PHSI3910 NOTES

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LECTURE 1 – SEXUAL REPRODUCTION: WHAT IS IT AND WHY?

WHAT IS SEXUAL REPRODUCTION?

Sexual reproduction is the method used by vertebrates to perpetuate their species as two separate sexes combine to produce new offspring. The existence of both sexes is conserved in diverse vertebrate taxa, meaning that all vertebrates (despite varying) have both sexes. This is necessary as it ensures that offspring are produced with the species perpetuated. To model this, zebrafish, cane toads, and mice are used with the efficacy of these models varying.

For this to occur, the process of meiosis is necessary as germ cells have their total number of chromosomes halved, ensuring that the recombination (male and female gametes combine) and subsequent fertilisation allows for the formation of a new organism. In meiosis, the human somatic cells have 46 chromosomes (diploid as two copies of each chromosome), with this halved to produce gametes with 23 chromosomes (haploid). The combination of these two haploids (male and female) form a fertilised cell with the 46 chromosomes.

Gametogenesis occurs with the formation of male and female gametes. The first stage in gametogenesis is mitotic and given the primary spermatocytes and oocytes are diploid, the next stage maintains the 46 chromosomes. However, the division from mitosis involves the doubling of the chromosomal arm, with a split down the middle from where they move into each cell. Rather than splitting in the centromere, each chromosome moves to a new cell meaning that there is halving of the number of chromosomes but since the initial germ cell had doubled the copies (mitosis), there are still two copies in each but the only change is that they are just from one chromosome, meaning that they only have 23 chromosomes (just duplicated). In females, this process involves the meiosis of a primary oocyte (diploid) into a secondary oocyte and first polar body, with this secondary oocyte forming ovum that is needed to form a new zygote.

Overall, the haploid gametes produced by the male are called sperm whilst the haploid gametes produced by the female are eggs. Once fused (fertilised), a diploid zygote is formed.

ADVANTAGES

- Ensures recombination of genetic material so genetic variation and evolutionary flexibility can occur: Genetic variation is essential in preventing a catastrophe, with diseases capable of wiping out those that are "weaker" genetically and hence variation is essential for the propagation of the species.
- Can be viewed as "bet-hedging' for maximising an individual's (and population's) survival
- Hybrid vigour against disease and parasites
- Allows for responses to rapid changes in environmental conditions

DISADVANTAGES

- Both sexes must be present in a population: This is most clearly noted through the case of the short-nosed wombat, which had only one male remaining. As such, there were debates over whether it should be culled or not given that it was unable to reproduce without a female.

  Given this, some species (over 80 species of fish, amphibians, and lizards) have evolved at points of critical environmental collapse to produce offspring without the need for another sex. It allows for flexibility under environmental conditions, and this proved essential at times of mass extinction.

There are three methods that have developed to overcome a potential species catastrophe:

- **Parthenogenesis:** In this, the cells duplicate in the final stage of mitotic division, allowing for chromosomal duplication without separation (it can also be done in the second meiotic event) allowing for a full set of chromosomes. This ensures that the female passes on the combined genetic material completely, without the need for a male.
- **Gynogenesis:** In this, the female produces eggs with the full complement of chromosomes but given the presence of the zona pellucida around the egg of the female, it requires the presence of sperm to attach to it. This changes its morphology, ensuring fertilisation can occur. However, the source of the sperm is irrelevant as the eggs produced by the female contain all genetic material that is to be passed on.
- **Hybridogenesis:** In this, the organism formed is a hybrid with the egg produced by the female and fertilised by a male from a closely related species. Despite this, only the maternal genetic material is passed on to the gametes in the gonads.

BASIC METHODS OF SEXUAL REPRODUCTION IN VERTEBRATES

The production of a zygote is highly dependent on the vertebrate group (taxa) and the environmental conditions, allowing for offspring to develop independently of the mother.

AMNIOTES VS ANAMNIOTES (IMPACT OF THE VERTEBRATE GROUP)

Some species have developed embryonic specialisation called the amnion, with this referring to the membrane that surrounds the developing embryo; it is filled with cushioning amniotic fluid. Its formation stems from the extraembryonic membranes from parts of fertilised eggs that don’t form embryos; it is from the endoderm and mesoderm.

Anamniotes: These developing embryos do not have this membrane (fish and amphibians). As such, they rely on simple secondary secreted coats that are done down the reproductive tract. However, because these egg cell coatings are rudimentary, the eggs must be laid in water. During this process, many oocytes or spermatozoa are released into the water (referred to as spawning). This however, has its consequences with changes in pH and other extinction events (including predation) capable of drastically inhibiting reproduction. Also, fertilisation is external to the body in most cases. Holistically, the probability of survival is much smaller, with this counteracted through the release of many gametes.

Amniotes: These developing embryos have the membrane (reptiles, birds, and mammals). These differ as they have internal fertilisation, and the presence of the amnion ensures that a cleidoic egg is formed, allowing for amniotes to escape the aquatic larval stage that anamniotes are confined to. This egg also ensures that other extra-embryonic membranes can be formed with gas exchange occurring with the outside (or with the mother's reproductive tract). It is through the ability for amniotes to escape the aquatic larval stage that animals have been able to disperse across the globe. Furthermore, the use of internal fertilisation ensures that fewer eggs are required, meaning it is a less energy expensive method.

OVIPARITY VS VIVIPARITY (IMPACT OF THE ENVIRONMENT)

Because of the harsh external conditions of land, amniote reproduction has changed with various protection mechanisms employed. These include the varying egg shell formations in birds and reptiles (reptiles have a hard, rubbery shell to prevent against desiccation and pH changes). On average, these shells are comprised of 7-8 layers of the reproductive tract, ensuring they protect the young. Most mammals, as well as some reptiles, fish, and amphibians use internal incubation of the developing embryo so that it does not have to deal with the harsh exterior. Fish and amphibians use mouthbrooding, where they scoop the developing eggs into the mouth, stop gastric secretion, and remove the eggs when they are tadpoles. Sea horses use brood pouches under the same hormonal control as pregnancy in mammals, with females laying the eggs into the pouches of the male who will hold it until they are fully formed.

Oviparity: This refers to the ability to lay eggs, with it used by all birds, most fishes, amphibians and reptiles. It is known that birds evolved from dinosaurs and yet dinosaurs laid live young (viviparous), meaning that the dinosaurs that eventually evolved into the birds we know today must have been oviparous. The eggs produced are generally large, with this done to ensure that it contains all nutrients required to support the developing embryo.

Ovoviviparity: This is where the embryo develops inside the mother, but doesn't rely on her for nourishment. It is also called aplacental viviparity, with it essentially where the mother is used as a reservoir until eggs hatch and pop out as developed.

Viviparity: This refers to the ability to give birth to live young (resulting in smaller eggs), with it used by cartilaginous fishes (sharks), some teleost fish, some amphibians, reptiles, and most mammals. Amniotic sharks don't form an attachment with the mother, but are developed inside, allowing for live birth. It is more complex in mammals, with different nutrition and maternal secretion but in all cases, nutrients are sourced from the mother (placental viviparity). However, there is a continuum from minimal to invasive embryo dependence on the mother. This mechanism has been used by vertebrates for many years (before mammals). Fossil evidence of the ichthyosaur has found that they gave birth to live young, with the same mechanism (tail first to prevent drowning). Similarly, the Archosauromorphs have embryos in their pelvis and despite being reptiles, they are use live birth.

Placental evolution has occurred many (>150) times, with it undergoing continual evolution in most cases. However, the zygotes can develop slower due to the lack of evolutionary pressure; this is because the embryo is internal and hence, there is less predation risk. Whilst having fewer eggs (as explained above) reduces the overall energy expenditure, internal fertilisation ensures that gestation is required (nutrient transfer from mother to child). An example of this is through the production of milk, with parental care paramount. As such, the demand for nutrients during pregnancy is high; this often results in failed pregnancies. We are examples of eutherian mammals as we use a placenta, and we use a hemochorial placenta (invasive). Cats and dogs do not have an invasive form, with the structure much more like marsupials who use endotheliochorial placentation. They all have an amniotic sac, amnion, and two layers of extraembryonic material. The Allantois is an extraembryonic development that allows for gas exchange with the individual. The yolk sac is not the same as the yolk from an egg as it is secreted by the ovary of the mother and is used in nutrient exchange and the transfer/storage of materials. The yolk sac placenta embeds in the endometria of the lining. The chorionallantoic placenta has a chorion that is in attachment and the transfer of material. Humans have three layers with chorionic villi which are used to aid in the transfer.

Monotremes are a mammalian oddity as, unlike most mammals, they lay eggs (oviparous rather than viviparous like mammals), produce milk, and are endothermic with hair. However, they share many anatomical features that are more similar to birds and reptiles than other mammals; these include the jaw and pelvis which are like reptiles.

Generally, mammals have evolved to use a high-investment, low-volume reproductive strategy; this refers to the fact that whilst energy requirements are high during gestation, fewer eggs are produced with much higher rates of reproductive success. The energy expenditure is not limited to gestation, with the production of the amniotic membrane, as well as the regular parental care further examples.

SEX DETERMINATION

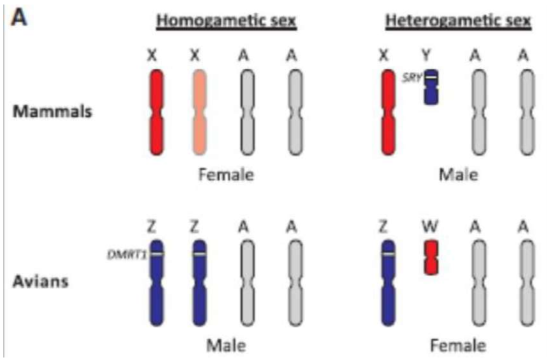
The two sexes in vertebrates have been strongly conserved over time, with this necessary to ensure sexual reproduction can occur. The two methods used to maintain the two sexes are:

GENOTYPIC SEX DETERMINATION IN VERTEBRATES

As the name suggests, the sex is genetically determined at the time of fertilisation. This means that the males and females have a different chromosomal karyotype (essentially different chromosomes) with this arising from the different sex chromosomes. This promotes the formation of sex-specific reproductive tracts.

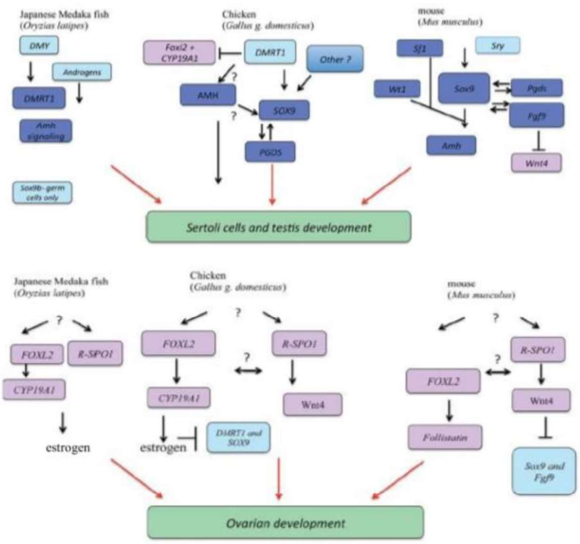
Male mammals are heterogametic (XY + autosomes) whilst females are homogametic (XX + autosomes). The use of X and Y arose from their appearance under the microscope. In birds and snakes, this differs as the males are the homogametic sex (ZZ + autosomes) whilst females are heterogametic (ZW + autosomes). Lizards vary, with some using the XY system, some ZW, and others using environmental sex determination. Fish use different systems too, with some using a single master gene system like mammals, others using multifactorial gene systems on several chromosomes, whilst others use environmental sex determination. It must be noted that much like humans, there is degeneration of the avian W chromosome (sex determining as it determines female sex). This is because there are only four genes that vary across the Z and W chromosomes in avians.

For genotypic sex determination to work, the method varies between mammals and birds. Mammals use **SRY** (Sex Determining Region of the Y Chromosome) whilst birds use **DMRT1** (Doublesex and mad-3 related transcription factor). In mammals, the Y chromosome is small (some lose it completely) as its sole purpose is sex determination and hence, should other mechanisms be used, it proves redundant.



In the image on the right, we can note that the second X chromosome in the female mammals is pale. This is because of a process called **Dosage Compensation** whereby the second chromosome is surplus to requirements, meaning it is switched off in the blastocyst stage (60-100 cells) and in eutherian mammals, this process is random. Once done, cells lines will continue with the same X chromosome switched off (inheritance). In marsupials however, the paternal one is always switched off, with the mechanism underpinning it integral in understanding how chromosomal disorders such as Fragile X syndrome occur. Dosage compensation is not seen in avians, as they use DMRT1 and this is required to produce a male (one gives female).

In both sexes, genotypic sex determination is integral in ensuring that sex-specific reproductive tracts are formed. Chickens and fish use DMRT1, with it used less in mice. In males, the SOX genes (specifically SOX9) are used to produce Sertoli cells and develop the testes. In fish, DMRT1 is only used in germ cells but in chicken and mice, they are important in signalling for the Anti-Mullerian hormone which suppresses the female uterine development, allowing for the generation of the male reproductive tract.



In females, the FOX (Forkhead box) promote ovarian development, with this specifically seen through FOXL2, as it signals for CYP19A1 (Cytochrome p450, family 19, subfamily A, polypeptide 1a) or ovarian aromatase to convert androgens to oestrogens. R-SPO1 (R-spondin 1 gene) also promotes ovarian development, with Wnt-4 (wingless-type MMTV integration site family, member 4) promoting cellular proliferation.

The mechanism of action across SRY and DMRT1 vary, with SRY relying on downstream hormonal control of the developing reproductive tract whilst DMRT1 controls the genotypic sex in each cell (cell autonomous). Any aberration in this ensures that each cell is developed, but unresponsive to hormones of the opposite sex, leading to cases such as the Gynandromorph Chicken. It is believed that this may arise due to the polar body not splitting off, meaning that both males and females develop within the same organism. Essentially, DMRT1 specifies the gonads, but the hormones produced by the gonads do not and this is used to specify part of the sex as well.

Whilst mammals use the XY system of sex determination, some have lost the Y chromosome, including Voles, Asian Spiny Mice, and Field Mice. However, many have shortening of the same chromosome but haven't lost the ability to determine sex through the SRY gene, whilst others have not lost the DMRT gene. Monotremes do not have SRY, but have DMRT1 (again, similar to birds not mammals). However, they also have 10 sex chromosomes, with this system more complex than other organisms. Organisms vary with regards to the mechanism used to determine sex, with some using a combination of SRY and DMRT1, whilst others place SRY atop the X chromosome; this is because the Y chromosome is not necessary with its sole purpose in determining sex.

This same trend is seen with great apes and humans, with reductions in Y chromosomes and polymorphisms (2+ phenotypes) occuring. The male-specific region of the Y chromosome (MSY) accumulates single nucleotide polymorphisms more rapidly than other chromosomes. In great apes, the Y chromosomes have lost most genetic material except the MSY genes. This again, arises from the fact that they are only used for sex determination. To construct a timeline of the y chromosome using single nucleotide polymorphisms, ancient DNA was used from a 45,000 year old man at the Siberian Steps. These polymorphisms occur more frequently in Y chromosmes but not in the tightly controlled X as the sole role of the Y chromosome is in sex determination. However, it must be noted that the important part of the Y chromosome (SRY gene) is not lost.

Despite this, hermaphrodism is seen in fish, with Protogynous fish (females first) including sea basses, wrasses, and parrot fish, as well as Protandrous fish (males first) such as porgies, flatheads, and barramundi. This occurs through the sequential expression of the genes that regulation CYP19a1. Fish do not have androgen, but have 11-ketotestosterone. It is noted that there is a surge in cortisol in both, with it believed that this surge in cortisol switches off the CYP19a1 gene and hence goes towards a male.

It has been found that in the Medaka fish, DMRT1 is expressed in the testis whilst Foxl2 and aromatase (CYP19a1a) is in the ovaries. However, they have a master sd gene (GSDF) which is from the TGF-β family.

ENVIRONMENTAL SEX DETERMINATION

This is primarily seen in fish and reptiles, with it including temperature dependent sex determination (TSD), as well as social interaction dependent sex determination. Whilst the mechanism appears unclear for most species, it is believed that the temperatures required for a female induce the expression of CYP19a1a, with this ovarian aromatase converting androgens into estrogens within the developing gonads. In the case of turtles, the steroids in the yolk sac can also influence the sex of the young.

In alligators and crocodiles, temperatures of incubation between 29 and 31 produce females but 32-34 produces males. However, this differs with turtles and lizards with the temperature dependence there, but it varies across species with some relying on higher temperatures to produce males and vice versa. As such, it is believed that other enzymes and steroid receptors may be involved. The temperature ranges vary, with the Tuatara producing all males at 22 degrees, all females at 20, and mixed at 21. However, this temperature is lethal to most lizards. The effect of climate change is negative, with the increasing temperatures possibly causing the production of only one gender, potentially giving rise to the issue of extinction through lack of reproduction.

Whilst TSD has been observed in reptiles, it is known that bird embryos develop eggs at incubation temperatures between 36-40 degrees, with higher temperatures lethal whilst lower temperatures prevent development. To ensure the temperature is kept within the appropriate range, some birds modify their incubation nests; these include Australian megapods such as the brush turkeys and malleefowl. In the case of the Brush Turkey, they make a special nest and use their beak to measure the temperature. They want to ensure that the eggs are of a certain size before sitting on them to allow them to develop. They all hatch simultaneously as that is the temperature that is ideal for growth. The malleefowl may aim to cool down the temperature of the nest as the outside temperatures are lethal.

With birds, temperature has proved to alter the phenotypes but the sex is easily reversed using estrogens (male → female) or aromatase (female → male). In the avian ZW system, there no no SRY gene, and no female-determining gene on the W gene, nor is there a dosage sensitive male-determining gene on the Z. With regards to the presence of a sex-determining gene on the W chromosome, there is no certainty with the most promising study finding a W-borne dominant gene called HINTW which is in both the Z and W, but encodes an inactive enzyme that interferes with the active nucleotide hydrolase of the Z. However, the presence of DMRT1 on the Z chromosome is believed to aid in sex determination as above mentioned. The effects of DMRT1 were found by knocking it out, noting that decreased expression → both ovarian tissues and testis cords. In males however, knocking it out activates Foxl2, reprogramming the Sertoli cells into granulosa cells.

Other factors that affect the sex ratio include toxoplasmosis, with it favouring the male embryos. However, the interaction between the gene and environment are integral, with the bearded dragon at extremely high incubation temperatures → female gonads despite having the male ZZ genotype. It is also believed that the ancestral mode of sex determination in amniotes is the ESD.