October 04, 2021

Evolution & Behaviour

Equalizing strength among sexes: sexual fluidity in female moles

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This Break was edited by Olivier Kirchhoffer, *Scientific Editor* - TheScienceBreaker

ABSTRACT
Female moles develop as intersexual – they have ovotestes, containing both ovary and testicular tissues. Ovotestes produce testosterone that makes female moles stronger and more aggressive, so that they can survive a demanding underground lifestyle. We sequenced the mole genome and discovered that the shuffling of large DNA segments directed mole's evolution towards intersexuality.

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Female moles develop as intersexual individuals, a rather exceptional feature among mammals. But why did evolution shape the female mole body into an intersex one?

Most likely, this is related to the rough environmental conditions where moles live. Moles spend their entire lives digging tunnels, fighting over scarce food supplies and defending their territories. In such a scenario, the testosterone produced by the male testes confers an extra push of strength and aggressiveness, which can make the difference between eating and being eaten. In spite of this advantage, female moles also have powerful allies: the ovotestes. This dual organ composed of testicular and ovarian tissues represents evolution's solution to equalize strength among sexes. Ovotestes produce high levels of testosterone that masculinize the body of the female, making them as

strong and aggressive as their male counterparts. How intersex moles develop ovotestes has remained a mystery for decades, but we decided to solve it by digging into their genome.

Linking the evolution of a certain animal trait to a gene is complex. We aimed to move away from gene-centric approaches by considering changes that affect how the genome is organized in 3D. Recent breakthroughs in the field of genomic regulation have revealed that genes and other associated sequences, known as enhancers, are organized into spatial units denominated Topologically Associating Domains or TADs. We and others demonstrated that mutations affecting these domains' structure can originate developmental diseases, by altering the communication between genes and enhancers. We wondered if such mutations could also lead to the appearance of new traits in evolution, such as the intersexuality in moles.

To answer this, we studied the genome of the mole and created maps of its 3D structure. We also generated an encyclopedia of active genes and enhancers in male testes and female ovotestes. It is worth noting that moles cannot be kept in captivity, so all these samples were prepared from animal specimens captured in the wild. The most challenging and fun part of the project was exactly this, to go to the field and find these critters: a real-life version of the whack-a-mole game! Using all these datasets we developed a novel bioinformatic strategy to compare our mole's genome against those from species that develop regular ovaries, such as human or mouse. We specifically looked for differences in DNA sequence that could affect mole's genome 3D organization and gene regulation.

Our strategy identified a variation at the *CYP17A1* TAD, which resulted in the duplication of two existing enhancers. The *CYP17A1* gene, gatekeeper of testosterone production, is highly active in the mole gonads of both sexes in comparison to other mammals. Therefore, we hypothesized that the duplication of enhancers in moles might lead to increased gene expression and subsequently higher testosterone production. To prove this, we created a transgenic mouse carrying the mole enhancer. Mutant mice displayed increased testosterone levels and were stronger than their control siblings, supporting a key role for *CYP17A1* in the evolutionary adaptation of female moles.

In addition, we also identified a mole-specific variant on the *FGF9* TAD. *FGF9* is a known pro-testicular gene, specifically active in male testis of most mammals. In moles, we observed that this gene was also active in female ovotestes during early development. The identified variant relocates active enhancers to the 3D vicinity of the *FGF9* gene, thus explaining its female-specific activation. To prove the capacity of this gene to induce ovotestes formation, we created a transgenic mouse where we artificially activated the gene during mouse ovarian development. These mutants develop structures that resemble a male testis, demonstrating the capacity of this gene to masculinize female genital tissue.

In summary, we showed that mutations affecting the 3D structure of the genome can reshuffle genes and enhancers, in a way that contributes to the appearance, survival and maintenance of a species. Our study on mole's intersexuality also highlights the complexity of sexual development, generally constrained by the binomial categorization of individuals into male or female. For moles in fact, intersexuality represents the norm and the development of intersexual features does not seem to compromise their fertility. Instead, it rather affects how they interact with their environment, boosting possibilities of survival in harsh conditions.