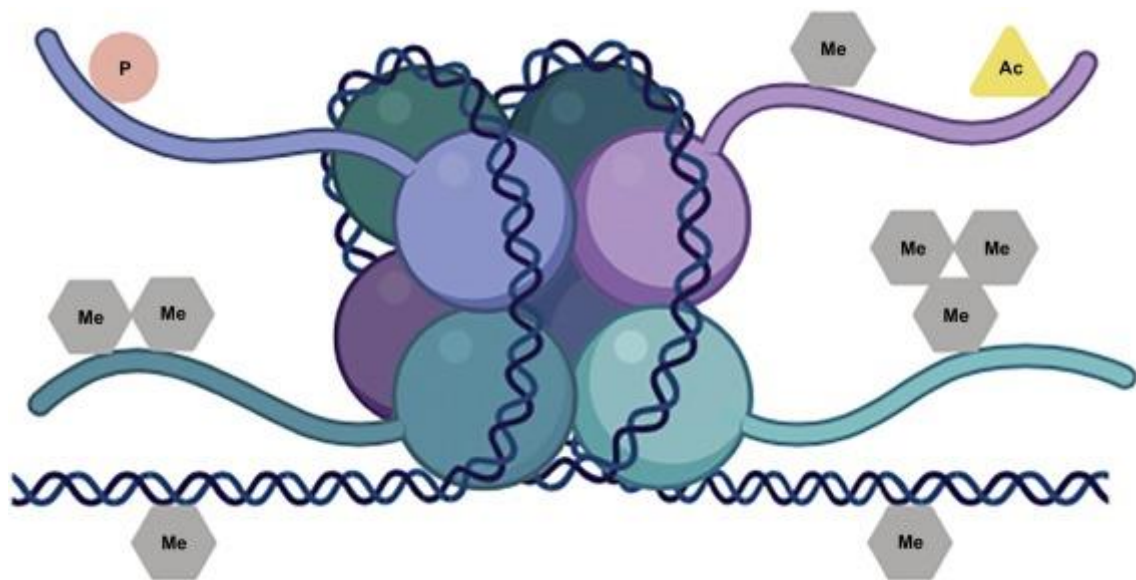


To what extent do epigenetic processes play a role in sex determination



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Foreword

I had the honour to perform my bachelor thesis under the supervision of Prof Dr L.W. Beukeboom. I learnt a lot about conducting a literature study especially since I had not performed a literature study in previous education. I would like to thank Prof Dr L.W. Beukeboom for the supervision, asking critical questions and giving me feedback that helped me understand the topic better. I think this knowledge and experience will be very useful in my later working life. In addition, delving into epigenetics has increased my interest even more. Because of this, I am sure I want to continue focusing both my courses and internships on this topic. I think by carrying out this bachelor thesis during my pre-master I will be even more prepared for my master's degree, which I hope to start next year.

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Abstract

Epigenetic is defined by change in gene expression without a change in nucleic acid sequence. Epigenetic modifications can be referred to markings on DNA which are heritable. Epigenetic modifications can put genes into a silenced state, resulting in less to no expression of these genes. Recent studies have shown that there is a correlation between epigenetics and sex determination. Sex determination is the biology in an organism that determines gender establishment. The most common sex-determination systems are the XX/XY sex-determination system and the ZW/ZZ sex determination systems. Most organisms possess a sex determination system where sex chromosomes are involved however fungi do not work with sex chromosomes, but they use mating types. There are also organisms in which the number of chromosomes sets determines sex determination. In recent years, there has been increasing evidence of the involvement of epigenetics in the regulation of key genes in sexual development. Epigenetics can influence sex determination through genomic imprinting of the X chromosome, regulation of *Sry* gene on the Y chromosome and mating type silencing in fungi. Sex determination is based on two major sex determinations systems: GSD and ESD in which ESD always has a non-genetic cue. Previously, researchers saw ESD and GSD as two separate systems because of this, sex determination was either genetic-based or environmental-based. Currently, it is thought that the combination of a specific epigenotype and genotype induces a particular phenotype. The main findings of this essay that epigenetic effects and positions can differ between and within species. In addition, some organisms possess multiple sexes and different sex determination. The sex determination of all organisms studied can be influenced by epigenetic processes. Sex determination is based on two major system which are GSD regulated by a particular gene or pathway and ESD regulated by environmental factors. It used to be seen as two separate systems but now many organisms are thought to have a combination of multiple sex determination systems. From the results and evidence gathered, it can be concluded that epigenetics does play a role in sex determination through ESD. However, epigenetic modifications itself will not be the deciding factor for sex determination. GSD and ESD should be seen as a combination of systems in which environmental and genetic factors exert their influence simultaneously on sex determination.

Abbreviations

CpGs	Cytosine located 5' from a guanosine
DNMTs	DNA methyltransferases
ESD	environmental sex determination
GSD	genotypic sex determination
TE	Transposable elements

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To what extent do epigenetic processes play a role in sex determination

Introduction

Epigenetic is defined by change in gene expression without a change in nucleic acid sequence. Epigenetic modifications can be referred to markings on DNA which are heritable. Inheritance from parent to offspring and inheritance during mitotic cell division. Epigenetics could potentially explain the correlations between lifestyle and risk of diseases (Hamilton, 2011). Epigenetic process can occur as a response to internal or external impulses from the environment. Lifestyle factors such as diet, smoking, psychological stress, and exposure to chemicals influence epigenetic patterns (Tachibana, 2016). Epigenetic mechanisms and positions can differ between and within species. Epigenetics is being looked at with great interest these days because it can be a novel biomarker for several purposes. Recent studies have shown that there is a correlation between epigenetics and sex determination (Tachibana, 2016). Sex determination is the biology in an organism that determines gender establishment. Sex determination is a complex and intricate process involving many different pathways and sex genes. The same applies to reproduction which can occur sexual and asexual and are different reproduction processes. In addition, the process itself can also differ between and within species. Therefore, the reproduction sex determination processes of animals (mammals, reptiles, and insects), plants and fungi will be compared. Sex determination systems determine the sexual characteristics of an offspring. There are two major sex determinations systems, these are genotypic sex determination (GSD) and environmental sex determination (ESD). Several studies have provided evidence that epigenetics has an indispensable role in both ESD and GSD. To better understand the link between epigenetics and sex determination, several studies are cited that provide increasing evidence for the role of epigenetics in sex determination. The main question answered in this essay is to what extent do epigenetic processes play a role in sex determination.

Epigenetics and mechanisms

Epigenetic modifications can put genes into a silenced state, resulting in less to no expression of these genes. Predominant epigenetic mechanisms such as DNA methylation and histone modification regulate gene expression at the transcription levels. Transcription can be regulated by preventing the binding of transcription factors or reducing the accessibility of DNA around histones. Due to epigenetic processes, two individuals sharing the same genotype may have a different phenotype (Tachibana, 2016). Epigenetics can be a novel biomarker for several purposes. Epigenetic screening can detect early cancer development and childhood asthma (Gomez, 2019) by targeting genetic pathways involved in methylation. Epigenetic modifications can be reversible which is an interesting feature, this allows them to be used as therapeutic interventions (Verma & Srivastava, 2002). There is still much ignorance, and much research is required before using epigenetics as therapeutic interventions. Epigenetic changes can directly and indirectly influence evolutionary processes because some epigenetic changes are inherited. In addition, there is evidence that there is a correlation between epigenetics and sex determination, which directly affects evolution (Ashe, Colot, & Oldroyd, 2021).

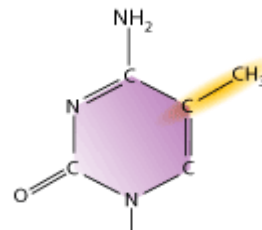
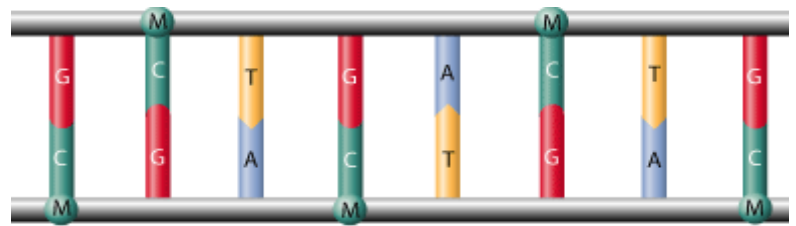
There are various epigenetic mechanisms, each mechanism targets a different molecule and adds a different additive. The table below briefly explains the different types of epigenetic mechanisms.

Table 1. Epigenetic mechanisms

Type epigenetic	Explanation
DNA methylation	A covalent transfer of a methyl group to a cytosine nucleotide facilitated by a group of enzymes called DNMTs. DNA methylation mostly inhibit the transcription of DNA.
Histone modification	A post-translational modification to histone proteins. Histone modification affect transcription by altering chromatin structure and so influence gene expression.
DNA acetylation	Addition of an acetyl group to a molecule. Enhancing transcription by removing positive charges which results in reduced affinity between DNA and histones.
Phosphorylation	Addition of a phosphate group to a molecule facilitated by a group of enzymes called kinases. Phosphorylation affects the structure and function of proteins.
Ubiquitination	Addition of a ubiquitin molecules to the lysine residues of target proteins. Increases transcription of DNA.

One of the predominant epigenetic mechanisms is DNA methylation, in this process a methyl group is covalently attached to a cytosine nucleotide, resulting in a 5-methyl-cytosine. The transfer of this methyl group is facilitated by a group of enzymes called DNA methyltransferases (DNMTs). DNA methylation influences gene expression on transcription level by affecting the transcriptional regulation or interfering with transcription factors. There are two types of methylation these are hypermethylation where there is an increased methylation intensity and hypomethylation where there is a decreased methylation intensity. Epigenetic mechanisms and positions can differ between and within species, this also applies to methylation. In animals, DNA methylation occurs only on cytosine located 5' from a guanosine, these sites are also called CpGs. Regions in which the CpG

content is high are called CpGs islands, these are mostly present in promoter regions of genes (Gibney & Nolan, 2010). DNA methylation in animals is mostly common in gene bodies rather than in their promoter region (Nai, Huang, Yen, & Chen, 2021). As in animals, methylation in plants also occurs in CG context. The function of DNA methylation in GC context in plants is not clear yet. Plants also contain two non-CG methylation systems. These non-CG methylation systems are called CHG and CHH, wherein H represents an A, C or T. High content of these regions are present in heterochromatic transposable elements (TE) (Gallego-Bartolomé, 2020). Like in plants, DNA methylation in fungi occurs in both CG methylation context and non-CG methylation (CHG and CHH) contexts. Fungi differ from plants and animals because DNA methylation in fungi hardly occurs in the gene body but in TEs and repeats of the genome. Compared to plants and animals, gene expression in fungi is not directly influenced by methylation of the promoter or gene body but indirectly by the methylation of surrounding TEs (Nai et al., 2021).



DNA methylation is the addition of a methyl group (M) to the DNA base cytosine (C).

Figure 1 DNA Methylation. Schematic illustration of a methyl group that can be covalently attached to a cytosine nucleotide in DNA sequence. (GRACEYIM, 2004)

The second predominant epigenetic mechanism is histone modification. Histone modification is involved in regulation of the *Sry* gene on the Y sex-chromosome and directly affects sex determination. Histone modification is a type of post-translational modification and affects the availability of DNA to the transcription machinery. In histone modification, a chemical group is covalently attached to the DNA, facilitated by specific enzymes. There are different types of histone modification. The histone modification locations are indicated by the histone number followed by the amino acid being targeted, histone 3 lysine residue 9 is noted as H3K9. Unlike to methylation where the methylation position differs between organisms, histone modification in most organisms is located at the N-terminal tails of histone. Compared to methylation, histone modification does not always inhibit transcription. Some types of histone modification can enhance transcription.

Reproduction and sex determination

Sex determination is the biology in an organism that determines gender establishment. Sex determination is a complex and intricate process involving many different sex genes and pathways. Epigenetic modifications can arise in the genes and pathways of these sex determination systems during reproduction and effect sex determination of organisms. Most reproduction occurs sexually in which a zygote cell is created by the fusion of two gametes both containing a single set of chromosomes. Besides sexual reproduction, there is also asexual reproduction in which no fusion of gametes occurs. In asexual reproduction, the offspring receives the entire chromosome set from one parent, therefore the offspring is genetically similar to the parent (Tabata, Ichiki, Tanaka, & Kageyama, 2016).

In mammals and reptiles, most reproduction takes place sexually and is based on the sex chromosomes with many involved sex genes (Tachibana, 2016). The most common sex-determination system is the XX/XY sex-determination system. XY individuals will develop a testis and become a male. Males contain both sex chromosomes XY and can pass on one of the chromosomes (X or Y) to the offspring. Females contain two XX chromosomes and will develop an ovary. An offspring will always inherit an X chromosome from its mother. The XX/XY sex-determination system of mammals and reptiles is Y centred, meaning that the presence or absence of the Y chromosome determines whether an individual develops into male or female. In reptiles, reproduction occurs sexually through the XX/XY sex-determination or the ZW/ZZ sex determination systems (Mittwoch, 1996). In the ZW/ZZ sex determination systems females contain the ZW chromosomes and males the ZZ chromosomes. Unlike in the XX/XY sex-determination system where the sperm determines the sex, in the ZW/ZZ sex determination system the ovum determines the sex of the offspring (Traut, Sahara, & Marec, 2007).

Insects are the most diverse group of organisms on the planet so there is also a large variation in sex determination present within this group. Like mammals and reptiles, some insects contain the XX/XY sex-determination system Y centred. However, some insects contain the XX/XY sex-determination X centred. This means that the ratio of X chromosomes to autosomes determines whether an individual develops into a male or female. Nevertheless, some insects contain the ZW/ZZ sex determination system (Laura Hake, Ph.D. & Clare O'Connor, Ph.D., 2008). In addition, insects could be haplodiploid this is type of asexual reproduction. In haplodiploid organisms, the number of sets of chromosomes determines sex determination. In haplodiploidy, male individuals are arising from a process called parthenogenesis in which eggs are unfertilised and are haploid, female insects are arising from the fusion of a sperm cell and an egg cell are diploid.

Plants contain a large variation in sex systems and its mechanisms can vary between and within genus and species. Plants use different mechanisms for asexual reproduction such as fragmentation, budding, spore formation and vegetative propagation. In asexual reproduction, the offspring is genetically identical to its parent. Sexual reproduction occurs in flowering plants by pollination. The male sex organs called stamens and female sex organs called pistils must come into contact through pollination for reproduction to occur. Plants exhibit a wide variety of sexual systems and sexes, both XX/XY sex-determination and ZW/ZZ sex determination systems are known to be present in plants. Most plants are co-sexual and possess male sex organs and female sex on the same organism (Leo W. Beukeboom, 2014). Co-sexual plants identifying as monoecious contain only purely male or purely female organs (Leite Montalvão, Kersten, Fladung, & Müller, 2021). A minority of plants are dioecious and contain male and female flowers but separated on different flowers. Hermaphrodite plants are also co-sexual but compared to the other two systems, hermaphrodite flowers contain both male and female on the same floral organs.

Fungi can reproduce both sexually and asexually. Fungi use sexual reproduction to increase genetic variation. During sexual reproduction of fungi, the nuclei of two haploid hyphae fuse together. For asexual reproduction, fungi use mechanisms such as spores, budding and fragmentation. In fungi, there are no sexes such as male or female but different mating types are used. Mating types are often referred to as (+) and (-) or (A) and (a) (Idnurm, Walton, Floyd, & Heitman, 2008)

Table 2. Sex-determination systems and details

Species	Sexual/ Asexual production	Sex-determination system
Mammals	Sexual	XX/XY (Y centred)
Reptiles	Sexual	XX/XY (Y centred) ZW/ZZ
Insects	Sexual/ Asexual	XX/XY (X centred) ZW/ZZ Haplodiploidy
Plants	Sexual/ Asexual	XX/XY (Y centred) ZW/ZZ
Fungi	Sexual/ Asexual	Mating type loci

Epigenetic mechanisms and sex determination

In recent years, there has been increasing evidence of the involvement of epigenetics in the regulation of key genes in sexual development. In insects such as *Drosophila melanogaster*, genomic imprinting of the X chromosome plays a role in sex determination. Genomic imprinting is a process in which changes are made to a gene or chromosome by methylation. The methylated allele depends on the sex of the individual transmitting the gene or chromosome. The allele which is methylated will be inactive and the unmethylated allele will be active. In insects, genomic imprinting can cause the inactivation of an entire chromosome (Anaka, Lynn, McGinn, & Lloyd, 2009). As mentioned earlier, some insects contain the XX/XY sex determination system X centred this also applies to *Drosophila melanogaster* (Slee & Bownes, 1990). This means that the ratio of X chromosomes to autosomes determines whether an individual develops into a male or a female. Inactivation of an X chromosome by means of methylation changes the ratio of X chromosomes to autosomes. As a result, genomic imprinting of the X chromosome will directly affect sex determination. Unlike insects, genomic imprinting in mammals and reptiles does not always cause inactivation of an entire chromosome but only a gene or groups of genes. In addition, genomic imprinting of the X chromosome in mammals does not directly affect sex determination (Anaka et al., 2009). The XX/XY sex determination system of mammals is Y centred, meaning that the presence or absence of the Y chromosome determines whether an individual develops into male or female. As a result, the inactivation of the X chromosome by means of methylation does not directly cause changes in sex determination of mammals and reptiles.

Despite modifications on the X chromosome of mammals and reptiles do not directly affect sex determination, modifications on the Y chromosome do. The presence or absence of the Y chromosome determines whether an individual develops into male or female in the Y centred XX/XY sex determination system. This is caused by the presence of the sex determining 1 gene (human) on the Y chromosome (Tachibana, 2016). Expression of *Sry* induces formation of testes and development of male sex characteristics. Recent research has shown that epigenetic mechanism such as histone methylation affects the regulation of the *Sry* gene. Through sodium bisulphite sequence analysis, DNA methylation levels at the *Sry* locus were investigated. It was shown that in tissue with little to no expression of *Sry*, high DNA methylation levels were present in the promoter region of *Sry*. In addition, low DNA methylation levels are found in tissue where *Sry* expression is high. This indicates that methylation negatively affects the expression of the *Sry* gene. The study shows that regulation of the *Sry* gene is under the control of epigenetic modification methylation. Expression of the *Sry* gene is necessary for inducing testis development. Epigenetic processes regulate the expression of the *Sry* gene thereby directly affecting sex determination (Nishino, Hattori, Tanaka, & Shiota, 2004). A second way in which epigenetic mechanisms affect the regulation of *Sry* is through the JMJD1A enzyme. JMJD1A catalyses the demethylation of H3K9, this histone modification is known to regulate the *Sry* expression. Studies show that in XY JMJD1A-deficient mice, male-to-female sex reversal occurred. Sex reversal is a process in which a determined sex is flipped towards the genetic pathway of the opposite sex. The expression of the *Sry* is significantly altered by JMJD1A deficiency. This suggests that the action of JMJD1A contributes to the expression of *Sry*. Epigenetic processes influence testis development and so sex determination by catalysing H3K9 demethylation (Kuroki et al., 2013).

Besides evidence about methylation in the XX/XY sex determination system, methylation also takes place in ZW/ZZ sex determination systems. In the ZW/ZZ sex determination systems females contain the ZW chromosomes and males the ZZ chromosomes. There is evidence that epigenetic modifications cause sex reversal in the ZW/ZZ sex determination systems and thus affect sex determination (Holleley et al., 2015). External stimuli have caused hypomethylation of *dmrt1*. This

gene plays a key role in male sex determination and testis development. Hypomethylation of the *dmrt1* promoter region causes masculinization of ZW females. These hypomethylated ZW females are genetically female but possess male sex characteristics (Shao et al., 2014). This is also evidence for the fact that the combination of a specific epigenotype and genotype induces a particular phenotype and determines sex determination.

Sex determination in plants can be influenced by epigenetic this is mainly found in the XX/XY sex determination. Male XY *Silene latifolia* plants naturally bearing only male flowers were treated with demethylating agent (5-azacytidine). The demethylating treatment caused the XY male *S. latifolia* to bear both male and bisexual flowers. However, explanation for the sex reversal is not yet clear it is possible that demethylating induced inhibition of Y-linked female-suppressing genes. In addition, it is also possible that demethylating affected autosomal female sex genes that induced the formation of bisexual sex characteristics. To better understand the cause-effect relationship between methylation and sex determination in sex determination systems involving sex chromosomes more research is needed (Janousek, Siroký, & Vyskot, 1996).

Epigenetics processes can influence sex determination in fungi by mating type silencing. In fungi, different sexes are indicated as mating types. When a particular mating type is silenced by methylation, it is no longer expressed, and this will affect the sexual characters of a fungus. Methyltransferase enzyme DOT1L is known to catalyse methylation of H3K79. Methylation of H3K79 is involved in the silencing of the mating-type loci HM in the yeast *Saccharomyces cerevisiae* (van Leeuwen, Gafken, & Gottschling, 2002). The expression of the mating-type will be changes due to silencing the mating-type loci HM by means of methylation, this directly affects the sex determination of this fungus.

Sex determination systems

The two major sex determination systems are GSD and ESD. In GSD sex determination is based on genetic information in DNA (Renn & Hurd, 2021). In ESD there is no change in nucleic acid sequence though there is a change in gene expression due to changes in the internal or external environment. Epigenetic mechanisms have a major role in integration of modifications in ESD. Epigenetic mechanisms are susceptible to environmental change and able to change a phenotype (Ashe et al., 2021). ESD always has a non-genetic cue such as, temperature, location, chemical exposure and, nutrient availability. Environmental factors must have exerted their influence shortly after fertilisation to be effective. ESD occurs in both animals, plants, and fungi. But even within these groups, different forms of ESD may be present.

The most studied environmental factor is temperature. Temperature-dependent sex determination (TSD) is a type of ESD in which temperature during the embryonic phase affects the sex of an individual. TSD is found in a select group of organisms such as reptiles. In a laboratory study aimed to sex determination in *Emys orbicularis* (turtle), eggs were incubated at different temperatures. Eggs incubated at 30 degrees only produced female *Emys orbicularis*. While male *Emys orbicularis* developed under a temperature of 25 degrees, showing that TSD is present in *Emys orbicularis* (Claude Pieau, Marc Girondot, Noëlle Richard-Mercier, Gisèle Desvages, Mireille Dorizzi, Patrick Zaborski, 1994). TSD is also known to be present in insects. In a study where *Talaeoporia tubulosa* (moth) eggs were incubated at warmer temperatures, more female individuals were found to develop. Colder temperatures had greater production of male individuals (Traut et al., 2007). This indicates that temperature affects sex determination and that TSD is present in those reptiles and insects. Further explanation of TSD and examples of ESD in the other species can be found in Appendix 1. Studies targeting TSD should be critically examined because it has been shown that TSD is far less widespread than currently believed. Observed sex ratio differences are often rather the effect of temperature influence on GSD as actual TSD (Ospina-Alvarez & Piferrer, 2008). Animals are exposed to extreme temperatures instead the temperature they would be incubated in the wild. Although the results of a studies showing TSD occurs in these animals may be true, it is less likely that TSD will also occur in the wild. However, it could be inferred from these studies that GSD and ESD factors influence each other. Previously, researchers saw ESD and GSD as two separate systems because of this, sex determination was either genetic-based or environmental-based. The type of system sex determination was based on was determined by the type of stimulus, which could be genetic or environmental. In GSD, this stimulus is a particular gene or pathway that is either turned on or off. An example is the sex determining gene *Sry* (human) on the Y chromosome (Tachibana, 2016). Expression of this gene causes development of male sex characteristics. In ESD, several factors can trigger the stimulus one of these is temperature as shown in the examples previously mentioned.

Recent research has revealed that the influence of environmental influences on sex determination in systems that appear to be fully GSD should not be ruled out because they may have played a larger evolutionary role than currently thought and has been proven (Schenkel, Billeter, Beukeboom, & Pen, 2022). Therefore, nowadays researcher look critically at the idea that sex determination is determined by a single system. Currently, many organisms are thought to have a combination of multiple sex determination systems. In which both environmental and genetic factors simultaneously influence sex determination. The combination of a specific epigenotype and genotype induces a particular phenotype. In a combined sex determination system there is an equilibrium, genetic effects can push the equilibrium in a certain direction which induces the development of female or male individuals. However, environmental factors can also influence the equilibrium, either in the same direction or in the opposite direction, resulting in a change of sex (Holleley et al., 2015). As can be seen from previous examples GSD and ESD factors influence each other. Therefore, GSD and ESD

should not be seen as two separate systems but as a combination of systems in which environmental and genetic factors exert their influence simultaneously.

Conclusion & discussion

The main question which will be answered in this essay is to what extent do epigenetic processes play a role in sex determination. First, knowledge of epigenetic mechanisms has been broadened. DNA methylation is one of the predominant epigenetic mechanisms and methylation positions can differ between and within species. In all organisms studied (animals, plants, and fungi), methylation occurs in CG context. Only in plants and fungi does methylation also occur on non-CG contexts (CHG and CHH contexts). A major difference in methylation regulation is that in animals and plants DNA methylation mainly takes place in the gene body while DNA methylation in fungi mainly takes place in TEs and repeats of the genome. In contrast to animals and plants, methylation in fungi does not directly change gene expression, but is indirectly caused by the methylation of surrounding TEs. Even though the methylation position differs between different organisms, no differences are seen from this in gene expression related to sex determination.

To determine the influence of epigenetics on sex determination, the different reproductive types and sex determination systems were investigated. All animals and plants studied possess sex determination systems involving sex chromosomes. This can be either the XX/XY sex-determination system or the ZW/ZZ sex determination system. Some insects are haplodiploid, in which gender is determined by the number of chromosomes sets an individual possesses. Unlike animals and plants where there are only two sexes, fungi have multiple sexes referred to as mating types. From this, it can be concluded that some organisms possess multiple sexes and that there are different sex determination systems that differ between and within species.

In recent years, there has been increasing evidence of the involvement of epigenetics in the regulation of key genes in sexual development. An example of this is genomic imprinting of the X chromosome by methylation. Genomic imprinting of the X chromosome directly affects sex determination in insects (*Drosophila melanogaster*) which containing the XX/XY sex determination X centred. This contrasts with mammals where genomic imprinting of the X chromosome does not directly affect sex determination because they contain the XX/XY sex determination Y centred. Besides epigenetic modifications on the X chromosome, there are also epigenetic modifications on the Y chromosome that can affect sex determination. Besides epigenetic modifications on the X chromosome, there are also epigenetic modifications on the Y chromosome that can influence sex determination. These epigenetic modifications usually relate to the expression of the *Sry* gene that induces formation of testes and development of male sex characteristics. From this it can be concluded that in XX/XY sex determination systems both the X and Y chromosome are sensitive to epigenetic modifications and sex determination can be influenced via both pathways. In addition, there is evidence that methylation can cause sex reversal in ZW/ZZ sex determination systems. As a result, it can be concluded that both sex determination systems involving sex chromosomes (XX/XY sex-determination and ZW/ZZ sex determination systems) can be influenced by methylation. Sex reversal has been found to occur due to epigenetic modifications on sex determination in plants. In addition, epigenetic processes influence sex determination by mating type silencing in fungi. By evidence in these studies, it can be concluded that the sex determination of all organisms studied are influenceable by epigenetic processes.

Sex determination is based on two major sex determinations systems which are GSD and ESD. Epigenetic mechanisms have a major role in integration of modifications in ESD. The most studied environmental factor in sex determination is temperature (TSD) and is found in some reptiles and insects. TSD studies should be critically examined because it has been shown that TSD in is far less widespread than currently believed. Observed sex ratio differences are often rather the effect of temperature influence on GSD as actual TSD.

The main question which is answered in this essay is to what extent do epigenetic processes play a role in sex determination. The main findings of this essay that epigenetic effects and positions can differ between and within species. In addition, some organisms possess multiple sexes and different sex determination. The sex determination of all organisms studied can be influenced by epigenetic processes. Sex determination is based on two major system which are GSD regulated by a particular gene or pathway and ESD regulated by environmental factors. It used to be seen as two separate systems but nowadays many organisms are thought to have a combination of multiple sex determination systems. From the results and evidence gathered, it can be concluded that epigenetics does play a role in sex determination through ESD. However, epigenetic modifications itself will not be the deciding factor for sex determination. Genetic effects can push the equilibrium of male or female development in a certain direction. At the same time environmental factors can influence the equilibrium, either in the same direction or in the opposite direction, resulting in a change of sex. Therefore, GSD and ESD should be seen as a combination of systems in which environmental and genetic factors exert their influence simultaneously on sex determination.

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Appendix 1

Temperature-dependent sex determination

TSD is a type of ESD in which temperature during the embryonic phase affects the sex of an individual. Three patterns have been discovered within TSD: pattern I, pattern II and pattern III. In pattern I males develop at cool incubation temperatures and female development at high temperatures. In pattern II, this is the exact opposite males develop at high incubation temperatures and female development at low temperatures. In pattern III, males develop at medium incubation temperatures and females develop at both high and low incubation temperatures (Pezaro, Doody, & Thompson, 2017).

Despite TSD being found only in reptiles and insects, ESD takes place in all organisms. Below are two examples that show evidence that ESD occurs in both plants and fungi. ESD is known to be present in plants and may depend on location. The presence of potential mates determines whether gametophytes develop into males or females. This has been proven by research on spore-bearing ferns (*Ceratopteris richardii*) (Banks, 1994). The spore-bearing fern itself will develop into either male or female gametophytes but in the absence of a potential mate, spore-bearing ferns produce both male and female gametes. Location determines whether potential mates are present in this way ESD affects the sex determination of the spore-bearing ferns (Pannell, 2017). Little research is known about ESD in fungi. The studies done about the influence of environmental conditions on asexual sporulation, zygospore formation and zygospore germination draw conflicting conclusions. A study showed evidence of the sex determination changes at certain environmental conditions in a zygospore (*Syzygites megalocarpus*). However, a duplicate study never managed to prove the influence of environmental factors (Idnurm, 2011). Based on current data it is not reliable to draw conclusions about ESD in fungi based on current data.

Appendix 2

Genes and enzymes

Table 3. Full description of gene abbreviations

Gene	Full name
<i>Sry</i>	Sex determining Region on the Y chromosome
<i>JMJD1A</i>	Jumonji domain containing 1A
<i>dmrt1</i>	Doublesex And Mab-3 Related Transcription Factor 1
<i>DOT1L</i>	Disruptor of telomeric silencing 1-like