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IL RUOLO DELLA PARTENOGENESI NEI SERPENTI

THE ROLE OF PARTHENOGENESIS IN SNAKES

Relatore:
Chiar.mo Prof. Francesco DI IANNI

Laureanda:
Anna D’Ardes

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Summary

Abstract

Parthenogenesis, or the production of embryos from unfertilized eggs, is a form of reproduction that occurs predominantly in different taxa of invertebrates where various types of such reproduction are recognized. Some animal species consist exclusively, or nearly so, of females that reproduce by parthenogenesis, generation after generation; others tend to alternate between parthenogenic and sexual generations or always have the ability to reproduce in both ways. Among vertebrates, parthenogenesis is a rare phenomenon and true parthenogenetic lineages can only be found in reptiles. Except for a single species which consists entirely of parthenogenetic females, snakes show the greatest amount of cases where parthenogenesis occurs occasionally in species that normally reproduce sexually (facultative or occasional parthenogenesis). Although some cases of snakes suspected of parthenogenetic reproduction have been reported in the past, the first documentation of facultative parthenogenesis (FP) occurred in 1997, following the application of molecular methods for parentage analyses. Since then, it has been described in different species of both viviparous and oviparous snakes. These cases have received a lot of scientific attention because, on the one hand, they can allow a better understanding of the general mechanisms that lead to parthenogenesis, on the other hand they can have important consequences for the breeding programs of reptiles. However, the evolutionary and adaptive role of FP in snakes, as well as its real diffusion in nature, requires further investigation in future studies.

Introduction

Sexual reproduction is the dominant mode of reproduction among multicellular eukaryotes; fertilization (or syngamy), which involves the fusion of two specialized cells (or gametes), is an essential condition for the continuation of life in all sexually-reproducing organisms (Suomalainen, 1950; Janko *et al.*, 2018; Fusco and Minelli, 2019; Dedukh *et al.*, 2020). Meiosis is the process that allows the production of those haploid gametes which merge to give rise to diploid individuals. Although the molecular mechanisms underlying meiosis are highly conserved, gametogenesis has been repeatedly modified in different ways over the course of evolution, leading to independent emergences of "asexual" lineages in different taxa (Janko *et al.*, 2018; Dedukh *et al.*, 2020). With the exception of the most evolved taxa such as mammals, parthenogenetic lineages are scattered all over the tree of life: both plants and animals include such examples which approximately represent up to 1% of the total number of species (Koivisto and Braig, 2003; Janko *et al.*, 2018).

In the first chapter, after giving the definition of parthenogenesis, its main forms will be described together with the main cytological mechanisms, making examples among some invertebrates and vertebrates. Parthenogenesis in plants will not be discussed since the terminology and mechanisms are quite different, but only a few examples will be cited. The second chapter will deal with parthenogenesis in reptiles and specifically in snakes, describing various cases reported in the literature.

Chapter 1. Parthenogenesis

Definition and historical aspects

The term parthenogenesis derives from the two Greek words "*parthenos*" meaning virgin and "*genesis*" meaning origin: it refers to the natural phenomenon whereby even virgins can give birth (Avise, 2008). Consequently, parthenogenesis has been defined as a form of "asexual" reproduction in which a female reproduces without the participation of a male, or, in other words, an embryo forms without the need to be fertilized by sperm (Lynch, 1984; Kearney, 2005; Avise, 2008; Gasanov and Katz, 2020). These embryos do not always develop into new individuals because they often suffer from high mortality, therefore the definition of parthenogenesis does not consider the formation of "new individuals" (Suomalainen, 1950) but only the formation of "embryos" (Mittwoch, 1978).

The whole process is made possible by the production of special egg cells that do not follow the typical meiosis and do not need to be fertilized (Stenberg and Saura, 2009). Some authors prefer to define it as a form of sexual rather than asexual reproduction because it involves the formation of egg cells with meiotic recombination of the genetic material coming from parents, unlike asexual reproduction in which new individuals are formed from somatic cells; therefore, parthenogenesis is seen as an "incomplete form of sexual reproduction" (Mittwoch, 1978; Fusco and Minelli, 2019). However, in most cases there is no genetic recombination and the progeny is genetically identical to its virgin mother, thus parthenogenesis has also been considered as a mode of "clonal" reproduction (Vrijenhoek, 1984, 1989; Avise, 2008). Terms like "unisexual" or "uniparental" are often used in place of "parthenogenesis" in contrast to "bisexual" or "biparental" which refer to sexual (or amphigonic; Fusco and Minelli, 2019) reproduction; in addition, also the terms "all-female" or "parthenogenetic" (as adjective) are used for the same purpose, while an individual resulting from unfertilized eggs is variously referred to as "parthenogenone", "parthenogen" or "parthenote" (Cuellar, 1977; Glesener and Tilman, 1978; Mittwoch, 1978; Lynch, 1984; Hörandl, 2006; Vrijenhoek and Parker, 2009). In this paper, all these terms will be used interchangeably as synonyms.

The suspicion that the eggs of certain animals developed without copulation had been known for a long time under the expression “*Lucina sine concubitu*” (Owen, 1849; Siebold, 1857; Mittwoch, 1978), but it was Charles Bonnet (1745) with his experiments on aphids who attracted the attention of the academic community to this subject: he was able to keep females segregated from other conspecifics and, under strict observation, to obtain several generations of aphids from them (Lynch, 1984). However, the interpretation initially given to the phenomenon was substantially different: parthenogenetic individuals were considered as results of vegetative propagation similarly to plants or as hermaphrodites, capable of reproducing without mating (Owen, 1849; Lynch, 1984). Although some hermaphrodites are capable of self-fertilization, it must be emphasized that parthenogenesis, which affects only female gametes, is clearly distinct from hermaphroditism in which the same organism produces both male and female gametes (Mittwoch, 1978).

The term “parthenogenesis” was introduced a century later by Richard Owen (1849) to mean “procreation without the immediate influence of the male”: he thought that a single “ancestral” mating was enough to give rise to all subsequent generations of aphids because the "spermatic virtue" was like a force that lasted several generations before it became exhausted; moreover, under the term he coined, he grouped a series of various reproductive processes including budding and fission as well as the development of “unimpregnated” ova (Siebold, 1857; Mittwoch, 1978). Later Siebold (1857) suggested to restrict its meaning to the "true parthenogenesis" corresponding to the production of “eggs capable of development without previous copulation and in an unfecundated condition”; he also confirmed that parthenogenetic aphids were actually females with perfectly developed reproductive organs and he provided new evidence of this phenomenon in moths (Lepidoptera) and bees (Hymenoptera). At the end of the 19th century, the cytological works of August Weismann allowed a better understanding of the mechanisms underlying parthenogenesis (Lynch, 1984).

Following the discovery of other similar forms of reproduction, such as gynogenesis and hybridogenesis, the term “metasexuality” has been introduced to refer to all these modes of reproduction, including parthenogenesis, that derive from amphigony but deviate in a variable way from it (Fusco and Minelli, 2019).

Parthenogenesis does not necessarily imply that females have no access to males: in some animals, such as aphids, sexual generations alternate with parthenogenetic generations; in others, such as bees, the eggs can always be both sexually or parthenogenetically produced; others as in lizards constitute populations or “species” formed entirely by females that reproduce exclusively by parthenogenesis (Mittwoch, 1978; Avise, 2008; Fusco and Minelli, 2019).

Regarding the use of the term “species” referring to parthenogenetic lineages, it is not possible to apply the biological species concept to them, since there are no males and no mating events; for this reason, the assignment of species names to unisexual taxa is often problematic and the word “biotype” has been employed to define a particular collection of unisexual lineages; however, asexual biotypes in nature often are distinct and recognizable entities, so taxonomists have assigned Latin binomials to these asexual biotypes in the same manner as sexual species (Avise, 2008; Fusco and Minelli, 2019).

Since parthenogenesis can occur in different ways within the life cycle of various animals, different classifications have been suggested according to various criteria (Suomalainen, 1950; Mittwoch, 1978). On the basis of the oldest classifications proposed by Winkler (1920), Thomsen (1927) and Ankel (1929) revised by Suomalainen (1950), three main criteria are distinguished: the mode of reproduction, sex determination and cytological mechanisms.

Taking into consideration the first criterion, i.e. the mode of reproduction, parthenogenesis can be classified into:

- occasional or accidental parthenogenesis (or tychoparthenogenesis), in which unfertilized eggs occasionally develop through parthenogenesis;
- facultative parthenogenesis, when eggs may either be fertilized or develop parthenogenetically;
- obligate parthenogenesis, when eggs develop always parthenogenetically; this latter mode can be further defined as:
    - constant or complete parthenogenesis, if all generations are parthenogenetic;
    - cyclical parthenogenesis, if one or more parthenogenetic generations alternate with bisexual generations;

    - paedogenesis (closely connected with cyclical parthenogenesis), if individuals at larval stage develop parthenogenetic egg (Suomalainen, 1950; Mittwoch, 1978; Fusco and Minelli, 2019).

All these definitions can be utilized to refer to the mode of reproduction at the species, population or individual level (Fusco and Minelli, 2019).

In regard to the second criterion that is the sex of the offspring, parthenogenesis can be divided into three types:

- arrhenotoky (or arrhenotokous parthenogenesis) when the unfertilized eggs develop parthenogenetically into males;
- thelytoky (or thelytokous parthenogenesis) which results in exclusively female offspring;
- deuterotoky or amphitoky (or deuterotokous, or amphitokous parthenogenesis) when offspring of both sexes are produced from the unfertilized eggs (Suomalainen, 1950; White, 1977; Mittwoch, 1978; Fusco and Minelli, 2019).

As regards the cytological mechanisms, the classifications of Thomsen (1927) and Ankel (1929) were already based on the "zygoid" (diploid or polyploid) or "azygoid" (haploid) state of the new individual and on how the zygoid state was obtained. Following Suomalainen (1950) and the concepts of White (1977) of "meiotic" and "ameiotic" parthenogenesis, it can be distinguished:

- haploid parthenogenesis, where reduced eggs (i.e. haploid), produced by ordinary meiosis, develop parthenogenetically into haploid offspring;
- diploid (or polyploid) parthenogenesis (or somatic parthenogenesis), where unreduced eggs (i.e. diploid, or polyploid, depending on the starting ploidy level of the germ line) develop parthenogenetically into diploid (or polyploid) offspring; depending on the mechanisms used for the production of unreduced eggs, this type of parthenogenesis can be further divided into:
    - meiotic parthenogenesis (or automictic parthenogenesis, or automixis), in which meiosis still occurs and the unreduced eggs are produced by a premeiotic duplication of chromosomes or by fusion of two nuclei from the same meiotic event;

- ameiotic parthenogenesis (or apomictic parthenogenesis, or apomixis), in which meiosis has been suppressed and the unreduced eggs are obtained by mitosis (White, 1977; Fusco and Minelli, 2019).

Finally, unfertilized eggs can be artificially induced to develop parthenogenetically (or artificial parthenogenesis) with the help of different methods such as chemicals or temperature changes (Suomalainen, 1950; Fusco and Minelli, 2019).

Thelytokous parthenogenesis

Thelytoky, also known as thelytokous parthenogenesis, is the most common form of parthenogenesis in which females give rise exclusively to female offspring, as suggested by its etymology meaning “giving birth to a female child” (Mittwoch, 1978). The production of females can occur by automixis (also called automictic or meiotic thelytokous parthenogenesis) or by apomixis, also known as apomictic or ameiotic thelytokous parthenogenesis (Fusco and Minelli, 2019). Thelytoky often is obligate being the only mode of reproduction in a species or population, in other cases it occurs in species that are normally bisexual (facultative or occasional), or it may be cyclical alternating with a bisexual generation (White, 1977; Mittwoch, 1978). It is a common event in invertebrates like arthropods, annelids, flatworms, nematodes, molluscs, but it can also occur in vertebrates, such as fishes and some whiptail lizards (Cole, 1975; White, 1977; Neaves and Baumann, 2011).

Thelytoky is widespread in many orders of insects, particularly in certain Diptera, Orthoptera, Hymenoptera, Coleoptera and Lepidoptera (White, 1977; Mittwoch, 1978). Among dipterans, 17 species appear to reproduce solely by parthenogenesis, and many of these are triploid; in some of the species thelytoky is ameiotic, in others meiosis takes place: for example, in the diploid *Drosophila mangabeirai* (Drosophilidae) thelytoky is meiotic and it is achieved by the fusion of meiotic products resulting from the second meiotic division (White, 1977; Mittwoch, 1978; Fusco and Minelli, 2019). The well-studied diploid grasshopper *Warramaba virgo* (order Orthoptera) is composed only of females and the thelytoky is meiotic with a mechanism that involves the doubling of the chromosome number before meiosis (Mittwoch, 1978; Kearney, 2005). The beetle *Bromius obscurus* (order Coleoptera, family Chrysomelidae) is not entirely parthenogenetic, but it consists of diploid sexual populations in North America and of triploid apomictic populations in Europe (Mittwoch, 1978; Fusco and Minelli, 2019). Most of the known cases of thelytoky in Coleoptera occur in weevils (family Curculionidae) which show many forms of polyploidy (3n, 4n, 5n, 6n) associated with parthenogenesis (White, 1977; Fusco and Minelli, 2019). Complete thelytoky in Lepidoptera had already been described by Siebold (1857) in the family Psychidae. Unlike other insects, lepidopterans have a chromosomal sex determination system with female heterogamety (ZW or Z0 system) in which females may produce gametes with different sex chromosomes; some parthenogenetic routes will consequently result in males, so the production of females is ensured through those cytological mechanisms that preserve heterozygosity: in species like *Solenobia triquetrella*, consisting of diploid and tetraploid parthenogenetic forms, thelytoky is achieved by a form of meiotic parthenogenesis (or central fusion) with the fusion of two nuclei separated at the first meiotic division (White, 1977; Mittwoch, 1978; Fusco and Minelli, 2019).

Among crustaceans, one of the most studied instance is the genus *Artemia* which includes species with sexual as well as thelytokous populations in which females are produced by automictic or apomictic parthenogenesis; both diploid and polyploid forms are known, but the latter appear to be mostly ameiotic (White, 1977; Mittwoch, 1978; Fusco and Minelli, 2019).

Among annelids, oligochaetes, which include earthworms, are generally hermaphrodites with cross-fertilization between two hermaphroditic individuals; however, the thelytokous oligochaetes seem to have suppressed the testicular development, although sperm is still necessary to activate the egg in some cases. The family Lumbricidae of true earthworms includes sexual species with various ploidy levels and many polyploid thelytokous forms; among these, apomictic parthenogenesis is less common than automictic one in which meiosis is preceded by a doubling of the chromosome number, allowing a regular segregation of chromosomes even in forms with an odd ploidy number (White, 1977; Mittwoch, 1978; Fusco and Minelli, 2019).

Polyploidy combined with thelytokous parthenogenesis is also common in some flatworms that are normally hermaphrodites and, in some nematodes, which reproduce by apomictic thelytoky (White, 1977; Mittwoch, 1978).

Arrhenotokous (haploid) parthenogenesis

Arrhenotoky (or arrhenotokous parthenogenesis), from the Greek meaning “giving birth to a male child”, is another form of reproduction which leads to the production of exclusively male offspring instead of females from unfertilized eggs (Mittwoch, 1978; Fusco and Minelli, 2019). However, populations that reproduce through arrhenotoky are not usually made up of males only, due to coexistence with other forms of reproduction such as amphigony (Fusco and Minelli, 2019). This reproductive mode is closely related to the system of sex determination: it is observed particularly in hymenopterans and thysanopterans with the haplodiploid sex-determination system in which females are diploid and males are haploid; the diploid females are produced from fertilized eggs, whereas the haploid males develop from unfertilized eggs, produced by the diploid females, through arrhenotoky (Mittwoch, 1978; Fusco and Minelli, 2019). Therefore, females have two parents, whereas males have no fathers and are produced by haploid parthenogenesis (White, 1977).

It should be noted that closely related species may show different modes of reproduction: both arrhenotoky and thelytoky can be found in related species; furthermore, arrhenotokous females can coexist with thelytokous females within the same species (White, 1977; Fusco and Minelli, 2019).

This system is particularly studied in the honey bee, *Apis mellifera*, where the queen after mating is able to lay both fertilized and unfertilized eggs, apparently at will; the fertilized eggs may develop either into workers or queen bees, the unfertilized ones develop into drones (Mittwoch, 1978). In addition to Hymenoptera and Thysanoptera, arrhenotokous parthenogenesis has been described in other insects such as whiteflies and scale insects among the homopterans, in some coleopterans and also in arachnids, nematodes and monogonont rotifers, whereas it is unknown in plants (Suomalainen, 1950; White, 1977; Fusco and Minelli, 2019).

Spermatogenesis in these haploid males does not require a reduction in the number of chromosomes: meiosis can be replaced by a single mitotic division which produces only two spermatozoa from each primary spermatocyte, as occurs in some arrhenotokous mites, homopterans, hemipterans and beetles; or it can be a modified meiosis as in Hymenoptera, with a first division that separates a nucleus from an anucleate mass and a second division similar to a mitosis; this final division in bees is asymmetric, so only one spermatozoon is obtained (Suomalainen, 1950; White, 1977; Fusco and Minelli, 2019).

Over the last century, several models have been suggested to try explaining why a female could only develop from a diploid embryo and a male from a haploid one. After the discovery of diploid males in some highly inbred populations of hymenopterans, Whiting (1943) suggested that the sex determination was linked to a multiallelic locus: if an individual is heterozygous at this locus will develop as a female, whereas homozygotes and hemizygotes will develop as males; the homozygous males resulting from inbreeding are generally sterile. Since its discovery, the Whiting’s model has been confirmed in several species of hymenopterans: in the honey bee (*A. mellifera*), the single gene has been identified and sequenced; however in many cases multiple loci are involved rather than a single locus as in Whiting’s original system (Fusco and Minelli, 2019).

True arrhenotoky must be distinguished from pseudoarrhenotoky (figure 1) reported in some arthropods, in which both males and females develop from fertilized eggs, but modifications of the chromosome complement during embryonic stages result in the development of haploid males; this is due to the elimination or inactivation of the entire paternal chromosome set during early embryonic stage resulting in a functionally haploid blastula which will develop into a male; conversely, in females the diploid condition is maintained during development (Fusco and Minelli, 2019).

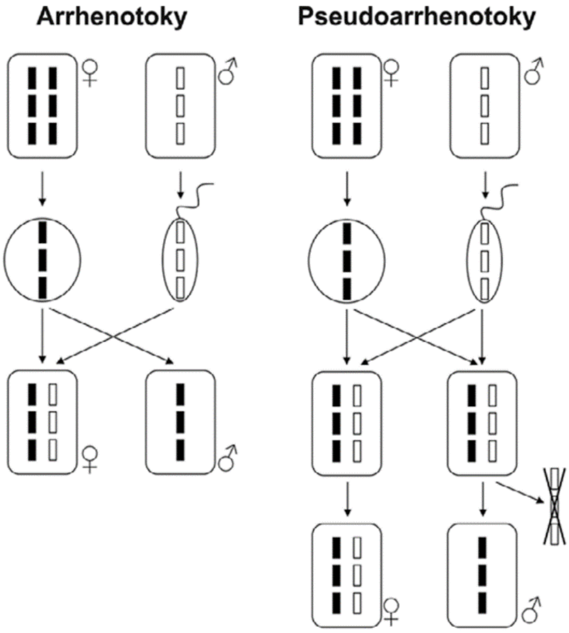


Figure 1. Comparison between true arrhenotoky and pseudoarrhenotoky (Fusco and Minelli, 2019).

Cytological mechanisms of animal parthenogenesis

Following the classification of Suomalainen (1950) a first distinction should be made between haploid and diploid parthenogenesis on the basis of cytological mechanisms.

In haploid parthenogenesis, the unfertilized eggs are reduced (or haploid): oogenesis is regular, during meiosis chromosomes pair in the usual way forming bivalents, crossing over occurs accordingly and the eggs undergo two meiotic divisions with a reduction of the chromosome number (Suomalainen, 1950). Because of the independent assortment and crossing over, the offspring will generally have genotypes different from the mother and from each other; therefore they are not clones of the mother (Fusco and Minelli, 2019).

Since meiosis proceeds regularly, these eggs might develop either through fertilization or without it: a single female can thus produce both fertilized and unfertilized eggs; moreover, these haploid unfertilized eggs develop into males in all those animals with a haplodiploid sex-determination system (already described in the previous paragraph). Therefore, haploid parthenogenesis is always facultative and arrhenotokous (Suomalainen, 1950).

In diploid parthenogenesis (or polyploid parthenogenesis in the case of polyploid species or populations), diploid (or polyploid) females produce unreduced eggs from which develop parthenogenetic individuals with the same ploidy level as the mother, that is diploid or polyploid; moreover, depending on the species the offspring can be of the same sex as the mother or of both sexes (Fusco and Minelli, 2019).

There are several cytological mechanisms that can lead to diploid parthenogenesis and they can be grouped into two main categories: meiotic or ameiotic parthenogenesis, depending on whether or not meiosis is maintained (White, 1977; Stenberg and Saura, 2009; Fusco and Minelli, 2019).

Meiotic parthenogenesis

Meiotic parthenogenesis, also known as automictic parthenogenesis or automixis, is the process in which meiosis is involved to produce parthenogenetic offspring (Suomalainen, 1950; White, 1977; Fusco and Minelli, 2019). Meiosis normally leads to the formation of haploid products and diploidy is restored at fertilization; since there is no fertilization in parthenogenetic individuals, different mechanisms are employed to restore the diploid condition (Stenberg and Saura, 2009). The early stages of meiosis in meiotic parthenogenesis are quite similar to the normal ones involving chromosomes pairing, crossing over and reduction of the chromosome number resulting in haploid products; however, in place of the fusion of two gametes deriving from two different individuals, meiotic parthenogenesis often involve the fusion of two meiotic products derived from a single individual and a single meiosis (Suomalainen, 1950; Stenberg and Saura, 2009; Fusco and Minelli, 2019).

If a population reproduce through this mode of parthenogenesis, the genetic variability depends on the exact cytological mechanism employed; in some cases, the genotype of the mother is transferred intact to the offspring, in others recombination which occurs during meiosis can produce a certain degree of genetic variability among the offspring (Stenberg and Saura, 2009; Fusco and Minelli, 2019). Unless other reproductive forms occur in this population, however, a loss of heterozygosity can be observed through many generations, up to a complete loss of heterozygosity; due to the lack of fertilization, meiotic parthenogenesis cannot produce genetic variations in the offspring if the mother is already homozygous at all loci (Fusco and Minelli, 2019).

All the main cytological mechanisms of meiotic parthenogenesis will be described below, following the descriptions of Stenberg and Saura (2009) and of Fusco and Minelli (2019).

In **gamete duplication** meiosis proceeds normally, but at the end of the second meiotic division, one of the four haploid products divide by mitosis forming two nuclei that eventually fuse to create a diploid nucleus; another option for restoring diploidy is the to replicate only the nuclear genome without cytokinesis (or endomitosis). This genome duplication is the reason why this mechanism produces solely offspring homozygous at all loci, regardless of the crossing overs that could have happened during meiosis (Figure 2).

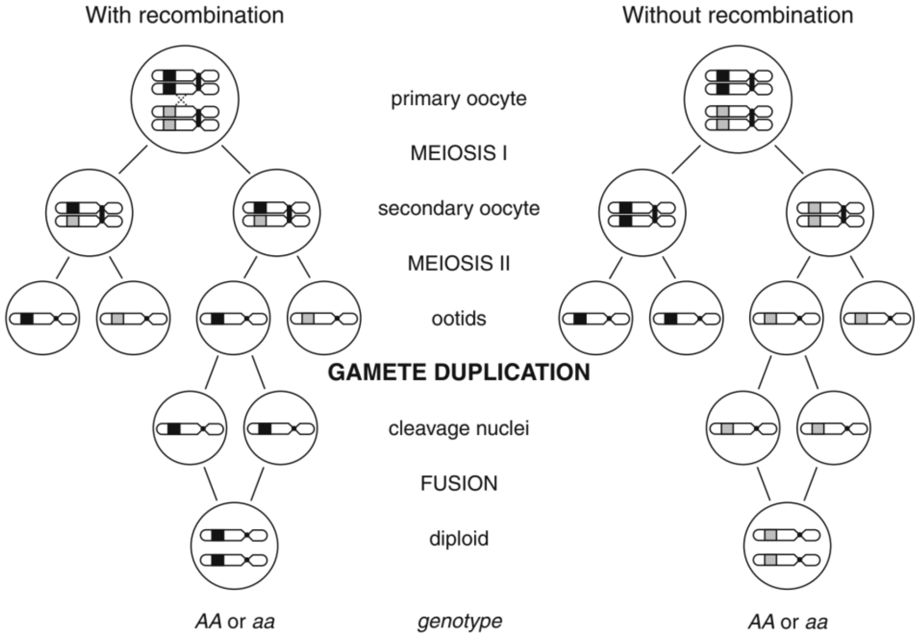


Figure 2. Meiotic parthenogenesis by gamete duplication, in which diploidy is restored through duplication and subsequent fusion of the two nuclei from a single meiotic product. For simplicity, a single pair of homologous chromosomes is shown. On the left, the case where crossing over occurred in the locus (A) of interest, whereas on the right the case in which no crossing over occurred in the same locus; in both cases the resulting genotype is homozygous (Stenberg and Saura, 2009; Fusco and Minelli, 2019).

Gamete duplication has been reported in crustaceans of the genus *Artemia*, in some mites and insects, including some parthenogenetic *Drosophila*.

In **terminal fusion**, the nucleus of the second polar body fuses with the nucleus of the egg cell. The second polar nucleus derives from the secondary oocyte like the egg cell and contains its other half of sister chromatids, therefore it can be considered as the “sister nucleus” of the egg cell. If a mother is heterozygous at a particular locus and no crossing over occurs, following terminal fusion the heterozygous locus will be exclusively homozygous in the offspring; on the other hand, if crossing over occurs in meiosis I heterozygosity can be maintained in the offspring (Figure 3).

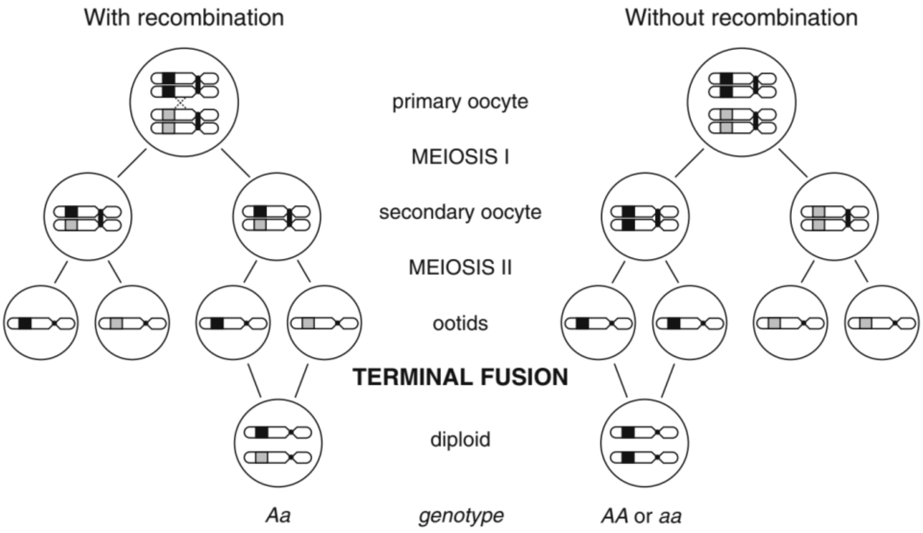


Figure 3. Meiotic parthenogenesis by terminal fusion, in which diploidy is restored through the fusion of two sister nuclei resulting from the second meiotic division. For simplicity, a single pair of homologous chromosomes is shown. In the case where crossing over occurred (on the left) the heterozygosity of the resulting genotype can be maintained; whereas in the case on the right where no crossing over occurred, the resulting genotype is always homozygous (Stenberg and Saura, 2009; Fusco and Minelli, 2019).

If the locus is close to the centromere, the chances of recombination are low, whereas if it is relatively distant from the centromere, the chances of crossing over are increased and the four alleles are randomly segregated among the four nuclei; in this situation, if one of the four nuclei carries an allele *A*, the probability that one of the three remaining nuclei carries the same allele *A* is 1/3. Therefore, the probability that a heterozygous locus (*Aa*) can be found in a homozygous state (*Aa* or *aa*) in the offspring is equal to 1/3.

In summary, each heterozygous locus has a probability of becoming homozygous from 1/3 (if it is far from the centromere) to 1 (if it is close to the centromere and cannot recombine); in a population reproducing by terminal fusion there will be a progressive reduction in average heterozygosity for each generation, at a rate that varies from locus to locus (between 1/3 and 1) and that is greater for those closer to the centromere.

Lastly, terminal fusion has been described in some nematodes, oligochaetes, tardigrades and arthropods.

Central fusion. The term “central fusion” refers precisely to the positions of the products deriving from the second meiotic division: the products occupying the central position merge to form the diploid zygote. Specifically, one haploid product deriving from the first polar body and another one deriving from the secondary oocyte are those which merge among the four haploid nuclei resulting from meiosis; they are therefore "non-sister" nuclei separated at the first meiotic division. In the absence of crossing over, the fusion of two non-sister products restores the heterozygosity, so the offspring are genetically identical to its mother. When crossing over occurs, alleles are rearranged among the four nuclei, so the fusion of two non-sister nuclei produces both homozygous (*AA* or *aa*) and heterozygous (*Aa*) genotypes in a ratio of 1/4 *AA*, 1/4 *aa* and 1/2 *Aa* (Figure 4).

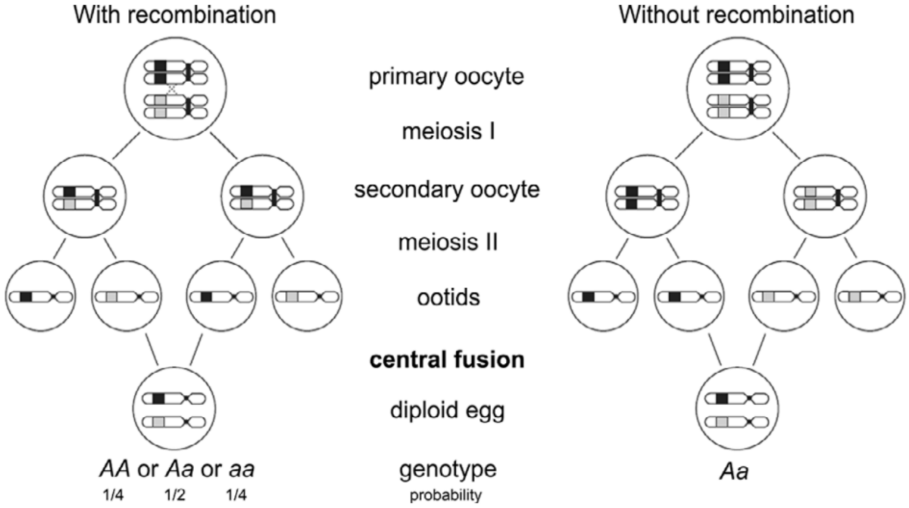


Figure 4. Meiotic parthenogenesis by central fusion, in which diploidy is restored through the fusion of two non-sister nuclei resulting from the second meiotic division. For simplicity, a single pair of homologous chromosomes is shown. In the case where crossing over occurred (on the left) both homozygous and heterozygous genotypes are produced; whereas in the case on the right where no crossing over occurred, the resulting genotype is always heterozygous (Stenberg and Saura, 2009; Fusco and Minelli, 2019).

Like in the previous case, assuming that one of the four nuclei carries an allele *A*, the probability that one of the three remaining nuclei carries the same allele *A* is 1/3, whereas the opposite probability that it carries a different allele (*a*) is 2/3 (Goudie and Oldroyd, 2014). In other words, the probability for a heterozygous locus (*Aa*) to become homozygous after the first meiotic division is 1/3, whereas the probability for this locus to remain heterozygous is 2/3. In summary, in one out of three events (1/3) a locus will become homozygous in the offspring and therefore the heterozygosity will be lost (Goudie and Oldroyd, 2014). Along the chromosomes, the probability that each locus becomes homozygous is almost zero if the locus is very close to the centromere since no recombination occurs (therefore the locus remains heterozygous); conversely, this probability gets closer to 1/3 if the locus is placed far from the centromere increasing the chances of recombination. In a population reproducing by central fusion, the average heterozygosity will gradually decrease for each generation at a maximum rate of 1/3 with differences across loci and higher values for those further from the centromere.

Central fusion has been reported in several species of insects, such as some dipterans, hymenopterans and lepidopterans which tend to maintain high levels of heterozygosity.

The term **“random fusion”** is used for the cases in which the egg nucleus fuses randomly with one of the three remaining nuclei of the haploid polar bodies. Considering a heterozygous locus, the same allele of the egg cell is carried only by one out of three polar bodies, whereas the alternative allele is carried by the other two polar bodies. Consequently, regardless of whether or not crossing over occurs, there is a 1/3 chance that a heterozygous locus will turn into homozygous after random fusion. As in the previous case, the average heterozygosity will tend to decrease by 1/3 in each generation, but equally for all loci.

Another particular mechanism is the “**fusion of the nucleus of the first polar body with the nucleus of the secondary oocyte**”; in this case the nuclei derived from the first meiotic division do not separate and fuse directly, or alternatively first separate and then fuse, producing a transitory tetraploid state which is reduced with the second meiotic division restoring diploidy. A heterozygous mother will produce both homozygous and heterozygous genotypes and recombination does not affect the progressive loss of heterozygosity, which is reduced by 1/3 for each generation in all loci.

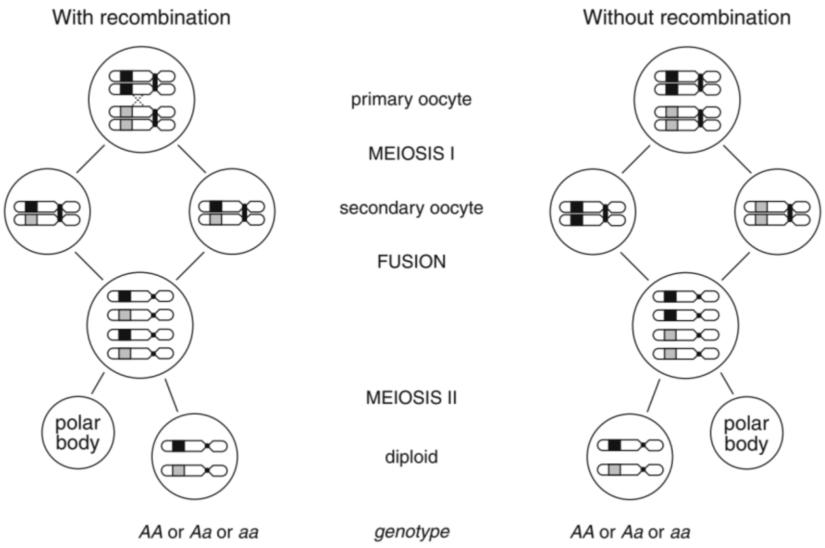


Figure 5. Meiotic parthenogenesis by fusion of the nucleus of the first polar body with the nucleus of the secondary oocyte; a reductional division of the tetraploid stage restore the diploid condition. For simplicity, a single pair of homologous chromosomes is shown (Stenberg and Saura, 2009; Fusco and Minelli, 2019).

In **premeiotic doubling** (or premeiotic endomitosis) an endomitosis or endoreplication occurs before meiosis, that is the genetic material replicates although the cell remains undivided (Figure 6). The primary oocyte has twice the number of chromosomes than normal and through meiosis the resulting product has the same level of ploidy as the germ cell from which it was produced. During the first meiotic division, theoretically the pairing of chromosomes could occur between pairs of homologous or sister chromosomes; however, in almost all cases the paired chromosomes (or bivalents) consist of sister chromosomes, not homologous ones (Lutes *et al.*, 2010). Since the sister chromosomes are identical to each other, the crossovers (i.e. crossing over) that take place between them do not alter the mother's genotype, maintaining the original heterozygosity at all loci. Although heterozygosity in sexual reproduction is ensured by recombination between bivalents of homologous chromosomes, in premeiotic doubling heterozygosity is preserved through recombination between identical sister chromosomes (Lutes *et al.*, 2010). Homologues pairing, in this case, would result in some loss of heterozygosity in the offspring. Thus, the resulting meiotic product is an egg cell genetically identical to a somatic cell of the mother that will generate offspring exactly like the mother. Considering the genetic consequences, this mode of meiotic parthenogenesis can be considered similar to ameiotic parthenogenesis.

Premeiotic doubling of chromosomes has been documented in angiosperms (e.g. in some species of Allium), among the parthenogenetic forms of flatworms and earthworms and is also known in many insects (e.g. grasshopper *Warramaba virgo*), mites, tardigrades and in parthenogenetic vertebrates.

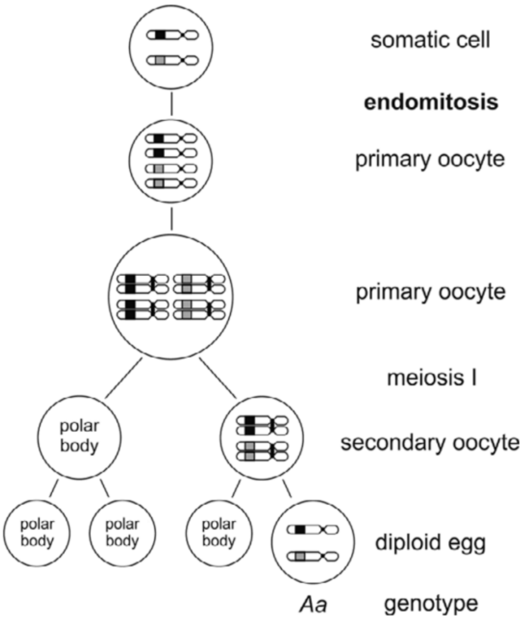


Figure 6. Meiotic parthenogenesis by premeiotic doubling, in which a tetraploid nucleus produced by endomitosis is reduced to a diploid one. A single pair of homologous chromosomes is shown. In this case, crossing over does not affect the genotypes of the meiotic products (Stenberg and Saura, 2009; Fusco and Minelli, 2019).

Ameiotic Parthenogenesis

The absence of meiosis is what distinguishes the ameiotic parthenogenesis, also called apomictic parthenogenesis, or apomixis. The egg cells are produced by a single cell division that is generally indistinguishable from a mitosis; consequently, the progeny is genetical identical to the mother (Stenberg and Saura, 2009). Source of genetic variability could be the possible mutations occurring during gametogenesis that are then transmitted unaltered to the offspring and are enhanced by the accumulation of other mutations; alternatively, another source of genetic variability could be ameiotic recombination (Fusco and Minelli, 2019).

This mode of parthenogenesis is the most common type and it is found in plants and in different animal groups: all animals showing cyclical parthenogenesis reproduce by apomixis, including monogonont rotifers, cladoceran crustaceans and aphids, in addition to many representatives of cnidarians, flatworms, nematodes, bdelloid rotifers, gastropods, oligochaetes, and arthropods of different groups; in plants, ameiotic parthenogenesis corresponds to different forms of apomixis (Suomalainen, 1950; Stenberg and Saura, 2009; Fusco and Minelli, 2019).

Sperm-dependent parthenogenesis

Parthenogenesis is often compared with other seemingly similar forms of unisexual reproduction such as gynogenesis and hybridogenesis which require the participation of sperm at some level (figure 7), whereas true parthenogenesis is sperm-independent (Vrijenhoek, 1984, 1989; Kearney, 2005; Neaves and Baumann, 2011).

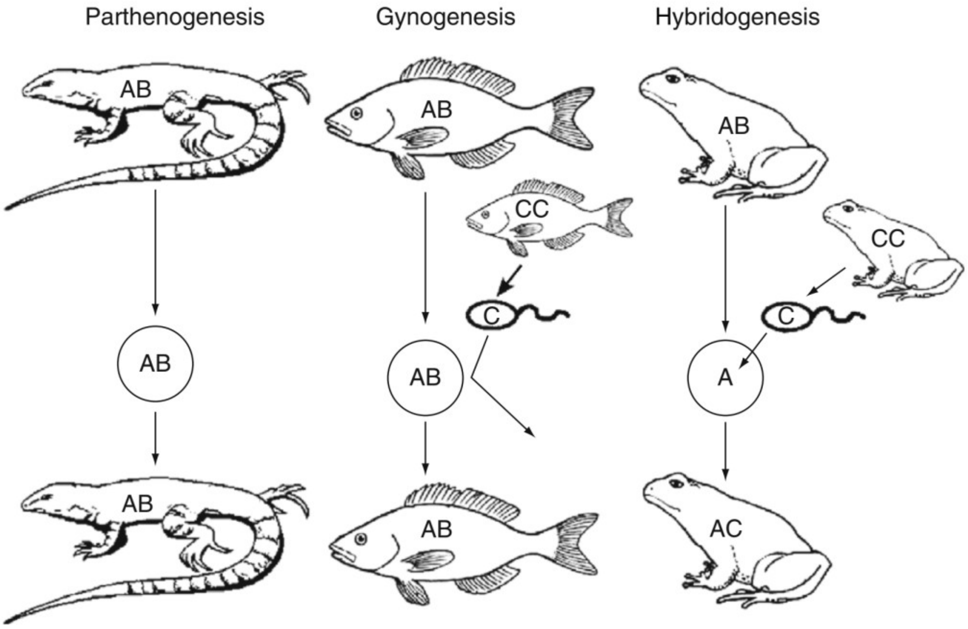


Figure 7. Comparison between three unisexual modes of reproduction. Parthenogenesis does not require sperm contribution; gynogenesis requires sperm to stimulate embryogenesis, but male gamete does not provide its genome (C) to the offspring; in hybridogenesis one parental genome (A) is transmitted to the egg, whilst the other (B) is lost during gametogenesis and replaced at each generation through fertilization (Ryskov, 2008).

Gynogenesis is considered a form of sperm-dependent parthenogenesis or pseudogamy (or pseudogamous parthenogenesis); but differently from parthenogenesis, gynogenesis requires the activation of the egg cell by sperm, although this one does not contribute genetically to the development of the new individual (Vrijenhoek, 1989; Kearney, 2005; Lampert, 2008; Neaves and Baumann, 2011). The gynogenic female produces unreduced eggs that are not properly fertilized, but they do not begin to develop until “activated” by a male; the male gamete may simply come into contact with the egg cell or it may penetrate this one, but it does not provide any genetic material to the genome of the egg cell and to the resulting offspring (Vrijenhoek, 1989; Beukeboom and Vrijenhoek, 1998; Avise, 2008; Fusco and Minelli, 2019).

The term gynogenesis, meaning literally “the origin of females”, implies to be descended from females; species that reproduce exclusively by gynogenesis are composed only by females (Beukeboom and Vrijenhoek, 1998; Avise, 2008). These all-female gynogenic species require the coexistence with a closely related sexual species or lineage, as the mating is necessary for the egg activation; however, if the females are also hermaphroditic (e.g. flatworms, annelids, nematodes), they don’t need for a different species or lineage as a sperm donor (Beukeboom and Vrijenhoek, 1998; Fusco and Minelli, 2019). Given their hermaphroditism, for these species the term pseudogamy should be preferred to the term gynogenesis which implies being generated only by females (Beukeboom and Vrijenhoek, 1998).

The resulting gynogenic daughters usually carry no genes from their father and are genetically identical to their mother, thus it has also been considered a form of clonal reproduction; however as in the case of true parthenogenesis, the effects of reproduction by gynogenesis on the genetic structure of the population depend on the specific cytological mechanism producing the unreduced eggs (Avise, 2008; Neaves and Baumann, 2011; Fusco and Minelli, 2019). Moreover, elements of the paternal genome may be occasionally incorporated into the genome of gynogenic offspring, an event known as “paternal leakage” (Fusco and Minelli, 2019).

Among invertebrates, gynogenesis is well known in various freshwater planarians and only in a few species of insects; amphibians and fishes provide all the known vertebrate examples (Avise, 2008).

For example, salamanders of the genus *Ambystoma* have reproduced in this way for over a million years, but probably new alleles have been introduced into the gene pool through paternal leakage (Avise, 2008; Neaves and Baumann, 2011; Fusco and Minelli, 2019). In goldfish (*Carassius auratus*), a normally amphigonic species, some gynogenic populations are known; in another fish genus, *Poeciliopsis*, some forms are gynogenic, while others hybridogenic, all requiring insemination by a related sexual form; in the genus *Poecilia*, the Amazon molly (*Poecilia Formosa*) was the first clonal vertebrate known to science discovered in 1932 by Carl and Laura Hubbs: it is the result of natural hybridization between two sexual species and its all-female progeny develop through gynogenesis in a process in which the first meiotic division is suppressed (Schultz, 1971; Avise, 2008; Fusco and Minelli, 2019).

Hybridogenesis, literally “the origin of hybrids”, has been defined as a hemiclonal form of reproduction, halfway between biparental and uniparental sexual reproduction (Avise, 2008; Fusco and Minelli, 2019). It differs from gynogenesis since sperm from a sexual male actually enters and fertilizes the eggs; moreover, each egg produced by a hybridogenetic female is reduced rather than unreduced and the fertilization actually restore the diploid condition; in addition, during gametogenesis, hybridogenic females, which are hybrids deriving from two parental species, produce haploid eggs which contain only the maternal set of chromosomes since the paternal set has been discarded (Avise, 2008; Lampert, 2008; Neaves and Baumann, 2011). The paternal genome may be removed from the egg cell through a particular meiosis in which no recombination occurs, so the only source of genetic variability, in addition to mutation, remains syngamy; another alternative mechanism, occurring for example in hybridogenic green frogs, is the exclusion of paternal chromosomes prior to meiosis that results in a haploid cell followed by an endomitosis to double chromosomes and a subsequent normal meiosis to restore the haploid condition (Avise, 2008; Fusco and Minelli, 2019).

In both cases, a hybrid female passes the genome of her mother unaltered to the next generation without any recombination between maternal and paternal chromosomes as normally occurs in sexual reproduction; given that these gametes are partial clones (or hemiclones) of the maternal genome, this form of reproduction is defined as hemiclonal. Each individual, therefore, expresses the genome of both maternal and paternal origin, but fails to transmit the paternal genome to daughters which is replaced by the genetic material of a new male in each generation (Lampert, 2008; Neaves and Baumann, 2011; Fusco and Minelli, 2019).

As stated by Avise (2008), since a female through hybridogenesis does not transmit the genes of her father to the offspring, her father cannot be considered the genetic grandfather of her progeny.

Hybridogenesis is known in some fishes of the genus *Poeciliopsis*, in green frogs of the genus *Pelophylax* (previously *Rana*) and in stick insects of the genus *Bacillus* (Bullini, 1994; Avise, 2008; Fusco and Minelli, 2019).

One of the most studied examples of hybridogenesis belongs to the genus *Pelophylax* of European green frogs and involves a complex of three species (*P. lessonae*, *P. ridibundus* and *P. esculentus*); *P. esculentus* is a hybrid of the other two species and it reproduces by hybridogenesis through the backcrossing with males of the parental species *P. lessonae* which provide the paternal genome; this genome is eliminated during gametogenesis by the hybridogenic females, so that they produce egg cells with only the *P. ridibundus* genome. Less frequently, some hybrid populations exclude *P. ridibundus* genome during gametogenesis, producing gametes with only the *P. lessonae* genome. Consequently, this system is possible only under sympatry between hybrids and one parental species (Avise, 2008; Fusco and Minelli, 2019).

Another similar example of this reproductive mode is the crossing between females of *Poeciliopsis monacha* and males of *Poeciliopsis lucida* that generates the diploid hybrid *P. monacha-lucida* represented only by females which reproduce with males of *P. lucida* or another related species (Schultz, 1971; Fusco and Minelli, 2019).

Some salamanders of the genus *Ambystoma* have complex genetic systems that cannot be considered strictly hybridogenic or gynogenic; it has been suggested the term “kleptogenesis” to indicate these reproductive systems in which females use as they please the sperm “stolen” from males of related sympatric species (Avise, 2008; Fusco and Minelli, 2019).

Cyclical parthenogenesis

Cyclical parthenogenesis is a type of heterogonic cycle characterized by the alternation of different generations with different modes of reproduction: one or more parthenogenetic generations alternate with a bisexual generation in which fertilization takes place (Suomalainen, 1950; Mittwoch, 1978).

In about 15,000 species of animals, thelytokous parthenogenesis alternates with amphigony: particularly, in most representatives of the monogonont rotifers, cladocerans, in some parasitic nematodes, cynipid hymenopterans, cecidomyiid dipterans, as well as in many aphids (Fusco and Minelli, 2019).

The life cycles of monogonont rotifers, cladocerans and aphids will be considered below as examples.

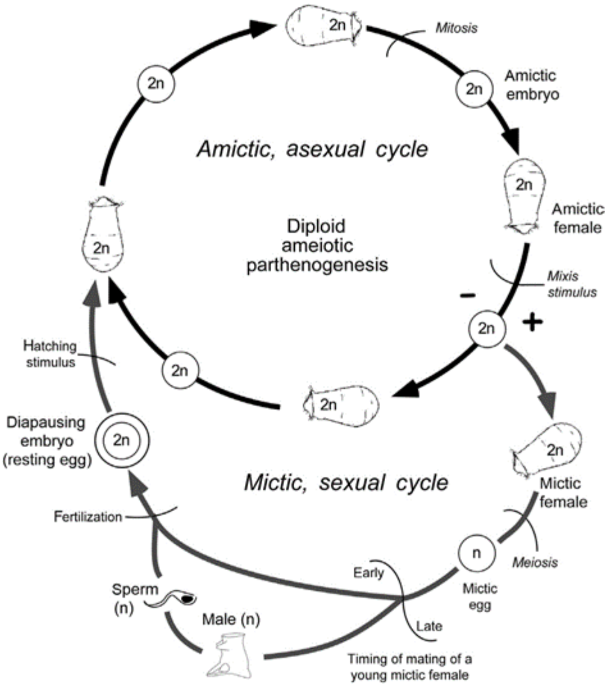


Figure 8. Life cycle of monogonont rotifers: an amictic (or asexual) phase alternates with a mictic (or sexual) phase (Wallace, Snell and Smith, 2015).

Monogonont rotifers are small invertebrates principally found in freshwaters, they have a single gonad, are generally oviparous and are haplodiploid; their life cycle involves an amictic (or asexual) phase and a mictic (or sexual) phase (Figure 8; Wallace, Snell and Smith, 2015). Amictic females are diploid and reproduce by ameiotic parthenogenesis generating diploid eggs (or amictic eggs) through a single equational division; from these unfertilized eggs hatch females usually within 24 hours: this is why they are often called “*subitaneous*” eggs (Fusco and Minelli, 2019). This amictic phase generally include several parthenogenetic generations and occurs in favourable conditions until, under specific stimuli (e.g. chemical or environmental signals), females begin to produce mictic daughters; these diploid mictic females, through a regular meiosis, produce haploid eggs (or mictic eggs) that can either be fertilized or unfertilized: those unfertilized will develop parthenogenetically into haploid males that will fertilize the mictic eggs (Wallace, Snell and Smith, 2015; Fusco and Minelli, 2019). These eggs fertilized by males in an early stage, will give rise to a diploid zygote whose development is arrested before maturation, resulting in a diapausing embryo (also termed “resting egg”); these resting eggs have a thick wall and are resistant to unfavourable environmental conditions, so they allow rotifers to survive harsh seasons (Wallace, Snell and Smith, 2015). After a variable dormant period, they hatch as diploid amictic females and the cycle begins again; stimuli such as changes in temperature, light, salinity and oxygen concentration, may trigger the hatching of resting eggs (Schröder, 2006; Wallace, Snell and Smith, 2015). In some species, the signals that trigger the transition from asexual to sexual phase are chemical compounds excreted by rotifers in the water, such as the mixis induction protein (MIP): this protein accumulates to a threshold concentration as the population increases and stimulates the amictic females by binding to their receptors and transmitting the signal to the oocyte (Snell *et al.*, 2006; Wallace, Snell and Smith, 2015).

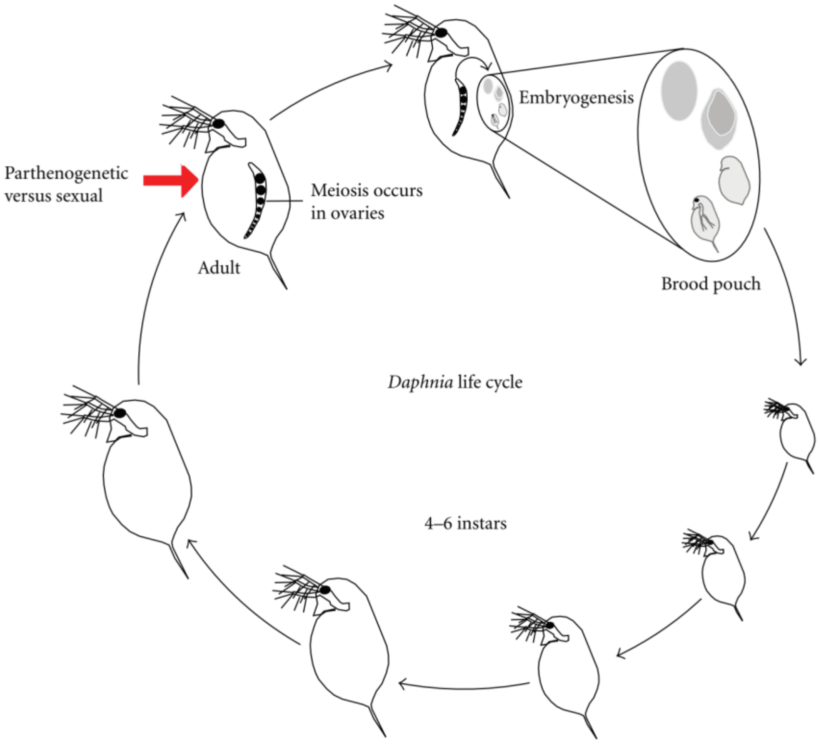


Figure 9. In the heterogonic cycle of the water flea Daphnia, the transition from parthenogenetic to amphigonic reproduction and the sex of the offspring is determined by endocrine signals in response to specific environmental cues (Fusco and Minelli, 2019). Sex determination and sexual reproduction are epigenetically determined; modified image from Harris, Bartlett and Lloyd, 2012.

Similar life cycles are exhibited by cladocerans (figure 9), small crustaceans commonly called water fleas, including especially the well-studied genus *Daphnia* (Smirnov, 2017; Fusco and Minelli, 2019; Toyota *et al.*, 2021). Some species, called monocyclic, show a single heterogonic cycle per year; whereas others, called polycyclic, show more than one heterogonic cycle per year (Smirnov, 2017). Under favourable conditions, cladocerans parthenogenetically reproduce building up a population consisting of only females with an exponential growth of this population (Toyota *et al.*, 2021). After several parthenogenetic generations, under unfavourable conditions, cladocerans produce diploid males asexually and shift to bisexual reproduction producing dormant or resting eggs, which are generally surrounded by a reinforced shell, or "*ephippium*", to tolerate extreme environmental conditions (Smirnov, 2017; Toyota *et al.*, 2021). Resting eggs will hatch as females when favourable conditions will be restored (Toyota *et al.*, 2021). Thus, ovaries can contain both parthenogenetic and sexual eggs (Harris, Bartlett and Lloyd, 2012) and the same female can produce haploid eggs by conventional meiosis or diploid eggs by parthenogenesis: the haploid ones are fertilized by males and develop into diploid females, whereas the diploid ones are unfertilized and can develop into diploid females and males, unlike monogonont rotifers (Banta and Brown, 1929; Ignace, Dodson and Kashian, 2011). Even after amphigonic reproduction, females can return to parthenogenesis and switch between the two types of reproduction (Deng, 1996). Sex determination as well as the production of sexual eggs are triggered by environmental factors: food abundance, crowding, photoperiod, temperature and chemicals have been tested and seem to influence their reproduction (Deng, 1996; Harris, Bartlett and Lloyd, 2012; Smirnov, 2017; Toyota *et al.*, 2021). As reported by Banta and Brown (1929) and Ignace, Dodson and Kashian (2011), sex determination occurs when the egg is still immature in the ovaries of adult females, not during the embryo development; therefore, environmental factors act on the immature oocyte regulating meiosis, although the exactly molecular mechanisms by which these signals are transduced are not well understood (Harris, Bartlett and Lloyd, 2012). Probably an analogue of the insect juvenile hormone, or methyl farnesoate (MF), is involved in the transduction of these signals causing the production of males by parthenogenesis and the switch to sexual reproduction (Ignace, Dodson and Kashian, 2011; Toyota *et al.*, 2021).

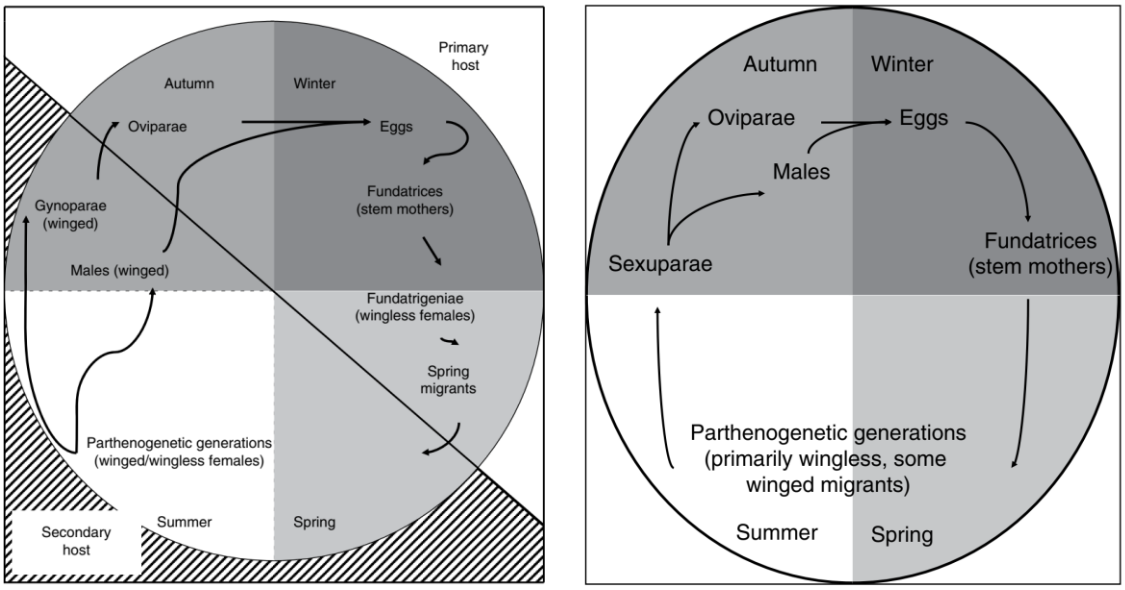


Figure 10. On the left a generalized life cycle of a host-alternating (heteroecious) aphid, on the right a generalized life cycle of a non-host alternating (monoecious) aphid (Hardie, 2017).

Among the insects, aphids (order Hemiptera, superfamily Aphidoidea), well known for their role as crop pests (Hardie, 2017; Singh and Singh, 2021), have a wide diversity in heterogonic cycles due to several factors. Firstly, they can spend their life on a single plant species (also called non-host alternating, monoecious or autoecious), or they can alternate the plant species that host them (host-alternating or heteroecious) living between a primary host in winter (usually woody plants) and a secondary host (usually herbaceous plants) in spring/summer (Hardie, 2017). Secondly, parthenogenesis and sexual reproduction can follow one another throughout the year (holocycle), or the cycle can extend over several years in some species (paracycle), or sexual reproduction may be lacking and they only reproduce by parthenogenesis (anholocycle) as occurs in country where the climate is mild during winter (Hardie, 2017; Fusco and Minelli, 2019). Moreover, they can alternate between winged forms, which migrate between the different host plants as the seasons change, and wingless forms which are sedentary (Hardie, 2017; Fusco and Minelli, 2019).

Considering a simplified annual cycle (figure 10), aphids reproduce sexually in autumn (on the primary host plant for heteroecious species) to lay diapausing eggs that overwinter and hatch in spring as parthenogenetic females, the so-called stem-mothers (or founders, or fundatrices) which are generally wingless (Suomalainen, 1950; Hardie, 2017; Fusco and Minelli, 2019). From these stem-mothers descend several generations of parthenogenetic females which can be wingless or winged and, thus, they can develop on the same primary host plant as the mothers or migrate to the secondary one (the so-called spring migrants) where they reproduce throughout the summer (Suomalainen, 1950; Hardie, 2017; Fusco and Minelli, 2019). When the autumn approaches (or in summer in some species), there is a parthenogenetic generation of *sexuparae* (so termed because their offspring sexually reproduce) composed by females that can produce individuals of both sexes: female-producing *sexuparae* are called *gynoparae*, whereas male-producing *sexuparae* are called *androparae* (Suomalainen, 1950; Fusco and Minelli, 2019). The winged forms of *sexuparae*, in heteroecious species, fly back to the primary host plant where they produce the sexual generation composed by amphigonic females (denominated *oviparae*) and males which mate producing sexual eggs (Suomalainen, 1950; Hardie, 2017; Singh and Singh, 2021). Males can be either winged or wingless (Singh and Singh, 2021) and in some heteroecious species they develop on the summer plant instead of the winter one where they later go back (Suomalainen, 1950; Hardie, 2017). The parthenogenetic generations are generally viviparous, while only the amphigonic females are oviparous (hence the term *oviparae*), even if some species are always oviparous (Suomalainen, 1950; Fusco and Minelli, 2019; Singh and Singh, 2021). Again, environmental factors such as lower temperatures, shorter day length, crowding, food quality and host availability influence the production of sexual forms together with an intrinsic “timer”, which seems genetically encoded (Hardie, 2017; Yan, Wang and Shen, 2020; Singh and Singh, 2021).

Aphids have an X0 (or XX/X0) chromosomal sex-determination system in which the heterogametic sex (male) has a single copy of heterochromosome, while the homogametic sex (female) has a double copy of it (Mittwoch, 1978; Fusco and Minelli, 2019). Although both males and females are diploid and can originate from parthenogenetic eggs, males have only one X chromosome (Suomalainen, 1950; Mittwoch, 1978; Singh and Singh, 2021). Thus, during spermatogenesis, males may develop two kinds of secondary spermatocytes which contain or not the X chromosome: those lacking it rapidly degenerate and those containing it are the only functional sperm cells (Suomalainen, 1950; Fusco and Minelli, 2019; Singh and Singh, 2021). When fertilization occurs, both haploid gametes provide an X chromosome; this is the reason why all fertilized overwintering eggs develop into females (XX), the founders of the following parthenogenetic generations (Suomalainen, 1950; Fusco and Minelli, 2019; Singh and Singh, 2021). Parthenogenesis is of apomictic nature with a single equational division (Suomalainen, 1950; Yan, Wang and Shen, 2020; Singh and Singh, 2021), so an X chromosome should be lost in order to obtain males asexually. Oocytes that will develop into males have a peculiar cell division: the autosomes divide equationally, while the X chromosomes pair forming a bivalent and undergo a reductional division, so that the egg cell receive a single X chromosome while the other goes to the polar body (Suomalainen, 1950; Mittwoch, 1978). On the contrary, oocytes that will develop into females maintain both heterochromosomes during maturation (Fusco and Minelli, 2019). The fate of the oocytes is controlled by the aforementioned external factors, in particular photoperiodism: photoreceptors located in the cephalic region are able to measure the night length and probably they induce endocrine signals involving the production of juvenile hormones, which are likely responsible for the transduction of these signals from the brain to ovaries (Trionnaire *et al.*, 2008; Yan, Wang and Shen, 2020).

The transition from the asexual to the sexual phase in cyclical parthenogens requires an adjustment of the secondary sex ratio (the ratio of males to females at birth) from nearly zero to an optimal level for the fertilization of sexual eggs; the timing of this adjustment together with the subsequent fertilization of eggs is the key for the success of sexual reproduction in cyclical parthenogenesis (Kleiven, Larsson and Hobæk, 1992). In all the cases so far described, the sex ratio as well as the shifting between the two phases are influenced by environmental and density dependent factors (Trionnaire *et al.*, 2008; Wallace, Snell and Smith, 2015; Smirnov, 2017; Yan, Wang and Shen, 2020; Toyota *et al.*, 2021). However, as observed by Kleiven *et al.* (1992), all these species differ in the cytological mechanisms determining sex among parthenogens which result in different strategies adopted to adjust the sex ratio: the haplo-diploid system of monogonont rotifers solves the problem of sex-ratio adjustment through a kind of feedback function by which fertilized eggs develop into diploid females while unfertilized ones develop automatically into males; in other words when the males are missing, new males are always produced since the eggs are not fertilized. On the other hand, cladocerans and aphids have both diploid females and males, hence in both taxa the mothers are the ones that "control" the sex of offspring under environmental signals; however, in aphids the sexual females generally constitute a generation distinct from those reproducing by parthenogenesis, whereas cladoceran females are capable of producing all kinds of offspring (Kleiven, Larsson and Hobæk, 1992). Despite the differences between species, the parthenogenetic generations allow a rapid propagation of the population during favourable seasons, whereas the sexual forms are the source of genetic variation that increase the survival rates during unfavourable seasons (Singh and Singh, 2021; Toyota *et al.*, 2021). The two phases of the cycle are, therefore, correlated with the seasonality and permanency of habitats: strongly seasonal habitats favour the recurrence of the sexual phase, while permanent ones make the occurrence of sexual reproduction rare and episodic (Kleiven, Larsson and Hobæk, 1992). In the latter condition, the sexual phase may be lost and the reproduction results exclusively by parthenogenesis (Suomalainen, 1950; Hardie, 2017). Therefore, compared to these asexual lineages, the preservation of sexual reproduction allows to take advantage of the changing environmental conditions (Yan, Wang and Shen, 2020; Toyota *et al.*, 2021). Moreover, cyclically parthenogenetic lineages usually have a greater genetic diversity than the obligate asexual ones and the occurrence of amphigony seems to purge the genome from the accumulation of deleterious mutations resulting from obligate parthenogenesis (Yan, Wang and Shen, 2020).

Paedogenesis

Paedogenesis is the parthenogenetic reproduction of individuals at larval or pupal stages (Suomalainen, 1950; Ibrahim and Gad, 1975; Hodin and Riddiford, 2000; Alejandra Perotti, Young and Braig, 2016). This is thought to occur through the precocious reproductive development in juveniles, which become able to reproduce while still being in a sexually immature stage (Ibrahim and Gad, 1975; Pierce and Smith, 1979; Achterkamp *et al.*, 2000; Hodin and Riddiford, 2000). This phenomenon was first discovered in 1861 by Nicholas Wagner (Russia) in dipterous insects of the genus *Miastor* of the family Cecidomyiidae (Achterkamp *et al.*, 2000). The term “paedogenesis” was introduced later by von Baer in 1866 to describe early reproduction in parthenogenetic insects (Pierce and Smith, 1979). The phenomenon is observed particularly in insects where it has evolved at least six times independently (Hodin and Riddiford, 2000; Fusco and Minelli, 2019). Dipterans of the family Cecidomyiidae (or gall midges) show the richest examples of either larval parthenogenesis and pupal parthenogenesis: *Miastor*, *Heteropeza* and *Mycophila* were the first best studied genera to reproduce parthenogenetically in the larval stage, whereas parthenogenesis in the pupal stage was described later in the species *Tecomyia populi* and *Henria psalliotae* (Wyatt, 1961). In their paper, Achterkamp *et al.* (2000) listed all the known cases of pedogenesis reported up to then in various animal taxa, re-evaluating and excluding many of them as possible cases of paedogenesis.

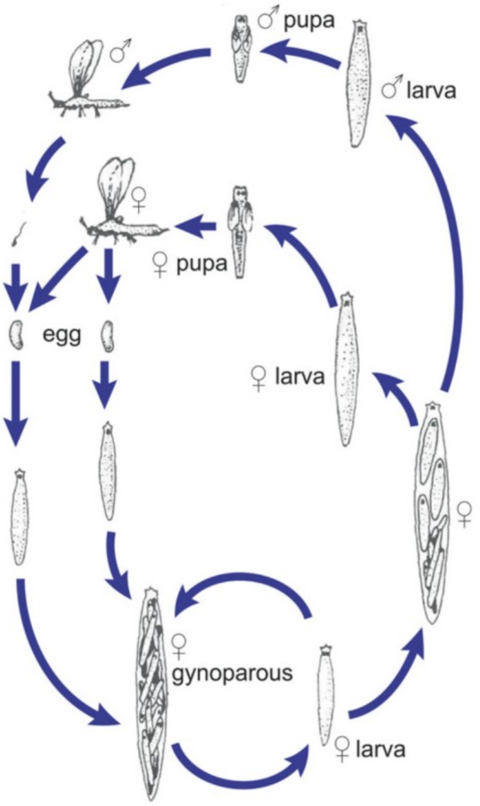


Figure 11. Life cycle of the midge Heteropeza pygmaea which can reproduce as a larva by paedogenesis, and it can reproduce as an adult by amphigony or parthenogenesis (Fusco and Minelli, 2019).

Following the description of Achterkamp *et al.* (2000), the life cycle of *Heteropeza pygmaea* can be considered as a typical example of larval parthenogenesis (figure 11). The adult female of *H. pygmaea*, which can reproduce by amphigony or parthenogenesis, lays a clutch of eggs which can be fertilized or not; from these eggs hatch “mother” larvae that reproduce by paedogenesis at larval stage: eggs start to develop in their ovaries from germ line cells, are released in the body cavity (or haemocoel) where new larvae develop until they mature and emerge from the body of their mothers whose tissues are completely histolysed for the benefit of the progeny. These “daughter” larvae can continue to produce paedogenetic larvae for several generations or they can give rise to adults of both sexes that will reproduce sexually. Therefore, different types of larvae can be recognized: thelytokous paedogenetic larvae (or thelygenic or gynogenic larvae) which give rise to other paedogenetic females similar to themselves, arrhenotokous paedogenetic larvae (also arrhenogenic or androgenic larvae) which produce male larvae destined to develop into adults, deuterotokous paedogenetic larvae (or amphigenic larvae) which produce both paedogenetic females and male larvae, and finally “super gynogenic” larvae which will develop into adult females (Achterkamp *et al.*, 2000; Fusco and Minelli, 2019). Parthenogenesis, in both adult and larval stages, seems to be ameiotic (Fusco and Minelli, 2019).

Specific environmental conditions, especially the availability of nutritional resources, seem to trigger the switching between paedogenetic and adult forms (Achterkamp *et al.*, 2000; Fusco and Minelli, 2019). Cecidomyiid dipterans are mostly phytophagous and many of them feed on mushrooms: when they colonize new fresh mushrooms, larval parthenogenesis is induced to exploit this abundant nourishment; whereas when food resources are scarce larvae will develop into adult females and males which fly away to find another food resource (Achterkamp *et al.*, 2000; Hodin and Riddiford, 2000). Moreover, hormonal control seems to strongly affect oogenesis: the steroid hormone 20-hydroxyecdysone (20 E) in association with the up-regulation of its receptors allow for the precocious ovarian differentiation in the paedogenetic development (Went, 1979; Went and Camenzind, 1984; Hodin and Riddiford, 2000).

Paedogenesis has also been described in *Micromalthus debilis*, a beetle of the family Micromalthidae, which show, as dipterans, different type of larvae: thelytokous (female producing), arrhenotokous (male producing) and amphiterotokous (male and female producing) paedogenetic larvae (Scott, 1938; Achterkamp *et al.*, 2000). However, the male-producing larva generates a single big egg and if this egg fails to develop, a small number of thelytokous larvae are instead produced; although larvae can develop into adult females and males, mating between them has never been observed and males are generally absent (Achterkamp *et al.*, 2000). As demonstrated by Alejandra Perotti, Young and Braig (2016), the adults are no longer reproductively functional: they were unable to copulate in their experiment, and the females were not able to produce progeny, either by sexual or parthenogenetic reproduction; moreover, the dissection of the adults provided evidence that they are no longer capable of sexual reproduction. Therefore, Alejandra Perotti, Young and Braig (2016) suggested that *M. debilis* is a special case in which sexual reproduction as well as adult stages have been lost for both sexes; it has probably lost the sexual part of its life cycle a long time ago and now it reproduces exclusively by larval thelytoky or paedogenesis. For this reason, the adults that are rarely produced by paedogenetic larvae are called “ghost adults” by Alejandra Perotti, Young and Braig (2016).

Paedogenetic reproduction has also discovered more recently in some species of microscopic marine invertebrates of the phylum Loricifera that have complex life cycles not fully understood involving adult stage (if any exist) and a succession of several larval forms with a paedogenetic life cycle (Ibrahim and Gad, 1975; Heiner and Kristensen, 2009).

In extreme cases of paedogenesis, as in some aphids, an individual can begin to reproduce when it is still in the body of its mother: a parthenogenetic female can contain in her body not only the embryo of her daughter but also that of her granddaughter which is developing within her daughter (Fusco and Minelli, 2019; Singh and Singh, 2021). Like matryoshka dolls, these generations are said to be "telescoped" into each other (Achterkamp *et al.*, 2000; Fusco and Minelli, 2019; Singh and Singh, 2021). However, as observed by Achterkamp *et al.* (2000), it only happens occasionally in aphids that daughters are born before the mother is adult, thus true paedogenesis is rare in these species and this term is not suitable to characterize them.

Geographical parthenogenesis

Starting from the fact that some species of animals and plants include both amphigonic and parthenogenetic populations, the French zoologist Vandel (1928) was the first to note that parthenogenetic forms had different ecological and geographical distributions than their sexual counterparts, so he coined the term "*parthénogènese géographique*” to refer to such differences. He came to this conclusion by investigating the distribution of *Trichoniscus elisabethae* (Crustacea, Isopoda): the parthenogenetic population was able to live in colder and drier climates than the bisexual population. Subsequently various authors have observed similar geographical patterns in additional invertebrates, vertebrates and plants concluding that parthenogens tend to be found at higher latitudes and altitudes, on islands instead of mainlands, in xeric opposed to mesic environments, and in disturbed rather than undisturbed habitats (Suomalainen, 1950; Wright and Lowe, 1968; Cuellar, 1977; Glesener and Tilman, 1978; Lynch, 1984; Beaton and Hebert, 1988). However, there are also cases of sexual and parthenogenetic populations that do not follow the general geographical pattern: for example, the lizard *Lepidophyma flavimaculatum* (Xantusiidae) has a parthenogenetic population in the less disturbed areas of American tropical forests; or two Psocoptera (*Reuterella helvimacula* and *Cerobasis guestfalicus*) have sexual populations at higher latitudes than the parthenogenetic ones (Glesener and Tilman, 1978; Fusco and Minelli, 2019). However, these exceptions received little attention in the scientific community.

Although many different and non-exclusive hypotheses have been suggested, the cause for such differences in the geographical distribution remain controversial (Beaton and Hebert, 1988; Kearney, 2005). A group of these hypotheses have focused on the differences in the modes of reproduction (asexual vs. sexual), others on the level of ploidy or on hybridization (Bierzychudek, 1985; Beaton and Hebert, 1988). Among the first ones, a usual explanation for these patterns is the increased likelihood for a unisexual population to become established compared to a bisexual population, since a single parthenogenetic female can reproduce autonomously and give rise to a new lineage; moreover, the potential rate of increase per generation is double that of a bisexual species since there is no waste of energy in producing males as all individuals are female (Cuellar, 1977; Glesener and Tilman, 1978). However, this does not explain why asexual forms do not replace sexual populations from which they derive (Glesener and Tilman, 1978). A conceptually similar hypothesis suggests that parthenogenesis can be favoured in those environments with very scattered populations where finding a mate is difficult; when populations reach respectable densities, parthenogenesis no longer has an advantage over sexual reproduction because the probability of finding a mate of the opposite sex in this circumstance is high (Tomlinson, 1966).

According to Cuellar (1977) parthenogenetic individuals could arise frequently, but they would not be able to become established without a suitable habitat giving them protection from their bisexual progenitors: the competition with them would prevent the expansion of the newly formed parthenogens; therefore, parthenogens are seen as “fugitives” which seek refuge far away from their ancestors in marginal and disturbed habitats. The view of parthenogens as competitively inferior was also supported by Glesener and Tilman (1978) who suggested that these geographical trends could best be understood by considering that sexual reproduction is favoured by the unpredictability of environments resulting from interspecific interactions, such as competition or predation; these interspecific interactions are altered in unpredictable ways by the continuous genotypic changes given by the meiotic process, so the sexuality of a competitor or a predator increase the selective pressure for sexuality rather than asexuality. Therefore, they came to the conclusion that this unpredictability could be sustained only by the sexual forms that tend to occupy areas with a greater biotic complexity than those occupied by the asexual forms, where abiotic factors are the main source of “uncertainty”.

Following this train of thought, Bell (1982) suggested the “tangled bank” model based on the idea that in a complex environment the genetically-diverse progeny of a sexual female is able to exploit a wide range of available niches, thus, reducing competition among themselves; conversely, the offspring of an asexual female, due to their genetic uniformity, will compete with each other for the same resources achieving less overall success. On the assumption that asexual clones are narrowly specialized, Bell concluded that asexuals, despite their greater reproductive efficiency, cannot replace a sexual population in these complex environments. Therefore, according to him, asexuals would be successful only in invariants habitats (Beaton and Hebert, 1988) in which biological interactions are relatively unimportant and a high reproductive capacity confer a greater advantage (Bierzychudek, 1985).

Lynch (1984) did not consider either of the two previous hypotheses sufficient to explain geographic parthenogenesis: the two hypotheses failed to explain why many sexual populations did not expand towards the range occupied by asexuals and they also did not take into account the examples of coexistence between parthenogens and their sexual relatives. He questioned the competitive inferiority of parthenogens, which would lead them to be outclassed by the sexual forms in areas of overlapping, in favour of those mechanisms that allow competing species to coexist. He also doubted the superior colonizing ability of parthenogens based on the fact that they generally have lower reproductive rates, which stem mainly from the poor hatching success of parthenogenetic eggs, than their sexual relatives. He did not rule out the possibility that narrowly adapted clones could arise, but they will survive as long as this narrow niche will be available. Therefore, Lynch (1984) came up with the “general-purpose genotype” hypothesis under which parthenogens are subjected to intense selection to promote, over evolutionary time, highly generalized genotypes. These genotypes have the greatest mean fitness and broad tolerance ranges, so they are able to survive in a wide variety of habitats. In addition, Lynch recognized that the selection for these “general-purpose genotypes” exist also in sexual populations, but in a limited way: as a consequence of genetic rearrangements in sexual reproduction, a broadly adapted sexual parent does not necessarily produce a broadly adapted progeny contrary to parthenogenetic offspring which are generally clones of their parent. Thus, according to him, the sexual forms tend to be more adapted to the immediate environment than parthenogens. The main distinction between this hypothesis and the previous ones is that the former considers geographical parthenogenesis as a by-product of the adaptations acquired through selection for generalism rather than as a simple consequence of the increased colonizing capacity of parthenogens (Lynch, 1984).

Another model that is considered the opposite of the "general-purpose genotype" is the “frozen niche variation” (FNV) model theorized by Vrijenhoek (1979, 1984) by observing the distribution of hybridogenetic and gynogenetic fishes of the genus *Poeciliopsis*, but later applied to truly parthenogenetic populations (Vrijenhoek and Parker, 2009). This model considers parthenogens as specialists rather than generalists, resembling Bell's (1982) “tangled bank” in this respect (Vrijenhoek and Parker, 2009). According to FNV model, when new parthenogens arise from genetically variable sexual ancestors, a portion of the genotypic variation of their ancestors is “frozen” in each of new clonal genomes along with its niche requirements; then, interclonal selection eliminates clones that have substantially overlapping niches with each other and with their sexual ancestors, leading to niche partitioning among specialized parthenogens (Vrijenhoek, 1979, 1984; Vrijenhoek and Parker, 2009). On the other hand, sexual ancestors tend to have broader niches because recombination, which occurs in sexual reproduction, tends to regress distributions towards the population mean, preventing niche diversification; so, a few specialized parthenogens should be able to coexist with their broad-niche sexual ancestors, at least as long as the origin rate of new parthenogens is not too high; a high clonal formation rate could lead to the competitive exclusion of sexual ancestors and to their extinction (Vrijenhoek and Parker, 2009).

Another hypothesis was given by Haag and Ebert (2004) who proposed to consider marginal habitats as less favourable and more fragmented, and consequently to consider the populations of these habitats as highly subdivided (or “metapopulations”) with an increased probability of local extinction and subsequent recolonization by a small number of individuals. On the contrary, they considered the populations of the “core” habitats as usually large and stable with low probabilities of extinction. According to them, the recolonization of marginal habitats would have stronger negative consequences in sexuals than in asexuals due to increased homozygosity and inbreeding depression in sexual but not in asexual populations; therefore, asexuals may replace sexuals in subdivided habitats (like the marginal ones), whereas they would not succeed in “core” habitats.

Alternatively, other theories have attributed the causes of such pattern to ploidy level variations rather than to differences in the modes of reproduction (Bierzychudek, 1985; Beaton and Hebert, 1988). These theories arise from the observation that the majority of parthenogenetic forms have been found to be polyploid, while their sexual relatives are generally diploid: Vandel (1940) himself had underlined this correlation between polyploidy and parthenogenesis. In light of this, Suomalainen (1950) hypothesized that polyploidy could make these animals hardier in unfavourable circumstances enabling them to widen their range. However, it is often difficult to tell whether their different distribution depends on their parthenogenetic reproduction or on their polyploidy (Suomalainen, 1950): polyploidy would normally interfere with the normal meiotic process needed for sexual reproduction, instead it is precisely parthenogenesis that permits it and its spread in new areas (Glesener and Tilman, 1978).

This is generally true in animals where polyploids are also parthenogenetic, while it is not true in plants where many polyploids are sexual (Bierzychudek, 1985; Kearney, 2005). Bierzychudek (1985) provided examples of polyploid plants that, regardless of the breeding system, showed a wider tolerance to extreme conditions than diploid relatives, that is, they have geographical distributions similar to parthenogenetic animals. Thus, she seems to support the above-mentioned view that considers the different geographical patterns as a consequence of the high ploidy level rather than of parthenogenesis itself. However, she emphasized the importance of experimental studies (e.g. with sexuals and asexuals of comparable ploidy levels) to discriminate among the alternative hypotheses.

Beaton and Hebert (1988) carried out an experimental study of this kind by examining the geographical distribution of different ploidy levels within an invariant breeding system. They collected asexually-reproducing clones of the cladoceran *Daphnia pulex* in different localities and they evaluated their ploidy levels. The results clearly showed that polyploid clones increased with latitude, supporting the hypothesis of Suomalainen (1950).

Kearney (2005) observed that polyploidy could not be necessary and sufficient for geographical parthenogenesis because similar geographical patterns were observed without polyploidy (e.g. in the asexual grasshopper *Warramaba virgo*). Instead, he showed that polyploid plants and animals, which had this geographical distribution, evolved as interspecific hybrids suggesting hybridization, rather than polyploidization, as the primary cause for geographical parthenogenesis (see below). Lundmark and Kearney (2006) rather suggested that hybridization and polyploidy could be two complementary phenomena that enhance the effect of each other.

Lastly, another explanation for geographical parthenogenesis involves hybridization which has often been mentioned by the authors as a possible cause, but it has not played a preponderant role in their hypotheses (Glesener and Tilman, 1978; Bierzychudek, 1985). The theories involving hybridization assume that most parthenogens have a hybrid origin (Wright and Lowe, 1968; Schultz, 1971; Lynch, 1984; Wetherington, Kotora and Vrijenhoek, 1987; Kearney, 2005). This assumption led Schultz (1971) to attribute the ecological success of unisexuality to heterosis (or “hybrid vigour”) resulting from high levels of heterozygosity in hybrids (Bulger and

Schultz, 1979). Since each of parental species carries alleles absent in the other, hybrids can have a great number of new allelic combinations which can improve fitness by increasing the range of conditions a hybrid can tolerate; therefore, heterosis may contribute to the ecological success by increasing niche width and providing greater fitness in any environment (Schultz, 1971; Bulger and Schultz, 1979). However, subsequent evidence have shown that hybrid superiority is not necessarily conferred upon hybridization, rather it is the result of the selection of the most fit clones; therefore, the ecological success of unisexuals would not derive directly from heterosis, but it would be the result of selection from a broad spectrum of genotypes that arose through hybridization (Wetherington, Kotora and Vrijenhoek, 1987). In addition to the "general-purpose genotype" hypothesis, Lynch (1984) provided another explanation involving hybridization, or "destabilizing hybridization" hypothesis. According to him, hybridization is on the contrary a process that tends to "destabilize" parthenogens because potential hybridization events between sexual and asexual could lead to the loss of their genetic identity and the rapid displacement of parthenogenesis by sexuality. In order to avoid backcrosses with their sexual ancestors, parthenogens that tend to occupy habitats different from those of their parental species will have a selective advantage over other parthenogens; thus, the association of parthenogenesis with marginal habitats would be a consequence of the difficulty in maintaining the genetic integrity in a habitat occupied by sexual ancestors (Lynch, 1984).

Kearney (2005) adopted a hybrid-centred view of geographical parthenogenesis suggesting that many of those cases might be better seen as part of a broader pattern for the hybrid advantage in certain environments. Starting from the fact that hybridization generates large amounts of genetic and phenotypic diversity at a rate much faster than mutations alone, he suggested that hybridization between two differently adapted populations or species could create a diverse range of new genetic combinations which might enable the colonization of new habitats. He provided examples of a hermaphroditic (sperm-dependent) parthenogenetic animal and also sexual polyploid animals that showed a similar distribution but all evolved through hybridization, suggesting that parthenogenesis might not be the key factor in explaining geographic parthenogenesis. He further linked parthenogenesis to glaciation (a link mentioned before by Vandel, 1940 and Suomalainen, 1950) and

Suomalainen) given that parthenogens commonly occupy those place (i.e. high latitudes, high altitudes and deserts) that were most strongly affected by cold and aridity during the last glaciation. Therefore, according to Kearney, the geographic patterns might be the result of the recolonization by parthenogens of those places after range contractions induced by glaciation; in this process, hybridization has played a decisive role by increasing the ecological success of parthenogenetic organisms through increased individual heterozygosity and increased genetic diversity within populations.

However, in response to Kearney's idea, Lundmark and Kearney (2006) suggested that there was insufficient evidence to attribute the geographic patterns solely and directly to hybridization, since there were also cases in which parthenogenesis had evolved without hybridization. Even according to Hörandl (2006), hybridization alone is not correlated with significant range expansions outside the areas of sexual relatives, but also polyploidy alone cannot explain geographical parthenogenesis. Perhaps, as Hörandl (2006) has pointed out, geographical parthenogenesis has a complex causality and depends on a combination of factors acting together: hybridization and polyploidization might generate opportunities for novel genotypes or phenotypes by inducing genetic or epigenetic changes that alter the reproductive pathway, the advantages of asexual reproduction might allow the colonization of new areas or certain habitats, niches differentiation among parthenogens and sexuals might help to maintain areas of coexistence. Therefore, parthenogenetic forms may prevail in certain areas, but the combination of these factors might not occur frequently enough to replace the well-established system of sexuality (Hörandl, 2006).

Parthenogenesis and hybrid origin

The evidence that many parthenogenetic animals and plants have a combined genome from two parental species has led many authors to suggest hypotheses explaining the link between asexual reproduction and interspecific hybridization (Cole, 1975; Bullini, 1994; Avise, 2008; Choleva *et al.*, 2012; Vallejo-Marín and Hiscock, 2016; Janko *et al.*, 2018; Dedukh *et al.*, 2020). These hypotheses converge in the theory of "hybrid origin" (or "hybrid theory"; Bullini, 1994) according to which parthenogenesis would originate from hybridization events, in contrast to “spontaneous” or “mutation origin” which considers parthenogenesis to arise from the accumulation of spontaneous mutations (Cole, 1975; Cuellar, 1977; Bullini, 1994; Avise, 2008; Choleva *et al.*, 2012). Under the latter theory, hybridization is not directly involved in causing parthenogenesis (Bullini, 1994): hybridization events would follow the spontaneous appearance of parthenogenesis in a bisexual species (i.e., hybridization comes second; Avise, 2009; Choleva *et al.*, 2012). On the other hand, the hybrid theory emphasizes the cause-and-effect relationship between interspecific hybridization and parthenogenesis (Avise, 2008): hybridization between bisexual species lead to hybrids capable of producing unreduced eggs which start development without true fertilization; parthenogenesis would, therefore, originate simultaneously with hybridization (Bulger and Schultz, 1979; Choleva *et al.*, 2012). Typically, hybrids are produced when two distinct allopatric species, each adapted to a particular environmental condition, come into contact before having achieved complete reproductive isolation (Cole, 1975; Bullini, 1994), or when ecological reproductive isolating mechanisms breaks down in disturbed habitats (Wright and Lowe, 1968; Glesener and Tilman, 1978; Bullini, 1994). Reproductive isolating mechanisms (or isolating barriers), such as ecological isolation, are important in the speciation process since they prevent gene flow (i.e. gene exchange) between incipient species impeding the mixing of the original genotypes and the possible disappearance of one or both parental species (Coyne and Orr, 2004; Vallejo-Marín and Hiscock, 2016; Janko *et al.*, 2018). Anthropological activities and climate change, by modifying natural habitats, have facilitated the removal of geographical barriers between genetically distinct species, thus increasing the likelihood of hybridization events (Jančúchová-Lásková, Landová and Frynta, 2015; Vallejo-Marín and Hiscock, 2016). Contacts between the two previously allopatric species often occurs in ecotones (i.e. a transition zone) where they hybridize forming a “hybrid zone” (Wright and Lowe, 1968; Cole, 1975; Bullini, 1994; Hörandl, 2006).

Interspecific hybrids are often inviable or sterile (Wetherington, Kotora and Vrijenhoek, 1987; Mallet, 2007) generally failing to develop functional gametes due to chromosomes pairing problems (Dedukh *et al.*, 2020). This low hybrid fitness acts as a postzygotic barrier (Janko *et al.*, 2018) that reduces or eliminates the extensive interbreeding between parental species before the evolution of effective premating isolating mechanisms which requires generations; thus, the production of unfit hybrids occurs for some time (Cole, 1975). However, Neaves (1971) and Cole (1975) hypothesized that an unusual hybrid could possess the combinations of gene that would allow the parthenogenetic development of unreduced egg cells, becoming reproductively successful. Fusco and Minelli (2019) observed that natural selection could act in favour of those cytological mechanisms that allow hybrids the production of functional gametes able to develop into new individuals. Also, Vallejo-Marín and Hiscock (2016) stated that asexual reproduction, selection for greater fertility and polyploidy are those mechanism that tend to “stabilize” newly formed hybrids allowing them to persist and spread. Basically, single hybrids (generally females) could acquire the capacity to reproduce either asexually, or by amphigony usually with their bisexual relatives giving rise to individuals that might have a higher ploidy level (Bullini, 1994; Fusco and Minelli, 2019); thus, polyploidy is often a consequence of hybridization events according to the hybrid theory (Choleva *et al.*, 2012). Hybrids are not always parthenogenetic since sexual reproduction can occur, but the most common modes of reproduction among them are parthenogenesis, gynogenesis and hybridogenesis (Schultz, 1971; Bulger and Schultz, 1979; Bullini, 1994; Jančúchová-Lásková, Landová and Frynta, 2015). Once these hybrids start to perpetuate themselves, they can eventually constitute new hybrid species, a process known as “speciation by hybridization” or “hybrid speciation” (Cole, 1975; Bullini, 1994; Mallet, 2007; Vallejo-Marín and Hiscock, 2016). As pointed out by Dedukh *et al.* (2020), parthenogenesis can be “a solution to hybrid sterility” and it can contribute to the speciation process. In accordance to the hybrid origin theory, these asexual species did not originate from the typical historical splitting of an ancestral lineage into two derived allopatric populations which cumulate changes; instead, they had an instantaneous origin from reproductively fit hybrids and experienced sympatric speciation (Neaves, 1971; Cole *et al.*, 2010).

During the years, the hybrid theory has been the subject of intense research; the main questions were how hybridization leads to asexuality and the reason for the coexistence of different types of reproduction among hybrids.

Already a century ago, Ernst (1918; cited in Janko *et al.*, 2018; Dedukh *et al.*, 2020) referring to plants, hypothesized that the type of reproduction in hybrids could depend on how divergent the hybridizing species are, from sexually reproducing hybrids between closely related species to obligate asexual hybrids between distant parental species. Starting from this assumption and having noticed a larger production of unreduced gametes in hybrids of distantly related species rather than in those closely related, Moritz *et al.*, (1989) suggested the “balance hypothesis” according to which asexuality in hybrids results from a delicate balance between the disruption of normal gametogenesis and the normal development of hybrids (Vrijenhoek, 1989): in other words, asexuality would arise when the hybridizing species are genetically divergent enough to accumulate incompatibilities that disrupt meiosis, but not so divergent to seriously compromise the viability and fertility of hybrids (Moritz *et al.*, 1989; Vrijenhoek, 1989; Moritz, Wright and Brown, 1992; Choleva *et al.*, 2012; Dedukh *et al.*, 2020). According to Dobzhansky (1936) and Muller (1942), the so-called Dobzhansky-Muller model, incompatible interactions between gene combinations occur in interspecific hybrids of diverging species: when two taxa diverge from a common ancestor, each of their genomes accumulate different substitutions which may be incompatible with each other when brought together by hybridization, leading to sterility or inviability of the resulting hybrids (Orr, 1996, 1997; Orr and Turelli, 2001; Delph and Demuth, 2016). Therefore, the rise of hybrid asexuality may be considered as a special case of the Dobzhansky-Muller genic model resulting from the accumulation of reproductive incompatibilities which impair sexual reproduction (Janko *et al.*, 2018; Dedukh *et al.*, 2020). Recent research work in gynogenetic fishes of the genus *Cobitis* (Choleva *et al.*, 2012; Janko *et al.*, 2018; Dedukh *et al.*, 2020), have proved that asexuality is a direct consequence of interspecific hybridization and it is also linked with the phylogenetic and genetic distance of parental species (Dedukh *et al.*, 2020): experimental crossings between closely related species produced fertile hybrids of both sexes, whereas distantly related species produced asexual females and sterile males (Choleva *et al.*, 2012; Janko *et al.*, 2018). Similarly among reptiles, asexual hybrids seem to appear between genetically distant parental species rather than closest relatives (Moritz, Wright and Brown, 1992; Jančúchová-Lásková, Landová and Frynta, 2015). Based on these data, Janko *et al.* (2018) suggested that, at initial stages of speciation, hybridization leads to sexual hybrids which promote gene flow between parental species; at later stages, the gradual accumulation of reproductive incompatibilities between parental species disrupts gametogenesis leading to hybrid asexuality which effectively restricts interspecific gene exchange. Thus, according to them, the production of clonal gametes represents a form of postzygotic barrier that contributes to speciation and evolves earlier than other forms of reproductive barriers, such as hybrid sterility or inviability. Therefore, asexuality would constitute a transient intermediate stage in the speciation process and it is likely that many species could have produced in the past asexual lineages that are currently extinct (Janko *et al.*, 2018). These studies have also shown that, among hybrids, males are often sterile, while only females are able to overcome sterility by producing clonal gametes (Choleva *et al.*, 2012; Janko *et al.*, 2018; Dedukh *et al.*, 2020). The exact reasons for these differences between sexes are unclear, although they are probably related to the genic sex determination (Dedukh *et al.*, 2020). Such data appear to be consistent with the empirical observation made by Haldane (1922), known as Haldane's rule, which stated that the heterogametic sex is the one most affected in terms of infertility or inviability following hybridization. Haldane (1922) noticed this rule both in taxa with heterogametic (XY) males (e.g. Diptera, Mammalia) and in those with heterogametic (ZW) females, such as Lepidoptera and Aves (birds). Different theories have been suggested to explain Haldane’s rule (reviewed by Orr, 1997; Delph and Demuth, 2016); in summary, some of these are based on the hemizygosity of sex-linked (X or Z) genes in the heterogametic sex and the higher probability that such sex is affected by incompatible interactions between sex chromosomes and autosomes or in between sex specific chromosomes (X and Y, or Z and W), other hypotheses involve genes manipulating the meiotic process (known as meiotic drivers) or a faster evolution of genes expressed in the heterogametic sex so that one sex tends to acquires infertility or inviability earlier than the other (Orr, 1997; Delph and Demuth, 2016). Therefore, hybrid asexuality tends to emerge in a sex-specific manner at lower divergence than hybrid sterility or inviability (Dedukh *et al.*, 2020), with hybrid infertility evolving more rapidly than inviability (Choleva *et al.*, 2012). Summarizing recent research, it could be said that the origin of parthenogenetic lineages requires successful hybridizations between particular pairs of sexual species that are genetically neither too close nor too distant (Avise, 2008).

Parthenogenetic invertebrates of hybrid origin are found among gastropods, crustaceans, beetles, stick insects and orthopterans (Fusco and Minelli, 2019). For example, stick insects of the genus *Bacillus* show parthenogenetic hybrid species like *B. whitei* and *B. lynceorum*, with different levels of ploidy: the former species is diploid, while latter is triploid (Bullini, 1994). Among vertebrates, the gynogenetic or hybridogenetic lineages (in amphibians and fishes) are usually hybrids between different species (Schultz, 1971; Bulger and Schultz, 1979; Choleva *et al.*, 2012; Dedukh *et al.*, 2020; Kočí *et al.*, 2020) and the true parthenogenetic lineages are probably all of hybrid origin (Avise, 2008; Fusco and Minelli, 2019). As suggested by Bullini (1994), gynogenesis and hybridogenesis might be intermediate steps of evolution that lead to parthenogenesis; the switch of these forms to parthenogenesis would allow them to take full advantage of clonality and eliminate the need for a paternal contribution from parental species. The cause and effect relationship between hybridization and the origin of parthenogenesis seems particularly evident in reptiles which show several examples of hybrid speciation (Bullini, 1994; Avise, 2008). In some of these parthenogenetic species, all extant members appear to be “monoclonal”, that is they appear to have originated from a single hybrid; in other cases, they appear to be formed from different clonal lineages resulting from separate hybridization events (Avise, 2008). Among the squamate reptiles and primarily among all major lizard clades, hybridization events are widely distributed and have led to amphigony, in some fertile hybrids, together with parthenogenesis in hybrids that would otherwise be sterile, forming diploid or triploid lineages (Jančúchová-Lásková, Landová and Frynta, 2015).

In the American lizard genus *Aspidoscelis* (previously in part *Cnemidophorus*; Cole, 1975; Avise, 2008) belonging to the family Teiidae, approximately one-third of the species are parthenogens and all have arisen through hybridization (Moritz, Wright and Brown, 1992; Reeder, Cole and Dessauer, 2002). Historically, the first generation (F1) females resulting from hybridization established new all-female lineages by giving rise to parthenogenetic offspring that reproduce in the same way (figure 12); occasionally, rare fertilization events of these parthenogenetic eggs resulted in triploid clonal species (Cole *et al.*, 2014).

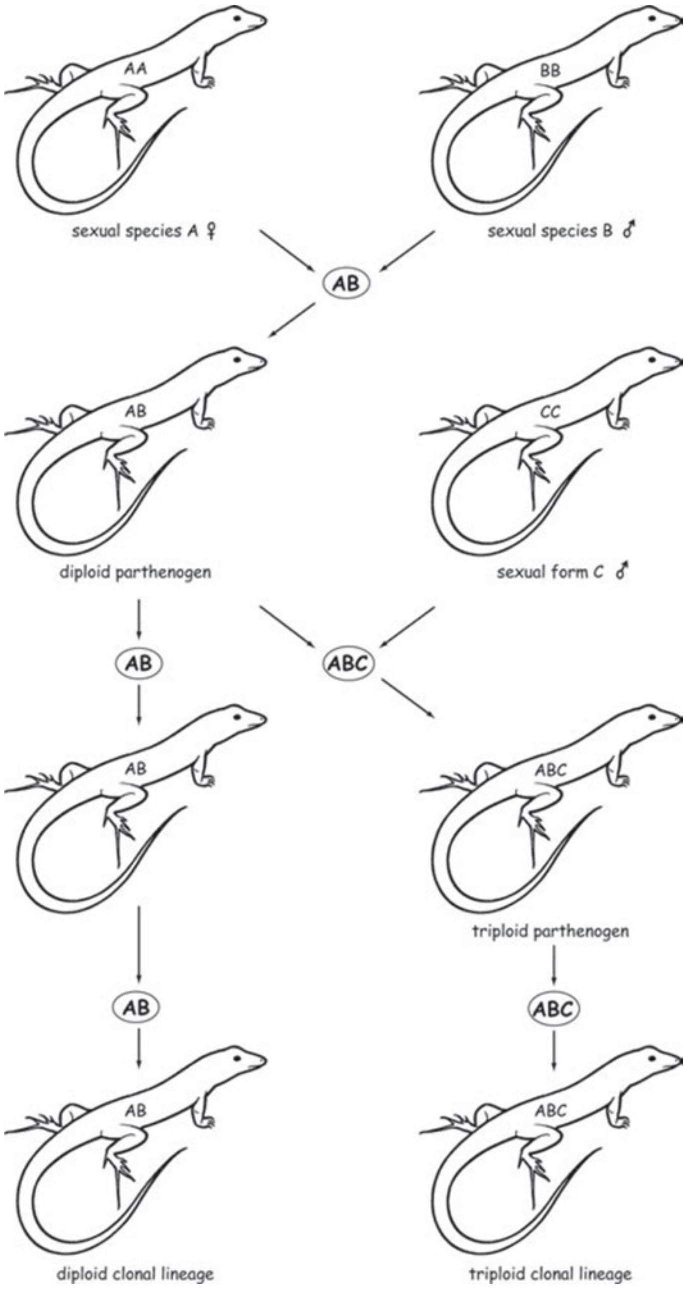


Figure 12. Typical examples of hybridization events involved in the genesis of diploid and triploid parthenogenetic lineages; hybridization between bisexual species (AA and BB) produces parthenogenetic offspring (AB) which establish diploid lineages; the fertilization of unreduced gametes (AB) by other sexual males (CC) produces triploid (ABC) individuals (Avise, 2008).

Therefore, these parthenogenetic species of hybrid origin in *Aspidoscelis* lizards show different degrees of ploidy, for example: *A. neomexicana* is a diploid species deriving from hybridization between the parental species *A. tigris* and *A. inornata* (figure 13); *A. tesselata* includes both diploid populations, arose by hybridization between the bisexual *A. tigris* and *A. septemvittata*, and triploid populations, resulted from further hybridization of these diploid hybrids with the bisexual *A. sexlineata*; *A.*

uniparens and *A. velox* are all triploid hybrid species in which an intermediate diploid parthenogenetic species backcrossed with the bisexual *A. inornata* (Cole, 1975; Reeder, Cole and Dessauer, 2002; Fusco and Minelli, 2019). In these triploid species, meiosis is generally preceded by an endoduplication of the chromosomes forming "pseudobivalents" of sister chromosomes, so that primary oocytes undergoing meiosis are hexaploidy and the resulting clonal eggs are triploid (Cole, 1975, 1979; Avise, 2008). Although a few tetraploid specimens of hybrid origin have been described, no tetraploid clonal species has been found in nature (Lowe *et al.*, 1970; Neaves, 1971; Cole, 1979). Cole *et al.* (2014, 2017) reported the first two known tetraploid species among tetrapods that reproduce by parthenogenetic cloning: *Aspidoscelis neavesi* and *Aspidoscelis priscillae*. These two all-female species originated in the laboratory from hybridization between a triploid parthenogen and the diploid bisexual *A. inornata* (now *A. inornatus*; Cole *et al.*, 2017). Although these species originated in captivity, Cole *et al.* (2014, 2017) hypothesized that these same hybridizations can also be found in nature and that have already been described in the past (Neaves, 1971). The F1 lizards obtained by Cole *et al.* (2014, 2017) were true hybrids with two parents of different species, whereas the subsequent generations were clones of only one parent; Cole *et al.* (2014) consider this is the "paradox" deriving from hybrid origin of unisexual species: only the F2 generation and the following ones are considered to form a named species, whereas the F1 female hybrids, of which they are clone, are not considered members of that species.

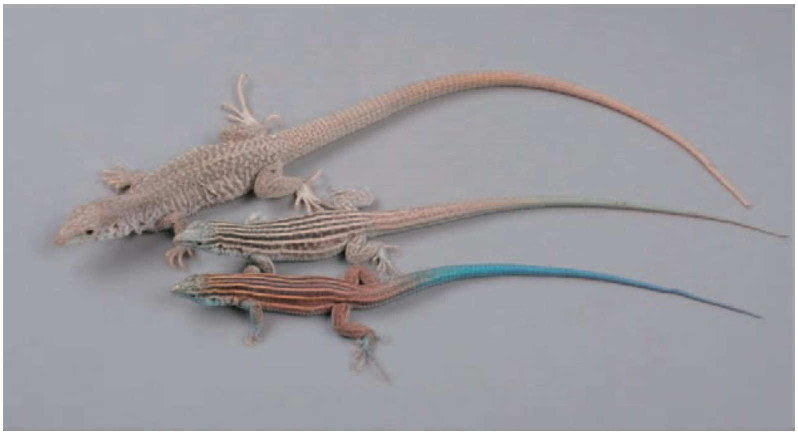


Figure 13. The diploid parthenogenetic species Aspidoscelis neomexicana (center) arose from a hybridization event between a male A. inornata (bottom) and a female A. tigris (top); photo by (Neaves and Baumann, 2011).

Infectious parthenogenesis

The only case in which the trigger for parthenogenesis is known with certainty is "infectious parthenogenesis" (Huigens *et al.*, 2000), in which clonal reproduction can be induced by infectious agents mainly included in the genera *Wolbachia*, *Cardinium* and *Rickettsia* (Stouthamer, Luck and Hamilton, 1990; Koivisto and Braig, 2003; Ma and Schwander, 2017). All these are endosymbiotic bacteria of invertebrates in which they are responsible for reproductive manipulations such as cytoplasmic incompatibility, feminization of genetic males, male-killing and thelytokous parthenogenesis (Koivisto and Braig, 2003; Zchori-Fein *et al.*, 2004). These endosymbionts are vertically transmitted from mothers to their offspring through the egg cytoplasm, but they are not transmitted by males which are considered evolutionary "dead-end"; since they are intracellular microorganisms, they tend to be inherited exclusively from the female egg cells which have a larger cytoplasm to room them than the smaller male gametes (Koivisto and Braig, 2003; Ma and Schwander, 2017). Consequently, by favouring the production of female offspring in their hosts (through the above-mentioned mechanisms including thelytokous parthenogenesis), these microorganisms increase their rate of transmission to future generations; thus, they are often called "sex ratio distorters" (Huigens *et al.*, 2000; Koivisto and Braig, 2003; Ma and Schwander, 2017). These bacteria do not necessarily benefit their host, so some authors define them also as "reproductive parasites" (Zchori-Fein *et al.*, 2001).

Stouthamer, Luck and Hamilton (1990) first reported that thelytokous strains of parasitoid wasps (genus *Trichogramma*) could produce males under antibiotic treatment or exposed to temperatures >30°, suggesting the involvement of maternally inherited microorganisms. The effect of high temperatures on sex ratio had been reported even before their findings, but it was generally accepted that sexual reproduction and primary sex ratios in many species of hymenopterans hinged on environmental cues, which indirectly had an effect on pre-embryonic stages by changing the sex of the offspring from female to male, or vice versa (Flanders, 1945, 1965; Koivisto and Braig, 2003). Stouthamer, Luck and Hamilton, (1990) also observed thelytokous lines of *Trichogramma* wasps that could not be reverted to bisexual reproduction by either temperature or antibiotic treatment, called “nonrevertible” parthenogenetic lines. The presence of microorganisms in the eggs of “revertible” lines, and the absence in the “nonrevertible” ones, was later confirmed cytologically by Stouthamer and Werren (1993) through a 2% lacmoid stain to visualize them. These microorganisms were subsequently identified as Proteobacteria of the genus *Wolbachia* by means of sequence analysis (Stouthamer and Werren, 1993). Interspecific transfer experiments of *Wolbachia* between two related species of hymenopterans (Grenier *et al.*, 1998), or between different orders of insects (e.g. from Hymenoptera to Diptera; Van Meer and Stouthamer, 1999), showed that *Wolbachia* could be successfully transferred from one species to another (or from one order to another) and in turn transmitted over several generations; but they also showed that a specific parthenogenesis-inducing *Wolbachia* strain could cause distinct effects in different taxa, e.g. a partial induction of parthenogenesis or no apparent effect on host reproduction. Perhaps, the limited expression of thelytoky in different hosts is a result of the low density of endosymbionts, or it is linked to unfavourable interactions either with the new genome or between host-endosymbiont (Grenier *et al.*, 1998; van Meer and Stouthamer, 1999). In addition to interspecific transfer that appear to be an extremely rare event in nature (Koivisto and Braig, 2003), Hulgens *et al.* (2000) reported horizontal transmission of parthenogenesis-inducing *Wolbachia* demonstrating that it was a natural and frequent mode of intraspecific transmission other than the main vertical mode. Other than *Wolbachia*, a new genus of bacteria observed by Zchori-Fein *et al.* (2001) in parasitoid wasps, and proposed as “*Candidatus* Cardinium” by Zchori-Fein *et al.* (2004), showed similar effects on reproduction.

Although research initially focused on Hymenoptera, infectious parthenogenesis has also been discovered in other arthropods. Currently, it has been reported in over 50 species of hymenopterans (belonging to different families such as Pteromalidae, Aphelinidae, Platygastridae, Encyrtidae, Scelionidae, Trichogrammatidae, Figitidae, Cynipidae; Koivisto and Braig, 2003; Fusco and Minelli, 2019), in a few species of thysanopterans and in some mites of the genus *Brevipalpus* and *Bryobia* (Koivisto and Braig, 2003; Ma and Schwander, 2017). Most of these arthropods have a haplodiploid sex-determination system in which normally unfertilized (haploid) eggs develop into males, whereas fertilized (diploid) eggs give rise to females; following the infection, the unfertilized eggs that should develop into males, instead develop parthenogenically giving rise to females (Ma and Schwander, 2017; Fusco and Minelli, 2019). Gamete duplication is the main cytological mechanism by which haploid eggs turn into diploid ones and develop into parthenogenetic females (Koivisto and Braig, 2003; Ma and Schwander, 2017). The high frequency of this mechanism in infectious parthenogenesis is interesting because it is rare among species with genetically determined parthenogenesis; probably gamete duplication is mechanistically easy to induce by endosymbionts (Ma and Schwander, 2017).

At least half of the above-mentioned species comprise both infected (parthenogenetic) and uninfected (sexual) females: since endosymbiont transmission to eggs is typically < 100%, some eggs probably remain uninfected, are fertilized and develop into sexual females which maintain sexual reproduction in the population; thus it is likely that many other species considered to be fully sexual have unknown parthenogenetic populations which are infected (Ma and Schwander, 2017).

The discovery of infectious parthenogenesis mainly in haplodiploid species has promoted research towards other taxa with a different sex determination system, such as weevils (Coleoptera: Curculionidae), scale insects (Hemiptera: Coccoidea), booklice (Psocoptera), springtails (Collembola) and Ostracoda (Koivisto and Braig, 2003; Ma and Schwander, 2017). In these taxa both males and females are diploid (or “diplodiploid”) and sexual differentiation depends on sex chromosomes (Ma and Schwander, 2017). The cytological mechanisms that lead to the production of infected parthenogenetic females can vary depending on whether the heterogametic sex is male or female. Gamete duplication could in theory induce thelytoky in all arthropod species except those with heterogametic females (Koivisto and Braig, 2003), but not enough cases have been investigated in this group to define which cytological mechanism is the most common (Ma and Schwander, 2017).

As observed by Ma and Schwander (2017), infectious parthenogenesis is often harder to demonstrate in diplodiploid taxa than haplodiploid one (figure 14): parthenogenetic females of haplodiploid species, when treated with antibiotics or heat, produce haploid sons instead of diploid daughters, providing direct evidence for the involvement of endosymbionts in causing parthenogenesis. Conversely, after the treatment of diplodiploid parthenogenetic females, they cannot produce on their own new individuals from haploid eggs as these require fertilization; therefore, treated females should be mated with sexual males to verify the production of both male and female offspring. However, the ability to fertilize eggs is often lost by these treated females since the presence of symbionts is needed for successful oogenesis, so the crosses with sexual males may result in nonviable eggs, or no eggs at all, making it difficult to interpret these results (Ma and Schwander, 2017). Moreover, detecting the presence of endosymbionts does not necessarily imply their involvement in parthenogenesis, so many reported cases in diplodiploid species have not been formally confirmed (Ma and Schwander, 2017).

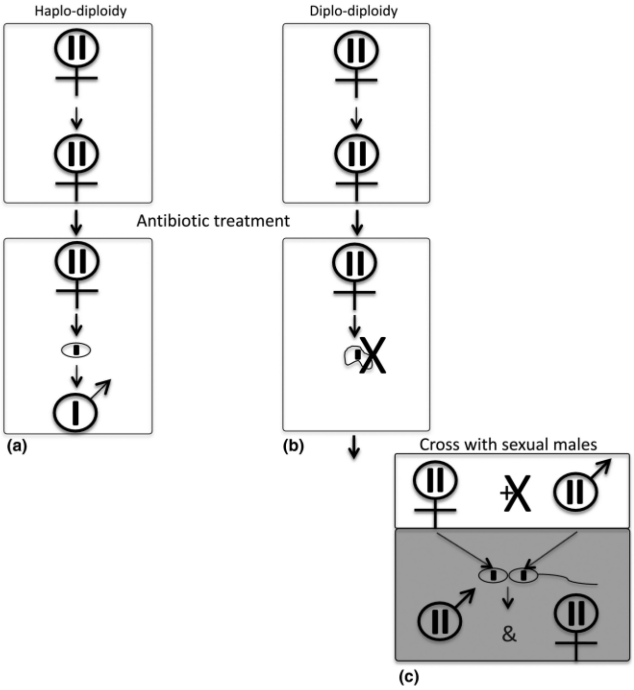


Figure 14. Consequences of endosymbiont removal under haplodiploid (a) and diplodiploid (b) species with infectious parthenogenesis. In the first case (a), endosymbiont removal leads to females producing haploid males; following the same treatment in the case (b), no offspring are produced by diplodiploid females because haploid eggs do not develop without fertilization; after mating with sexual males (c), the production of both female and male offspring can be a direct evidence for infectious parthenogenesis (Ma and Schwander, 2017).

It should be noted that, although vertically transmitted viruses or protozoans are common in insects, all the known cases involve bacteria, probably because they are easier to detect (e.g., by removal with antibiotic treatment; Ma and Schwander, 2017). The identification of bacterial symbionts has long been hampered by the inability to culture them (Stouthamer *et al.*, 1993) but the availability of polymerase chain reaction (PCR) technology has allowed to reveal the widespread occurrence of these bacteria (van Meer and Stouthamer, 1999). However, tests for endosymbionts typically cover bacteria already known to induce parthenogenesis, impeding the discovery of other endosymbionts capable of reproductive manipulation (Ma and Schwander, 2017); nevertheless, a considerable number of new and undefined endosymbionts has been described so far (Koivisto and Braig, 2003; Ma and Schwander, 2017). Among these, a new genus (*Xiphinematobacter*) has been suggested as potentially involved in thelytokous parthenogenesis of plant nematodes (Vandekerckhove *et al.*, 2000), but subsequent studies have shown the role of this symbiont as a nutritional mutualist rather than as a reproductive manipulator (Brown *et al.*, 2015). Data by Ma and Schwander (2017) suggest that 56-75% of cases with infectious parthenogenesis involve *Wolbachia* as endosymbiont, 6-13% *Cardinium*, 4% *Rickettsia* and 15-28% other unidentified bacteria.

Lastly, it seems possible that an increasing number of intracellular bacteria capable of inducing parthenogenesis as well as a wider range of taxa will be involved in future research, perhaps revealing that infectious parthenogenesis is also widespread in other diplodiploid species (Koivisto and Braig, 2003).

Chapter 2. Parthenogenesis in snakes

Parthenogenesis has been documented in a number of different vertebrate taxa: birds, elasmobranch fishes, lizards, and snakes (Kearney, Fujita and Ridenour, 2009; Booth and Schuett, 2016; Shibata *et al.*, 2017; Cubides-Cubillos *et al.*, 2020; Gasanov and Katz, 2020). Only two types of such reproduction have been described among these vertebrates: obligate and facultative (Lampert, 2008).

As already stated in the first chapter, obligate parthenogenesis (OP) is characterized by organisms that exclusively reproduce through asexual reproduction; it occurs in several populations made up of unisexual all-female parthenogens in which paternal genetic contribution is unnecessary; it is mostly widespread in plants and invertebrates, but within vertebrates true parthenogenesis occurs exclusively in different species of squamate reptiles (mostly lizards; Cole, 1975; Bell, 1982; Kearney, Fujita and Ridenour, 2009; Sinclair *et al.*, 2010; Neaves and Baumann, 2011; Sites, Reeder and Wiens, 2011). Among squamate reptiles, approximately 30 unisexual species have been formally named and they belong to different families such as Lacertidae, Gekkonidae, Teiidae, Gymnophthalmidae, Xantusiidae, Scincidae and Typhlopidae (Cole, 1975; Avise, 2008; Fusco and Minelli, 2019).

In snakes, the only known obligate parthenogenetic lineage is the brahminy blind snake (*Indotyphlops braminus*) previously known as *Ramphotyphlops braminus* (figure 15); despite its worldwide distribution attributed to the plant trade (hence, its common name “flower pot snake”), little is known about its mechanism of reproduction (Booth and Schuett, 2016).

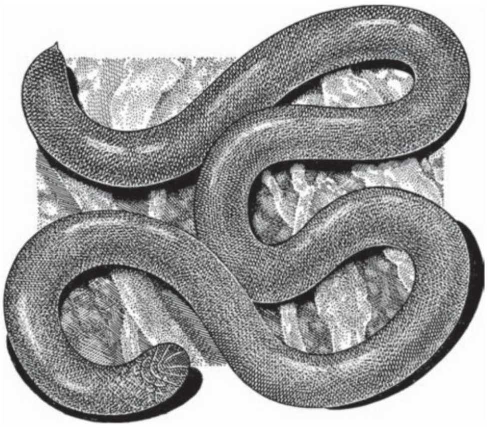


Figure 15. The only known obligate unisexual species of snakes (the brahminy blind snake) is composed of small burrowing snakes that look almost like earthworms with a rounded head and vestigial eyes covered by scales (Avise, 2008).

Different studies have focused on these obligate unisexual populations to understand their evolutionary origin, phylogenetic distribution, the consequences of a reduced genetic variability and of a potentially decreased fitness in the offspring (Lutes *et al.*, 2010; Sinclair *et al.*, 2010; Fujita *et al.*, 2020). With a single exception (Sinclair *et al.*, 2010), all cases of OP in squamate reptiles (lizards) appear to be the result of interspecific hybridization (Moritz *et al.*, 1989; Cubides-Cubillos *et al.*, 2020; Gasanov and Katz, 2020).

Facultative parthenogenesis (FP), where a female can produce offspring either sexually or parthenogenetically, is rare among vertebrates and it has been reported in less than 0.1% of vertebrate species (figure 16); on the contrary, a more widespread phenomenon is accidental parthenogenesis, that is the production of a very small proportion of unfertilized eggs in species that normally reproduce sexually (Booth, Million, *et al.*, 2011; van der Kooi and Schwander, 2015). However, without screens for hatching success of eggs laid by parthenogenetic females, it is difficult to distinguish between accidental and facultative parthenogenesis; therefore, the various examples of rare parthenogenesis in vertebrates reported in the literature are typically interpreted as facultative instead of accidental parthenogenesis (Watts *et al.*, 2006; Chapman *et al.*, 2007; van der Kooi and Schwander, 2015). According to van der Kooi and Schwander (2015) accidental parthenogenesis might be the necessary step to give rise to new facultative or obligate parthenogenetic lineages through the accumulation of gradual changes.

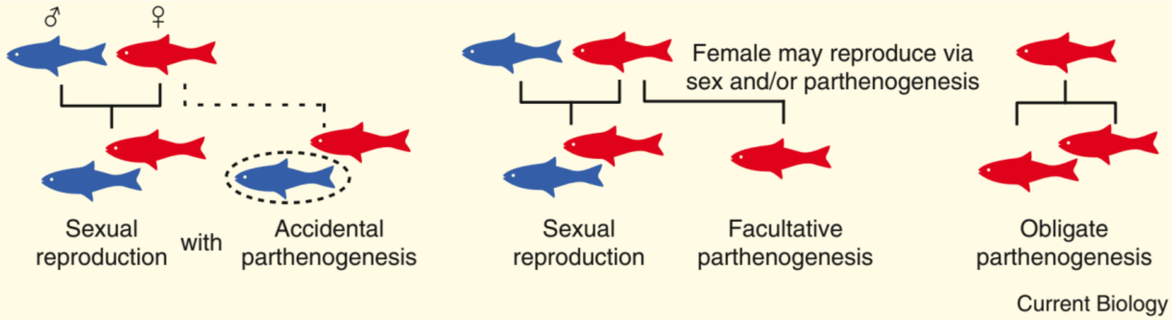


Figure 16. Accidental parthenogenesis is the rare hatching of unfertilized eggs in sexual populations; it has generally a very low hatching success and it is often not adaptive. Under facultative parthenogenesis a female may reproduce sexually or parthenogenetically, so this reproductive mode combines the advantages of sexual and parthenogenetic reproduction. Under obligate parthenogenesis, females reproduce solely through parthenogenesis (van der Kooi and Schwander, 2015).

Facultative (or occasional) parthenogenesis is characterized by a variable developmental success depending on the vertebrate lineage (Lampert, 2008; Booth and Schuett, 2016; Gasanov and Katz, 2020). Among vertebrates, it was first discovered in turkeys and chickens in the 1950s (Neaves and Baumann, 2011; Ramachandran and McDaniel, 2018). After about 50 years, new cases of FP have been described in other vertebrate species (snakes, lizards, elasmobranch fishes) validating them by molecular analyses not available in the early studies on fowl (Dubach, Sajewicz and Pawley, 1997; Schuett *et al.*, 1997; Chapman *et al.*, 2007; Fields *et al.*, 2015). The increasing detection of FP cases in vertebrates over the last twenty years suggests that new cases can be found by studying an increasing number of species. Among reptiles, this type of parthenogenesis has been reported in lizards limited to varanids (Watts *et al.*, 2006) and snake families including Pythonidae (Groot, Bruins and Breeuwer, 2003; Booth *et al.*, 2014), Boidae (Booth, Johnson, *et al.*, 2011; Booth, Million, *et al.*, 2011; Kinney *et al.*, 2013; Shibata *et al.*, 2017; Seixas *et al.*, 2020), Acrochordidae (Dubach, Sajewicz and Pawley, 1997), Colubridae (Schuett *et al.*, 1997; Germano and Smith, 2010; Reynolds *et al.*, 2012; Gasanov and Katz, 2020), Elapidae (Allen, Sanders and Thomson, 2018), and Viperidae (Schuett *et al.*, 1997; Booth and Schuett, 2011; Booth *et al.*, 2012; Jordan, Perrine-Ripplinger and Carter, 2015; Cubides-Cubillos *et al.*, 2020). In snakes and elasmobranch fishes, FP has been documented in both viviparous and oviparous taxa.

Therefore, the greatest amount of genetically confirmed and anecdotal cases of FP has been reported in snakes, and this amount might be actually greater (Booth and Schuett, 2016). FP attested by molecular markers or captivity data has been detected in a total of 28 snake species: *Crotalus horridus*, *C. unicolor*, *C. viridis*, *Agkistrodon contortrix*, *A. piscivorus*, *Bothrops asper*, *B. atrox*, *B. insularis*, *B. moojeni*, *B. leucurus* (Viperidae), *Oxyuranus scutellatus*, *Acanthophis antarticus* (Elapidae), *Boa constrictor*, *Epicrates maurus*, *E. cenchria*, *Eunectes murinus*, *Chilabothrus angulifer* (Boidae), *Python bivittatus*, *P. regius*, *P. brongersmai*, *Malopython reticulatus* (Pythonidae), *Acrochordus arafurae* (Acrochordidae), *Thamnophis elegans vagrans*, *T. marcianus*, *T. radix*, *T. couchii*, and *Nerodia sipedon* and *Nerodia rhombifer* (Colubridae) (Cubides-Cubillos *et al.*, 2020; Gasanov and Katz, 2020).

Unconfirmed cases of FP and long-term sperm storage

There are some published cases in which bisexual snakes have been suspected of producing offspring without sperm, but they have not been confirmed genetically (Schuett *et al.*, 1997); among them, there are reports on the natricine snakes *Nerodia Sipedon* (Scalka and Vozenilek, 1986; Smith, Thiss and Chiszar, 2000) and on *Acrochordus* species (Magnusson, 1979).

Magnusson (1979) reported the case of a gravid female *Acrochordus javanicus* that had been isolated from males for seven years; nine yolked ova and a fully-formed embryo were produced, however the author pointed out that the evidence was insufficient to support parthenogenetic reproduction. In reptiles, isolation for long periods from males is not a sufficient condition to affirm that reproduction took place without the involvement of sperm: in fact, females can store sperm and utilize it for long periods of time after a successful mating, a condition known as long-term sperm storage or LTSS (Schuett *et al.*, 1997; Avise, 2008).

There are many accounts in literature of offspring resulted from long-term sperm storage (Booth and Schuett, 2011); with the exception of a few cases, no critical evidence was provided to demonstrate unequivocally that the offspring were produced from sperm stored for long periods (Schuett *et al.*, 1997; Booth and Schuett, 2011). Appropriate analyses should be conducted to interpret reproductive events as a result of long-term sperm storage and to distinguish them from parthenogenesis; therefore, a re-evaluation of these cases could reveal new evidence for facultative parthenogenesis in snakes (Schuett *et al.*, 1997; Groot, Bruins and Breeuwer, 2003).

Booth and Schuett (2011) reconsidered past published cases of LTSS in snakes and they provided a list of those that appeared to be the result of FP.

Booth and Schuett (2016) later provided several cases of FP which appear to be confirmed based only on captivity data (e.g., the absence of males), but the phenomenon was not confirmed with molecular data in any of these cases.

Recently, Guedes and Guedes (2020) reported an uncertain case in the Caatinga's black snake (*Boiruna Sertaneja*), a rare Brazilian species of the Colubridae family. An adult captive female from the collection of Museu Vivo Répteis da Caatinga was placed together with an adult male. The female laid three clutches after a single event of copula: the first oviposition occurred two months after copulation, the second and third ones took place almost one year later. The long-time gap between the first oviposition and the others suggested prolonged sperm storage in their oviducts. Alternatively, facultative parthenogenesis could explain this long gap, but no molecular analysis was carried out to distinguish between the two. As suggested by the author, future studies could be performed to confirm parthenogenesis given that the specimens are kept in captivity and constantly monitored.

Genetically confirmed cases of FP

Nowadays, the number of FP cases have increased thanks to the technological advancement in molecular tools and their ease of application (Booth and Schuett, 2011). There are several molecular techniques available, but it is generally accepted that analyses of hypervariable regions of nuclear DNA provide the most reliable information to study parthenogenesis, parentage, and for the determination of LTSS; these techniques include mini- and microsatellite analyses which are methods of DNA fingerprinting used to compare the genetic profiles of individuals (Schuett *et al.*, 1997; Booth and Schuett, 2011).

The study of Schuett *et al.* (1997) was the first to present reliable evidence for FP in squamate reptiles. They reported four cases in four different species. In the first case *Thamnophis elegans*, the dam was collected as a young adult in wild in 1983 and then isolated from other conspecifics. The dam produced 4 litters from 1988 to 1994: in two litters the offspring were alive, in the other two litters the stillborn offspring were fully-formed or partially-formed or had minor abnormalities; in addition, in all four litters many yolked ova were expelled. The authors did not determine the sex of offspring in any of these cases. They performed minisatellite analysis (figure 17) using blood or brain tissue from the dam and her four stillborn offspring (from one litter): after DNA extraction from tissue samples, two multilocus DNA probes were used with restriction endonucleases and DNA profiles compared to each other by calculating the percentage of bands shared with the dam (or PBS). The four offspring shared a high percentage (87-100%) of their bands with the dam and between each other and they had a lot fewer bands than the dam, but each of them had 1 or 2 bands absent in the profile of the dam: these bands were interpreted as result of anomalies of hybridization or mutations, not as bands of paternal origins. Since a high similarity in band-sharing can also occur in highly inbred bisexual populations with low genetic diversity, they tested eight unrelated adults from the population of the dam and they compared bands of one male adult with the offspring's ones. In this case, the PBS values were significantly lower than in offspring-dam comparison and were in the range for nonrelatives, so Schuett *et al.* (1997) excluded that the high levels of band-sharing between the dam and the offspring were the result of a highly inbred population.

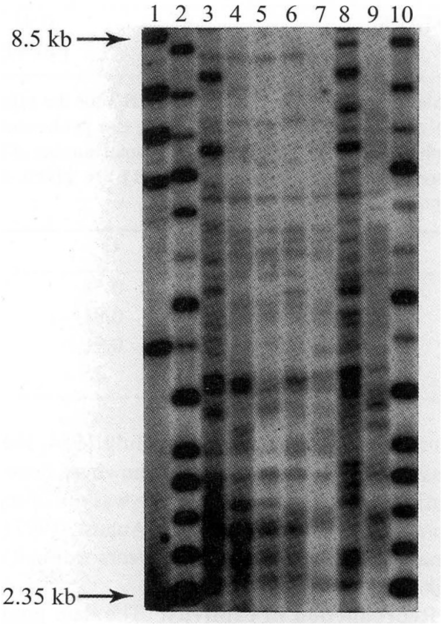


Figure 17. DNA profiles (fingerprints) of Thamnophis elegans. Lanes are denoted by numbers as follow: (1) visual standard lanes; (2,10) molecular weight sizing standard; (3) dam; (4-7) offspring; (8-9) nonrelative adult males (Schuett et al., 1997).

The second case involved a female *Crotalus horridus horridus* maintained in isolation since her birth. After 14 years of isolation, the dam produced one litter consisting of a single live offspring, two dead ones and a few yolked ova; the two stillborn offspring after the autopsy were determined to be male and probably the live one was also male. Minisatellite analysis of the dam and the live offspring was performed and the latter shared a high percentage of its bands with the dam as in the previous case (Schuett *et al.*, 1997).

The third case involved a female *Crotalus unicolor* that was born in captivity and reared in absence of males; after 8 years, abnormal growth of the posterior region revealed a mass of eggs which was removed from the oviducts by surgery: two partially-formed embryos, whose sex could not be determined, and a few yolked ova were discovered. No abnormalities (e.g., male tissues) were found in the reproductive system (Schuett *et al.*, 1997).

The fourth case involved a wild-collected female of *Thamnophis marcianus* placed in a zoo with no contact with other snakes; after 5 years the female gave birth to three offspring: one was alive, another was stillborn and the last died several days after birth. The sex of all three offspring was determined to be male by probing.

Based on captive history and long-term isolation, DNA analyses and surgical inspections, Schuett *et al.* (1997) rejected the hypothesis of amphigonic reproduction resulting from long-term sperm storage or hermaphroditism and they considered all four cases as a result of parthenogenesis. Since the offspring were not genetically identical to the dams but had no external genes, the authors considered meiotic parthenogenesis or automixis the reasonable mechanism involved in the two cases investigated molecularly. In the initial versions of their paper they did not proposed a mechanism for parthenogenetic development: their data differed from those available on lizards where the parthenogenetic offspring are female mainly produced by premeiotic doubling of chromosomes; they ruled out premeiotic doubling because the offspring would have been genetically identical to the dams, then, by comparing their findings with those of turkeys which produce mostly males through parthenogenesis and which have a ZW sex-determination system, they hypothesized that the usual route (figure 18) for the production of parthenogenetic males in snakes was the fusion of the second polar body with the egg cell (i.e., terminal fusion) which produce ZZ males (Schuett *et al.*, 1998).

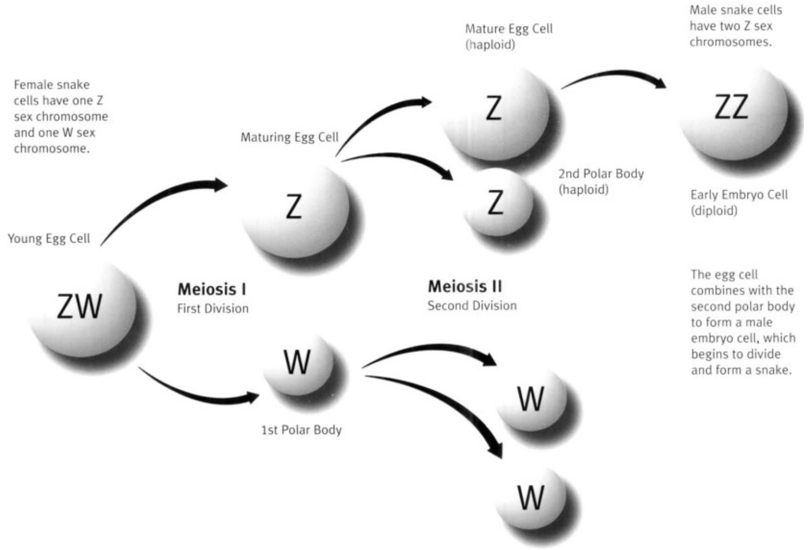


Figure 18. The usual route for the production of parthenogenetic males in ZW snakes: a ZZ individual (male) is produced when an egg cell, which carries a Z sex chromosome, fuse with the second polar body which carries another Z chromosome (Schuett et al., 1998).

The first demonstration of parthenogenesis in snakes by Schuett *et al.* (1997) al pioneered the use of molecular biology tools to distinguish between parthenogenesis and long-term sperm storage.

Some years later, another case was reported by Groot, Bruins and Breeuwer (2003) in a Burmese snake, *Python molurus bivittatus* and it was the first confirmed case in the family Pythonidae; a virgin female in an Amsterdam zoo generated a clutch of eggs every spring for five consecutive years with a low percentage of developing embryos. Based on the presence of ovaries at dissection, the authors identified all offspring as female. They conducted molecular analysis comparing the dam and seven embryos by using AFLP (amplified fragment length polymorphism) and microsatellite markers; in addition, two other adult female snakes from the same zoo were sampled. Some of the microsatellite markers were developed by the authors for this species, others were developed previously for different snake species; however, they all gave little information to confirm parthenogenesis because all individuals were homozygous for the same allele, probably due to inbreeding, except for a single microsatellite locus which showed allele variation in the other two adult females.

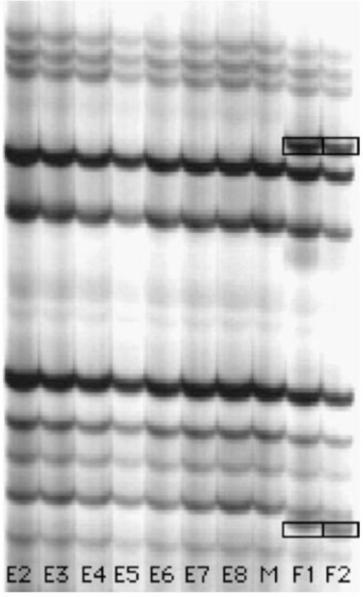


Figure 19. An example of AFLP fingerprinting of P. m. bivittatus, 692 AFLP markers were scored in total. All seven offspring (E2-E8) had identical fingerprints, whereas female 1 (F1) and female 2 (F2) had markers (marked in the image) absent in the mother (M) and in the offspring (Groot, Bruins and Breeuwer, 2003).

AFLP (a tool of DNA fingerprinting) allowed them to confirm parthenogenetic reproduction (figure 19): every marker of the offspring was also found in their dam and vice versa, and no marker was found suggesting the involvement of a father. They rejected the hypothesis of mating with a related male (e.g., a brother) because this putative father should have had the same markers as the dam to obtain a progeny with the same fingerprints. Differently from the previous studies by Schuett *et al.* (1997) and Dubach, Sajewicz and Pawley (1997) which reported a loss of genetic information in the offspring, in this case the offspring were genetically identical to the dam. Therefore, they excluded those cytological mechanisms, such as terminal fusion, which result in homozygous male (ZZ) individuals with a loss of genetic markers; in its place they suggested those mechanisms which are expected to result in “clones” of their mother (ZW) such as central fusion, premeiotic doubling, or fusion of the first polar nucleus with the nucleus of the secondary oocyte, but they were unable to determine which.

New confirmed cases had not been reported until Germano and Smith (2010) investigated possible reproduction by parthenogenesis in the bisexual Sierra garter snake (*Thamnophis couchii*), a viviparous species native to California. They reported the case of a female *T. couchii* that was collected as a juvenile when she was brought into captivity in a museum and she was never housed with a male; after 6 years of isolation, the female produced a litter with one living offspring. They performed microsatellite analysis on the dam, her offspring, an additional female *T. couchii* from the same museum and an unrelated male (*T. ordinoides*).

Unlike the previous cases, the products of molecular analysis were sequenced and analysed by software to determine the exact size of the microsatellite fragments; two out of the four microsatellites analysed were uninformative because three individuals were homozygous for the same allele, whereas the other two microsatellite loci fully supported parthenogenetic reproduction: the dam and her offspring were homozygous for the same allele, the other two adults were either heterozygous or homozygous for different alleles (Germano and Smith, 2010). Therefore, the authors confirmed parthenogenesis since the female and her offspring were identical for all four microsatellite loci and unique paternal alleles were not found, but they did not propose a cytological mechanism for the formation of this parthenogenetic offspring. As observed by Booth and Schuett (2016), the probable mechanism was automixis, but the molecular data were inconclusive to further investigate the underlying mechanisms.

Booth, Johnson, *et al.* (2011) and Booth, Million, *et al.* (2011) provided the first evidence of parthenogenesis in two species of viviparous snakes of the family Boidae: boa constrictor (*Boa constrictor imperator*) and rainbow boa (*Epicrates maurus*). The female Boa constrictor imperator was born in captivity and produced a small litter through sexual reproduction with a male; after four years from the removal of the male, the female produced two litters made up of many live offspring which exhibited a rare recessive colour trait (Booth, Johnson, *et al.*, 2011). Booth, Johnson, *et al.* (2011) determined the gender of all offspring to be female by cloacal probing, then they confirmed it by subjecting a randomly selected representative to exploratory surgery. Eight microsatellite markers were tested in the dam, all offspring and all males with which the dam was housed, except the first; they found that all offspring were homozygous at each heterozygous locus of the dam and genetically identical at all loci for which the mother was homozygous. Since the probability that all individuals received identical alleles from their parents across all loci was infinitesimally low, they rejected the hypothesis of sexual reproduction. According to the authors, the high homozygosity of the offspring suggested that the parthenogenetic mode was terminal fusion automixis. However, in all previous studies, terminal fusion had led to the development of only ZZ males, while the chromosomal arrangement WW had failed to yield viable offspring; on the contrary, they suggested that all these female offspring were WW females and they speculated that the dam was hemizygous for the W chromosome (i.e., W0) to explain the absence of male offspring. This was an important discovery because WW females had previously been produced only through experimental manipulations in fishes and amphibians, so their findings described the first evidence of viable, non-experimentally induced WW females in a vertebrate lineage (Booth, Johnson, *et al.*, 2011).

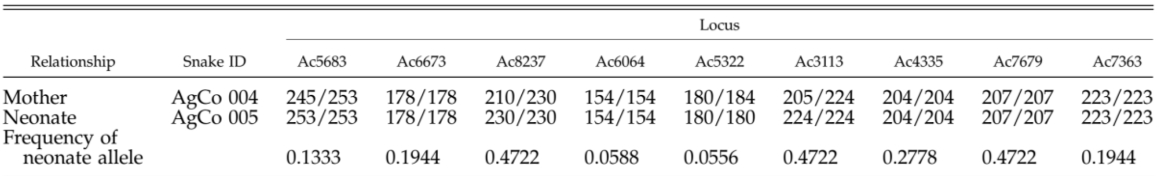
The other example was reported by Booth, Million, *et al.* (2011) in a Colombian rainbow boa (*Epicrates maurus*). Unlike the record in *B. c. imperator*, the female *E. maurus* was isolated from conspecifics from birth and, at the age of 19, she gave birth to two litters in two consecutive years composed of six alive offspring (four died later), two deformed, five stillborn and an unrecorded number of unfertilized ova.

The gender of offspring was confirmed to be female following dissection of the deceased specimens. Given the captive history and the long period of time prior to the production of the litters, Booth, Million, *et al.* (2011) ruled out sperm storage as a viable possibility; however, parthenogenesis was confirmed by the application of 22 microsatellite markers to the dam and seven of her offspring. As in the case of *B. c. imperator*, all offspring were differentially homozygous for a maternal allele and the most likely parthenogenetic mechanism was considered to be terminal fusion automixis with the production of WW females; however, the lack of male offspring in the litters of both boas challenged the dynamics of genetic sex determination in these lineages of snakes (Booth, Million, *et al.*, 2011).

Booth and Schuett (2011) described the first record of FP in a viviparous North American pitviper species, the copperhead *Agkistrodon contortrix*. The female was collected from the wild as a juvenile and housed with another female of the same species; after 5 years, the female copperhead produced a litter of four offspring and several unfertilized ova. They screened the dam and two offspring at 12 microsatellite loci of which only 8 successfully amplified; the mother resulted heterozygous at these loci, while the offspring were homozygous for a maternal allele. Since all offspring were male, they considered this case as a typical example of terminal fusion automixis following the model of Schuett *et al.* (1997) in which only homogametic males (ZZ) are produced.

Kinney *et al.* (2013) described another case of FP in the Boidae family: it was the first report in a Brazilian Rainbow Boa (*Epicrates cenchria cenchria*). The female boa was born in captivity and housed together with a male since three months of age in a zoological collection with no other animals; the female sexually reproduced four times giving birth to viable offspring, then the male was vasectomized and, fifty-nine months following vasectomy, the female gave birth to four offspring (three alive and one stillborn) at the age of 22. They sexed, by cloacal probing, the three live offspring which resulted to be all female. They considered four hypotheses to explain the birth of this litter: sexual reproduction as a result of failed vasectomy, recanalization of the vas deferens, prolonged sperm storage and parthenogenesis; the first two hypotheses were rejected after the death of the adult male 16 months later, since necropsy and histopathology revealed fibrosis and complete occlusion of the vas deferens; prolonged sperm storage seemed unlikely after five years, but it wasn't excluded. They collected samples (blood and tissues) from the dam, the male and all four offspring to perform genetic analysis. They tested DNA extracted from samples with 37 microsatellite markers by PCR amplification; however, some markers failed to amplify, others were uninformative since they were homozygous in all samples, so only nine markers were diagnostic at the end. The results of their analysis showed that all offspring were homozygous for all loci and no one had a paternal allele, thus supporting parthenogenetic reproduction. Regarding the cytological mechanism, they excluded apomixis and premeiotic doubling since offspring were not genetically identical to the dam and they considered automixis as the most likely mechanism; since all four offspring were homozygous females, they hypothesized these offspring were WW females produced through terminal fusion by following the explanation given by Booth, Johnson, *et al.* (2011).

Jordan, Perrine-Ripplinger and Carter (2015) reported another confirmed case in the Copperhead *(Agkistrodon contortrix)*. The female was captured from the wild and never housed with other snakes; after 9 years of isolation from males, she produced four nonviable ova and a stillborn offspring whose gender was determined to be male. They suspected parthenogenetic reproduction because the isolation period of the female exceeded the LTSS maximum time reported in viperids; to confirm it, samples were obtained from the stillborn offspring, the mother and 18 wild snakes captured as potential sires. After DNA extraction, they correctly amplified 10 out of the 23 microsatellite loci evaluated; the genetic analysis showed high levels of heterozygosity in the wild sample, heterozygosity at 4 maternal loci and homozygosity for maternal alleles at all offspring's loci, in accordance with terminal fusion automixis (figure 20; Jordan, Perrine-Ripplinger and Carter, 2015)



		Locus								
Relationship	Snake ID	Ac5683	Ac6673	Ac8237	Ac6064	Ac5322	Ac3113	Ac4335	Ac7679	Ac7363
Mother	AgCo 004	245/253	178/178	210/230	154/154	180/184	205/224	204/204	207/207	223/223
Neonate	AgCo 005	253/253	178/178	230/230	154/154	180/180	224/224	204/204	207/207	223/223
Frequency of neonate allele		0.1333	0.1944	0.4722	0.0588	0.0556	0.4722	0.2778	0.4722	0.1944

Figure 20. Genotypes of the captive mother and the stillborn neonate at nine loci; the stillborn neonate is homozygous for maternal alleles at all loci. The population frequencies of the alleles found in the neonate are in the bottom row (Jordan, Perrine-Ripplinger and Carter, 2015).

Rather than comparing all offspring alleles with a potential sire, they developed a new approach based on allele frequencies (figure 20), that is, the authors calculated the frequencies of offspring alleles in the wild population above-mentioned to obtain the probability that these alleles came from a randomly chosen sire in the population. This probability was very low compared to parthenogenesis probability, so they ruled out the hypothetical paternal contribution to the alleles of the stillborn offspring.

In all FP reports considered so far, females were captive bred or they remained in a long isolation after being removed from wild; FP was therefore considered as a consequence of the captivity condition (Booth *et al.*, 2012; Booth and Schuett, 2016). Even if FP seemed widespread in squamate reptiles (snakes, lizards and amphisbaenians), its detection in nature was complicated by the efforts required; in general terms, a progeny and its mother should be detected and genotyped by identifying alleles inherited from the mother and those from a putative male (Booth *et al.*, 2012).

Booth *et al.* (2012) showed the first cases of FP in wild females of two species: copperhead (*Agkistrodon contortrix*) and cottonmouth (*Agkistrodon piscivorus*). They selected two male offspring from the wild pregnant females *A. contortrix* and *A. piscivorus* for genetic analysis; after DNA extraction, seven (*A. contortrix*) and eight (*A. piscivorus*) microsatellite loci were screened. While the two dams showed heterozygosity at some loci, the offspring of both species showed homozygosity at all loci for one of the maternal alleles. Sexual reproduction would require the contribution of a male with the same allele as the female to obtain homozygous loci; to exclude this possibility, they collected samples from wild adults of both species (n= 63 for *A. contortrix* and n= 45 for *A. piscivorus*). They evaluated frequencies of offspring alleles within these wild populations to calculate the probability that a randomly chosen male contributed same alleles as the dam; this extremely low probability combined with the offspring features (male-only individuals, lots of unfertile ova and offspring homozygosity) supported FP. The homozygous genotype, in contrast to the maternal heterozygosity, indicated terminal fusion automixis. In addition, the authors questioned about the evolutionary role of FP in wild squamates and the reproductive competence of parthenogens in the future; while the reproductive competence of the cottonmouth parthenogen wasn't evaluable due to the release after birth, the copperhead parthenogen remained healthy. Regarding the role of FP in nature, their study showed that FP could no longer be considered only as a result of a long isolation from males (Booth *et al.*, 2012).

Until 2017, facultative parthenogenesis was validated by DNA analysis in three species of Boidae: *Boa constrictor* (Booth, Johnson, *et al.*, 2011), *Epicrates maurus* (Booth, Million, *et al.*, 2011) and *Epicrates cenchria cenchria* (Kinney *et al.*, 2013). Even though cases in the green anaconda (*Eunectes murinus*) were reported before, Shibata *et al.* (2017) provided the first case of facultative parthenogenesis in *E. murinus* confirmed by molecular evidence. One month after the arrival of a wild female at Ueno Zoo (Japan), several offspring were born (two of them sampled for this study); after an insolation period of eight years, the female died for pneumonia and in her oviduct were found two dead female offspring fully developed and 17 unfertilized eggs (figure 21; Shibata *et al.*, 2017).

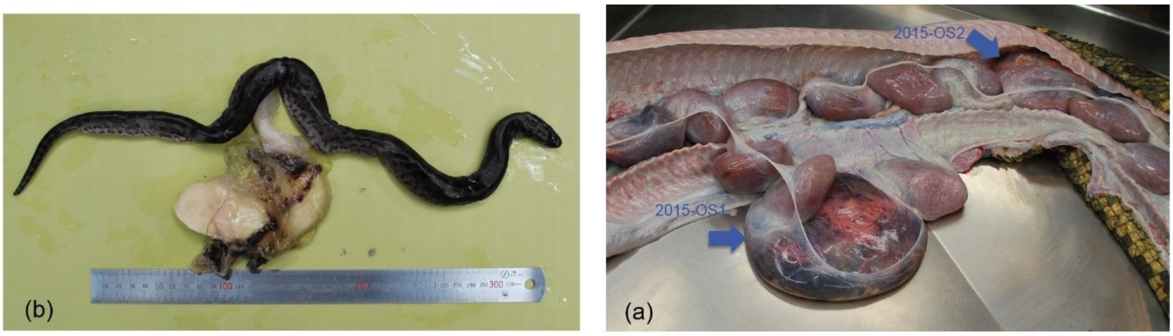


Figure 21. Developed foetuses (2015-OS1 and 2015-OS2 shown with arrows) found in the oviduct of the dam Eunectes murinus isolated from other snakes for seven years; also multiple undeveloped eggs were found; in (b) one of the two foetuses (2015-OS1) is shown (Shibata et al., 2017).

Shibata *et al.* (2017) extracted DNA from tissues of the mother, the four offspring and five additional unrelated individuals for molecular analysis. They personally searched for microsatellite sequences in the maternal genome selecting 18 sequences with trinucleotide motifs as microsatellite markers; then, they developed PCR primers for these marker regions. After amplification and fragment analysis, only 16 microsatellites were informative. They found that the dam was heterozygous for 14 microsatellites and the two dead offspring were homozygous for maternal alleles; there were no paternal alleles suggesting the two offspring were produced by parthenogenesis. In contrast, they found that the elder offspring produced 8 years earlier showed paternal alleles, indicating that the mother switched from sexual reproduction to parthenogenetic reproduction during the isolation period.

To exclude the possibility of LTSS, they evaluated allele frequencies in the five unrelated individuals and at each locus they calculated the probability of having two homozygous offspring. The product of these probabilities across all loci gave the probability that the two homozygous neonates were produced by sexual reproduction which was a very low number. Regarding the possible parthenogenetic mechanism, they advanced a new hypothesis after the discovery of XY sex chromosomes in Boidae and Pythonidae (Gamble *et al.*, 2017).

In all snake species, females were considered to be the heterogametic sex (ZW) so the production of homogametic males was the result of terminal fusion automixis whereas the production of females resulted from premeiotic doubling, central fusion or terminal fusion automixis; females produced by terminal fusion would be WW homozygous individuals that were not considered to be viable except in rare cases (Booth, Johnson, *et al.*, 2011; Kinney *et al.*, 2013). Exclusively female offspring were reported from Alethinophidia families of Boidae and Pythonidae (Groot, Bruins and Breeuwer, 2003; Booth, Johnson, *et al.*, 2011; Booth, Million, *et al.*, 2011; Kinney *et al.*, 2013), whereas exclusively male offspring were reported from Caenophidia families (Dubach, Sajewicz and Pawley, 1997; Schuett *et al.*, 1997; Germano and Smith, 2010; Booth and Schuett, 2011; Jordan, Perrine-Ripplinger and Carter, 2015). The recent discovery of XY sex chromosomes in snakes suggested that the offspring's sex differences observed in different families could correspond to two different modes of sex determination: Shibata *et al.* (2017) suggested that exclusively female offspring occurred in those species with homogametic females (XX) to give XX parthenogenetic females, whereas exclusively male offspring occurred in species with heterogametic females (ZW) to give ZZ parthenogenetic males. In species with homogametic females, such as *E. murinus*, central fusion was expected to produce only heterozygous female offspring; terminal fusion produced homozygous females with some heterozygous loci due to recombination during meiosis; gametic duplication produced completely homozygous female offspring, even if recombination took place (Shibata *et al.*, 2017). Due to limited informative markers, previous studies in Boidae could not distinguish clearly between terminal fusion and gametic duplication (Booth, Johnson, *et al.*, 2011; Booth, Million, *et al.*, 2011; Kinney *et al.*, 2013). In the study of Shibata *et al.* (2017), the female offspring were systematically homozygous at all 16 microsatellite markers, including 14 markers that were heterozygous in the mother. According to the authors, this exclusive homozygosity indicated that parthenogenesis occurred by gametic duplication, not terminal fusion; a wide genome analysis, such as whole genome sequencing, should be performed to distinguish between the two mechanisms.

Shibata *et al.* (2017) also suggested the possibility that the offspring of *E. murinus* were haploid individuals developed from unfertilized eggs; however, this kind of parthenogenesis with the reduction of ploidy is rare in vertebrates and had been reported only in a shark species. This possibility could be excluded studying karyotypes by cytological analysis or quantifying nuclear DNA; unfortunately, the parthenogenetic offspring described in their study were dead and the authors were unable to obtain live cells for cytological analysis, so they could not eliminate the possibility of haploid offspring.

Despite the increasing number of FP reported cases, no one until 2018 was reported from the elapid (Elapidae) snake family, which include well-known snakes such as mambas, cobras, taipans and sea snakes. Allen, Sanders and Thomson (2018) reported for the first time FP in two elapid species: the oviparous coastal taipan (*Oxyuranus scutellatus*) and the viviparous common death adder (*Acanthophis antarcticus*). The former can be found in coastal regions of Australia and in the island of New Guinea, while the latter occurs in eastern and southern Australia; both species are highly venomous (Allen, Sanders and Thomson, 2018). They selected three taipan females (two captive and one wild) and a wild death adder female that were housed individually; all four females reproduced sexually with males before producing parthenogenetic litters. According to the authors, these litters were atypical compared to previous records for the species: low fertility rates, many unfertilized eggs, two stillborn offspring and some morphological abnormalities (malformed scales and a deformed eye). They sampled all four females, their offspring and captive males as potential sires; double digest restriction-site associated DNA markers (ddRAD-seq) were created to valuate single nucleotide polymorphisms (SNP) among DNA samples.

DdRAD-seq is a recent sequencing protocol used for SNP discovery and genotyping (figure 22): in this method, genomic DNA is digested with a restriction enzyme and a first barcoded adapter (P1) is ligated to the fragments; the ligated fragments from different samples are combined and the DNA digested by a second restriction enzyme; the fragments are size-selected and a second adapter (P2) is then ligated to the fragments, containing now both adapters, that are amplified by PCR. The obtained libraries are then sequenced (Peterson *et al.*, 2012).

Figure 22. A ddRAD sequencing protocol in which a double digestion of genome is followed by amplification of the resulting fragments (https://emea.illumina.com/science/sequencing-method-explorer/kits-and-arrays/ddradseq.html).

Allen, Sanders and Thomson (2018) then utilized several software (STACKS, GBSX, BBTools) to process the sequences of each species, to identify from which samples came the sequences and to obtain stacks of putative alleles; by comparing allele stacks, a set of putative loci (RAD loci) was provided and SNPs were detected at each locus. They were able to evaluate a large number of loci through this method (up to 3715 loci). The authors calculated three indices through another statistical software to confirm parthenogenetic origins of litters: the internal relatedness (IR), the standardized heterozygosity (SH) and the homozygosity by loci (HL).

IR could assess inbreeding from multilocus heterozygosity using allele frequencies; SH was calculated as the proportion of heterozygous loci divided by the population's heterozygosity mean for these loci; HL weighed the contribution of each locus to the homozygosity value depending on its allelic variability. As expected, parthenogens had positive IR values (greater than 0.152), low SH values (0.302–0.539) and high HL values (0.694–0.884) compared to that of mothers and putative fathers, which was indicative of parthenogenesis (Allen, Sanders and Thomson, 2018).

They determined the sex of parthenogens to be male by probing and manually everting hemipenes, but they were unable to confirm the gender genetically due to the lack of known sex-specific ddRAD markers. Therefore, they could not genetically confirm the lack of females, but the parthenogens were most likely ZZ male individuals. The authors did not mention the mechanism of FP, but the high values of HL and SH suggested terminal fusion automixis. Regarding the health of parthenogens, all taipans remained alive, were robust and developed normally (although two of them had minor physical malformations), but the two death adders died about 2 months after birth. Moreover, the litter of death adders included stillborn offspring with developmental abnormalities (like those previously reported in the other studies).

Seixas *et al.* (2020) reported the first case of FP in a female Cuban boa (*Chilabothrus angulifer*), kept in captivity in a Portuguese zoo; the female was isolated from males for seven years, then she gave birth to a stillborn (preserved in formalin) and many unfertilized eggs. After 4 years another similar litter (a stillborn with multiple unfertilized eggs) was generated. They performed histopathological and molecular analysis on both stillborn offspring (one fixed in formalin and another fresh). Macroscopically, both stillborn offspring were fully developed, but the second one showed a morphological alteration, specifically bilateral anophthalmia (figure 23). Microscopically, organs did not show any alterations. The examination of reproductive system revealed that both offspring were female (Seixas *et al.*, 2020).

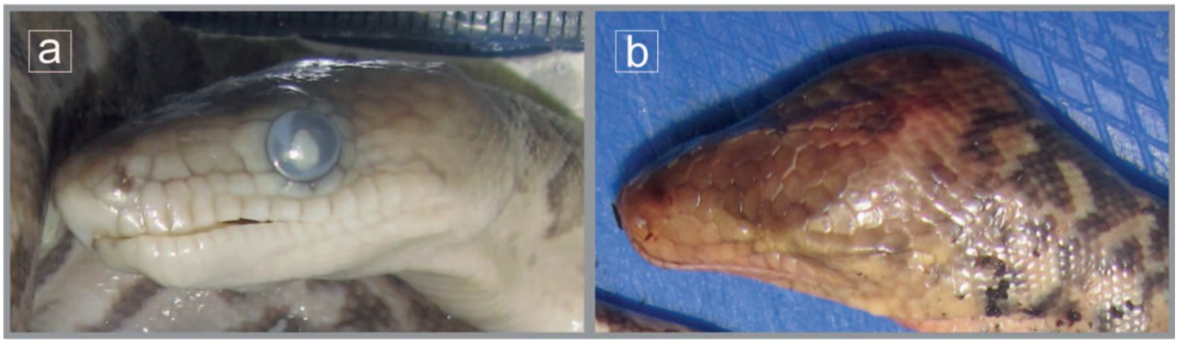


Figure 23. Offspring of the Cuban boa (Chilabothrus angulifer); the first offspring (a) had a normal head; (b) the second one (b) showed bilateral anophthalmia (Seixas et al., 2020).

After extracting DNA from both the mother and the offspring, Seixas *et al.* (2020) analysed thirteen microsatellite markers for boid species; two of these markers did not amplify or gave non-specific PCR products. A pre-screening of microsatellite variations was performed using high-resolution melting (HRM) analysis, a method that allows to assess genotypic variations between individuals detecting differences in PCR melting temperatures by returning melting curves. They identified four loci with allelic variability among mother and offspring through HRM analysis (figure 24); the other loci did not show any allelic variability. They performed electrophoresis to determine allele sizes and to confirm HRM results. As expected, the mother turned out to be heterozygous at those four loci and homozygous at the remaining loci analysed; both offspring were homozygous in all microsatellite loci, carrying only maternal alleles. The offspring's homozygosity and the long isolation period from males (13 years for the second litter) supported a reproduction through parthenogenesis. They ruled out long term sperm storage due to the lack of paternal alleles at all loci. The high level of homozygosity suggested FP by terminal fusion automixis. However, according to Booth and Schuett (2016) and Shibata *et al.* (2017) gamete duplication could not be excluded as an alternative mechanism without confirmation. Both stillborn offspring were female, but the authors did not mention whether they were WW or XX individuals. As previously stated by the work of Shibata *et al.* (2017), it is reasonable to assume that these stillborn were XX individuals.

Ultimately, the bilateral anophthalmia founded in the second offspring was related to the high homozygosity level that led to the expression of disadvantageous recessive genes.

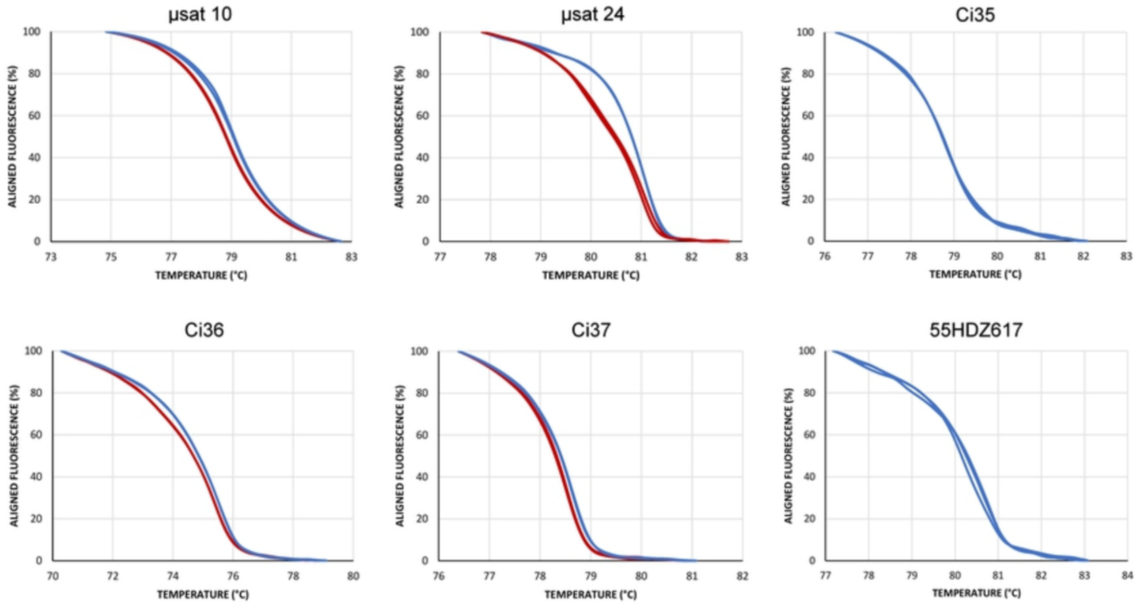


Figure 24. Melting curve profiles obtained in the pre-screening of the microsatellite loci using HRM analysis. The fluorescence differences allowed to identify four loci (µsat 10, µsat 24, Ci36 and Ci37) with allelic variability among the dam and offspring, while the other two loci (Ci35 and 55HDZ617) showed no differences in fluorescence, i.e. they were all homozygous at these loci (Seixas *et al.*, 2020).

Cubides-Cubillos *et al.* (2020) investigated facultative parthenogenesis in three viviparous pitvipers species of neotropical genus *Bothrops*: *B. atrox*, *B. moojeni* and *B. leucurus*. *B. atrox* occurs in the northern part of South America, *B. moojeni* occupies central and southeastern Brazil, Paraguay and Argentina and *B. leucurus* is mainly distributed throughout northeastern Brazil (Cubides-Cubillos *et al.*, 2020). A female of *B. atrox*, two females of *B. moojeni* and one female of *B. leucurus* were analysed molecularly in their study. All the females were housed in different institutes and were kept in isolation from males for several years (seven years in total for the two former species and nine years for the latter one).

The *B. atrox* female gave birth to three litters composed of two live males (one died a few months later, the other had malformations), a malformed embryo, a stillborn and several yolked ova; the two males were both sampled by the authors.

The first *B. moojeni* female gave birth in three times to some fully formed offspring, a partially formed embryo, four stillborn offspring and several yolked ova; three offspring among all were analysed molecularly in their study.

The three litters of the second *B. moojeni* female comprised two live offspring (one died 3 hours after birth), two embryos, and a lot of yolked ova; they analysed molecularly the two live offspring, two embryos, and six ova.

The *B. leucurus* female gave birth to a single litter of several yolked ova and a malformed embryo; they analysed the latter and the content of one ovum.

The gender of all checked offspring was found to be male and they confirmed it by microscopy when possible; in some cases, the gender was not detected for different reasons (i.e. malformed or disintegrated offspring or eaten by the mother).

For the molecular analyses, Cubides-Cubillos *et al.* (2020) extracted DNA from mothers' scales or livers, from embryo fragments and yolked ova; polymerase chain reactions (PCR) were performed using nine heterologous microsatellite primers developed for other different species (*Agkistrodon contortrix*, *Bothrops marmoratus* and *Bothrops insularis*) from which only four amplified. PCR products were loaded in an electrophoresis apparatus and the resulted images were saved as digital pictures (Cubides-Cubillos *et al.*, 2020).

They found that the *B. atrox* female was heterozygous for three loci, whilst the offspring was homozygous; the fourth locus was not informative because both the mother and sons were homozygous (figure 25).

They found also that the first *B. moojeni* female and its offspring were homozygous for all loci analysed, so parthenogenesis could not be confirmed. The second *B. moojeni* female was heterozygous for two out of four loci, whilst the two offspring, the two embryos and the six ova were homozygous; in the other two loci the mother and the offspring showed the same electrophoretic band, thus they were all homozygous. In the case of *B. leucurus*, the mother was heterozygous for three loci and homozygous in the other one; the offspring and an ovum shared one band with the mother for each locus, so they were homozygous for all markers (Cubides-Cubillos *et al.*, 2020). In summary, Cubides-Cubillos *et al.* (2020) found that each offspring shared a band with the mother for all loci.

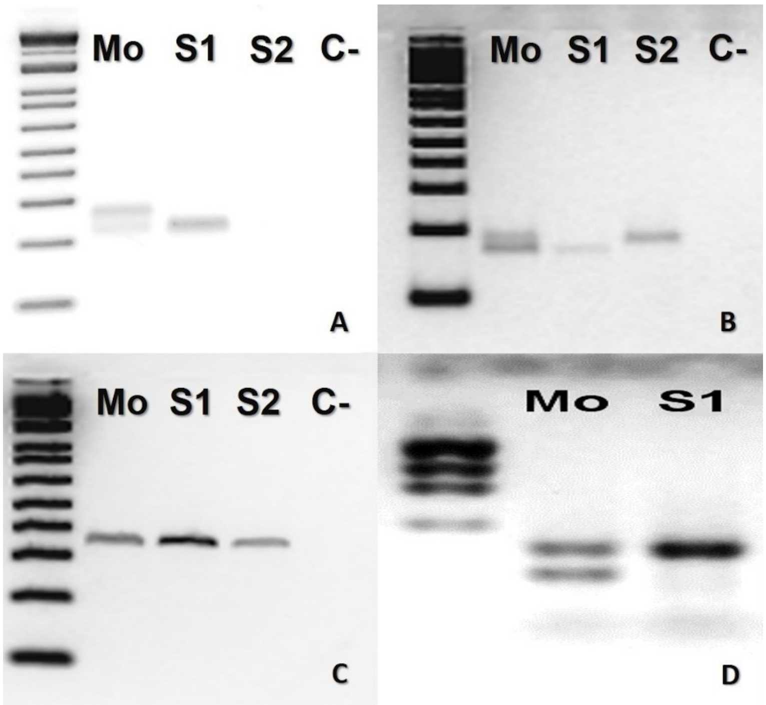


Figure 25. Microsatellite bands of Bothrops atrox in electrophoretic agarose gels, where Mo is the parthenogenetic mother, S1 and S2 her male offspring and C- is the negative control. The first lane is a molecular weight standard and A-D are the four different microsatellite loci. Mo is heterozygous at three loci (A, B, D), whilst the offspring is always homozygous (Cubides-Cubillos *et al.*, 2020).

Cubides-Cubillos *et al.* (2020) then estimated the sizes of amplified bands (or alleles sizes) through an analysis software by comparing sizes of gel bands to molecular weight markers; so, the authors confirmed previous observations of gel bands, i.e. the offspring were homozygous and showed only maternal alleles. By considering captivity information (none of mothers had been housed with males ruling out prolonged sperm storage), litter features (large number of undeveloped ova, malformations and stillborn offspring) and molecular data (only maternal alleles in the offspring), they confirmed for the first time facultative parthenogenesis in at least three out of four mothers studied: the offspring of *B. atrox*, second *B. moojeni* and *B. leucurus* females showed homozygosity for heterozygous loci in the mother. The first *B. moojeni* mother and its offspring were found homomorphic in all amplified loci so there was no confirmation for this single case, even if the absence of additional alleles in the offspring and the captivity condition of the mother were in agreement with the facultative parthenogenesis hypothesis. In addition, the low probabilities of obtaining the same offspring with sexual reproduction could rule out the hypothesis of long-term sperm storage, thus confirming parthenogenesis for all the cases reported (Cubides-Cubillos *et al.*, 2020).

Given that *Bothrops* genus belongs to Caenophidia clade, most of Caenophidian snakes exhibit females as the heterogametic sex (ZW) and males as the homogametic (ZZ) sex (Booth and Schuett, 2016). Cubides-Cubillos *et al.* (2020) confirmed the karyotype at least for one *B. moojeni* mother to be like those already reported in *Bothrops* genus (2n= 36 with ZW sex pair). Given that terminal fusion automixis is the most common mode of facultative parthenogenesis in snakes (Booth and Schuett, 2016), the authors proposed it as the most likely mechanism considering that terminal fusion in Caenophidian ZW snakes brings to litters composed only of males like those in their study.

Gasanov and Katz (2020) described a case of facultative parthenogenesis in a diamondback water snake (*Nerodia rhombifer*) of Colubridae family. Within Colubridae, facultative parthenogenesis had been described in several natricine species of the genus *Thamnophis* (Schuett *et al.*, 1997; Germano and Smith, 2010; Reynolds *et al.*, 2012) and also in at least two species of the genus *Nerodia* (Conant, 1965; Smith, Thiss and Chiszar, 2000; Booth and Schuett, 2011, 2016).

In the study of Gasanov and Katz (2020), an adult captive female *N. rhombifer* produced four unfertilized ova, one stillborn and one live neonate. The female was kept in isolation since its arrival and had no contacts with other snakes during the entire period of observation. The offspring exhibited morphological abnormalities (spinal deformities; figure 26) that were noticeable in the stillborn and moderate in the viable one; in addition, both offspring had irregularities in the head scales and asymmetries in several ventral ones. The sex of both offspring was determined to be male by manual eversion of hemipenes, a common method for sex determination in colubrid snakes. They collected skin and tissue samples for DNA extraction from the female, the two offspring, four unrelated individuals of the same species and an outgroup individual of *N. fasciata* species. They performed polymerase chain reactions (PCR) using twelve specific microsatellite markers as primers and the resulting products were analysed through electrophoresis. Six microsatellite markers did not amplify or gave specific products only for *N. fasciata*; two other markers were only helpful to distinguish between two different species like *N. fasciata* and *N. rhombifer* considering that all *N. rhombifer* gel bands were the same. One marker gave weakly distinguishable products for all samples. Overall, only three out of twelve microsatellite markers gave different products within samples: one locus showed a fully corresponding banding pattern between the mother and the offspring, the other two were only partially corresponding; the mother was thus heterozygous for these two loci, while the offspring were homozygous for a maternal allele (Gasanov and Katz, 2020).

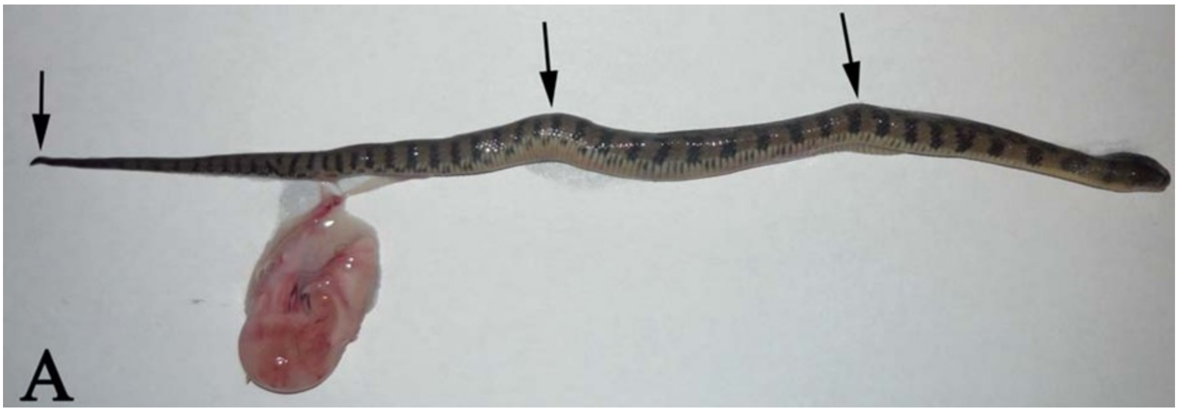


Figure 26. Spinal deformities (marked by arrows) in the stillborn offspring (A) of the captive female Nerodia rhombifer (Gasanov and Katz, 2020).

Long term sperm storage in past studies had been described in natricine snakes of the genus *Thamnophis*, but it was not confirmed in the genus *Nerodia* (Gasanov and Katz, 2020). Gasanov and Katz (2020) ruled out the possibility of prolonged sperm storage by considering the captivity condition of the female: it was obtained as a juvenile prior to being sexually mature and was kept in isolation for more than two years. By considering the female's history, the morphological abnormalities of the offspring, the litter's characteristics (lots of unfertilized ova, low offspring viability) and the genotypic pattern, the most plausible explanation was considered to be facultative parthenogenesis (Gasanov and Katz, 2020). Although in the present study the karyotypes of snakes were not analysed, caenophidian snakes in previous studies had the ZZ/ZW sex determination system and offspring produced by facultative parthenogenesis were always males (ZZ), so it is reasonable to think that the most likely mechanism in this case was terminal fusion automixis (Booth and Schuett, 2011, 2016; Booth *et al.*, 2012; Reynolds *et al.*, 2012; Gasanov and Katz, 2020).

Considering the growing number of documented cases of parthenogenesis and its discovery in nature, Booth and Schuett (2016) analysed all the cases of FP reported in the literature up to then in snakes and reviewed the phylogenetic distribution by identifying common features and differences among the different lineages. They provided the first steps to understand the phylogenetic origin and the evolution of this phenomenon in snakes. They divided parthenogenesis into two groups (type A and type B) based on five characteristics commonly discussed in reports: the parthenogenetic mode (FP or OP), the sex of the parthenogens and their viability, the ploidy, the sex chromosome morphology and the mode of parity. These characteristics were interpreted in a phylogenetic point of view evaluating the phylogenetic trees of snake species built on the basis of molecular or morphological analyses. They obtained that these characteristics diverged clearly between the lineages of "ancient" and "advanced" snakes (figure 27).

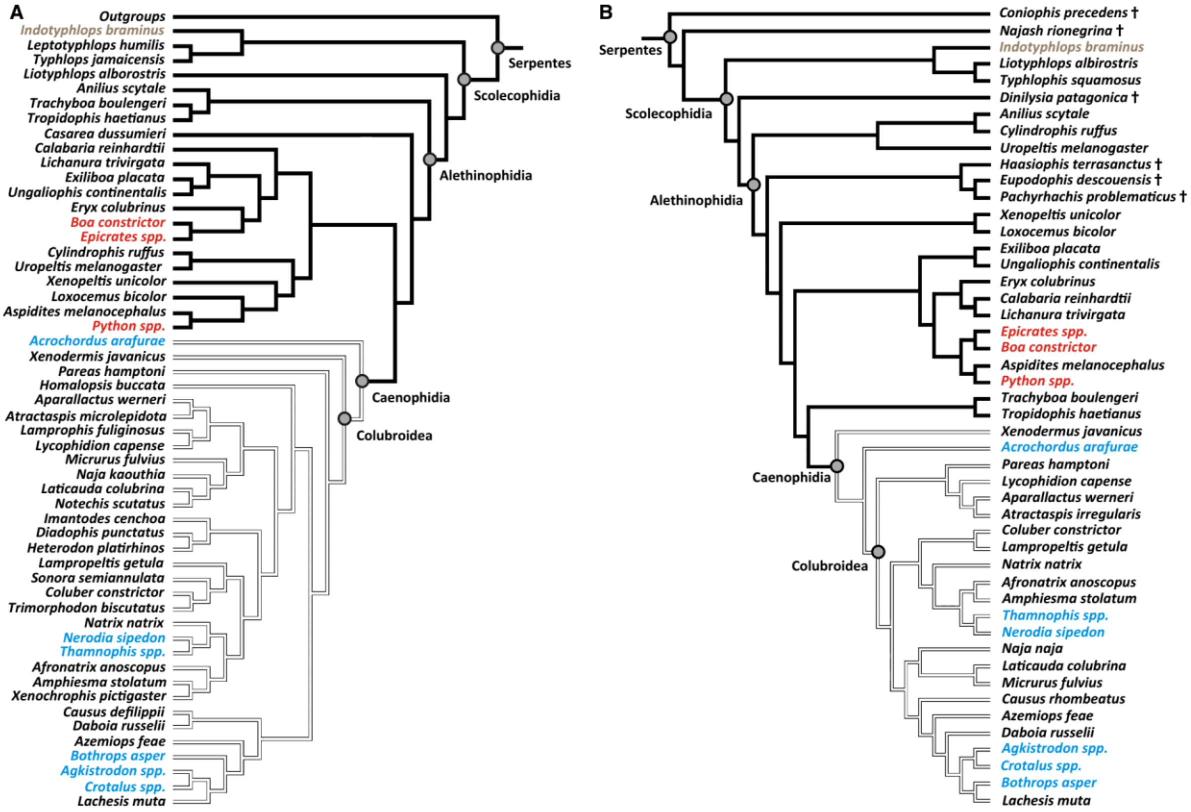


Figure 27. Phylogeny of snakes constructed from molecular characters (A) and from morphological and fossil characters (B) related to pattern of parthenogenesis. Species documented as being parthenogenetic are indicated in brown (obligate), red (facultative, type A), and blue for facultative, type B (Booth and Schuett, 2016).

Type A parthenogenesis was observed in basal alethinophidians (Boidae and Pythonidae): it was distinguished by terminal fusion automixis as the most likely mechanism and a female only progeny with an arrangement of sex chromosomes presumed to be WW (Booth, Johnson, *et al.*, 2011; Booth, Million, *et al.*, 2011; Kinney *et al.*, 2013; Booth and Schuett, 2016). However, as suggested by Gamble *et al.* (2017) and Shibata *et al.* (2017) at least in some "primitive" lineages (Alethinophidia) the sex determination is of the XX/XY type with XX viable females and YY unviable individuals (Guedes and Guedes, 2020).

In Alethinophidia, ZW female parthenogens had been reported only in a single research by Groot, Bruins and Breeuwer (2003) who suggested as possible mechanisms apomixis, premeiotic doubling of chromosomes and central fusion automixis. However, this apparent clonal reproduction opposed to all other cases of automixis described in alethinophidian and caenophidian taxa, so as suggested by Booth and Schuett (2016) the actual mechanism may not have been accurately assessed. In the case reported by Groot, Bruins and Breeuwer (2003) the adult female may have been produced herself by parthenogenesis representing an example of secondary parthenogenesis (Booth *et al.*, 2014; Booth and Schuett, 2016). In secondary parthenogenesis, if a female was produced by facultative parthenogenesis showing an extensive genome homozygosity and she, in turn, reproduces by parthenogenesis, the offspring will appear as a clone of the mother regardless of the mechanism involved; this could be the reason why the progeny of *P. bivittatus* was a clone of the mother (Booth and Schuett, 2016). Unpublished data obtained by personal communications between Booth and Groot (see Booth and Schuett, 2016) showed that P. bivittatus produced a second parthenogenetic clutch with characteristics similar to those described in all other alethinophidians, confirming the hypothesis given by Booth and Schuett (2016). However, additional researches should be performed to better understand the mechanisms of FP in this taxon.

In contrast to type A FP, type B facultative parthenogenesis occurred in advanced snakes (Caenophidia) that showed terminal fusion automixis as the most likely mechanism and progeny composed only of males with ZZ sex chromosomes (Schuett *et al.*, 1997; Germano and Smith, 2010; Booth and Schuett, 2011, 2016;

Reynolds *et al.*, 2012). In advanced snakes there are no records of female offspring (ZW or WW) produced by FP (Booth and Schuett, 2011).

Another characteristic that appeared conserved was the viability of progeny: in alethinophidians (type A), the litters (or clutches) resulting from FP were large and apparently viable; in advanced lineages (type B), the progeny resulting from FP often exhibited morphological abnormalities, low viability and typically contained many unfertilized ova (Schuett *et al.*, 1997; Germano and Smith, 2010; Booth *et al.*, 2012; Reynolds *et al.*, 2012; Jordan, Perrine-Ripplinger and Carter, 2015).

According to Booth and Schuett (2016) the switch that triggers automixis may be identified across many ova in ancestral lineages of snakes, though in a few ova in advanced ones. Moreover, according to them, the deformities observed in type B are probably the result of the inbreeding depression caused by terminal fusion automixis. Regarding the reproductive competence, the existence of apparently healthy parthenogens have been reported in boas and python (basal alethinophidians) and also in some caenophidians; deformities of genitalia have been documented in some cases of FP type B (Schuett et al., 1997), but also apparently normal spermatozoa in other cases (Reynolds et al., 2012; Booth 2016).

Facultative parthenogenesis has not documented in any other basal alethinophidian species outside of Boidae and Pythonidae; the discovery of FP in other alethinophidian members (e.g., Scolecophidia, Aniliidae, Cylindrophiidae, Loxocemidae, Tropidophiidae, Uropeltidae, and Xenopeltidae) might help to understand better the phylogenetic distribution of FP and the mechanisms of sex determination. For example, it might help to figure out if FP type A, described for boids and pythonids, occurs also in other earliest snakes like basal scolecophidians; however, the main obstacle in studying these taxa is to obtain these animals themselves (Booth and Schuett, 2016).

Conclusions

Obligate parthenogenesis, although widespread among lizards and invertebrates, is rare in snakes and limited to a single species; future research is required to identify OP in other snake lineages. On the other hand, parthenogenesis in snakes occurs mainly in bisexual species that normally reproduce by amphigony. Facultative parthenogenesis in snakes seems to be more common than previously thought and probably new species will be revealed in the future with additional research. In the past, FP in snake species had been suspected in captive-bred animals based on the long period of isolation from males and on the characteristics of the litters, i.e. the production of many unfertilized eggs and stillborn offspring. It was therefore considered as a captivity syndrome restricted to animals isolated from males. However, the technological advancement of molecular techniques has made easier the detection and demonstration of facultative parthenogenesis. Its discovery in females housed with conspecific males and in wild vertebrates suggests that the presence of males does not necessarily exclude parthenogenetic reproduction and that it should no longer be seen as a mere curiosity outside the vertebrate evolution. Despite recent findings, the causes that lead to parthenogenetic reproduction in snakes, and in general in all other animals, are not fully understood and more investigations should be conducted to understand the triggers of parthenogenesis. Although single parthenogenic events may retain high levels of heterozygosity in the offspring, habitual parthenogenetic reproduction potentially has detrimental effects due to the reduction of genetic diversity and the fixation of deleterious alleles. Therefore, the increasing discovery of FP in different species or taxa could have important consequences on breeding programs of endangered species that aim to promote genetic diversity; molecular genetic analyses might be required to maintain high levels of genetic diversity in these populations. However, future studies are needed to understand the spread of this phenomenon on wild populations and the real consequences it has on population genetics.

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