SAGE-Hindawi Access to Research
International Journal of Evolutionary Biology
Volume 2011, Article ID 972457, 7 pages
doi:10.4061/2011/972457

Review Article

Boule and the Evolutionary Origin of Metazoan Gametogenesis: A Grandpa's Tale

José M. Eirín-López[1] and Juan Ausió[2]

[1] *CHROMEVOL-XENOMAR Group, Departamento de Biología Celular y Molecular, Facultade de Ciencias, Universidade da Coruña, Campus de A Zapateira s/n, E15071 A Coruña, Spain*
[2] *Department of Biochemistry and Microbiology, University of Victoria, V8W 3P6 Victoria, BC, Canada V8W 3P6*

Correspondence should be addressed to José M. Eirín-López, jeirin@udc.es

Received 16 January 2011; Revised 18 April 2011; Accepted 9 May 2011

Academic Editor: Rob Kulathinal

The evolution of sex remains a hotly debated topic in evolutionary biology. In particular, studying the origins of the molecular mechanisms underlying sexual reproduction and gametogenesis (its fundamental component) in multicellular eukaryotes has been difficult due to the rapid divergence of many reproductive proteins, pleiotropy, and by the fact that only a very small number of reproductive proteins specifically involved in reproduction are conserved across lineages. Consequently, during the last decade, many efforts have been put into answering the following question: did gametogenesis evolve independently in different animal lineages or does it share a common evolutionary origin in a single ancestral prototype? Among the various approaches carried out in order to solve this question, the characterization of the evolution of the DAZ gene family holds much promise because these genes encode reproductive proteins that are conserved across a wide range of animal phyla. Within this family, *BOULE* is of special interest because it represents the most ancestral member of this gene family (the "grandfather" of *DAZ*). Furthermore, *BOULE* has attracted most of the attention since it represents an ancient male gametogenic factor with an essential reproductive-exclusive requirement in urbilaterians, constituting a core component of the reproductive prototype. Within this context, the aim of the present work is to provide an up-to-date insight into the studies that lead to the characterization of the DAZ family members and the implications in helping decipher the evolutionary origin of gametogenesis in metazoan animals.

1. Preliminary Considerations on the Evolution of Sexual Reproduction

The appearance of sexual reproduction constituted an important breakthrough with critical genetic, cellular, physiological, and evolutionary implications. This is mainly due to three reasons [1, 2]. Firstly, it provided a mechanism for DNA crossing-over and recombination during meiosis [3] leading to the generation of genetically diverse gametes [4, 5]. Second, it permitted the differentiation of a germinal cell lineage in multi-cellular eukaryotes, responsible for the generation of haploid gametes through a sequential process known as gametogenesis [6]. Third, sexual reproduction involved the differentiation of two sexes in which male- and female-specific gametes are generated by means of a sequential process involving sex determination, mitotic proliferation, meiosis, and gamete differentiation [7, 8].

The evolution of sexual reproduction has represented an important milestone in evolutionary biology due to its relevance to the genetic diversification of individuals within a species and its consequences for speciation. Many aspects of the unique cell division process of meiosis associated with sexual reproduction are well known to have been highly conserved across eukaryotes (i.e., the key components of the meiotic machinery for chromosomal synapses and recombination), sharing a common evolutionary origin [9–12]. However, the complexity of sexual reproduction goes beyond meiosis ultimately leading to the differentiation of sexually dimorphic male sperm and female eggs through a process known as gametogenesis. In metazoan animals, sex-specific gametes are produced, displaying distinct differentiation patterns that result in different size, motility and gamete morphologies. These are sex-specific or sex-biased, with no conserved male- or female-specific gametogenic

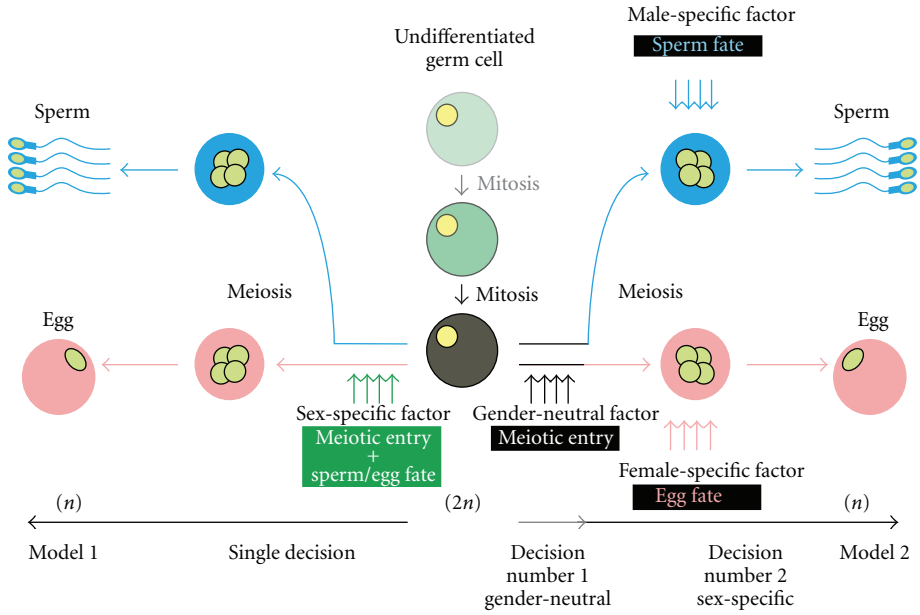

Figure 1: Schematic representation of the germ cell fate decisions following two alternative models (adapted from [13]) involving either a single sex-specific decision (model 1) or two decisions (gender neutral and sex specific, model 2). Uncommitted, male-committed, and female-committed germ cells are indicated in grey, blue, and pink, respectively.

factors [13, 14]. Furthermore, genes for sex-specific traits usually diverge very rapidly in males and females, and, with very few exceptions, only a very small number of reproductive proteins specifically involved in reproduction (broadly defined as those that act after copulation and that mediate gamete usage, storage, signal transduction, and fertilization) are conserved across lineages [15, 16]. Such dichotomy is in sharp contrast with the requirement of meiosis by both males and females, raising an important question as to what extent the features of sexual reproduction can be conserved. In other words, did gametogenesis evolve independently multiple times in different animal lineages (achieving a similar functional goal through convergent evolution) or can its origin be traced back to a single prototype sharing a common evolutionary origin (ensued by a rapid divergence of most of components of the reproductive machinery)? In this latter instance, it would be reasonable to expect a prevalence of a few core components from the ancient prototype [7].

Among the different studies attempting to address such an interesting issue (see [6, 16, 17] for review), those carried out by E. Y. Xu and collaborators during the last decade stand out due to the identification of a very conserved family of reproductive proteins (the deleted in azoospermia (DAZ) gene family) across a wide range of animal phyla. Particularly, the gene *BOULE* is of critical interest within this family since it seems to be invariably conserved both in protostome and deuterostome lineages during metazoan evolution. Furthermore, molecular evolutionary analyses indicate that *BOULE* escapes from the process of rapid adaptive evolution that is typically operating in many reproductive genes expressed postmeiotically [18] as well as in many premeiotic and meiotic genes [19–21]. Hence, *BOULE* has been the object of detailed studies, constituting a potentially conserved male-gametogenic requirement that provides support for a common evolutionary origin for spermatogenesis in metazoan animals [7].

2. Gametogenesis Involves Tough Decisions

During the early stages of gametogenesis (premeiotic gametogenesis), animal germ cells proliferate through mitotic divisions while kept on an undifferentiated state mediated by RNA-binding proteins [13]. During the process, the first critical decision that germ cells must take involves the arrest of mitotic divisions in order to enter into the meiosis stage that will lead to the subsequent formation of the haploid sex-specific gametes. To this end, germ cells must be informed (via somatic cell signaling) of whether they are in a male or in a female body in order to subsequently differentiate into male-specific spermatozoa or female-specific eggs, with this constituting a second critical decision. The timing of the first of these decisions (mitosis/meiosis) is often sex-specific and closely related with the second decision (sperm/egg). The molecular mechanisms underlying such critical choices during early (premeiotic) gametogenesis have been a subject of debate during the last ten years, leading to the proposal of two main models that differ in the steps involved in how the two basic decisions are made (Figure 1). The first model (model 1) involves a single sex-specific regulator responsible for both the entrance in meiosis and the determination of sperm or egg cell differentiation. A second model calls for the presence of a gender-neutral regulator responsible for the switch from mitosis to meiosis, as well as a second sex-specific regulator involved in the sperm/egg decision [22].

Recent reports have provided evidence favoring model 1 over model 2, arguing that RNA-binding proteins are responsible for keeping germ cells on an undifferentiated

state during mitotic proliferation [23–25], while maintaining the reversibility of such process that would allow for a transient regulation in a yet unknown fashion. Indeed, RNA-interference experiments on key regulators carried out in nematodes have revealed that sexual identity is labile, with adult females generating eggs that are amenable to switch and follow a spermatogenic development [26] and vice-versa [27]. Notably, the decision about the sexual fate of germ cells appears to be adopted at around the same time they exit mitosis to enter meiosis, underscoring the connection between the two processes [28]. On the other hand, while certain sex-specific decisions occur around the time when germline cells undergo meiosis, other decisions happen far earlier [29]. Although it is still premature to determine the universality of such mechanism, the model in which a conserved factor directs the main decisions within the core mechanism of germ cell development, may provide a very interesting insight into the evolution of the gametogenesis and sex reproduction processes.

Once the choices for mitosis/meiosis and male/female have been taken, germ cells undergo a postmeiotic cell differentiation leading to the formation of sex-specific gametes. Many of the structures and proteins appearing during these late stages seem to be related to the establishment of reproductive barriers that have critical implications for the process of speciation [30]. Therefore, it is not surprising that in many instances they evolve rapidly. However, it is important to note that although the alteration of any of these genes often leads to intraspecific infertility, none of the trends associated with them appear to be critical for proper zygote formation nor pose a roadblock for the differentiation process. This suggests that while genes involved in gamete specialization are very important for different aspects of intra-and interspecific fertilization [31] they are not the core determinants of gametogenesis.

3. The DAZ Gene Family Encodes Widely Conserved Gametogenic Factors

Although the major steps of dimorphic (male/female) gametogenesis in animals are very similar, the identification of male- and female-specific gametogenic factors common to all lineages of animals has shown to be anything but obvious [13, 14]. Reproductive proteins are often involved in other general cellular processes (besides reproduction itself), and, thus, the relevant question pertains as to whether such conservation is due to their crucial role in gametogenesis or whether it is the result of the pleoitropic functions they perform in their reproductive and somatic functions [32–34]. The key to solve this paradox probably lies in the identification of remnants of an ancient gametogenic core, lending support to a common origin of sexually dimorphic traits among animals, or in other words, the identification of conserved male- or female-specific gametogenic proteins across large evolutionary ranges. According to Xu and collaborators [7, 8], the components of such gametogenic core in the reproductive prototype should fulfill four requirements, including: (a) presence in most of major lineages of multicellular animals with sexual reproduction, (b) evolutionary origin at the same time as sexual reproduction, (c) conservation of sequence expression and function in different phyla; and most importantly (d) being selectively involved in gametogenesis in one of the two sexes and being excluded from any other pleiotropic process outside reproduction that could condition their evolutionary conservation.

Among all reproductive-associated genes known to date, the Deleted in AZoospermia (DAZ) gene family has probably been the group of reproductive factors fulfilling the above criteria that have attracted more interest from researchers during the last ten years. In humans, the DAZ gene family encompasses three genes referred to as *BOULE*, *DAZ*-like (*DAZL*), and *DAZ* encoding translational regulators with common features, including the presence of a RNA-binding domain with signature (ribonucleoprotein, RNP) RNP-1/RNP-2 motifs, as well as DAZ repeats rich in N, Y, and Q residues ([7, 8, 35], Figure 2(a)). The *BOULE* gene maps to chromosome 2 in humans and to chromosome 3 in mouse, at a region where a male sterile mutation is located which is syntenic to human chromosome 2 [8]. Human *BOULE* shares identical RNP-1 and RNP-2 motifs with fly and nematode *boule* [36, 37]), acting as a meiotic regulator expressed during late stages of male-specific meiosis (Figure 2(b)). Although defects in BOULE cause meiotic arrest predominantly in males, interference with meiosis in females has been also indicated in the nematode *boule* homolog known as *daz-1* [37]. In contrast, mutations on the *DAZL* gene (located at chromosome 3 in humans) interfere with both male and female germ cell development [8], with an expression pattern that begins early in development and continues through the meiotic divisions of gametogenesis (Figure 2(b)). Finally, the *DAZ* gene emerged on the Y chromosome in humans showing structural (protein 95% similar) and functional similarity to *DAZL* (Figure 2(b)). However, in contraposition to *DAZL*, *DAZ* expression is restricted to males [8, 35], albeit is not essential for the completion of spermatogenesis [38, 39], and its deletion is linked to infertility [7, 8].

The evolutionary conservation observed in the DAZ family members across different animal lineages, together with the lack of evidence of positive Darwinian selection acting on their members, have sparked the interest in this family as potential remnants of an ancient gametogenic core providing support to a common origin for gametogenesis in metazoans. However, and most importantly, the relevance of the study of the DAZ gene family within this context is further supported by the fact that all DAZ members are restricted to germ cells, eliminating the potential masking effects of pleiotropy in the study of the evolution of reproductive mechanisms.

4. The Conservation of *Boule* Supports a Common Evolutionary Origin of Gametogenesis from an Ancestral Prototype in Metazoans

Molecular evolutionary and phylogenetic analyses indicate that the *DAZ* gene family consists of two subfamilies

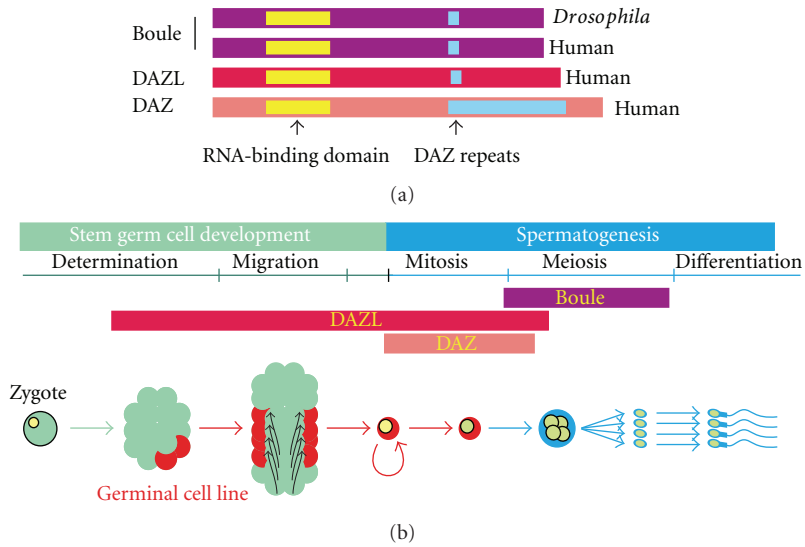

Figure 2: Molecular structure and expression patterns of DAZ gene family members during germ cell development and differentiation [8]. (a) diagram of *BOULE*, *DAZL*, and *DAZ* genes displaying the RNA-binding domains (yellow) and the DAZ repeats (blue). (b) expression patterns of *BOULE*, *DAZL*, and *DAZ* genes represented by horizontal lines during gametogenesis. *BOULE* is an ancient meiotic regulator conserved in all metazoans, giving rise to a gene family required for novel vertebrate germ cell functions. While *BOULE* encompasses a meiotic expression, *DAZL* and *DAZ* evolved novel premeiotic functions related to stem germ cell proliferation and differentiation later on during evolution.

(*DAZL* and *BOULE*) involved in different stages of germ cell development ([8], Figure 3). *BOULE* represents the ancestral single copy gene founder of the *DAZ* family, (the "grandfather" of *DAZ*) giving rise to *DAZL* (the "father" of *DAZ*) through gene duplication events most likely in the ancestral lineage of bony fishes before the emergence of tetrapods [7, 8]. A gene duplication process seems to be also responsible for the emergence of *DAZ* from *DAZL*, appearing in the Y chromosome of primates after the divergence between New World monkeys and Old World monkeys (Figure 3), approximately 30–40 MYA [8]. Y-linked *DAZ* went through two more gene duplication events as recently as 55,000 years ago, giving rise to a cluster of four *DAZ* genes [40–42]. While it seems clear that both *BOULE* and *DAZL* are subject to purifying selection, the selective process guiding *DAZ* evolution has been controversial as purifying selection as well as positive Darwinian selection of *DAZ* have both been described depending on the lineage of primates analyzed [43]. Perhaps, as suggested by Xu and colleagues, *DAZ* has yet to evolve a function essential for the completion of spermatogenesis, maybe by probing different evolutionary possibilities of nature [8].

Among all *DAZ* members, only *Boule* homologs have been identified in protostomes (fly and nematode [36, 37]) and deuterostomes (humans [7, 8, 35]), displaying a high degree of conservation across a large evolutionary time. Additionally, *Boule* exhibits functional similarity in both lineages, as revealed by the ability of a human *Boule* transgene to partially rescue the *boule* function in *Drosophila* [44]. Consequently, *Boule* has attracted a great deal of interest as one strong candidate to represent a conserved reproductive factor in the core machinery of sexual reproduction from metazoans. Indeed, recent studies suggest that *Boule* is restricted to animals, with homologs across cnidarians and bilaterian species encompassing a high degree of conservation both at the RNA-recognition motif (RRM) as well as in their genomic structure (intron-exon boundaries). These data suggest that *Boule* was already present 600 MYA in urbilaterians as well as in eumetazoans, evolving under a strong purifying selection process, whose intensity is not even relieved by the presence of a partially redundant *Dazl* function [7, 45].

In order to completely fulfill the requirements defined in the previous section for a general gametogenic factor, it remained to be demonstrated that the functional constraints acting on *Boule* were restricted to one sex. In the case of protostomes, the analysis of *Boule* expression revealed a different requirement for male (*Drosophila*) and female (nematode) reproduction [36, 37]. In deuterostomes, the expression of a *Boule* homolog was also reported both in testes and ovaries of the fish medaka [46]. The answer to this apparent contradiction came from recent studies carried out in mouse, unveiling the presence of 2 different *Boule* transcripts as a result of alternative splicing that result in a major male-specific type and a secondary type expressed in males but also in early embryonic male and female gonads [7]. Thus, while urbilaterian *Boule* was important for gametogenesis ancestrally, experimental data seems to suggest that the predominant expression of *Boule* in animals was restricted to testes. Indeed, and although exceptions to the male-specific function of *Boule* also happened during evolution in a lineage-specific manner such as in nematodes, the functional relevance of *Boule* in male gametogenesis has been further assessed using mice mutants producing

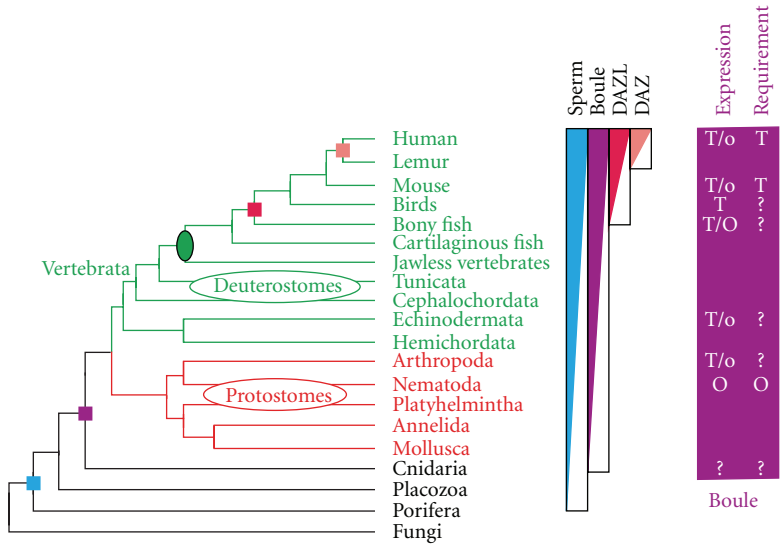

Figure 3: Evolutionary distribution of motile sperm and members of the *DAZ* family among major lineages of animals adapted from [7, 8]. Motile sperm is found in all major phyla of metazoan animals (evolutionary origin indicated by a blue box). The ancient gene *Boule* in the common ancestor of bilateria is indicated by a purple box in the tree topology, likely originated during the evolution of eumetazoans. Its function was spermatogenesis specific based on the predominance of a testis-biased expression in diverse bilaterian lineages as well as in the conservation of male reproductive function in the fly and in mice. *DAZL* arose from the ancestral *BOULE* through gene duplication events, most likely in the ancestral lineage of bony fishes after the divergence from cartilaginous fishes (indicated by a red box) but before the emergence of tetrapods, and is lacking in protostomes. *DAZ* arose from *DAZL* in the primate lineage (pink box) becoming integrated in the Y chromosome later on during primate evolution, after the divergence between New World monkeys and Old World monkeys, approximately 30–40 MYA. Testis expression/functional requirement (T) or ovary expression/functional requirement (O) for *Boule* in different metazoans is indicated in the right-hand side of the tree in purple background. Low levels of *Boule* expression in the ovary are indicated by the lowercase letter "o", in order to distinguish them from abundant expression referred to as "O".

a truncated Boule protein. The resulting male homozygous phenotype matches exactly the mutant *boule* phenotype in *Drosophila*, supporting the role of *Boule* as a reproductive factor widely conserved (structurally and functionally) across eumetazoan animals [7].

5. Concluding Remarks and Future Perspectives

The evolution of sex has constituted a very important subject in evolutionary biology since the very beginning of this discipline, and the study of the evolution of the molecular mechanisms underlying sexual reproduction has proven to be quite challenging due to the rapid divergence of an important part of the reproductive proteins and to the masking effect of pleiotropy (somatic functions in addition to reproductive functions). Within this complex scenario, the characterization of conserved gametogenic factors encompassing reproductive-exclusive roles within the DAZ family has been critical in order to help decipher the evolutionary origin of gametogenesis in metazoan animals. *BOULE* represents the most ancestral *DAZ* member, encompassing an essential reproductive-exclusive requirement in urbilaterians and a high degree of conservation across metazoan animals resulting from purifying selection. This provides support to its role as an ancient male gametogenic factor whose function has been conserved over 600 MY of evolution. Interestingly, the members of the DAZ family share a common molecular nature (RNA-binding proteins) with the molecular signals mediating the entrance of germ cells into meiosis and the differentiation of sperm/eggs. The functional prevalence of *BOULE* throughout metazoan evolution, together with the increasing support favoring a single decision model (responsible for both the entrance to meiosis and the determination of sperm or egg cell differentiation), seem to suggest that male- and female-specific gametogenesis evolved from a common somatic ancestral prototype. This likely took place early in metazoan evolution, instead of arising independently in different lineages.

Although the key role of *Boule* constituting a core component of the metazoan reproductive prototype seems to be well established, further studies will be necessary in order to clarify certain aspects pertaining its evolutionary origin, including detailed analyses of *Boule* expression in additional protostomes and deuterostomes, its characterization in outgroup of bilaterians, as well as the characterization of its subcellular expression. Furthermore, and as previously suggested by Xu and collaborators, the search for a female-specific core component of the reproductive prototype that performs a role as a gametogenic factor (similar to *Boule* in males) will be of an outmost importance [7, 8]. From a more general perspective, the studies reviewed in the present work raise several new questions regarding the specific mechanisms involved in gametogenesis and their evolution. For instance, how do novel regulators of gametogenesis such as the (inhibitor of growth) ING2 protein, a potential tumor suppressor involved in the regulation

of human spermatogenesis through p53- and chromatin-mediated mechanisms [47], fit into the picture?, are these regulators evolutionarily conserved or are they circumscribed to certain mammalian lineages?, if so, when and how were they recruited into spermatogenesis?. The answers to such questions will help to increase our knowledge on the origin and the functional evolution of sexual reproduction, probably one of the most important processes in Biology.

Nonstandard Abbreviations

DAZ: Deleted in azoospermia
DAZL: *DAZ*-like
MY: Million years
RRM: RNA-recognition motif.

Acknowledgments

The authers would like to thank Ana Meira for critical reading of the preliminary version of the manuscript and Rob Kulathinal and two anonymous reviewers for numerous comments on this work. This work was supported by a contract within the Ramon y Cajal Subprogramme from the Spanish Government-MICINN, a Travel Grant from the Xunta de Galicia (INCITE 2006–2010, European Social Fund) and a Research Grant from the Xunta de Galicia (10-PXIB-103-077-PR) awarded to J.M.E.-L. Support was also provided by a Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC-OGP-0046399-02) (to J.A.).

References

[1] J. F. Crow, "Advantages of sexual reproduction," *Developmental Genetics*, vol. 15, no. 3, pp. 205–213, 1994.
[2] A. D. Peters and S. P. Otto, "Liberating genetic variance through sex," *BioEssays*, vol. 25, no. 6, pp. 533–537, 2003.
[3] T. Allers and M. Lichten, "Differential timing and control of noncrossover and crossover recombination during meiosis," *Cell*, vol. 106, no. 1, pp. 47–57, 2001.
[4] S. C. Barrett, "The evolution of plant sexual diversity," *Nature Reviews Genetics*, vol. 3, no. 4, pp. 274–284, 2002.
[5] G. S. Roeder, "Meiotic chromosomes: it takes two to tango," *Genes & Development*, vol. 11, no. 20, pp. 2600–2621, 1997.
[6] H. Ellegren and J. Parsch, "The evolution of sex-biased genes and sex-biased gene expression," *Nature Reviews Genetics*, vol. 8, no. 9, pp. 689–698, 2007.
[7] C. Shah, M. J. Vangompel, V. Naeem et al., "Widespread presence of human BOULE homologs among animals and conservation of their ancient reproductive function," *PLoS Genetics*, vol. 6, no. 7, Article ID e1001022, 2010.
[8] E. Y. Xu, F. L. Moore, and R. A. Pera, "A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 13, pp. 7414–7419, 2001.
[9] M. A. Handel and J. C. Schimenti, "Genetics of mammalian meiosis: regulation, dynamics and impact on fertility," *Nature Reviews Genetics*, vol. 11, no. 2, pp. 124–136, 2010.
[10] R. Kumar, H. M. Bourbon, and B. de Massy, "Functional conservation of Mei4 for meiotic DNA double-strand break formation from yeasts to mice," *Genes & Development*, vol. 24, no. 12, pp. 1266–1280, 2010.
[11] M. A. Ramesh, S. B. Malik, and J. M. Logsdon, "A phylogenomic inventory of meiotic genes: evidence for sex in Giardia and an early eukaryotic origin of meiosis," *Current Biology*, vol. 15, no. 2, pp. 185–191, 2005.
[12] A. M. Villeneuve and K. J. Hillers, "Whence meiosis?" *Cell*, vol. 106, no. 6, pp. 647–650, 2001.
[13] J. Kimble and D. C. Page, "The mysteries of sexual identity: the germ cell's perspective," *Science*, vol. 316, no. 5823, pp. 400–401, 2007.
[14] M. M. Matzuk and D. J. Lamb, "Genetic dissection of mammalian fertility pathways," *Nature Cell Biology*, vol. 4, pp. S41–S49, 2002.
[15] W. Haerty, S. Jagadeeshan, R. J. Kulathinal et al., "Evolution in the fast lane: rapidly evolving sex-related genes in Drosophila," *Genetics*, vol. 177, no. 3, pp. 1321–1335, 2007.
[16] W. J. Swanson and V. D. Vacquier, "The rapid evolution of reproductive proteins," *Nature Reviews Genetics*, vol. 3, no. 2, pp. 137–144, 2002.
[17] G. J. Wyckoff, W. Wang, and C. I. Wu, "Rapid evolution of male reproductive genes inn the descent of man," *Nature*, vol. 403, no. 6767, pp. 304–309, 2000.
[18] J. M. Good and M. W. Nachman, "Rates of protein evolution are positively correlated with developmental timing of expression during mouse spermatogenesis," *Molecular Biology and Evolution*, vol. 22, no. 4, pp. 1044–1052, 2005.
[19] J. A. Anderson, W. D. Gilliland, and C. H. Langley, "Molecular population genetics and evolution of Drosophila meiosis genes," *Genetics*, vol. 181, no. 1, pp. 177–185, 2009.
[20] A. Civetta, S. A. Rajakumar, B. Brouwers, and J. P. Bacik, "Rapid evolution and gene-specific patterns of selection for three genes of spermatogenesis in Drosophila," *Molecular Biology and Evolution*, vol. 23, no. 3, pp. 655–662, 2006.
[21] D. J. Obbard, F. M. Jiggins, D. L. Halligan, and T. J. Little, "Natural selection drives extremely rapid evolution in antiviral RNAi genes," *Current Biology*, vol. 16, no. 6, pp. 580–585, 2006.
[22] A. E. Baltus, D. B. Menke, Y. C. Hu et al., "In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication," *Nature Genetics*, vol. 38, no. 12, pp. 1430–1434, 2006.
[23] J. R. Huynh and D. St Johnston, "The role of BicD, Egl, Orb and the microtubules in the restriction of meiosis to the Drosophila oocyte," *Development*, vol. 127, no. 13, pp. 2785–2794, 2000.
[24] R. Mendez and J. D. Richter, "Translational control by CPEB: a means to the end," *Nature Reviews Molecular Cell Biology*, vol. 2, no. 7, pp. 521–529, 2001.
[25] B. E. Thompson, D. S. Bernstein, J. L. Bachorik, A. G. Petcherski, M. Wickens, and J. Kimble, "Dose-dependent control of proliferation and sperm specification by FOG-1/CPEB," *Development*, vol. 132, no. 15, pp. 3471–3481, 2005.
[26] M. Otori, T. Karashima, and M. Yamamoto, "The Caenorhabditis elegans homologue of deleted in azoospermia is involved in the sperm/oocyte switch," *Molecular Biology of the Cell*, vol. 17, no. 7, pp. 3147–3155, 2006.
[27] P. J. Chen, A. Singal, J. Kimble, and R. E. Ellis, "A novel member of the tob family of proteins controls sexual fate in Caenorhabditis elegans germ cells," *Developmental Biology*, vol. 217, no. 1, pp. 77–90, 2000.
[28] M. K. Barton and J. Kimble, "fog-1, a regulatory gene required for specification of spermatogenesis in the germ line of Caenorhabditis elegans," *Genetics*, vol. 125, no. 1, pp. 29–39, 1990.

[29] A. Casper and M. Van Doren, "The control of sexual identity in the Drosophila germline," *Development*, vol. 133, no. 15, pp. 2783–2791, 2006.

[30] S. R. Palumbi, "Speciation and the evolution of gamete recognition genes: pattern and process," *Heredity*, vol. 102, no. 1, pp. 66–76, 2009.

[31] J. D. Lewis, Y. Song, M. E. de Jong, S. M. Bagha, and J. Ausió, "A walk though vertebrate and invertebrate protamines," *Chromosoma*, vol. 111, no. 8, pp. 473–482, 2003.

[32] D. S. Chu, H. Liu, P. Nix et al., "Sperm chromatin proteomics identifies evolutionarily conserved fertility factors," *Nature*, vol. 443, no. 7107, pp. 101–105, 2006.

[33] S. Dorus, S. A. Busby, U. Gerike, J. Shabanowitz, D. F. Hunt, and T. L. Karr, "Genomic and functional evolution of the Drosophila melanogaster sperm proteome," *Nature Genetics*, vol. 38, no. 12, pp. 1440–1445, 2006.

[34] K. McGuigan, L. Rowe, and M. W. Blows, "Pleiotropy, apparent stabilizing selection and uncovering fitness optima," *Trends in Ecology & Evolution*, vol. 26, pp. 22–29, 2011.

[35] K. Kee, V. T. Angeles, M. Flores, HA. N. Nguyen, and R. A. Reijo Pera, "Human DAZL, DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation," *Nature*, vol. 462, no. 7270, pp. 222–225, 2009.

[36] C. G. Eberhart, J. Z. Maines, and S. A. Wasserman, "Meiotic cell cycle requirement for a fly homologue of human deleted in Azoospermia," *Nature*, vol. 381, no. 6585, pp. 783–785, 1996.

[37] T. Karashima, A. Sugimoto, and M. Yamamoto, "Caenorhabditis elegans homologue of the human azoospermia factor DAZ is required for oogenesis but not for spermatogenesis," *Development*, vol. 127, no. 5, pp. 1069–1079, 2000.

[38] R. Reijo, R. K. Alagappan, P. Patrizio, and D. C. Page, "Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome," *The Lancet*, vol. 347, no. 9011, pp. 1290–1293, 1996.

[39] R. Reijo, T. Y. Lee, P. Salo et al., "Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene," *Nature Genetics*, vol. 10, no. 4, pp. 383–393, 1995.

[40] C. Carani, J. Gromoll, M. H. Brinkworth, M. Simoni, G. F. Weinbauer, and E. Nieschlag, "cynDAZLA: a cynomolgus monkey homologue of the human autosomal DAZ gene," *Molecular Human Reproduction*, vol. 3, no. 6, pp. 479–483, 1997.

[41] J. Gromoll, G. F. Weinbauer, H. Skaletsky et al., "The old world monkey DAZ (Deleted in AZoospermia) gene yields insights into the evolution of the DAZ gene cluster on the human Y chromosome," *Human Molecular Genetics*, vol. 8, no. 11, pp. 2017–2024, 1999.

[42] R. Saxena, J. W. de Vries, S. Repping et al., "Four DAZ genes in two clusters found in the AZFc region of the human Y chromosome," *Genomics*, vol. 67, no. 3, pp. 256–267, 2000.

[43] J. P. Bielawski and Z. Yang, "Positive and negative selection in the DAZ gene family," *Molecular Biology and Evolution*, vol. 18, no. 4, pp. 523–529, 2001.

[44] E. Y. Xu, D. F. Lee, A. Klebes, P. J. Turek, T. B. Kornberg, and R. A. Reijo Pera, "Human BOULE gene rescues meiotic defects in infertile flies," *Human Molecular Genetics*, vol. 12, no. 2, pp. 169–175, 2003.

[45] D. W. Houston, J. Zhang, J. Z. Maines, S. A. Wasserman, and M. L. King, "A Xenopus DAZ-like gene encodes an RNA component of germ plasm and is a functional homologue of Drosophila boule," *Development*, vol. 125, no. 2, pp. 171–180, 1998.

[46] H. Xu, Z. Li, M. Li, L. Wang, and Y. Hong, "Boule is present in fish and bisexually expressed in adult and embryonic germ cells of medaka," *PLoS One*, vol. 4, no. 6, Article ID e6097, 2009.

[47] M. Saito, K. Kumamoto, A. I. Robles et al., "Targeted disruption of Ing2 results in defective spermatogenesis and development of soft-tissue sarcomas," *PLoS One*, vol. 5, no. 11, Article ID e15541, 2010.