Is there evidence of sexual reproduction (meiosis) in *Acanthamoeba*?

Naveed A. Khan, Ruqaiyyah Siddiqui

Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan

Evolution of independently breeding species into males and females (gametes) has remained a puzzle. Given the significant advantages of sexual reproduction over asexual reproduction as a long-term species survival strategy; here, we pose the question whether there is some form of meiosis in *Acanthamoeba* species, which represents our ancient lineage. The recently available *Acanthamoeba* genome revealed several genes implicated in meiosis in sexual eukaryotes such as *Spo11*, *Mre11*, *Rad50*, *Rad51*, *Rad52*, *Mnd1*, *Dmc1*, *Msh*, and *Mlh*, suggesting that *Acanthamoeba* is capable of some form of meiosis, inferring the presence of sexual reproduction in *Acanthamoeba*, and that meiosis evolved early in eukaryotic evolution.

Keywords: Sexual reproduction, *Acanthamoeba*, Gender

Sexual Reproduction (Meiosis) in Asexual *Acanthamoeba*?

Evolutionary biology studies have shown that amoebae evolved into intermediate organisms, which then evolved into higher organisms such as fish, reptiles, amphibians, birds, mammals, and finally humans. Despite notable similarities in the molecular and cellular architecture of various cell types of eukaryotic lineage, the origin of male and female sexes has remained unclear. Among lower eukaryotes such as *Acanthamoeba*, the mode of reproduction is generally considered to be asexual, i.e., mother cell divides into two daughter cells via binary fission or in simple terms, amoebae do not need any genetic material from another organism to reproduce. In this context, *Acanthamoeba* are 'God's favorite holy creation' as they can reproduce without needing a partner of the same species. There is only one amoeba and all others are clones. This is a remarkable property to increase the progeny of a particular species, without the need of a 'male' counterpart. It simplifies the life cycle of the species and ensures species survival under solitary conditions without the fear of species termination. How and when independently breeding species evolved into males and females (gametes) has been puzzling evolutionary biologists for centuries. Giving up reproductive autonomy and selecting the evolutionary path of acquiring greater genetic diversity, where both males and females are required, together with far more

ATP-dependency in order to ensure the procreation to further the existence of a particular species is a difficult path, yet it is widespread. Given the significant advantages of sexual reproduction over asexual reproduction, can there be male and female amoeba? In support, the evolutionary theory suggests that the species, which reproduce by cloning (do not mix genetic material) do not cope well with varied environments or may not survive when exposed to noxious agents¹ and this is a short-lived strategy, while sexual reproduction (exchange of genetic material) allows them to evolve quickly and develop new characteristics to adapt to changing environments. As *Acanthamoeba* represents an ancient lineage (over a billion years old),² which has survived catastrophic events, thus it must have developed mechanisms to endure harsh conditions. Here, we pose the question whether there is some form of meiosis, i.e., exchange of genetic material resulting in genetic variation in *Acanthamoeba*.

Evidence of Meiotic Genes in *Acanthamoeba*

The significant event of sexual reproduction in eukaryotes is generation of gametes (haploid cells), a processes known as meiosis, followed by fusion of gametes or nuclei to bring about new combinations of genetic material. Thus, the presence of meiosis can be considered as a hallmark of sexual reproduction in eukaryotes. There is no evidence of meiotic division cycle or genetic recombination in *Acanthamoeba*. However, using the recently available genome information,³ we identified several genes implicated in meiosis such as *Spo11*, *Mre11*, *Rad50*, *Rad51*, *Rad52*, *Mnd1*, *Dmc1*, *Msh*, and *Mlh* (Table 1), which are widely present among sexual

Correspondence to: Naveed Ahmed Khan, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan. Email: naveed5438@gmail.com

Table 1 Meiosis genes in *Acanthamoeba*

Gene	Protein	NCBI accession
<i>Spo11</i>	Topoisomerase Spo11 protein	XM_004334324
<i>Mre11</i>	DNA repair protein	XP_004339664
<i>Rad50</i>	Rad50 subfamily protein	XP_004339639
<i>Rad51</i>	DNA repair protein	XP_004341892
<i>Rad52</i>	DNA repair and recombination protein	XP_004337923
<i>Mnd1</i>	Meiotic nuclear division protein	XP_004340260
<i>Dmc1</i>	Meiotic recombinase	XP_004353078
<i>Msh2</i>	DNA mismatch repair protein	XP_004356558
<i>Msh4/Msh5</i>	Meiosis-specific MutS-like protein	ACA13171
<i>Msh6</i>	MutS domain V domain containing protein	XP_004337484
<i>Mlh1/2/3</i>	DNA mismatch repair protein, C-terminal domain containing protein	ELR20129

eukaryotes. The selection of meiotic genes was based on the fact that they have major meiotic functions in animals, fungi and plants and some protists.⁴ These data indicate that *Acanthamoeba* genome encodes homologous of key genes required for meiosis. Among animals, fungi, and plants, these genes are largely known for meiosis. Among meiotic genes, *Acanthamoeba* does not appear to have homologous of *Hop* or *Pms* genes;⁵ however, it is not clear whether this is because of the absence of these genes in *Acanthamoeba* genome or whether they were lost during evolution. Regardless, the presence of several genes that are involved in meiosis suggests the presence of meiosis in *Acanthamoeba*. The ability of *Acanthamoeba* to undergo meiosis warrants further experimental investigations for the identification of meiosis signature proteins and these are under investigation. The available genomic data³ (<http://eupathdb.org/eupathdb/>) support the presence of meiotic genes in *Acanthamoeba*. Given that the surveyed genes are known for meiosis in animals, fungi and plants, their role in non-meiotic function in *Acanthamoeba* is unlikely. Based on the presence of these meiotic genes, it is reasonable to hypothesise that *Acanthamoeba* is capable of some form of meiosis. Additionally, these findings suggest that meiosis evolved early in eukaryotic evolution. This is not an entirely surprising finding as sexual reproduction is quite common even among prokaryotes (e.g., via sex pili) as well as lower eukaryotes such as ciliates and complex sexual systems have been described for various ciliate species. Among many lower eukaryotes, sexual and asexual reproduction processes coexist within the same species.⁶ In addition to *Plasmodium* and Kinetoplastids, the presence of meiotic genes in *Entamoeba*, *Encephalitozoon*, *Giardia*, *Naegleria*,^{5–10} and now *Acanthamoeba* suggest the presence of meiosis across various eukaryotic domains and the likely presence of ‘sexual cycles’ in these species. Notably, the meiosis signature genes in *Acanthamoeba* described

here are assigned functions based on sequence information, and further identification of meiosis genes based on orthologous relationships with known meiotic organisms, rather than sequence homology, and their experimental investigation will provide a complete understanding of meiosis, inferring the presence of ‘sexual reproduction’ in this fascinating organism. Also, it is worth noting that meiosis signature proteins have been found in species with no meiotic pathway, such as *Candida albicans*, thus experimental studies at the functional level are needed to address this issue. If some form of meiosis is proven in *Acanthamoeba*, the next questions will be identification of molecular mechanisms of ‘sexual reproduction’ including secretion of ‘pheromones’ (mating signals), their receptors leading to morphogenetic changes in the participating cells. Being one of the most early diverging eukaryotes, *Acanthamoeba* represents an important species to provide a phylogenetic key to understanding the origin and evolution of meiosis. This coupled with the recently available genome sequence information together with high throughput genomics technology, axenic cultivation, and innovative approaches to make these parasites genetically tractable means that *Acanthamoeba* is an attractive model to scientists for phylogenomic studies.

In summary, the presence of meiotic genes suggests that (i) *Acanthamoeba* may be capable of meiosis, inferring the presence of sexual reproduction, (ii) meiosis evolved early in eukaryotic evolution, or (iii) they are adaptational strategies which were developed in recent millennia and/or transformational to a higher level of survival. Further experimental investigations will identify the conserved meiotic machinery of these genes that encode a variety of component proteins, including those involved in meiotic recombination. Being one of the most early diverging eukaryotes, *Acanthamoeba* represents an important species to provide a phylogenetic key to understanding the origin and evolution of meiosis.

Key findings

- Several meiotic genes were identified in *Acanthamoeba* genome, which are widely present among sexual eukaryotes.
- *Acanthamoeba* is capable of meiosis, inferring the presence of sexual reproduction.
- As *Acanthamoeba* represents our ancient lineage, meiosis evolved early in eukaryotic evolution.

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Author Contributions

Both authors contributed equally to this manuscript and both read and approved its final version.

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Contributors NAK mined the literature. RS examined genomes of different organisms. NAK and RS wrote the manuscript. Both authors read and approved the final manuscript.

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