2010.

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