THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En génétique et génomique

École doctorale GAIA

Unité de recherche CEFE, CNRS

Evolutionary causes and consequences of non-clonal asexuality in *Artemia*

Présentée par Loreleï BOYER
Le 7 février 2022

Sous la direction de Thomas LENORMAND et Christoph HAAG

Devant le jury composé de

Isabelle SCHÖN, Senior scientist, Royal Belgian Institute of Natural Sciences, Brussels, Belgium	Rapportrice
Tanja SCHWANDER, Associate professor, University of Lausanne, Switzerland	Rapportrice
Susana COELHO, Director, Max Planck Institute for Developmental Biology, Tübingen, Germany	Examinatrice
Nicolas BIERNE, Directeur de recherche, ISEM, CNRS, Montpellier, France	Examinateur
Thomas LENORMAND, Directeur de recherche, CEFE, CNRS, Montpellier, France	Directeur de thèse
Christoph HAAG, Chargé de recherche, CEFE, CNRS, Montpellier, France	Co-directeur de thèse

UNIVERSITÉ DE MONTPELLIER

Table of contents

Table of contents 3
Abstract (English) 5
Résumé (Français) 6
Introduction 7
I. How does asexuality evolve from sex? 9
a. Pathways of transition from sex to parthenogenesis 9
b. Mechanistic basis 10
c. Male production and sex 11
II. Evolution of asexuals 12
a. Can the Paradox of sex be resolved by non-clonal asexuality? 12
b. Evolutionary fate of clonal and non-clonal asexuals 12
III. Artemia: a model to study sex-asex transitions 14
References 15
Chapter 1 – Not so clonal asexuals: Unraveling the secret sex life of *Artemia parthenogenetica* 19
Abstract 21
Introduction 22
Methods 23
Results 24
Discussion 27
References 29
Supplementary materials 32
Chapter 2 – Questioning the preeminence of clonality among parthenogenetic animals 47
Abstract 49
Introduction 50
Main text 52
Asexual reproductive modes with high LOH 52
Central fusion-like parthenogenesis 53
Mitosis 56
Premeiotic doubling 57
Erroneous methods and misconceptions 58
Discussion 63

References 66
Supplementary data 74
Chapter 3 – Asexual production of males by ZW recombination in *Artemia parthenogenetica* 83
Abstract 85
Introduction 86
Methods 89
Results 95
Discussion 97
References 101
Supplementary materials 104
Discussion 109
I. How does the transition from sex to asexuality explain the distribution of clonal and non-clonal asexuals? 111
a. Non-clonal asexuals are more common than previously thought 111
b. LOH: A barrier to the evolution of asexuality 111
II. Asexual genome evolution 112
a. What is the effect of low LOH? 112
b. Genomic regions are more or less clonal 112
III. The role of rare sex 113
a. Genetic conflict and male production 113
b. Does inter-population sex have a significant impact? 114
c. How frequent is sex in asexuals? 115
Conclusion 115
References 116
Summary of the thesis in French 119
Références 125
Annex – The origin of asexual brine shrimps 129
Abstract 131
Introduction 132
Methods 137
Results 144
Discussion 153
References 161

Abstract (English)

The majority of parthenogenetic species are often thought to be clonal. Clonality is costly in the long term, as it can result in accumulation of deleterious mutations and lower adaptability. However, cases reporting non-clonal asexuals are accumulating. Non-clonal asexuality has very different genomic and fitness consequences compared to clonality, and may be a key intermediate step in the transition from sex to asexuality. Additionally, asexuality may be often non-obligate, with events of cryptic sex. These events may also shape the genome and evolution of asexual lineages. In this PhD, I investigated the reproductive mode of *Artemia parthenogenetica* and its role in the transition from sex to asexuality and the evolution of asexual lineages. Specifically, I used the capacity of asexually produced males ("rare males") to cross with sexual females and transmit asexuality to their offspring (contagious asexuality), to experimentally generate new lineages. I showed that diploid asexual *Artemia* have a non-clonal reproductive mode, in which recombination results in loss of heterozygosity (LOH) in the offspring. LOH is costly as it can reveal recessive deleterious mutations. Perhaps due to selection caused by the deleterious consequences of LOH, the recombination rate in these asexuals was lower than in a closely related sexual species. I also found that sex-asex hybrids had a mixed sexual and asexual reproduction, and that asexual females from natural populations were capable of rare sex. This means that rare events of sex in asexual *Artemia* could occur between a rare male and an asexual female reproducing sexually. In a review of how asexual reproductive modes were identified in the literature, I found that there was a bias in the identification and general perception of asexuals toward clonality, as an important part of the asexual species reviewed were in fact non-clonal, and evidence for clonality was often missing. Furthermore, the majority of non-clonal asexuals had reproductive modes that resulted in low LOH. This suggests that non-clonal asexuals often evolve secondarily toward a more clonal-like reproduction, so that even clonal species may not have been clonal throughout their evolutionary history. Finally, using genomics on contagion-generated lineages, I found that in *Artemia*, rare males are produced asexually through recombination and thus LOH on the ZW sex chromosomes. We know that contagious asexuality, and possibly between-lineages crosses, occurred in the evolutionary history of *A. parthenogenetica*. Perhaps, contagious asexuality and/or within asexual sex events provide opportunities for the gene(s) controlling asexuality to escape declining lineages into new ones. In this case, contagious asexuality through rare males may be the reason why recombination persists in asexual *Artemia*. Whether non-clonal asexuality and sex events occur in many parthenogenetic species is still unclear, and requires thorough investigation. Theoretically, there is a strong need for models taking into account the genomic consequences of non-clonal and non-obligate asexuality, and their role in the transition from sex to asexuality and the maintenance of sex.

Keywords: Non-clonal asexuality, Recombination, Contagious asexuality, Cryptic sex, *Artemia,* Experimental crosses, Sex-asex transition

Résumé (Français)

La majorité des espèces parthénogétiques sont souvent perçues comme clonales. La clonalité est coûteuse à long terme, car elle peut entraîner l'accumulation de mutations délétères et une moins bonne capacité d'adaptation. Cependant, les cas d'espèces asexuées non clonales s'accumulent. L'asexualité non-clonale génère des conséquences génomiques et de fitness très différentes de la clonalité, et pourraient représenter une étape-clé dans la transition du sexe vers l'asexualité. De plus, l'asexualité peut être souvent non-obligatoire, avec des événements de sexe cryptiques. Ces évènements peuvent aussi façonner le génome et l'évolution des lignées asexuées. Dans cette thèse, j'ai étudié le mode de reproduction d'*Artemia parthenogenetica,* et son rôle dans la transition du sexe vers l'asexualité et l'évolution des lignées asexuées. En particulier, j'ai utilisé la capacité des mâles produits par voie asexuée ("mâles rares") à se croiser avec des femelles sexuées et à transmettre l'asexualité à leurs descendants (asexualité contagieuse), pour générer expérimentalement de nouvelles lignées. J'ai montré que les *Artemia* asexués diploïdes ont un mode de reproduction non-clonal, dans lequel la recombinaison entraîne une perte d'hétérozygotie (LOH, pour "*loss of heterozygosity*") chez les descendants. Le LOH est coûteux car il peut révéler des mutations délétères récessives. Peut-être en raison de la sélection causée par les conséquences délétères du LOH, le taux de recombinaison chez les *Artemia* asexués était plus faible que chez une espèce sexuée apparentée. J'ai également constaté que les hybrides sexués avaient une reproduction mixte sexuée et asexuée, et que les femelles asexuées issues de populations naturelles étaient capables de sexe rare. Cela signifie que des événements rares de sexe chez les *Artemia* asexués pourraient se produire entre un mâle rare et une femelle asexuée se reproduisant sexuellement. En effectuant une revue de la façon dont les modes de reproduction asexués sont identifiés dans la littérature, j'ai constaté que l'identification et la perception générale des asexués étaient biaisées en faveur de la clonalité, car une grande partie des espèces asexuées examinées étaient en fait non-clonales, et les preuves de la clonalité étaient souvent insuffisantes. En outre, la majorité des asexués non-clonaux avaient des modes de reproduction qui entraînaient de faibles taux de LOH. Cela suggère que les asexués non-clonaux évoluent souvent secondairement vers une reproduction plus clonale. Ainsi, même les espèces clonales pourraient ne pas avoir été clonales au cours de leur histoire évolutive. Enfin, avec une analyse génomique sur de nouvelles lignées générées par contagion, j'ai démontré que chez *Artemia*, les mâles rares sont produits asexuellement par recombinaison et donc LOH sur les chromosomes sexuels ZW. Nous savons que l'asexualité contagieuse, et peut-être des croisements entre lignées, ont eu lieu au cours de l'histoire évolutive d'*A. parthenogenetica*. L'asexualité contagieuse et/ou des événements sexuels chez les asexués constituent peut-être des opportunités pour que le(s) gène(s) contrôlant l'asexualité s'échappe(nt) des lignées en déclin vers de nouvelles lignées. Dans ce cas, l'asexualité contagieuse par le biais de mâles rares pourrait être la raison pour laquelle la recombinaison persiste chez les *Artemia* asexués. Chez de nombreuses espèces, l'identification de l'asexualité non clonale et des événements de sexe n'est toujours pas claire et nécessite une étude approfondie. Théoriquement, il y a un fort besoin de modèles prenant en compte les conséquences génomiques de l'asexualité non-clonale et non-obligatoire, et leur rôle dans la transition du sexe vers l'asexualité et la maintenance du sexe.

Mots-clés : Asexualité non-clonale; Recombinaison; Asexualité contagieuse; Sexe cryptique; *Artemia*; Croisements expérimentaux; Transition sex-asex

Introduction

Sexual reproduction is the ancestral reproductive mode in eukaryotes, with most species reproducing exclusively sexually (Simon *et al.* 2003). Eukaryotic sex generally implies segregation and recombination of chromosomes during meiosis, and requires fertilization (*i.e.*, fusion of the products of two separate meioses). Asexuality is a reproductive mode that does not necessitate fertilization. Parthenogenesis is a form of asexual reproduction through oogenesis, where unreduced oocytes are produced, which develop into an embryo without fertilization. In this thesis I will use both terms, but focus essentially on parthenogenesis. Obligate parthenogenesis in particular is rare, although it has evolved independently multiple times across the eukaryotic tree from sexual ancestors (Schön *et al.* 2009). To explain the distribution of parthenogenesis in eukaryotes, it is necessary to understand how asexually reproducing species can evolve and are maintained. To this end, research has mainly focused on how arising asexual lineages can invade sexual populations, and on the long-term consequences and costs of asexuality. The majority of parthenogens are considered clonal, which means that the produced offspring are genetically identical to the parent, barring mutations (Suomalainen 1950). The underlying mechanism is generally thought to be mitosis, where recombination and segregation do not take place, leading to complete genetic linkage. However, there is a diversity of parthenogenetic mechanisms derived from meiosis (still with no fertilization) in which segregation and recombination can occur more or less frequently. These non-clonal processes affect the genome of asexuals by generating loss of heterozygosity (LOH) and breaking up genetic associations within individuals. These consequences may differ greatly from what is expected under clonality. Moreover, some "obligate" parthenogenetic species are capable of rarely reproducing sexually (in particular if they can produce males), either within asexuals or with related sexual species (Simon *et al.* 2003; Schurko *et al.* 2009). The role of non-clonal and non-obligate asexuality on the emergence of parthenogenesis and their long-term consequences (especially if these events are rare) are not well known.

I. How does asexuality evolve from sex?

As mentioned above, sex is an ancestral character in eukaryotes, meaning that asexual species evolved from sexual ancestors. The actual process of transition from sex to parthenogenesis may thus plays a crucial role in determining the distribution and features of asexual species in eukaryotes.

a. Pathways of transition from sex to parthenogenesis

A possible transition pathway to asexuality is spontaneous evolution, in one or a few mutational steps (Simon *et al.* 2003). Still, several other transition pathways for asexuality have been reported: (1) Hybridization seems to be involved in the origin of asexuality in many cases (Avise *et al.* 1992; Avise 2008; Neaves and Baumann 2011), although its precise role in the transition from sex to parthenogenesis is still unclear. It could be the direct cause of evolution to asexuality through the disruption of meiosis (Moritz *et al.* 1989). However, it is unclear how incompatibilities stemming from hybridization can specifically modify how meiosis takes place so that it results in parthenogenesis. Alternatively, this frequent association between hybridization and parthenogenesis may be a result of the costs of hybridization. According to this theory, hybridization does disrupt meiosis, but rather than directly resulting in

parthenogenesis, this causes sterility, and thus a strong selective pressure for any change in the reproductive mode that can bypass this cost (Vrijenhoek 1998; Neiman *et al.* 2014). (2) Another transition pathway to asexuality is manipulation by a parasite (Weeks *et al.* 2002). (3) The last known pathway of transition to asexuality occurs in sexual species related to parthenogenetic lineages that produces males. Several asexual species are capable of producing males (Blackman 1972; Innes and Hebert 1988; van der Kooi and Schwander 2014). In some cases, males can cross with females of the sexual species and transmit asexuality to their offspring: this phenomenon is called contagious asexuality (Simon *et al.* 2003; Sandrock and Vorburger 2011; Jaquiéry *et al.* 2014, see part I.c). Theoretically, females with a mixed sexual-asexual phenotype may also be capable of contagion by crossing with a male from a sexual species.

Additionally, the evolution of parthenogenesis, because it is a transition from sex, can involve facultative or cyclical forms (Simon *et al.* 2003). In this case, obligate parthenogenesis can evolve secondarily, as described in *Daphnia* (Innes and Hebert 1988). This would mean that potentially, asexuals observed in nature differ greatly from emerging lineages. It is also possible that during the transition to asexuality, both obligate and facultative asexual lineages emerge, and that only one type of reproduction is selected and prevails through time. Still, for most species, the role of facultative sex in the emergence and evolutionary history of parthenogens remains enigmatic.

b. Mechanistic basis

Asexuals are often reported as reproducing mainly clonally through mitosis (Suomalainen 1950; Stenberg and Saura 2009). Yet, there are many other ways to achieve parthenogenesis than mitotic reproduction. Several meiosis modifications can result in unreduced daughter cells, which can develop into an embryo without fertilization (Suomalainen *et al.* 1987; Archetti 2010): Products of the same meiosis can fuse, with either a fusion of products of the first (central fusion automixis) or second (terminal fusion automixis) meiotic division. Meiotic divisions can also be suppressed (suppression of the first or second meiotic division). An extra replication can occur before meiosis (premeiotic doubling). Replication can also happen after meiosis (gamete duplication). All these modifications result in unreduced meiotic products. These meiosis modifications have diverse genetic consequences. Some do not differ from the consequences of mitotic reproduction (clonality), while others are non-clonal. Non-clonal asexuality results in loss of heterozygosity (LOH) from parent to offspring. The genomic patterns of LOH vary according to the asexual reproductive mode, from very rare and localized in the telomeric region to complete LOH in a single generation (Archetti 2010). Under central fusion automixis and suppression of the first meiotic division, which are the main asexual reproductive modes we focus on in this thesis, heterozygosity is maintained, unless recombination happens (Figure 1). Recombination results in LOH from the point of the crossing-over to the telomeres (or the next point of crossing-over). Note that since the genetic consequences of central fusion automixis and suppression of the first meiotic division are the same, both asexual reproductive modes will be referred to as central fusion-like parthenogenesis hereafter. In other reproductive modes, LOH can be caused by segregation (premeiotic doubling) or replication itself (gamete duplication). Yet, LOH can also be completely absent under some non-mitotic asexual reproductive modes. For instance, under central fusion-like

parthenogenesis in absence of recombination, there will be no LOH (Stenberg and Saura 2009; Archetti 2010; Nougué *et al.* 2015; Engelstädter 2017). Generally, the evolutionary consequences and prevalence of each type of asexuality are not well known.

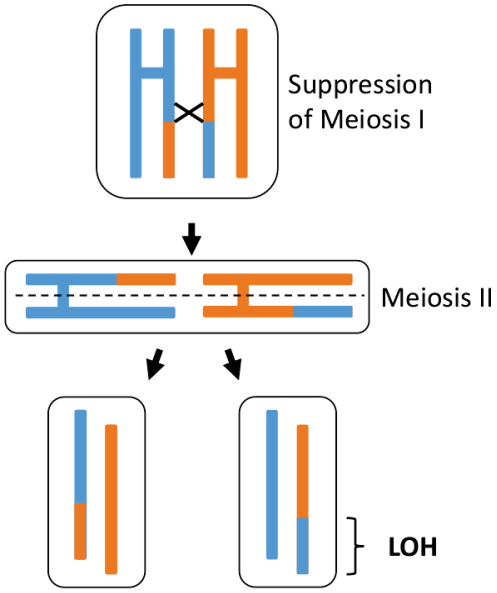


Figure 1. Central fusion-like parthenogenesis (suppression of the first meiotic division) with recombination. The first steps of meiosis I still occur. In particular, during chromosomal pairing, homologous chromosomes can recombine (black cross). Then, meiosis II occurs normally and sister chromatids are separated. In recombinant offspring, this can result in loss of heterozygosity (LOH), downstream from the point of recombination.

Non-clonal asexuality results in homozygosity. This is contrary to clonality, where mutation accumulation should result in high heterozygosity (Mark Welch and Meselson 2000, but see Butlin 2002). Similarly to inbreeding depression, LOH may unmask recessive deleterious alleles and is therefore thought to be costly, at least on the short term. Thus, there might be selection for a lower LOH rate among asexuals, although this may not be equally achievable for all asexual reproductive modes (Archetti 2010; Engelstädter 2017). Under central fusion-like automixis, as LOH results from recombination, a lower LOH rate can be achieved by either a reduced number of crossing overs, or by a more telomeric localization of these crossing-overs. Such a reduced recombination rate (compared to the closest sexual species) has been reported in ants (Rey *et al.* 2011) and Cape honeybees (Goudie *et al.* 2012).

c. Male production and sex

Asexuality is not necessarily obligate, especially in early transition stages (see above, Simon *et al.* 2003). In fact, most asexual species are actually facultative or cyclical (Bell 1982). Moreover, rare events of sex are reported in lineages otherwise reproducing asexually. (Schurko *et al.* 2009; D'Souza and Michiels 2010). The genetic and evolutionary consequences of facultative or cyclical asexuality differ greatly from obligate asexuality (D'Souza and Michiels 2010; Hartfield 2016; Stelzer and Lehtonen 2016), as sex generates highly diverse allele combinations through recombination, segregation and outcrossing.

Whether male production by some parthenogenetic species has an effect on their evolution depends on male survival and fecundity. If males can reproduce sexually and transmit asexuality to their offspring, this results in contagious asexuality (Simon *et al.* 2003; Engelstädter *et al.* 2011). This phenomenon generates new asexual lineages with introgressed sexual genome. However, because hybrids between sexuals and asexuals resulting from these crosses inherit traits that evolved under both reproductive modes, they might display

maladaptive phenotypes. Moreover, the exact asexual reproductive mode of the parental species might not be transmitted faithfully (especially if different aspects of asexuality are controlled by different genes), which could result in the generation of new or intermediate reproductive modes and different genomic consequences compared to the parental asexual reproductive mode.

II. Evolution of asexuals

a. Can the Paradox of sex be resolved by non-clonal asexuality?

Because asexuality does not imply male production nor mating, asexuals should have a demographic advantage over sexuals (that should pay the "twofold cost of males", Maynard Smith 1978). Additionally, asexuals do not need to find a partner, and can thus colonize new habitats easily (reproductive insurance, although is not necessarily limited to asexuals, as self-fertilizing species also benefit from this advantage). For these reasons, asexuality is usually perceived as advantageous compared to sex. This generally admitted advantage is paradoxical due to the rarity of (especially obligate) asexuality (Maynard Smith 1978). The "Paradox of sex" is generally resolved by the long-term costs asexuals are expected to pay, such as the accumulation of deleterious mutations (although these costs apply especially to clonal asexuals see part II.b.).

However, the emergence of asexuality may itself be rarely successful, especially if it is non-clonal. As seen in part I., mechanistically diverse asexual reproductive modes can emerge from sexually reproducing species, with diverse genomic features depending on the ancestral sexual species and the transition pathway. In the short term, non-clonal asexuality may be costly due to LOH, in which case lineages with reproductive modes that generate high LOH should quickly go extinct and not be observed in nature (Asher 1970; Archetti 2010). Additionally, evolution toward asexuality may be particularly costly in some cases due to other limitations, such as remnant male production and epigenetic reset in metazoans (Engelstädter 2008; Lenormand *et al.* 2016). Finally, although evolution toward reducing the costs of non-clonal parthenogenesis may be expected (for instance, reduction in recombination rate under central fusion-like parthenogenesis, Nougué *et al.* 2015; Engelstädter 2017), not all reproductive modes may allow such change in phenotype. Emerging asexual lineages might thus be more or less likely to succeed, and the asexuals we observe in nature may not represent the whole possible diversity of asexuality. The conditions of emergence of non-clonal asexual lineages are however not well known, and the theories suggesting non-clonal consequences should impede the evolution of asexuality remain to be tested.

b. Evolutionary fate of clonal and non-clonal asexuals

Due to the absence of recombination and segregation, clonality results in large genetic associations, which can maintain beneficial alleles associations. Eventually however, it may be costly as no new combinations of alleles can appear, so that selection will have a weaker effect (due to a large-scale Hill-Robertson effect, Hill and Robertson 1966). Moreover, clonal lineages compete with each other and one usually prevails, drastically decreasing diversity (clonal

interference, Gerrish and Lenski 1998). The absence of recombination can lead to an accumulation of deleterious mutations by the successive stochastic extinction of the least loaded class of individuals in the population (Muller's ratchet, Muller 1964). Finally, the lack of diversity in clonal asexuals due to the absence of recombination makes adaptation more difficult, especially in the context of parasitism (Red Queen hypothesis, Van Valen 1973). The negative long-term effects of clonality should result in the extinction of clonal lineages.

The impact of non-clonal asexual reproductive modes, especially considering their diversity, on the genome and fitness of asexuals compared to clonality has not been thoroughly investigated. The above processes expected under clonal asexuality depend on selective forces (mutation and genetic drift) that significantly affect relatively large populations only on the long term. In non-clonal asexuals, these may be hindered or suppressed in regions where recombination and/or LOH occurs, perhaps even at a low frequency. LOH may allow a more efficient purging of recessive deleterious alleles, break up genetic associations and generate diversity. This could mean that non-clonal asexuals can avoid the costs of clonality. However, the great majority of asexual species, clonal and non-clonal alike, are of recent origin (although other factors than the costs of asexuality can explain this phenomenon, Schwander and Crespi 2009). The effect of LOH on asexual genomes remains to be investigated.

Similarly, the role of sex, and rare sex in particular, in the evolutionary history of asexual lineages, is not well known. Events of sex, even rare, result in segregation which may generate many new allele combinations, and thus diversity on which selection can act (D'Souza and Michiels 2010). The case of contagious asexuality is even more complex as it implies introgression of sexual genome in asexuals, which could generate high diversity but also maladapted hybrids. Contagious asexuality could also explain the apparent youth of asexual lineages, where most of their genomes would appear closely related to sexual species while regions controlling for asexuality would be much more divergent (Rode *et al. in press*, this paper is available in the Appendix of the thesis).

Although we know they should differ from clonality, consequences of non-clonal and non-obligate asexuality remain unexplored. In particular, we do not know what frequency of non-clonal or sex events is needed to affect the evolution of parthenogens. Moreover, the prevalence of clonal versus non-clonal asexuality in nature is unclear. In general, clonality is considered as the most frequent asexual reproductive mode, but more and more asexual species reported as clonal turn out not to be (Svendsen *et al.* 2015; Simion *et al.* 2021, Schwander 2021). This is further complicated by the fact that the reproductive mode and LOH rate of an asexual lineage may evolve through time. Therefore, it is possible that the perception of asexuals is biased toward clonality. Furthermore, LOH and sex may be difficult to detect if they occur rarely.

Asexuals have been studied for a long time by evolutionary biologists. Still, many questions regarding their distribution and evolution remain. Especially, evolution toward asexuality may be more complex and diverse, and non-clonal asexuality may be more frequent than previously thought, generating diverging genomic and evolutionary consequences compared to clonality.

III. Artemia: a model to study sex-asex transitions

Artemia sp. or Brine shrimps is a genus of small crustaceans adapted to hypersaline environments. This species complex is composed of several sexual species both in the old and new world. *A. parthenogenetica* (hereafter *Ap*) is widespread in the old world and regroups all populations of obligate asexual *Artemia*, which are related to Central Asian sexual species (*A. urmiana*, *A. tibetiana*, *A. sinica*, and *A. sp. Kazakhstan*, hereafter *Akaz*, which is undescribed, Muñoz *et al.* 2010; Asem *et al.* 2016, Rode *et al. in press*). *Ap* populations display several ploidy levels, from 2n to 5n. Polyploid *Ap* are described as clonal in the literature (Barigozzi 1974). Diploid *Ap* (hereafter *Ap2n*) reproduce through a modified meiosis where the first meiotic division is suppressed (central fusion-like parthenogenesis, Nougué *et al.* 2015). *Ap2n* also produce males (called rare males) at a small rate, which are capable of contagious asexuality if crossed with a Central Asian sexual species (Maccari *et al.* 2014). This means that new asexual hybrid lineages can be generated through sexual reproduction. The mechanism of rare male production is thought to be recombination on the ZW sex chromosomes, which would generate ZZ offspring (Browne and Hoopes 1990; Abreu-Grobois and Beardmore 2001), but this hypothesis remains to be verified. Based on nuclear and mitochondrial markers, all *Ap* populations were inferred to originate from a single emergence of asexuality in *Artemia*, and new lineages and ploidy levels were probably generated by events of sex with Central Asian species (see figure 9 in Rode *et al.*, *in press*). This suggests that all lineages of *Ap* could have the same asexual reproductive mode, contrary to what is reported in the literature. Interestingly, Rode *et al.* (*in press*) reinterpreted cytological observations by Goldschmidt (1952), suggesting that pairing does occur in polyploid *Ap*. Therefore, the departure of *Ap* (especially *Ap2n*) from clonality makes *Artemia* an interesting model to study the transition to asexuality and its genomic and evolutionary consequences. Moreover, the possibility to experimentally generate new asexual lineages using contagious asexuality gives the opportunity to study the very first steps of these transitions.

In this thesis, I investigated the reproductive characteristics of diploid asexual *Artemia* and the genomic consequences of non-clonal asexuality, in particular central fusion-like parthenogenesis. In the first chapter, I studied whether hybrids between sexuals and asexuals produced by contagious asexuality in the lab could reproduce asexually and/or sexually by using cross-experiments and paternity analysis. I also investigated whether newly-generated asexual *Artemia* lineages went through LOH by genotyping microsatellite markers in successive generations. The second chapter of the PhD is a review of the literature that evaluates if there is a bias in the perception and report of parthenogenesis toward clonal reproduction, and whether the cytological and genetic evidence put forward to support clonality in parthenogenetic species is usually sufficient. In the third chapter, I used RAD-seq data to investigate the hypothesis that rare males in *Ap2n* are produced by recombination, and thus LOH, on the ZW sex chromosomes. The results of my thesis show that it is crucial to study the genomic impact of non-clonal asexuality and rare sex, in particular in early transition stages, as they may play an important role in the evolution of parthenogenesis and sex.

References

Abreu-Grobois, F. A., and J. A. Beardmore. 2001. The generation of males by diploid parthenogenetic Artemia cannot occur in the way Stefani suggested. P. *in* Proceedings of the 4th international large branchiopod symposium (Vol. 1).

Archetti, M. 2010. Complementation, genetic conflict, and the evolution of sex and recombination. J. Hered. 101:1–13.

Asem, A., A. Eimanifar, and S. C. Sun. 2016. Genetic variation and evolutionary origins of parthenogenetic Artemia (Crustacea: Anostraca) with different ploidies. Zool. Scr. 45:421–436.

Asher, J. H. 1970. Parthenogenesis and genetic variability. II. One-locus models for various diploid populations. Genetics 66:369–391.

Avise, J. 2008. Clonality: the genetics, ecology, and evolution of sexual abstinence in vertebrate animals. Oxford University Press.

Avise, J. C., J. M. Quattro, and R. C. Vrijenhoek. 1992. Molecular clones within organismal clones. Evolutionary biology. Evol. Biol. 225–246.

Barigozzi, C. 1974. Artemia: A Survey of Its Significance in Genetic Problems. Evol. Biol. 221–252.

Bell, G. 1982. The Masterpiece of Nature. University of California Press, Berkeley.

Blackman, R. L. 1972. The inheritance of life-cycle differences in *Myzus persicae* (Sulz.) (Hem., Aphididae). Bull. Entomol. Res. 62:281–294.

Browne, R. A., and C. W. Hoopes. 1990. Genotype Diversity and Selection in Asexual Brine Shrimp (Artemia). Evolution 44:1035.

Butlin, R. 2002. The costs and benefits of sex: New insights from old asexual lineages. Nat. Rev. Genet. 3:311–317.

D'Souza, T. G., and N. K. Michiels. 2010. The costs and benefits of occasional sex: Theoretical predictions and a case study. J. Hered. 101:1–8.

Engelstädter, J. 2017. Asexual but Not Clonal : Evolutionary Processes in Automictic Populations. 206:993–1009.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–1150.

Engelstädter, J., C. Sandrock, and C. Vorburger. 2011. Contagious parthenogenesis, automixis, and a sex determination meltdown. Evolution 65:501–511.

Gerrish, P. J., and R. E. Lenski. 1998. The fate of competing beneficial mutations in an asexual population. Genetica 102:127–144.

Goldschmidt, E. 1952. Fluctuation in chromosome number in *Artemia salina*. J. Morphol. 91:111–133.

Goudie, F., M. H. Allsopp, M. Beekman, P. R. Oxley, J. Lim, and B. P. Oldroyd. 2012. Maintenance and loss of heterozygosity in a thelytokous lineage of honey bees (*Apis mellifera capensis*). Evolution 66:1897–1906.

Hartfield, M. 2016. Evolutionary genetic consequences of facultative sex and outcrossing. J. Evol. Biol. 29:5–22.

Hill, W. G., and A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genet. Res. 8:269–294.

Innes, D. J., and P. D. N. Hebert. 1988. The Origin and Genetic Basis of Obligate Parthenogenesis in Daphnia pulex. Evolution 42:1024–1035.

Jaquiéry, J., S. Stoeckel, C. Larose, P. Nouhaud, C. Rispe, L. Mieuzet, J. Bonhomme, F. Mahéo, F. Legeai, J. Gauthier, N. Prunier-leterme, D. Tagu, and J. Simon. 2014. Genetic Control of Contagious Asexuality in the Pea Aphid. PLoS Genet. 10:1–10.

Lenormand, T., J. Engelstädter, S. E. Johnston, E. Wijnker, and C. R. Haag. 2016. Evolutionary mysteries in meiosis. Philos. Trans. R. Soc. B Biol. Sci. 371.

Maccari, M., F. Amat, F. Hontoria, and A. Gómez. 2014. Laboratory generation of new parthenogenetic lineages supports contagious parthenogenesis in *Artemia*. PeerJ 2:e439.

Mark Welch, D., and M. Meselson. 2000. Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. Science 288:1211–1215.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge Univ. Press.

Moritz, C., W. M. Brown, L. D. Densmore, J. W. Wright, D. Vyas, S. Donnellan, M. Adams, and P. Baverstock. 1989. Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekkonidae). Pp. 87–112 *in* R. M. Dawley and J. P. Bogart, eds. Evolution and ecology of unisexual vertebrates.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. Mol. Mech. Mutagen. 1:2–9.

Muñoz, J., A. Gómez, A. J. Green, J. Figuerola, F. Amat, and C. Rico. 2010. Evolutionary origin and phylogeography of the diploid obligate parthenogen *Artemia parthenogenetica* (Branchiopoda: Anostraca). PLoS One 5.

Neaves, W. B., and P. Baumann. 2011. Unisexual reproduction among vertebrates. Trends Genet. 27:81–88.

Neiman, M., T. F. Sharbel, and T. Schwander. 2014. Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. J. Evol. Biol. 27:1346–1359.

Nougué, O., N. O. Rode, R. Jabbour-zahab, A. Ségard, L. M. Chevin, C. R. Haag, and T. Lenormand. 2015. Automixis in Artemia: Solving a century-old controversy. J. Evol. Biol. 28:2337–2348.

Rey, O., A. Loiseau, B. Facon, J. Foucaud, J. Orivel, J. M. Cornuet, S. Robert, G. Dobigny, J. H. C. Delabie, C. D. S. F. Mariano, and A. Estoup. 2011. Meiotic recombination dramatically decreased in thelytokous queens of the little fire ant and their sexually produced workers. Mol. Biol. Evol. 28:2591–2601.

Rode, N. O., R. Jabbour-Zahab, L. Boyer, É. Flaven, F. Hontoria, G. Van Stappen, F. Dufresne, C. Haag, and T. Lenormand. *in press*. The origin of asexual brine shrimps. Am. Nat.

Sandrock, C., and C. Vorburger. 2011. Single-Locus recessive inheritance of asexual reproduction in a parasitoid wasp. Curr. Biol. 21:433–437.

Schön, I., K. Martens, and P. Van Dijk. 2009. Lost Sex, The Evolutionary Biology of Parthenogenesis.

Schurko, A. M., M. Neiman, and J. M. Logsdon. 2009. Signs of sex: what we know and how we know it. Trends Ecol. Evol. 24:208–217.

Schwander, T., and B. J. Crespi. 2009. Twigs on the tree of life? Neutral and selective models for integrating macroevolutionary patterns with microevolutionary processes in the analysis of asexuality. Mol. Ecol. 18:28–42.

Simion, P., J. Narayan, A. Houtain, A. Derzelle, L. Baudry, E. Nicolas, R. Arora, M. Cariou, C. Cruaud, F. R. Gaudray, C. Gilbert, N. Guiglielmoni, B. Hespeels, D. K. Kozlowski, K. Labadie, A. Limasset, M. Lliros, M. Marbouty, M. Terwagne, J. Virgo, R. Cordaux, E. G. Danchin, B. Hallet, R. Koszul, T. Lenormand, J.-F. Flot, and K. Van Doninck. 2021. Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer *Adineta vaga*. Sci. Adv. 7.

Simon, J. C., F. Delmotte, C. Rispe, and T. Crease. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. Biol. J. Linn. Soc. 79:151–163.

Stelzer, C. P., and J. Lehtonen. 2016. Diapause and maintenance of facultative sexual reproductive strategies. Philos. Trans. R. Soc. B Biol. Sci. 371.

Stenberg, P., and A. Saura. 2009. Cytology of asexual animals. Pp. 63–74 *in* Lost Sex: The Evolutionary Biology of Parthenogenesis.

Suomalainen, E. 1950. Parthenogenesis in Animals. Adv. Genet. 3:193–253.

Suomalainen, E., A. Saura, and J. Lokki. 1987. Cytology and evolution in parthenogenesis. CRC Press.

Svendsen, N., C. M. O. Reisser, M. Dukić, V. Thuillier, A. Ségard, C. Liautard-Haag, D. Fasel, E. Hürlimann, T. Lenormand, Y. Galimov, and C. R. Haag. 2015. Uncovering cryptic asexuality in *Daphnia magna* by RAD sequencing. Genetics 201:1143–1155.

van der Kooi, C. J., and T. Schwander. 2014. On the fate of sexual traits under asexuality. Biol. Rev. 89:805–819.

van Valen, L. 1973. A new evolutionary law. Evol. Theory 1–30.

Vrijenhoek, R. C. 1998. Animal clones and diversity: Are natural clones generalists or specialists? Bioscience 48:617–628.

Weeks, A. R., K. Tracy Reynolds, and A. A. Hoffmann. 2002. Wolbachia dynamics and host effects: What has (and has not) been demonstrated? Trends Ecol. Evol. 17:257–262.

Chapter 1

Not so clonal asexuals: Unraveling the secret sex life of *Artemia parthenogenetica*

Published.

doi:10.1002/evl3.216

Evolution Letters

Not so clonal asexuals: Unraveling the secret sex life of *Artemia parthenogenetica*

Loreleï Boyer,[1,2] Roula Jabbour-Zahab,[1] Marta Mosna,[1] Christoph R. Haag,[1] and Thomas Lenormand[1]

[1]*CEFE, Univ Montpellier, CNRS, Univ Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France*

[2]*E-mail: lorelei.boyer@cefe.cnrs.fr*

Received July 2, 2020
Accepted January 15, 2021

The maintenance of sex is paradoxical as sexual species pay the "twofold cost of males" and should thus quickly be replaced by asexual mutants reproducing clonally. However, asexuals may not be strictly clonal and engage in "cryptic sex," challenging this simple scenario. We study the cryptic sex life of the brine shrimp *Artemia parthenogenetica*, which has once been termed an "ancient asexual" and where no genetic differences have ever been observed between parents and offspring. This asexual species rarely produces males, which can hybridize with sexual females of closely related species and transmit asexuality to their offspring. Using such hybrids, we show that recombination occurs in asexual lineages, causing loss-of-heterozygosity and parent-offspring differences. These differences cannot generally be observed in field-sampled asexuals because once heterozygosity is lost, subsequent recombination leaves no footprint. Furthermore, using extensive paternity tests, we show that hybrid females can reproduce both sexually and asexually, and transmit asexuality to both sexually and asexually produced offspring in a dominant fashion. Finally, we show that, contrary to previous reports, field-sampled asexual females also rarely reproduce sexually (rate ~2‰). Overall, most previously known facts about *Artemia* asexuality turned out to be erroneous. More generally, our findings suggest that the evidence for strictly clonal reproduction of asexual species needs to be reconsidered, and that rare sex and consequences of nonclonal asexuality, such as gene flow within asexuals, need to be more widely taken into account in more realistic models for the maintenance of sex and the persistence of asexual lineages.

KEY WORDS: Artemia, asexuals, automixis, contagious asexuality, rare sex, recombination.

Impact summary

Although supposedly advantageous, asexual reproduction is rare in nature, compared to sexual reproduction. Most models explaining the maintenance of sex, "the queen of problems" in evolutionary biology for decades, include a sex-asex contrast. In the vast majority of models, asexuals are simplified as obligate and clonal, where the maternal genome is transmitted faithfully (barring new mutations). Even though other asexual reproductive modes exist and their population genetic consequences are starting to be understood, moving beyond this simplification is extremely challenging theoretically and in practice. In this article, we focused on a well-known asexual taxon, *Artemia parthenogenetica*, whose reproductive mode has been studied for over a century. We challenged supposedly established facts about its asexuality through experiments using "rare males," which are sometimes produced in asexual lineages. They were used to produce sex-asex crosses and backcrosses with a closely related sexual species. This allowed us to unravel characteristics that profoundly change the view on almost all aspects of *Artemia* asexuality: We show that these supposedly "obligate asexuals" can sometimes reproduce sexually and that asexuality can be sexually transmitted via both males and females. We also show that recombination was selected against, but not totally lost in asexual *Artemia*. These findings explain how asexuals can appear as clones, despite not being clonal. It appears reasonable to think that similar discoveries could be made in other asexual taxa through detailed investigations using nonstandard approaches. This is crucial, as evolutionary processes, including the advantage compared to sex, may strongly differ between clonal and

© 2021 The Authors. *Evolution Letters* published by Wiley Periodicals LLC on behalf of Society for the Study of Evolution (SSE) and European Society for Evolutionary Biology (ESEB).
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

nonclonal asexuals. Taken together, our study shows that real asexuals are far from the caricature used in current models. If accounted for, this could help understanding the maintenance of sex with a more comprehensive view of reproductive mode diversity.

The prevalence of sexual over asexual reproduction in eukaryotes is generally explained by the fact that, even though the costs of sex are high (including the famous putative twofold cost of males, Maynard Smith 1978, but see Meirmans et al. 2012), the costs of asexuality, particularly when clonal, are even higher (e.g., increased accumulation of deleterious mutations, slower rate of adaptation, Otto and Lenormand 2002; Otto 2009; Schön et al. 2009). However, clonality—the production of offspring genetically identical to their mothers, barring new mutations—may not be as ubiquitous as expected (Engelstädter 2008; Archetti 2010; Lenormand et al. 2016). In particular, rare events of recombination and sex in asexuals might be missed because they are difficult to detect. These occurrences of rare sex in "asexuals" may be especially frequent in young asexual lineages emerging within a population of sexual ancestors (i.e., when their relative fitness matters most). This would lead to an underestimation of nonclonal asexuality in nature and undermine the classical "paradox of sex" scenario, which considers that asexuality emerges within sexual species through strictly clonal mutants.

In this article, we study recombination and sexual reproduction in *Artemia parthenogenetica*, which was once described as an "ancient asexual" (Judson and Normark 1996). *Artemia parthenogenetica* is a heterogeneous group of brine shrimps encompassing diploid and polyploid asexuals. Here, we focus on diploids (hereafter *Ap2n*), whose reproductive mode has been debated throughout the 20th century (Barigozzi 1944; Narbel-Hofstetter 1964; White 1973; Cuellar 1987; Neiman et al. 2009). Recent genetic data suggest that they reproduce by "central fusion" automixis (Nougué et al. 2015). This type of automixis can correspond, at the cellular level, either to the fusion of meiotic products separated at meiosis I or to the abortion of meiosis I (sometimes called meiotic apomixis; Archetti 2010). Both cases have the same genetic consequences and maintain diploidy without fertilization (Asher 1970). With central fusion automixis, centromeric regions maintain maternal heterozygosity (i.e., are transmitted clonally), but if there is recombination, centromere-distant regions can become autozygous, that is, undergo loss of heterozygosity (hereafter, LOH; Stenberg and Saura 2009; Svendsen et al. 2015). Note that this expectation applies only to species with monocentric chromosomes, as is the case in *Artemia* (Yarmohammadi and Pourkazemi 2004). This reproductive mode was inferred indirectly from population genetic data (strongly contrasting F_{IS} levels among different markers in wild populations; Nougué et al. 2015). However, no genetic variation has ever been observed within isofemale lines of *Ap2n*. Browne and Hoopes (1990) found no change in allozyme genotypes in three heterozygous lines maintained for three years in the laboratory. Similarly, Nougué et al. (2015) found no genotypic changes at five heterozygous microsatellite loci in three isofemale lines maintained for 20–37 months. This has been considered as evidence for essentially clonal reproduction (or central fusion automixis without recombination, which is genetically equivalent; Abreu-Grobois 1983; Abreu-Grobois and Beardmore 2001). Yet this conclusion does not account for potential difficulties in detecting nonclonal reproduction: Indeed, centromere-distant loci that frequently recombine with the centromere and therefore have high rates of LOH may already have lost heterozygosity and hence no further LOH can be detected. In other words, centromere-distant loci are expected to be mostly homozygous (and have positive F_{IS}; Nougué et al. 2015), except for short periods of times following the occurrence of a new mutations (Engelstädter 2008). In contrast, loci close to the centromere may only rarely experience LOH and therefore have high heterozygosity (and negative F_{IS}; Nougué et al., 2015). In principle, the recombination and new LOH events could be detected at these loci. However, if LOH rate is low, they will, by definition, only rarely be observed. Hence, detecting recombination in automicts is methodologically challenging, irrespectively of the genomic location, with high or low LOH rates. This in turn suggests that erroneous inferences of clonality could easily occur.

In addition to this issue of recombination, *Ap2n* lineages are known for their production of "rare males" by parthenogenesis (0–1.7% of all offspring; Browne & Hoopes, 1990; Maccari et al., 2013). The contribution of these males to *Ap2n* reproduction remains unresolved. Their ability to cross with sexual females and transmit asexuality to their offspring is termed "contagious asexuality" and could in principle generate a large diversity of new asexual lineages. This mechanism has been demonstrated in a handful of asexual species that produce rare males (Aphids: Jaquiéry et al., 2014; *Daphnia*: Paland et al., 2005; Parasitoid wasps: Sandrock & Vorburger, 2011). Asexual hermaphrodites can also sexually transmit asexuality through their male function (Van Dijk, 2009). This happens in some animals (D'Souza et al., 2004) and potentially in many plants (Hörandl & Paun, 2007). However, the overall prevalence of contagious asexuality among extant asexuals is difficult to establish, as it requires the identification of rare males (or male function), successful crosses with closely related sexuals, and the assessment of the reproductive mode of sex-asex hybrids. The first studies on contagious asexuality in *Ap2n* found no evidence for transmission of asexuality after hybridization with closely related sexuals (Bowen et al., 1978). A more recent study showed that rare *Ap2n* males can transmit asexuality, but concluded that transmission is

recessive (Maccari et al., 2014). Recessivity would strongly limit the appearance of new asexual lineages, as no asexuals are generated in the F1. New asexual lineages may only occur after another rare cross, mating between rare F1 or a backcross with another rare, asexually derived male. Furthermore, although *Ap2n* populations are widely distributed, the geographical distribution of sexual species is narrow; however, they do currently overlap in central Asia in few locations (Browne & MacDonald, 1982; Agh et al., 2007; Muñoz et al., 2010). The relevance of contagious asexuality in this species is therefore unclear, and, given the divergent results of earlier studies, some doubts also remain regarding the mode of inheritance of asexuality.

The purpose of the present study was to revisit the reproductive biology of a supposedly well-known obligate asexual and to use this example to showcase the correspondence between real asexual species and their caricatures used in most models. In particular, we reconsidered features of *A. parthenogenetica* reproduction through a series of five critical experiments. First, we tested whether recombination occurs in *Ap2n*. To do so, we used contagious asexuality to experimentally generate new hybrid asexual lineages. Note that hybridization itself can sometimes induce asexuality ("balance hypothesis"; Moritz et al., 1989). However, in *Artemia*, experimental hybrid crosses between sexual species never resulted in asexual offspring (Clark & Bowen, 1976; Pilla & Beardmore, 1994; Abatzopoulos et al., 2002; Maccari et al., 2013), and several further lines of evidence indicate that the "balance hypothesis" was unlikely to operate in our crosses (see discussion). We crossed *Ap2n* "rare males" with females from the closest sexual species (*A. sp. Kazakhstan*, hereafter *Akaz*; Muñoz et al., 2010). Because of hybridity, these lineages are expected to show high heterozygosity, which is expected to greatly improve the likelihood to detect LOH events, if they occur at all. Second, we investigated the reproductive mode of these F1 sex-asex hybrid females by pairing them with *Akaz* males and subjecting the resulting offspring to paternity tests. Third, we asked whether contagious asexuality occurs only via "rare males" or may also happen through females carrying asexuality genes, while, at least partially, retaining sexual function. We assessed this possibility by crossing laboratory-produced F1 sex-asex hybrid females with *Akaz* males and testing whether some of the resulting offspring females were able to reproduce asexually. Fourth, we tested whether females from relatively older, field-sampled *Ap2n* asexual lineages can sometimes reproduce sexually (they were hitherto thought to be 100% obligate asexuals). To answer this question, we conducted mass-cross experiments combined with paternity tests. Finally, we asked whether recombination had evolved in asexual lineages. To this end, we used the proportion of males produced during asexual reproduction (i.e., the frequency of rare males) as a proxy for recombination rate in *Ap2n* asexuals, F1 sex-asex hybrids, and several generations of backcrosses to *Akaz*. Overall, the results of these five experiments entirely change our view of *Artemia parthenogenetica* asexuality. Similar experimentation could lead to reappraisal in other systems, and our results highlight that models on the maintenance of sex may require to be updated, in particular by including more realistic assumptions about asexuality, beyond strict clonality.

Methods

In the different experiments, we used standard raising conditions for *Artemia*, as described in Lievens et al. (2018). Details are provided in Supporting Information part 1.

EXPERIMENT 1: LOH IN SEX-ASEX HYBRIDS

To investigate LOH, we generated hybrid lineages via contagious asexuality, crossing rare males from two *Ap2n* lineages, Aigues-Mortes (France), hereafter *P1*, and Urmia (Iran), hereafter *P2* (see Supporting Information part 1), with *Akaz* females. Asexual offspring were isolated and propagated asexually in 34 lineages for up to 13 generations. To screen for LOH, we genotyped last-generation individuals for seven microsatellite markers that were heterozygous in the F1. Events of LOH were then traced back to the generation in which they occurred (Supporting Information part 2, Fig. S1). We included five informative loci in the data that were analyzed using likelihood models in Mathematica version 9.0 (Wolfram Research, 2012), investigating effects of the cross (*P1*×*Akaz* or *P2*×*Akaz*), time (i.e., generation number at which LOH happened), and locus (Table S1).

EXPERIMENT 2: REPRODUCTIVE MODE OF F1 HYBRID FEMALES

The second experiment was aimed at identifying the reproductive mode of females produced by contagious asexuality. We used a previously established protocol (Maccari et al., 2014) with some modifications. We crossed a rare male from *P1* and a rare male from *P2* with *Akaz* females, isolated all F1 hybrids, and identified the sex of the offspring. During period 1, F1 females were kept isolated for 14 (*P2*×*Akaz*) or 30 days (*P1*×*Akaz*). During a period 2, we paired them with an *Akaz* male. A longer period 1 was applied to the *P1*×*Akaz* cross, which was performed after *P2*×*Akaz*, to increase the chance to observe asexual reproduction during isolation in this second cross. Period 1 was only used as a check that females could reproduce in isolation. Offspring produced during period 2 were genetically tested using microsatellites to determine whether they were produced sexually or asexually (Supporting Information part 3, Table S2). The proportion of sexually versus asexually produced offspring was estimated only using offspring produced

during period 2. Females that only produced asexual clutches during both periods were labeled as "asexual," those that only produced sexual clutches when paired and no clutches in isolation as "sexual." It is, however, possible that females capable of both modes of reproduction only displayed one reproductive mode during the experiment because of the limited number of clutches and offspring tested. To account for this uncertainty, data were analyzed using likelihood models in Mathematica version 9.0 (Wolfram Research, 2012), distinguishing the different categories of females, and testing the effects of the origin of the cross (*P1*×*Akaz* or *P2*×*Akaz*) on the reproductive mode (Table S3).

EXPERIMENT 3: CONTAGIOUS ASEXUALITY VIA HYBRID F1 FEMALES

The third experiment was designed to detect whether asexuality could be transmitted sexually by females (contagious asexuality through females). We used 12 clutches produced by the paired F1 females of *P1*×*Akaz* from experiment 2. Five of these were produced sexually, as verified by paternity testing. From each clutch, we isolated one to six female offspring for four weeks and recorded whether they were able to reproduce asexually (Supporting Information part 4, Table S4). The occurrence of such asexual female offspring would show that asexuality could be sexually and maternally transmitted, thus demonstrating contagious asexuality via females.

EXPERIMENT 4: RARE SEX IN *Ap2n* FEMALES

In the fourth experiment, we investigated whether rare sex could occur in field-sampled *Ap2n* females of the two populations studied in this article. We placed 115 *P1* females with 57 *Akaz* males and 52 *P2* females with 25 *Akaz* males in large tanks. We used *Akaz* males for practical reasons (easy availability of males with diagnostic loci) and because *Akaz* is the closest related sexual species to *Ap2n* (Muñoz et al., 2010). Eight and four male offspring were obtained among 1828 and 1061 offspring, respectively (Table S5). Any male offspring appearing in these tanks were therefore either produced asexually by *Ap2n* females (i.e., rare males) or produced sexually by hybridization between the *Ap2n* females and the *Akaz* males. We used paternity tests on all these male offspring to determine whether they were produced by sexual reproduction (Supporting Information part 5).

EXPERIMENT 5: ESTIMATING RECOMBINATION RATE IN AUTOMICTS

The fifth experiment was designed to assess whether recombination rate had evolved in *Ap2n* lineages, compared to their sexual *Akaz* ancestor. One of the few hypotheses explaining how rare males may be produced in *Ap2n* suggests that they result from LOH at the sex-determining locus during oogenesis (Stefani, 1964; MacDonald & Browne, 1987; Browne & Hoopes, 1990; Abreu-Grobois & Beardmore, 2001). Females are ZW (Bowen, 1963; Stefani, 1963; de Vos et al., 2013), thus LOH could result in ZZ or WW offspring, WW potentially being nonviable and ZZ being rare males. According to this, the rate of rare male production in a lineage would be a proxy for the recombination rate between the centromere and the sex locus in this lineage (Browne & Hoopes, 1990). This hypothesis has not been experimentally addressed to date, but is consistent with our observations (see Discussion). We used the rate of rare male production as a proxy for automictic recombination rate to investigate how recombination evolves in automictic lineages. We predicted that, because it leads to LOH that may expose deleterious recessive mutations, recombination may be selected against in automictic lineages. Compared to asexuals, F1 hybrids and further backcross generations to *Akaz* sexuals should thus show increased recombination rates, reaching saturation once backcrosses have integrated all recombination-controlling genes of the *Akaz* sexual species. We used a *P3*×*Akaz* cross using a rare male from another Aigues-Mortes population (*P3*), and an *Akaz* female. We then used repeated backcrosses on *Akaz* to introgress the asexuality genes of *Ap2n* into an increasingly *Akaz* (and thus sexually derived) genome. We maintained asexuality by selecting each generation males whose daughters were able to reproduce asexually. We recorded the rate of rare male production (denoted α) in asexually produced clutches from the F1 for up to four backcross generations (Table S6). Data were analyzed using likelihood models with Mathematica version 9.0 (Wolfram Research, 2012). The models investigated how mean α changed throughout subsequent generations of crossing and back-crossing (in a linear, quadratic, or step-wise fashion). The variance of α among lineages was also fitted either assuming that recombination rate was controlled by a major gene (monogenic models, where we expect two categories of females in the backcrosses) or that is was polygenic (polygenic models, where we expect a continuous distribution of recombination rates among females; Supporting Information part 6, Table S7).

Results

EXPERIMENT 1: LOH IN SEX-ASEX HYBRIDS

Microsatellite analysis of the hybrid *Ap2n* lineages showed that five out of seven loci (including the two loci later discarded from the statistical analysis; see Supporting Information part 2) that were initially heterozygous in F1 underwent LOH in at least one out of 34 asexual hybrid lineages within 1–13 generations (Fig. S1). Moreover, according to our best model (ΔAICc = 2.2; Table S1), LOH rates varied between the two populations of origin and among loci, with LOH occurring mainly at loci that show excess homozygosity (compared to Hardy-Weinberg proportions) in

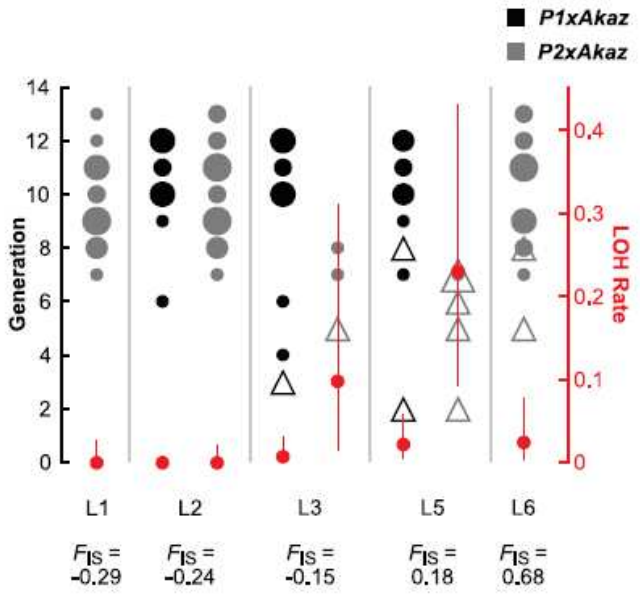


Figure 1. Loss and retention of heterozygosity at six microsatellite loci (L1 to L6) in asexual F1 hybrid lineages in experiment 1. Disks represent *P1xAkaz* (black) and *P2xAkaz* (gray) hybrid lineages that retained heterozygosity, and triangles lineages that lost heterozygosity. The generation at which heterozygosity was lost or, alternatively, the generation until which the lineage was followed without LOH is indicated for each disk and triangle on the left axis. The size of the symbols represents the number of lineages with the same value. Small and large triangles correspond to 1 or 2 lineages, respectively. Red dots and bars represent per-locus LOH rates and support limits (right axis) estimated from our best model. For each locus, the F_{IS} found in natural populations by Nougué et al. (2015) is indicated below. Note that L1 and L6 were not initially heterozygous in *P1* lineages and are therefore not represented. Note also that the representation of the generation in the figure does not account for partial nonindependence of some of the lineages due to sharing part of their ancestry (see Fig. S1 for the exact pedigree and LOH events of all lineages). The model estimates are, however, not affected by this as they account for partial nonindependence.

natural *Ap2n* populations (Fig. 1). With central fusion automixis, we expect such heterogeneity among loci, depending on their chromosomal position. Centromere-distal loci should lose heterozygosity and show heterozygosity deficit in natural populations, whereas loci close to the centromere should show the reverse pattern (Nougué et al., 2015; Svendsen et al., 2015). The close correspondence between LOH observed in our laboratory F1 crosses and heterozygosity patterns independently observed in asexuals sampled in the field indicate that our crosses qualitatively reflect recombination actually occurring in natural *Ap2n*.

EXPERIMENT 2: REPRODUCTIVE MODE OF F1 HYBRID FEMALES

In contrast to previous findings (Maccari et al., 2014), our crosses revealed that a large fraction of virgin F1 females were able to reproduce while isolated (i.e., asexual reproduction during period 1): 89% ± 5% (SE) of the *P1*×*Akaz* hybrids and 45% ± 5% (SE) of the *P2*×*Akaz* hybrids (Table S2). The different proportions between *P1*×*Akaz* and *P2*×*Akaz* hybrids may be explained by different duration of period 1 (see Methods and Discussion). When paired with an *Akaz* male, some females continued reproducing asexually, as verified by paternity testing (Table S2). Indeed, 66.1% ± 6% (SE) of females whose reproductive mode could be identified by paternity testing only ever produced asexual clutches throughout their lives (although the maximum number of clutches observed for a given female was five). Yet 25.4% ± 6% (SE) of females showed "mixed" reproduction, that is, they produced both asexual and sexual clutches (Table S2). Note that, within a given clutch, all offspring were produced by the same reproductive mode (Supporting Information part 3). There was only limited evidence for the existence of females with pure sexual reproduction: Among the 18 (of a total of 59) females that did not reproduce while isolated and whose reproductive mode could be identified by paternity testing, only five (all from the *P2*×*Akaz* cross) produced only sexual clutches when paired with a male (Table S2). However, each of them produced only one or two clutches, so that it is difficult to exclude that they would have been able to reproduce asexually in subsequent clutches. Accordingly, our most likely statistical model (Table S3) did not support the occurrence of purely sexual females (ΔAICc = 2.1) and included only two categories of F1 females (Fig. 2): 56% purely asexual and 44% mixed (not significantly different from 50% each, ΔAICc = 0.4 with a model where the proportion was fixed to 50%). Among the mixed F1 females, those from the *P2*×*Akaz* cross produced significantly (ΔAICc = 7.3 with a model where there is no cross effect) more sexual clutches than those from the *P1*×*Akaz* cross (79% vs. 29%; Table S3). The second-best model (ΔAICc = 1.1) also included two categories of females but mixed females had slightly heterogeneous rates of sexual reproduction, which differed between *P1*×*Akaz* and *P2*×*Akaz* crosses.

EXPERIMENT 3: CONTAGIOUS ASEXUALITY VIA HYBRID F1 FEMALES

The third experiment showed that contagious asexuality, which previously was thought to happen only via rare males, can also occur via females. Indeed, we found that asexual "mixed" females, when crossed with a sexual male, could transmit asexuality to some of their sexually produced daughters. Among the 12 clutches produced by *P1*×*Akaz* females paired with *Akaz* males, five were later identified as being the result of a sexual cross (i.e., being a first-generation backcross), whereas the

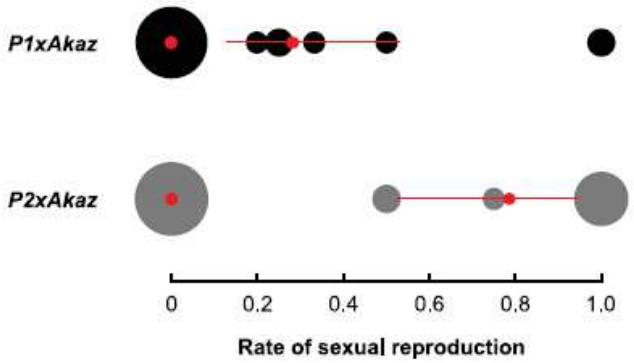


Figure 2. Frequency of sexual clutches in F1 hybrids in experiment 2. Disks represent *P1×Akaz* (black) and *P2×Akaz* (gray) F1 females, with the size of symbols proportional to the number of females with the same value. Red dots and bars represent the rate of sexual reproduction and support limits as estimated from our best model. The estimated proportion of females in the two categories (fully asexual vs. mixed) does not significantly differ between the two crosses and is estimated at 56 % asexual (support limits: 35–71%) and 44% mixed (support limits: 28–66%).

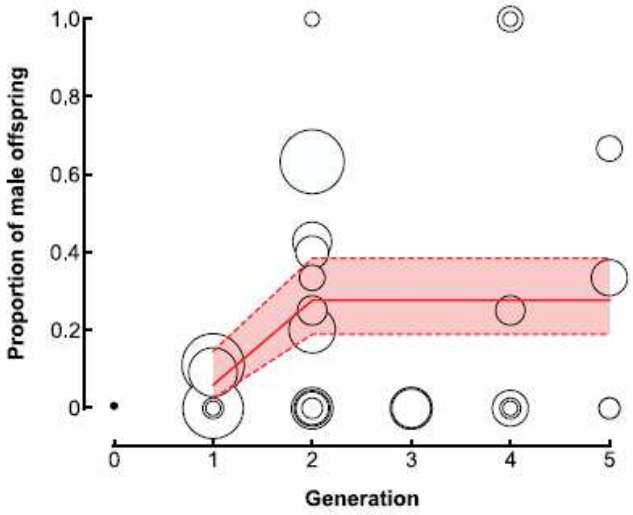


Figure 3. Proportion of asexually produced males in different *P3 ×Akaz* backcross generations in experiment 5. Empty disks represent sex ratios among asexually produced offspring per female, with the area of the circle being proportional to the number of offspring. The thick red line shows the mean proportion of male offspring as estimated from our best model (reaching a plateau at 27%), and the dashed red lines represent the confidence interval of this mean. The black dot at generation zero indicates the proportion of rare male production reported for *P3* (3.93‰; Maccari et al., 2013).

others were found to be asexually produced. A total of 10 virgin female offspring from these five clutches were isolated. Two of them successfully reproduced in absence of males (production of cysts; Table S4), showing that they were capable of asexual reproduction.

EXPERIMENT 4: RARE SEX IN *Ap2n* FEMALES

The fourth experiment showed that *Ap2n* females engage in rare sexual reproduction. The mass-cross between *P1* females and *Akaz* males produced 1828 offspring, of which eight were males. Paternity tests revealed that four of these were rare males, produced asexually, whereas four were *P1×Akaz* hybrids. The hybrids were possibly the result of a single copulation between an *Akaz* male and a *P1* female (in experiment 2, we found that, in the clutches produced by F1 hybrids, all offspring were produced by the same reproductive mode; Supporting Information part 3). They were found at the same time, likely had the same age, and, according to their genotype, it is possible that they had the same father (Table S5). Fertilization may either have resulted in diploid or triploid offspring, depending on whether the mother produced diploid or haploid ovules. To distinguish between these two hypotheses, we checked whether the sexually produced males inherited both maternal alleles at loci that were heterozygous in *P1* females. In three male offspring that could conclusively be tested (one was inconclusive due to shared null-alleles), the male inherited only one of the two female alleles (Table S5). It is therefore likely that the females from the *P1* population can (rarely) undergo normal meiosis and produce haploid gametes. The alternative explanation of fertilization of a diploid egg that underwent LOH is unlikely, as the estimated LOH rates at the same loci in experiment 1 are 0.021 and 0.025 per generation (L5 in *P1×Akaz* F1 and L6 in *P2×Akaz* F1, respectively; Fig. 1). In the second mass cross (involving *P2*), we found four males among 1061 offspring, but they were all rare *Ap2n* males.

EXPERIMENT 5: ESTIMATING RECOMBINATION RATE IN AUTOMICTS

We found that α, the rate of rare male production, was higher in F1 hybrids than in asexual populations (Fig. 3; Maccari et al., 2013). The model best fitting the data was a polygenic model with a step variation of the mean α, and a quadratic effect on the variance of α (Table S7). It was better than the best monogenic model (ΔQAIC = 6.3). This model shows that α further increased in the first backcross generation, but not significantly afterward, plateauing at a value of 27% (Fig. 3). The variance of α was found null among F1, increased in subsequent backcross, and returned to zero in the fourth backcross generation (Fig. S2). This is expected under a polygenic control of recombination, with variation introduced by the introgression of *Akaz* recombination genes until all *Ap2n* recombination genes are replaced by *Akaz* ones during successive backcrosses. The second-best model (ΔQAIC = 1.8) was similar but mean α followed a cubic variation with a qualitatively similar shape.

Discussion

We show that contagious asexuality can be used to generate hybrids and backcrosses and that this experimental approach provides excellent opportunities to investigate, in detail, the reproduction biology of asexuals, the inheritance of asexuality, as well as the mechanisms and genetic consequences of asexuality. Our results suggest that asexuality in hybrids is transmitted from the asexual parent, rather than caused by hybridization itself. In experiment 1, loci with a high LOH rate in hybrids tend to have high F_{IS} (homozygosity excess) in wild *Ap2n* (see Fig. 1 and Nougué et al., 2015), which indicates that the same type of asexuality (central fusion automixis) occurs in hybrids and their asexual parents (this correspondence is expected if asexuality is inherited but very unlikely if asexuality is caused by hybridization). Furthermore, in experiment 5, it was possible to maintain asexuality (by selecting males that were likely to carry asexuality genes) during up to four backcross generations. About 97% of the genome of these fourth-generation backcross individuals is of *Akaz* origin. This supports our assumption that asexuality is passed on from *Ap2n* to these individuals by the transmission of a small part of the genome that carries asexuality gene(s) rather than by hybridization per se, thus arguing against the balance hypothesis as a plausible explanation for our results. We found five major results, corresponding to the five experiments reported in this article. Each of these experiments revealed surprises, which were entirely new to *Artemia* biology (summarized in Table S8) and which have major implications for the evolution of asexuality, as they suggest that similar hidden features of asexuality could be found in many other asexual taxa if investigated in sufficient detail.

EVIDENCE FOR CENTRAL FUSION AUTOMIXIS IN *Ap2n*

We provide the first demonstration of genetic differences between parents and their asexually produced offspring in *Artemia*. The estimated per-locus LOH rates (up to 23.0% per generation) are much higher than typical gene conversion rates (Liu et al., 2018), and LOH therefore likely results from recombination. The direct observation of partial LOH ("partial" because only observed for some loci) provides strong evidence in favor of central fusion automixis in *Ap2n*. As the *Artemia* example shows, a lack of parent-offspring differences does not necessarily prove clonality, although it is frequently interpreted as such (Stenberg & Saura, 2009; Dukić et al., 2019). Indeed, central fusion automixis is genetically equivalent to clonality only in the complete absence of recombination. If recombination occurs, it has different genetic consequences (Engelstädter, 2017). Yet even in the presence of recombination, parent-offspring differences may remain unnoticed because, like in *Artemia*, and depending on genomic location, there is either no heterozygosity to lose (regions with high recombination likely have lost heterozygosity before) or there is a low chance to observe it (regions with low recombination can be heterozygous, but they are unlikely to undergo LOH). Indeed, no genetic differences were found in field-sampled *Ap2n* lineages across tens of generations (Browne & Hoopes, 1990; Nougué et al., 2015). Our approach to generate new asexual hybrids was key for the detection of LOH. The same or similar approaches could be used in other systems with rare males or in systems where crosses are possible by other means (e.g., partial asexuals, hermaphrodites).

RECOMBINATION RATE MAY BE SELECTED AGAINST IN AUTOMICTS

Recombination in automictic asexuals has different consequences compared to recombination in sexuals. Especially in newly formed asexuals, LOH exposes recessive deleterious mutations, leading to a phenomenon similar to inbreeding depression (loss-of-complementation; Archetti, 2010). This may explain the low rate of sex-to-asex transitions in eukaryotes with only few lineages being able to escape this early fitness decrease (Archetti, 2010; Engelstädter, 2017). Escaping LOH may also be achieved by bypassing meiosis altogether, but this is likely to pose other severe problems (e.g., perturb epigenetic reset, Lenormand et al., 2016; or other problems, Engelstädter 2008). Another possible way to avoid the deleterious consequences of LOH is to reduce the recombination rate, which may explain why many extant asexuals genetically behave like "clones" (Goudie et al., 2012; Engelstädter, 2017; Dukić et al., 2019). Our results support this hypothesis, as we show that (a) hybrids from the *P2*×*Akaz* lineage have substantially higher LOH rates than hybrids from the *P1*×*Akaz* lineage (Fig. 1), suggesting that recombination rate can vary and thus evolve in automictic lineages; (b) we estimated an increased rare male production between *P3*×*Akaz* F1 and the first generations of backcross to *Akaz*. This strongly supports that the recombination rate is lower in asexuals compared to their closest sexual relative *Akaz*, which already has an exceptionally low recombination rate compared to other sexual species (Haag et al., 2017). Still, recombination rate is not zero. A key limiting factor in preventing the complete loss of recombination in *Ap2n* could perhaps be positive selection for rare sex or contagion, which requires residual male production and therefore nonzero recombination.

CONTAGIOUS ASEXUALITY VERSUS RARE SEX VERSUS CYCLICAL PARTHENOGENESIS

Contagious asexuality has always been assumed to occur only via asexually produced males (in *Artemia* and other asexual species), mainly because asexual females are thought to be unable to reproduce sexually. We found, for the first time, that hybrid

females with a "mixed" reproductive mode can sexually transmit asexuality. This led us to ask whether *Ap2n* females were capable of sex, although Browne and Hoopes (1990) found no evidence for cyclical parthenogenesis. To our surprise, we found that females from field-sampled asexual lineages can rarely reproduce sexually, likely through normal meiosis, which is an entirely new result for asexual *Artemia*. It is, however, unlikely that these occurrences of rare sexual reproduction represent "cyclical parthenogenesis," where sex occurs periodically, triggered by environmental cues (Burt, 2000; Meirmans et al., 2012) and often is linked to the production of diapause stages. Our experiment was conducted in the laboratory under controlled and constant conditions, thus dependence on environmental cues is unlikely (although we cannot exclude it). Moreover, offspring produced were live nauplii, not diapause stages (cysts). Overall, it thus seems likely that our observations represent somewhat unpredictable events of rare sexual reproduction of *Ap2n* females rather than cyclical parthenogenesis. Although the frequency of these events is unknown, these findings challenge the robustness of the evidence for purely asexual reproduction also in other taxa: Identifying occasional sex via morphologically distinct rare males is far easier than detecting rare sexual events in otherwise asexual females. Hence, it is possible that the common view that contagious asexuality occurs mainly through males may be influenced by an ascertainment bias between sexes in the study of rare sex.

THE DIVERSIFICATION OF ASEXUAL LINEAGES

The demonstration that asexuality can be sexually transmitted also via females indicates that new asexual lineages may be created more easily than previously thought. Furthermore, contagion generates hybrids with mixed reproduction, which can in turn breed with other hybrids or backcross, thus potentially generating numerous new asexual lineages from just a single original hybridization event. Finally, rare sex may allow gene flow between different asexual lineages without the need to hybridize first with a sexual species. Even if extremely rare, this vastly expands the possibility of generating new asexual lineages as it does not require local co-occurrence of closely related sexual species whose geographic distributions are narrow (Muñoz et al., 2010). These possibilities, combined with LOH, could explain the high diversity of asexual lineages observed in *Artemia* (Browne & Hoopes, 1990). Many asexual taxa indeed show surprisingly high genetic diversity (Parker, 1979; Browne & Hoopes, 1990; Simonsen & Holmstrup, 2008; Bengtsson, 2009). It seems likely that part of this diversity may be explained by rare occurrences of sex and recombination, although other factors may contribute (e.g., the origin of asexual lineages and mutation accumulation; Simon et al., 2003).

THE GENETIC BASIS OF ASEXUALITY

The reproductive modes of sex-asex hybrid females are more complex than expected. Almost all F1 females were able to reproduce asexually, which radically differs from results by Maccari et al. (2014), which suggested recessive inheritance of asexuality. Although our methodology was similar, their assumption that only sexual reproduction occurs once F1 females are paired with males was clearly rejected by our paternity analyses. In addition, some clutches in their experiment (produced in the presence of males) showed low sex ratios typical of asexual reproduction, suggesting that at least some asexual reproduction did in fact occur. Although we cannot currently explain why none of their F1 females reproduced during the 14 days of isolation period in their experiment (a substantial fraction of F1 females did so during the same period in our experiment), it is nonetheless likely that the different results and conclusions are largely explained by methodology. Our results indicate that the capacity to reproduce asexually is dominant and was homozygous in the rare males used for the crosses. Dominant asexuality is mainly found in plants (Van Dijk, 2009; Neiman et al., 2014), whereas recessive asexuality is found in several animal systems (Jaquiéry et al., 2014; Sandrock & Vorburger, 2011; Yagound et al., 2020). Together with *Daphnia* (Lynch et al., 2008), *Artemia* seem to be an exception to this pattern. Dominant asexuality also allows reversal to sexuality if LOH occurs at a heterozygous asexuality-determining region (thus LOH is a possible explanation for the occurrence of rare sex in females as well as of the production of rare males). Surprisingly, we also found two distinct reproductive phenotypes within F1 females, which were either completely asexual or "mixed." This could be explained by a dominant factor, heterozygous in the *Akaz* females used for the cross or by an epistatic interaction with a second locus. The two traits (the capacity to reproduce asexually and the ability to switch between sexual and asexual clutches) displayed by F1 females thus could be controlled by different loci. The occurrence of at least two loci is further suggested by the observation that the propensity to reproduce asexually in "mixed" females differs between crosses. This finding indicates that the "asexuality" phenotype may be more complex, with a history of secondary modifications, involving more than a single gene.

SEX-TO-ASEX TRANSITIONS AND THE MAINTENANCE OF SEX

Over the last two decades, the classical view of regarding asexuality as largely synonymous with clonality has started to change (Gorelick, 2003; Gorelick & Carpinone, 2009; Dukić et al., 2019). Nonclonal asexual modes have been uncovered in an increasing number of taxa (Stenberg & Saura, 2009; Hiruta et al., 2010; Svendsen et al., 2015). In addition, it has become clear that even the ones that do show fully or largely clonal reproduction

from a genetic point of view often do so by modified meiosis (e.g., central fusion automixis with very little or no recombination) rather than by mitosis. This suggests that recombination may have been frequent during earlier phases of their asexuality evolution (Archetti, 2010). Especially during these phases, the evolutionary consequences of asexuality may have strongly differed from those of clonality, as a different set of costs and benefits apply to nonclonal asexual modes (Stenberg & Saura, 2009; Archetti, 2010; Meirmans et al., 2012; Engelstädter, 2017). These different costs and benefits may strongly affect the fitness of new asexual lineages compared to sexual ancestors, and hence the rate at which new lineages are produced as well as their diversity. For instance, a low rate of sex in asexuals may be sufficient to confer most advantages of sexual reproduction while minimizing the cost of sex (Bengtsson, 2009; Otto, 2009; Schurko et al., 2009; Engelstädter, 2017). However, as we show, nonclonal asexuality can appear as clonality, which is too often considered as the "default" asexuality mode. This causes our conceptions of sex-to-asex transitions and maintenance of sex to largely rely on contrasting the costs and benefits of sex with those of clonality (Maynard Smith, 1978; Hartfield & Keightley, 2012). If generalized, this means that the twofold advantage of an asexual mitotic mutant (Maynard Smith, 1978; Hartfield & Keightley, 2012) may often simply be irrelevant (see also Meirmans et al 2012). This calls for a more realistic consideration of sex-to-asex transitions in theories dealing with the maintenance of sex.

ACKNOWLEDGMENTS

The authors are grateful to G. Van Stappen for providing the *Akaz* sample, and to F. Amat for providing *Ap2n* from lake Urmia. We thank T. Schwander for useful comments. We also thank M.-P. Dubois and The Genomics, Molecular Ecology, and Experimental Evolution platform (GEMEX) at CEFE, genotyping and sequencing facilities of the Institut des Sciences de l'Evolution-Montpellier, and the Labex Centre Méditerranéen de l'Environnement et de la Biodiversité. This work was funded by the Grant ANR-17-CE02-0016-01, GENASEX, from the French National Research Agency.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

TL and CH acquired funding, conceived and supervised the study. TL, CH, and LB designed the experiments. RZ and LB performed the experiments. TL provided resources. LB, MM, and TL analyzed the data. LB and MM wrote the original draft of the manuscript. TL, CH, and LB reviewed and edited the manuscript. LB and MM produced figures. TL and RZ administered the project.

DATA ARCHIVING

Data for experiments 1 and 2 are available in Tables S9 and S10, respectively.

LITERATURE CITED

Abatzopoulos, T. J., I. Kappas, P. Bossier, P. Sorgeloos, and J. A. Beardmore 2002. Genetic characterization of *Artemia tibetiana* (Crustacea: Anostraca). Biological Journal of the Linnean Society 75 333–344.

Abreu-Grobois, F. A. 1983. Population genetics of Artemia. University College of Swansea, Swansea, U.K.

Abreu-Grobois, F. A. and J. A. Beardmore 2001. The generation of males by diploid parthenogenetic Artemia cannot occur in the way Stefani suggested. In Proceedings of the 4th international large branchiopod symposium (Vol. 1). La Paz, Mexico.

Agh, N., T. J. Abatzopoulos, I. Kappas, G. Van Stappen, S. M. Razavi Rouhani, and P. Sorgeloos 2007. Coexistence of sexual and parthenogenetic Artemia populations in Lake Urmia and neighbouring lagoons. International Review of Hydrobiology 92 48–60. https://doi.org/10.1002/iroh.200610909

Archetti, M. 2010. Complementation, genetic conflict, and the evolution of sex and recombination. Journal of Heredity 101 1–13. https://doi.org/10.1093/jhered/esq009

Asher, J. H. 1970. Parthenogenesis and genetic variability. II. One-locus models for various diploid populations. Genetics 66 369–391.

Barigozzi, C. 1944. I fenomeni cromosomici delle cellule germinali in Artemia salina leach. Chromosoma 2 549–575.

Bengtsson, B. O. 2009. Asex and evolution: a very large-scale overview. Pp. 1–19 in I. Schön, K. Martens, and P. Dijk, eds. Lost sex: the evolutionary biology of parthenogenesis. Springer, Dordrecht, The Netherlands.

Bowen, S. T. 1963. The genetics of Artemia salina. II. White eye, a sex-linked mutation. The Biological Bulletin 124 17–23. https://doi.org/10.2307/1539564

Bowen, S. T., J. P. Durkin, G. Sterling, and L. S. Clark 1978. Artemia hemoglobins: genetic variation in parthenogenetic and zygogenetic populations. Biological Bulletin 155 273–287.

Browne, R. A. and C. W. Hoopes 1990. Genotype diversity and selection in asexual brine shrimp (Artemia). Evolution 44 1035–1051. https://doi.org/10.1111/j.1558-5646.1990.tb03824.x

Browne, R. A. and G. H. MacDonald 1982. Biogeography of the brine shrimp, Artemia: distribution of parthenogenetic and sexual populations. Journal of Biogeography 9 331–338.

Burt, A. 2000. Perspective: sex, recombination, and the efficacy of selection—was Weismann right? Evolution 54 337–351.

Clark, L. S. and S. T. Bowen 1976. The genetics of Artemia salina: VII. Reproductive isolation. Journal of Heredity 67 385–388. https://doi.org/10.1093/oxfordjournals.jhered.a108758

Cuellar, O. 1987. The evolution of parthenogenesis: a historical perspective. Pp. 43–104 in P. Moens, ed. Meiosis. Academic Press, Cambridge, MA.

D'Souza, T. G., M. Storhas, H. Schulenburg, L. W. Beukeboom, and N. K. Michiels 2004. Occasional sex in an "asexual" polyploid hermaphrodite. Proceedings of the Royal Society B: Biological Sciences 271 1001–1007. https://doi.org/10.1098/rspb.2004.2675

de Vos, S., P. Bossier, G. van Stappen, I. Vercauteren, P. Sorgeloos, and M. Vuylsteke 2013. A first AFLP-based genetic linkage map for brine shrimp Artemia franciscana and its application in mapping the sex locus. PLoS ONE 8 1–10. https://doi.org/10.1371/journal.pone.0057585

Dukić, M., D. Berner, C. R. Haag, and D. Ebert 2019. How clonal are clones? A quest for loss of heterozygosity during asexual reproduction in *Daphnia magna*. Journal of Evolutionary Biology 32 619–628. https://doi.org/10.1111/jeb.13443

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30 1138–1150. https://doi.org/10.1002/bies.20833

———. 2017. Asexual but not clonal: evolutionary processes in automictic populations. Genetics 206: 993–1009. https://doi.org/10.1534/genetics.116.196873/-/DC1.1

Gorelick, R. 2003. Transposable elements suppress recombination in all meiotic eukaryotes, including automictic ancient asexuals: a reply to Schön and Martens. Journal of Natural History 37 903–909. https://doi.org/10.1080/0022293021000007705

Gorelick, R. and J. Carpinone 2009. Origin and maintenance of sex: the evolutionary joys of self sex. Biological Journal of the Linnean Society 98 707–728. https://doi.org/10.1111/j.1095-8312.2009.01334.x

Goudie, F., M. H. Allsopp, M. Beekman, P. R. Oxley, J. Lim, and B. P. Oldroyd 2012. Maintenance and loss of heterozygosity in a thelytokous lineage of honey bees (*Apis mellifera capensis*). Evolution 66 1897–1906. https://doi.org/10.1111/j.1558-5646.2011.01543.x

Haag, C. R., L. Theodosiou, R. Zahab, and T. Lenormand 2017. Low recombination rates in sexual species and sex-asex transitions. Philosophical Transactions of the Royal Society B: Biological Sciences 372 1–11. https://doi.org/10.1098/rstb.2016.0461

Hartfield, M. and P. D. Keightley 2012. Current hypotheses for the evolution of sex and recombination. Integrative Zoology 7 192–209. https://doi.org/10.1111/j.1749-4877.2012.00284.x

Hiruta, C., C. Nishida, and S. Tochinai 2010. Abortive meiosis in the oogenesis of parthenogenetic *Daphnia pulex*. Chromosome Research 18 833–840.

Hörandl, E. and O. Paun 2007. Patterns and sources of genetic diversity in apomictic plants: implications for evolutionary potentials and ecology. Pp. 169–194 in E. Hörandl, U. Grossniklaus, P. Van Dijk, and T. Sharbel, eds. Regnum vegetabile. Lubrecht & Cramer Limited, Port Jervis, NY.

Jaquiéry, J., S. Stoeckel, C. Larose, P. Nouhaud, C. Rispe, L. Mieuzet, et al. 2014. Genetic control of contagious asexuality in the pea aphid. PLoS Genetics 10 1–10. https://doi.org/10.5061/dryad.7215m.Funding

Judson, O. and B. Normark 1996. Ancient asexual scandal. Trends in Ecology and Evolution 11 41–46. https://doi.org/10.1016/0169-5347(96)81040-8

Lenormand, T., J. Engelstädter, S. E. Johnston, E. Wijnker, and C. R. Haag 2016. Evolutionary mysteries in meiosis. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20160001. https://doi.org/10.1098/rstb.2016.0001

Lievens, E., J. Perreau, P. Agnew, Y. Michalakis, and T. Lenormand 2018. Decomposing parasite fitness in a two-host, two-parasite system reveals the underpinnings of parasite specialization. Evolution Letters 2 390–405.

Liu, H., J. Huang, X. Sun, J. Li, Y. Hu, L. Yu, et al. 2018. Tetrad analysis in plants and fungi finds large differences in gene conversion rates but no GC bias. Nature Ecology and Evolution 2 164–173. https://doi.org/10.1038/s41559-017-0372-7

Lynch, M., A. Seyfert, B. Eads, and E. Williams 2008. Localization of the genetic determinants of meiosis suppression in *Daphnia pulex*. Genetics 180 317–327. https://doi.org/10.1534/genetics.107.084657

Maccari, M., A. Gómez, F. Hontoria, and F. Amat 2013. Functional rare males in diploid parthenogenetic Artemia. Journal of Evolutionary Biology 26 1934–1948. https://doi.org/10.1111/jeb.12191

Maccari, M., F. Amat, F. Hontoria, and A. Gómez 2014. Laboratory generation of new parthenogenetic lineages supports contagious parthenogenesis in Artemia. PeerJ 2: e439. https://doi.org/10.1038/ismej.2014.199

MacDonald, G. H. and R. A. Browne 1987. Inheritance and reproductive role of rare males in a parthenogenetic population of the brine shrimp, *Artemia parthenogenetica*. Genetica 75 47–53.

Maynard Smith, J. 1978. The evolution of sex. Cambridge Univ. Press, Cambridge, U.K.

Meirmans, S., P. G. Meirmans, and L. R. Kirkendall 2012. The costs of sex: facing real-world complexities. Quarterly Review of Biology 87 19–40.

Moritz, C., W. M. Brown, L. D. Densmore, J. W. Wright, D. Vyas, S. Donnellan, et al. 1989. Genetic diversity and the dynamics of hybrid parthenogenesis in Cnemidophorus (Teiidae) and Heteronotia (Gekkonidae). Pp. 87–112 *in* R. M. Dawley and J. P. Bogart, eds. Evolution and ecology of unisexual vertebrates. New York State Museum, Albany, NY.

Muñoz, J., A. Gómez, A. J. Green, J. Figuerola, F. Amat, and C. Rico 2010. Evolutionary origin and phylogeography of the diploid obligate parthenogen Artemia parthenogenetica (Branchiopoda: Anostraca). PLoS ONE 5:e11932. https://doi.org/10.1371/journal.pone.0011932

Narbel-Hofstetter, M. 1964. Les altérations de la méiose chez les animaux parthénogénétiques. Springer-Verlag, Berlin, Germany.

Neiman, M., S. Meirmans, and P. G. Meirmans 2009. What can asexual lineage age tell us about the maintenance of sex? Annals of the New York Academy of Sciences 1168 185–200. https://doi.org/10.1111/j.1749-6632.2009.04572.x

Neiman, M., T. F. Sharbel, and T. Schwander 2014. Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. Journal of Evolutionary Biology 27 1346–1359. https://doi.org/10.1111/jeb.12357

Nougué, O., N. O. Rode, R. Jabbour-zahab, A. Ségard, L. M. Chevin, C. R. Haag, et al. 2015. Automixis in Artemia: solving a century-old controversy. Journal of Evolutionary Biology 28 2337–2348. https://doi.org/10.1111/jeb.12757

Otto, S. P. 2009. The evolutionary enigma of sex. American Naturalist 174:S1-S14. https://doi.org/10.1086/599084

Otto, S. P. and T. Lenormand 2002. Resolving the paradox of sex and recombination. Nature Reviews Genetics 3 252–261. https://doi.org/10.1038/nrg761

Paland, S., J. K. Olbourne, and M. Lynch 2005. Evolutionary history of contagious asexuality in *Daphnia pulex*. Evolution 59 800–813.

Parker, E. D. 1979. Ecological implications of clonal diversity in parthenogenetic morphospecies. Integrative and Comparative Biology 19 753–762. https://doi.org/10.1093/icb/19.3.753

Pilla, E. J. S. and J. A. Beardmore 1994. Genetic and morphometric differentiation in old world bisexual species of Artemia (The brine shrimp). Heredity 73 47–56. https://doi.org/10.1038/hdy.1994.97

Sandrock, C. and C. Vorburger 2011. Single-locus recessive inheritance of asexual reproduction in a parasitoid wasp. Current Biology 21 433–437. https://doi.org/10.1016/j.cub.2011.01.070

Schön, I., K. Martens, and P. Van Dijk 2009. Lost sex: the evolutionary biology of parthenogenesis. Springer, Berlin, Germany.

Schurko, A. M., M. Neiman, and J. M. Logsdon 2009. Signs of sex: what we know and how we know it. Trends in Ecology and Evolution 24 208–217. https://doi.org/10.1016/j.tree.2008.11.010

Simon, J. C., F. Delmotte, C. Rispe, and T. Crease 2003. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. Biological Journal of the Linnean Society 79 151–163. https://doi.org/10.1046/j.1095-8312.2003.00175.x

Simonsen, V. and M. Holmstrup 2008. Deviation from apomictic reproduction in *Dendrobaena octaedra*? Hereditas 145 212–214. https://doi.org/10.1111/j.0018-0661.2008.02045.x

Stefani, R. 1963. La Digametia Femminile in Artemia Salina Leach e la Costituzione del Corredo Cromosomico Nei Biotipi Diploide Anfigonico e Diploide Partenogenetico. Caryologia 16 625–636. https://doi.org/10.1080/00087114.1963.10796102

———. 1964. The origin of males in parthenogenetic populations of Artemia salina. Rivista Di Biologia, 57: 147–156.

Stenberg, P. and A. Saura 2009. Cytology of asexual animals. Pp. 63–74 *in* I. Schön, K. Martens, and P. Dijk, eds. Lost sex: the evolutionary biology of parthenogenesis. Springer, Dordrecht, The Netherlands.

Svendsen, N., C. M. O. Reisser, M. Dukić, V. Thuillier, A. Ségard, C. Liautard-Haag, et al. 2015. Uncovering cryptic asexuality in daphnia magna by RAD sequencing. Genetics 201 1143–1155. https://doi.org/10.1534/genetics.115.179879

Van Dijk, P. 2009. Apomixis: basics for non-botanists. Pp. 47–62 *in* I. Schön, K. Martens, and P. Dijk, eds. Lost sex: the evolutionary biology of parthenogenesis. Springer, Dordrecht, The Netherlands.

White, M. J. D. 1973. Animal cytology and evolution. Cambridge Univ. Press, Cambridge, U.K.

Wolfram Research, Inc. 2012. Mathematica (9.0). Wolfram Research, Inc., Champaign, IL.

Yagound, B., K. A. Dogantzis, A. Zayed, J. Lim, P. Broekhuyse, E. J. Remnant, et al. 2020. A single gene causes thelytokous parthenogenesis, the defining feature of the cape honeybee *Apis mellifera capensis*. Current Biology. https://doi.org/10.1016/j.cub.2020.04.033

Yarmohammadi, M. and M. Pourkazemi 2004. Cytogenetic study of Artemia from Urmiah, Maharloo and Incheborun Lakes. Hydrobiologia 529 99–104. https://doi.org/10.1007/s10750-004-5236-x

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Hybrid asexual lineages used in experiment 1 and LOH events.
Figure S2. Variance in rare male production among the asexual females generated by hybridization and backcross of experiment 5.
Table S1. Models fitted to the data of experiment 1 and ΔAICc.
Table S2. Reproductive mode of F1 hybrid females in each of the two crosses of experiment 2.
Table S3. Likelihood models fitted to the reproductive mode data of F1 hybrid females in experiment 2 and their ΔAICc
Table S4. Reproduction of females isolated from each candidate clutch in experiment 3.
Table S5. Microsatellite genotypes of potential parents (*P1* females and *Akaz* males) and male offspring from the *P1* mass-cross in experiment 4.
Table S6. Sample sizes of individuals used for crosses and asexuality tests in each generation of experiment 5 and sex ratios of asexually produced offspring.
Table S7. Likelihood models fitted to the data on the proportion of asexually produced males in experiment 5 and their QAIC.
Table S8. Summary of the five experiments conducted in this study and their main results.
Table S9. Data for experiment 1.
Figure S10. Data for experiment 2.

Supplementary materials

1. Origin of laboratory populations and culturing conditions

The *P1* population was established from a single female sampled in the Aigues-Mortes salterns, France by Nougué et al. (2015) (referred to as PAM7 in that publication). A second population, *P3*, also sampled from Aigues-Mortes but from a different location and not isofemale was used in experiment 5. The *P2* population originated from Lake Urmia, Iran, with cysts obtained from F. Amat (Instituto de Acuicultura de Torre de la Sal, Spain). Note that this is an *Ap2n* population, different from the bisexual species *A. urmiana*. The mitochondrial haplotypes of P1 (unpublished data) and P2 (Maccari et al., 2013) are closest to *Akaz*. The *Akaz* population originated from Kazakhstan, and cysts were obtained from the *Artemia* Reference Center (Ghent University, Belgium, code of the cyst sample: ARC1039). The *P2* and *P3* populations were maintained in the laboratory for <1 year (<12 generations), and the *P1* and *Akaz* populations for >5 years (>60 generations) before the beginning of the experiments. Population cultures of >100 individuals were maintained at 21 ± 1 °C in 5 L aquaria in a solution of brine diluted with deionized water to a salinity of 80 to 90 g/L. They were fed *ad libitum* with freeze-dried microalgae *Tetraselmis chuii* diluted in deionized water. *Ap2n* population cultures were regularly scanned for males (recognizable by their claspers). These males were crossed with virgin *Akaz* females. Individual juveniles, adults, pairs and their offspring were kept in plastic jars in 100 mL of brine solution (as above). They were fed three times a week with 1-2 mL of 1 g/L freeze-dried *Tetraselmis chuii* diluted in deionized water.

2. Experiment 1: LOH in sex-asex hybrids

We produced three crosses between *P1* rare males and *Akaz* females (two using the same rare male, Fig. S1), and two crosses between *P2* rare males and *Akaz* females (Fig. S1). From each cross, one F1 female was isolated at juvenile stage and kept individually until she produced offspring, which were in turn isolated as well. Lineages, which were sometimes split into sub-lineages, were propagated in this way for one to few generations. One lineage from each cross was selected for the experiment, which lasted up to 13 asexual generations (Fig. S1).

DNA extractions for microsatellite analysis was carried out using the digestive tracts of dissected females, which contain sufficient DNA for microsatellite analysis. Extraction methods and protocols for microsatellite analyses followed those described by Nougué et al. (2015), which were based on two previous studies (Muñoz et al., 2009; Nougué et al., 2015). DNA was extracted in 15 µL HotShot extraction buffer (Sigma), in a thermocycler at 95 °C for 10 min and 20 °C for 10 min. The extraction product was then diluted with 25 µL sterile water. We added 1 µL of DNA extract to 9 µL of a PCR mix containing 5 µL of Multiplex buffer (Qiagen), 1 µL of forward and 1 µL of reverse primers [2 µM], and 2 µL of sterile water. PCR amplification was conducted in a thermocycler with an initial denaturation step at 95 °C for 15 min, 30 cycles consisting of 30 s at 94 °C, 90 s at 60 °C and 60 s at 72 °C, and finally a supplementary extension step of 30 min at 60 °C.

We added 3 µL of diluted PCR product (20 to 200X) to 15 mL of HI-DI TM formamide (Applied Biosystems) and 0.2 mL of GeneScan-500 LIZ size standard. Samples were analysed

on an ABI 3500XL 24 capillary sequencer DNA Analyzer (Applied Biosystems) at the LabEx CeMEB sequencing platform (Montpellier, France). Fragment analysis and scoring were carried out using GeneMapper v. 3.7 (Soft Genetics, State College, PA, USA). The DNA fragment sizes measured from the peaks were converted into discrete alleles by comparison with reference lists of allele sizes.

Only six loci were informative in our study: L1: Appm20, L2: Apcpm1, L3: Aupm16, L4: Aupm21 L5: AP02, and L6: AP01, with locus numbers corresponding to Figure 1 and Figure S1 and locus names to those given in Nougué et al. (2015). Note that locus 4 was only initially heterozygous in one F1 female and was lost at the third generation, thus it was not included in the statistical analyses. We also observed three LOH events in *P2xAkaz* lineages for a seventh locus, AP03, which was heterozygous with a null allele. This locus was non-informative as homozygosity was only detectable for the null allele. In some cases, the loci were analyzed in simplex reactions rather than in the multiplex reactions described in Nougué et al. (2015).

To test whether LOH rate depends on the population of origin of the rare male ("*pop*"), differs among loci ("*loc*"), or changes across subsequent asexual generations ("*gen*"), we considered several likelihood models, written and then fitted using Mathematica V.9.0 (Wolfram Research, 2012; Table S1). The likelihood of each model was written by supposing that the loss or retention of heterozygosity from one generation to the next resulted from a Bernoulli draw. In the first model, the rate of LOH was assumed to be constant across generations and loci. In the second model, it was allowed to vary with the number of asexual generations in a logistic way. The third and fourth models allowed for different LOH rates depending on the origin of the rare male (*P1* or *P2*) and/or depending on the locus, and subsequent models included combinations of these effects as well as interactions. We compared models using Akaike's information criterion with a correction for small sample sizes (AICc). Models were checked for, but did not show overdispersion ($\hat{c} = 0.39$).

3. Experiment 2: Reproductive mode of F1 hybrid females

We crossed each of two rare males, one from *P1* and one from *P2*, with virgin *Akaz* females. All F1 hybrids were individually isolated at juvenile stage to assure that they remained unmated. They were sexed according to morphological characters (claspers for males, ovisac for females). Adult females were kept isolated to check for asexual reproduction. The generation time in *Artemia* is about a month. F1 Females from the *P2*x*Akaz* cross were kept isolated (period 1) for 14 days, while females from the *P1*x*Akaz* cross were isolated for 30 days to increase the possibility of asexual reproduction. After the isolation period, they were each paired with an *Akaz* male (period 2), and couples were kept together for up to 70 days (dead males were replaced by new ones). Offspring produced during period 2 were subjected to paternity testing to determine whether they were produced sexually or asexually.

In sexual and asexual *Artemia*, offspring are produced in clutches of either liveborn nauplii or cysts (Browne, 1992), which in this experiment were treated similarly (i.e., both were considered successful reproduction events). Pairs were checked three times a week for clutches, and each individual clutch produced was collected. Live nauplii were grown to juvenile stage and then preserved in 96 % ethanol at -20 °C. Cysts were dried and kept at -3 °C. Once a pair

had produced three (*P2*x*Akaz* cross) or six (*P1*x*Akaz* cross) clutches, or if the female displayed signs of poor viability, adults were also sampled and preserved in 96 % ethanol at -20 °C. A higher clutch number threshold was applied to the *P1*x*Akaz* cross, which was performed after *P2xAkaz*, to increase the chance of observing both reproductive modes (sexual and asexual) during pairing in this second cross. DNA extraction for microsatellite paternity tests were carried out using whole juveniles or cysts for the offspring and half bodies or digestive tracks for the adults.

We conducted microsatellite paternity tests on a total of 63 pairs and 167 clutches (Table S2). We used the same protocols as described in section 1. For extractions, we used whole samples when they were small (such as juveniles, digestive tracts and cysts), and half of large samples (adult individuals). Before extraction, cysts were rehydrated during one hour in 100 µL sterile water, then crushed directly in the extraction buffer. We used the multiplex reaction described in Muñoz et al. (2009), which includes the loci with highest divergence between *Akaz* and *Ap2n* and hence most likely to be informative regarding paternity. For a subset of 21 clutches (with three to eight offspring each), all offspring were analyzed, and the results indicated that, within a clutch, all offspring were produced by the same reproductive mode (sexual or asexual). We assumed this to be the case also for the remaining clutches and therefore, carried out paternity tests on only two offspring per clutch for each of the remaining clutches (in every case, both were indeed produced by the same reproductive mode).

Paternity testing accounted for the possible presence of null alleles. For instance, a locus with a homozygous profile for different alleles in the two parents and a homozygous profile for the maternal allele in a given offspring were counted as non-informative, as it is possible that the father was, in fact, heterozygous for a null allele and passed that null allele via sexual reproduction to its offspring. Due to these uncertainties, we could reliably infer the mode of reproduction for only 81 % of all clutches that were produced. But for all except four of the 63 females that were paired with a male, the mode of reproduction could be inferred for at least one of their clutches.

To analyze the proportions of the different modes of reproduction in F1 females, we concentrated only on the period when females were paired with a male because only during this period both reproductive modes were possible. We conducted a likelihood analysis, which accounted for sampling effects. We considered three categories of models (Table S3), written and then fitted using Mathematica V.9.0 (Wolfram Research, 2012). Models of category A assumed that all F1 females were 'mixed', and that the proportion of asexually produced clutches was a constant. The probability of observing a given number of asexual clutches then followed a binomial distribution. Because the data shows a large proportion of asexual clutches (Table S2), models of categories B and C considered two types of F1 females, asexual ones and mixed ones. We introduced a parameter to measure the proportion of these two types of females. In models of category B, the proportion of asexually produced clutches in mixed females was constant, but in models of category C, we assumed that mixed females were heterogeneous and that the proportion of asexually produced clutches followed a beta distribution among females. In all cases, we further considered a possible effect of the cross (*P1* or *P2*) on the probability to reproduce asexually. In model C.3, we considered an effect of the cross on the mean of the beta

distribution but not on the variance. In models of categories B and C, we also considered an effect of the cross on the probability to be in the 'mixed' category. We then compared models using AICc. The best model, model B.2 was slightly underdispersed ($\hat{c}$ = 0.88). An additional model suggested that there was no evidence for the existence of F1 females with 100% sexual reproduction: the model was identical to model B.2, except that it included three categories of females (mixed, 100% asexual and 100% sexual). To avoid bias, both model B.2 and the three-categories model were fitted to a dataset omitting the six mixed females that reproduced asexually during period 1 but only reproduced sexually during period 2 (Table S2). Again, model B.2 performed better (ΔAICc=2.1).

4. Experiment 3: Contagious asexuality *via* hybrid F1 females

In order to determine whether asexuality could be sexually transmitted by mixed hybrid F1 females, we kept large clutches of nauplii produced by F1 females from the *P1*x*Akaz* cross in experiment 2 after these F1 females were paired with *Akaz* males to test for sexual reproduction. As we did not yet know which clutches were produced sexually, we used the sex ratio of these clutches (as morphologically assessed once the offspring reached pre-adulthood) as a first indication. Indeed, sexually produced clutches tend to have a much higher proportion of male offspring than asexually produced ones. Twelve clutches with one or more males were selected as candidates. After paternity testing, five of these were found to be indeed sexually produced. One to six virgin females of each of these clutches were isolated, and checked weekly for offspring production for four weeks. Two females originating from the same clutch produced cysts in isolation, demonstrating that they were able to reproduce asexually (Table S4). The fact that contagious asexuality was observed in only one out of five clutches tested, is possibly explained by the nature of the cross, a backcross to the sexual species, which may, in some cases, lead to a loss of asexuality-conferring genes. This experiment was not conducted on the *P2xAkaz* cross, which was performed before the *P1xAkaz* cross, because at that time we did not know that some F1 females had a mixed reproductive mode.

5. Experiment 4: Rare sex in *Ap2n* females

We grew large *P1* and *P2* isofemale lines (i.e., populations of asexual descendants of a single female), and placed all healthy females in 5L aquaria (one aquarium per line). We added young *Akaz* males so that the ratio of *Akaz* male to *Ap2n* female was approximately equal for each population. The first aquarium contained 115 females from the *P1* isofemale line with 57 *Akaz* males, while the second contained 52 females from the *P2* isofemale population with 25 *Akaz* males. The experiment lasted for four weeks, and each week we moved all parents to a new aquarium to allow their offspring to grow with reduced competition. At the end of the experiment, all parents were preserved in 96 % ethanol at -20 °C. The offspring produced during the experiment were morphologically sexed once they reached pre-adulthood. In total, sexing revealed 1820 female and 8 male offspring from the *P1* isofemale population and 1057 female and 4 male offspring from the *P2* isofemale population. After sexing, offspring were also preserved in 96 % ethanol at -20 °C.

All male offspring as well as eight female offspring, two female parents and five male parents from each population were used for microsatellite analyses, using the same protocols

as described in section 1 and the same multiplex reaction as in section 2. We used half bodies for DNA extraction. We determined whether the offspring were produced sexually or asexually using the same criteria as described in section 2.

6. Experiment 5. Estimating recombination rate in automicts

From an initial cross between a *P3* rare male and an *Akaz* female, we obtained a F1 and four backcross generations on *Akaz*, using two to three *P3xAkaz* males per each generation, crossed again to *Akaz* females (Table S6). These males were either sexually produced during the backcross itself or asexually by females from a given backcross generation. We maintained asexuality throughout these generations, by using males that were most likely to carry asexuality genes, as indicated by the fact that sisters of these males were able to reproduce asexually. This was assessed by isolating females before they reached adulthood and by keeping them isolated for 30 to 45 days once adult to check for asexual reproduction. We confirmed that the males actually transmitted asexuality-conferring genes by verifying that some of their daughters were also able to reproduce asexually. Sub-lineages in which asexuality was not transmitted (or where results were inconclusive) were discarded. Note that maintaining asexuality throughout these generations was only possible by these selection procedures. Indeed, in every generation, a majority of sub-lineages were discarded, though to prove that discarded females were sexuals would have required pairing them with a genetically distinct male and carrying out paternity tests, which was not done. In each generation, all offspring produced during the asexuality tests were sexed using morphological characters once they reached pre-adulthood. We refer to the proportion of males in these data as the proportion of asexually produced males (α) and used it as a proxy for recombination rate in each generation.

To analyze the sex-ratio data, we fitted 21 likelihood models to the data and assessed their relative fit (Table S7). We used two categories of models: In the first category, “monogenic models” (category A in Table S7), we assumed that recombination rate differences between *Ap2n* and *Akaz* are governed by a single major locus with two alleles. In the second category, “polygenic models” (category B in Table S7), we assumed that these differences are under a polygenic control. In monogenic models, we assumed that F1 are heterozygous (+/-), with the allele for higher recombination (allele +) coming from *Akaz* and the allele for lower recombination (allele -) from *Ap2n*. Hence, in backcross females, there are only two possible recombination genotypes (+/- or +/+), and thus two possible sex-ratio phenotypes in their progeny, p_1 for (+/-) females and p_2 for (+/+) females. In the model we thus considered that offspring sexes were sampled from either of two binomial distributions $B(\Phi n, p_1)$ or $B((1-\Phi)n, p_2)$, with n being the total number of offspring, Φ being the probability to sample from one or the other distribution (i.e., whether an offspring is +/- or +/+), p_1 being the probability to produce a male for (+/-) females and p_2 for (+/+) females, re-parameterized such that $p_2 = p_1+f(1-p_1)$, where parameter f is defined within a constant range $0 < f < 1$. Different variants of this model considered that parameters were either constant or varied across generations, in a linear or stepwise manner (the latter corresponding to an initial increase in sex ratio, from generation F1 to backcross 1, followed by a plateau). The polygenic models assumed that offspring sexes were also binomial samples, but the proportion of this binomial was assumed to vary among females at each generation. Specifically, this proportion was assumed to follow

a Beta distribution (as in BetaBinomial models often used to model extra-binomial variation for overdispersed data; Harrison, 2015). In this way, different females could exhibit different offspring sex-ratio (i.e., different rates of rare male production) in a continuous way, as if the trait was polygenic and continuously varying among females at a given generation. In these models, the Beta distribution was parameterized with its mean (μ, the average offspring sex-ratio among females) and variance (V, the heterogeneity among females for this sex-ratio). Different constraints on parameter values were investigated to determine if the mean (μ) and variance (V) of sex-ratio changed across backcross generations following a linear, step, quadratic and/or cubic variation. For each model, the likelihood of the data was written and then fitted using Mathematica V.9.0 (Wolfram Research, 2012). Model comparison was done using quasi Akaike information criterion (QAIC). The best model (B.6) explained 44 % of the total deviance and was moderately overdispersed ($\hat{c}$ = 1.7).

Given the proportion of males observed among asexual offspring of a female (α), it is possible to compute the proportion of heterozygosity loss (k) that would explain the observed rate of male production

$$\boldsymbol{\alpha = \frac{(k/2)}{(1-k)+(k/2)}} \quad \textbf{(1)}$$

The numerator represents the proportion of males in the zygotes. This is given by the rate of the loss of heterozygosity divided by two, as only half of the homozygous individuals are ZZ and thus, males. This proportion is divided by the total number of surviving individuals, which is given by one minus the loss of heterozygosity, representing the proportion of heterogametic female individuals (ZW, assuming the WW genotype generated by recombination is lethal), plus the males (ZZ). This ratio provides the proportion of male offspring produced given a level k of heterozygosity loss. Under automixis, it is possible to compute the expected loss of heterozygosity for a given genetic distance from the centromere (Svendsen et al., 2015). Under central fusion automixis, this LOH rate is zero at the centromere and increases up to 33 % for a large genetic distance. When sexual genome was introgressed in asexual *Artemia*, α reached the value of 0.27, which gives an estimate of 42% loss of heterozygosity. This LOH rate exceeds the maximal LOH rate expected under central fusion automixis (i.e., for a locus at a large genetic distance from the centromere), although not significantly (Figure 3).

References of the supplementary materials

Harrison, X. A. (2015). A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. *PeerJ*, *2015*(7). https://doi.org/10.7717/peerj.1114

Maccari, M., Amat, F., & Gómez, A. (2013). Origin and Genetic Diversity of Diploid Parthenogenetic Artemia in Eurasia. *PLoS ONE*, *8*(12). https://doi.org/10.1371/Citation

Muñoz, J., Green, A. J., Figuerola, J., Amat, F., & Rico, C. (2009). Characterization of polymorphic microsatellite markers in the brine shrimp Artemia (Branchiopoda, Anostraca). *Molecular Ecology Resources*, *9*(2), 547–550. https://doi.org/10.1111/j.1755-0998.2008.02360.x

Nougué, O., Flaven, E., Jabbour-Zahab, R., Rode, N. O., Dubois, M. P., & Lenormand, T. (2015). Characterization of nine new polymorphic microsatellite markers in *Artemia parthenogenetica*. *Biochemical Systematics and Ecology*, *58*, 59–63.

Nougué, O., Rode, N. O., Jabbour-zahab, R., Ségard, A., Chevin, L. M., Haag, C. R., & Lenormand, T. (2015). Automixis in Artemia: Solving a century-old controversy. *Journal of Evolutionary Biology*, *28*(12), 2337–2348. https://doi.org/10.1111/jeb.12757

Svendsen, N., Reisser, C. M. O., Dukić, M., Thuillier, V., Ségard, A., Liautard-Haag, C., Fasel, D., Hürlimann, E., Lenormand, T., Galimov, Y., & Haag, C. R. (2015). Uncovering cryptic asexuality in *Daphnia magna* by RAD sequencing. *Genetics*, *201*(3), 1143–1155. https://doi.org/10.1534/genetics.115.179879

Wolfram Research, I. (2012). *Mathematica* (9.0). Wolfram Research, Inc.

Supplementary tables and figures

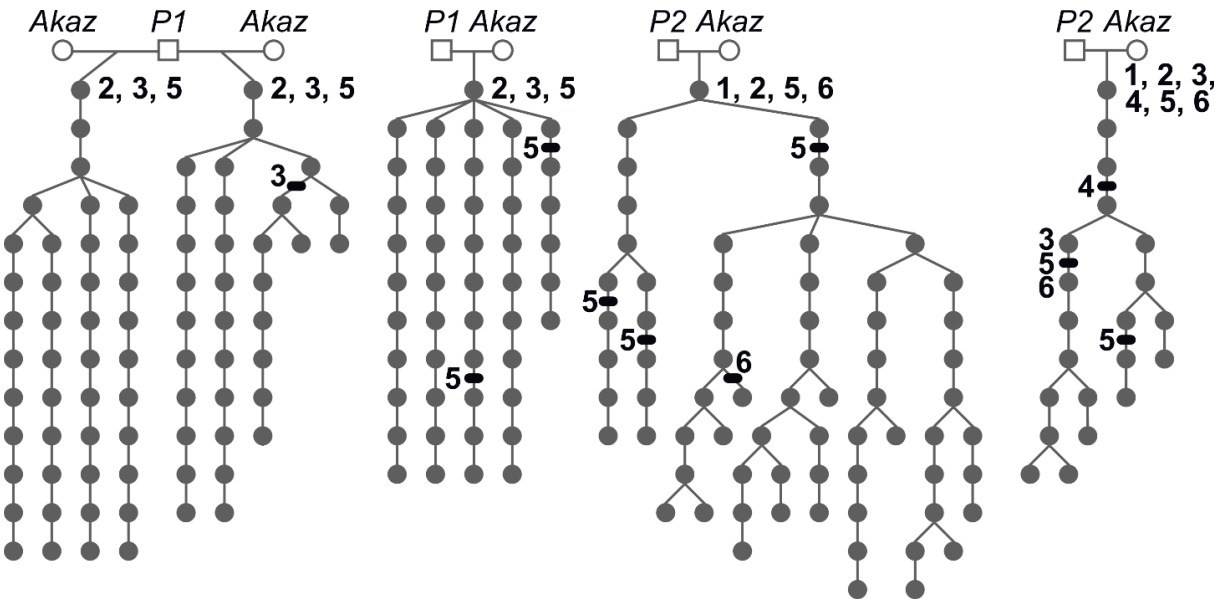


Figure S1. Hybrid asexual lineages used in experiment 1 and LOH events. The figure shows the genealogy of all lineages and sub-lineages starting from *P1* or *P2* rare males (white squares) crossed with *Akaz* females (white circles). Each grey dot corresponds to an F1 female and asexually produced further-generation descendant of the F1 females. Some lineages had not reached 13 generations at the time of the analysis or they were stopped earlier. Informative loci (1 to 6) that were initially heterozygous are indicated next to each F1 female. The seventh locus is not represented due to its ambiguity. LOH events are represented by black bars with the corresponding locus number next to them.

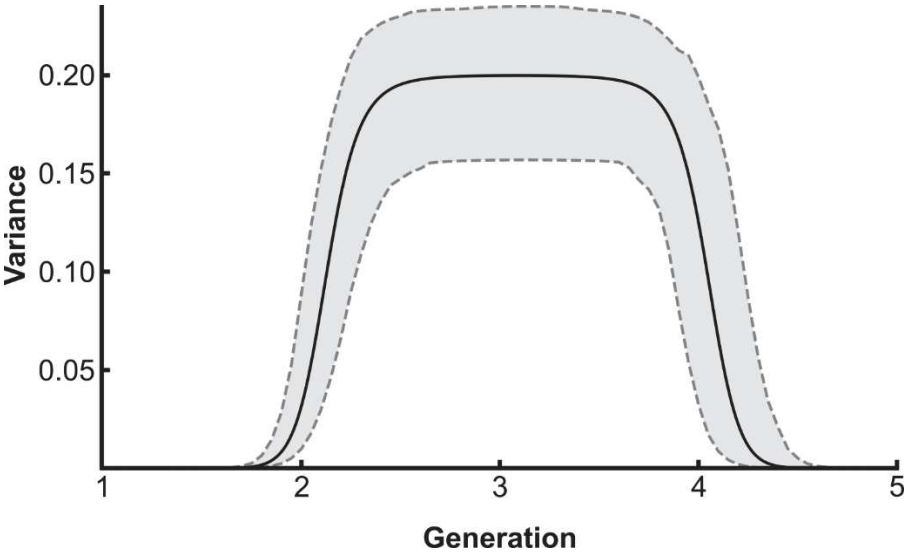


Figure S2. Variance in rare male production among the asexual females generated by hybridization and backcross of experiment 5. The thick black line represents the variance as estimated by the best model and the grey dotted lines represent the support limits.

Name	Model	ΔAICc
1	$p_i \sim$ constant	19.2
2	$p_i \sim gen$	19.5
3	$p_i \sim pop$	17.7
4	$p_i \sim loc$	11.0
5	$\boldsymbol{p_i \sim loc + pop}$	**0**
6	$p_i \sim loc * pop$	9.5
7	$p_i \sim loc + gen + pop$	2.2
8	$p_i \sim loc * gen + pop$	11.3
9	$p_i \sim loc + gen * pop$	3.6

Table S1. Models fitted to the data of experiment 1 and ΔAICc. p_i represents the LOH rate, "*gen*" is the number of generations since the F1, "*pop*" is the population of origin of the rare male (*P1* or *P2*), and "*loc*" is the locus. The best model, model 5, is highlighted in bold. ΔAICc are given relative to this model.

		Cross	
		P1x*Akaz*	P2x*Akaz*
Number of F1 offspring	Males	61	84
	Females	51	85
Isolation period	Reproduction	39	38
	No reproduction	5	47
Pairing with *Akaz* males	Asexual reproduction	19	20
	Sexual reproduction	2	9
	Mixed reproduction	5	4
	NA	2	2

Table S2. Reproductive mode of F1 hybrid females in each of the two crosses of experiment 2. The first section (Number of F1 offspring) indicates the number of male and female F1 individuals produced by each cross. The second section (Isolation period) refers to the number of females that reproduced or not during the isolation period (30 days for the *P1*x*Akaz* cross, 14 days for the *P2*x*Akaz* cross), conditional on survival for the entire isolation period. The third section (Pairing with *Akaz* males) indicates the number of F1 females that produced clutches only by asexual reproduction, only by sexual reproduction or that produced subsequent clutches by both modes of reproduction ('mixed') when paired with *Akaz* males. NA refers to the number of F1 females that produced clutches when paired with *Akaz* males, but for which the mode of reproduction could not reliably be assessed. The females that did not reproduce in the presence of a male are not included in the third section. Of the females that only produced sexual clutches wile paired, both females from the *P1xAkaz* cross and four of the nine females from the *P2xAkaz* cross did reproduce asexually while isolated. These females thus had a mixed reproductive mode.

Model	Proportion of mixed F1 females			Proportion of sexually produced clutches in mixed F1 females						ΔAICc
				Constant		Beta				
	1	*q*	*q*~cross	*p*	*p*~cross	*a*	*β*	*a*~cross	*β*~cross	
A.1	X			X						19.8
A.2	X				X					12.0
B.1		X		X						7.3
B.2		***q* = 0.44**			***P1*: *p* = 0.29; *P2*: *p* = 0.79**					**0**
B.3			X	X						6.5
B.4			X		X					2.1
C.1		X						X	X	3.9
C.2			X			X	X			3.6
C.3		***q* = 0.52**						***P1* Mean *p* = 0.25 ; *P2* Mean *p* = 0.70 ; Variance in *p* = 0.05**		**1.1**

Table S3. Likelihood models fitted to the reproductive mode data of F1 hybrid females in experiment 2 and their ΔAICc. Models of category A allow for mixed females only, while models from categories B and C allow for asexual and mixed females, with *q* being the proportion of mixed females. In mixed females, *p* is the proportion of sexually produced clutches, and *a* and *β* are the parameters of the beta distribution modelling the distribution of *p*, when applicable. The effect of the cross (*P1xAkaz* or *P2xAkaz*) is represented by *~cross*. The best model (in bold) is B.2. The parameter estimates of this model are indicated, with *P1 and P2* being F1 females from the *P1* and *P2* crosses, respectively. Model C.3 (also in bold) fits the data only slightly worse, with ΔAICc < 2 compared to B.2. Its interpretation is however very close, simply adding some biological heterogeneity in the rate of asexuality among mixed females (their fitted average is very close to the point estimate in model B.2). For each model, the cross X indicates parameters that are estimated in each model. Empty cells indicate parameters that were not estimated and greyed-out zones indicate that the parameters are not relevant for the model.

Candidate clutch number	Clutch produced by	N females isolated	N female that reproduced
1	Asexual reproduction	1	1
2	Asexual reproduction	1	1
3	Asexual reproduction	2	2
4	Asexual reproduction	1	1
5	Asexual reproduction	1	1
6	Asexual reproduction	6	6
7	Asexual reproduction	3	0
8	Sexual reproduction	3	0
9	Sexual reproduction	1	0
10	Sexual reproduction	3	0
11	**Sexual reproduction**	**2**	**2**
12	Sexual reproduction	1	0

Table S4. Reproduction of females isolated from each candidate clutch in experiment 3. Clutches later identified (by paternity analysis) as sexual are shown in grey. Females from clutch 11 (in bold) were able to reproduce asexually (production of cysts).

Microsatellite marker	AP01		AP02		AP03		Offspring produced by
P1 female 1 (potential mother)	165	0	221	224	0	0	
P1 female 2 (potential mother)	165	0	221	224	0	0	
Akaz male 1 (potential father)	185	185	221	221	202	214	
Akaz male 2 (potential father)	187	187	221	236	214	214	
Akaz male 3 (potential father)	187	187	221	236	214	214	
Akaz male 4 (potential father)	187	187	221	221	214	214	
Akaz male 5 (potential father)	187	187	221	236	214	214	
Male offspring 1	165	0	221	224	0	0	Asexual reproduction
Male offspring 2	**165**	**185**	**221**	**221**	**202**	**0**	**Sexual reproduction**
Male offspring 3	165	0	221	224	0	0	Asexual reproduction
Male offspring 4	**185**	**0**	**221**	**224**	**202**	**0**	**Sexual reproduction**
Male offspring 5	**185**	**0**	**221**	**224**	**214**	**0**	**Sexual reproduction**
Male offspring 6	**165**	**185**	**221**	**224**	**202**	**0**	**Sexual reproduction**
Male offspring 7	165	0	221	224	0	0	Asexual reproduction
Male offspring 8	165	0	221	224	0	0	Asexual reproduction
Female offspring 1	165	0	221	224	0	0	Asexual reproduction
Female offspring 2	165	0	221	224	0	0	Asexual reproduction
Female offspring 3	165	0	221	224	0	0	Asexual reproduction
Female offspring 4	165	0	221	224	0	0	Asexual reproduction
Female offspring 5	165	0	221	224	0	0	Asexual reproduction
Female offspring 6	165	0	221	224	0	0	Asexual reproduction
Female offspring 7	165	0	221	224	0	0	Asexual reproduction
Female offspring 8	165	0	221	224	0	0	Asexual reproduction

Table S5. Microsatellite genotypes of potential parents (*P1* females and *Akaz* males) and male offspring from the *P1* mass-cross in experiment 4. The reproductive mode by which each offspring was produced, according to paternity testing, is indicated in the last column. Males produced sexually are shown in bold. In three of the four sexually produced males, one of the maternal alleles was absent (allele 224 was missing in male offspring 2 at the AP02 locus and allele 165 was missing in male offspring 4 and 5 at the AP01 locus), suggesting their mother produced haploid ovules. The genotype of the fourth male (male offspring 6) was inconclusive regarding ploidy of the ovule, due to null-alleles and shared alleles between males and females.

Generation	N fathers	N females tested	N asexually produced female offspring	N asexually produced male offspring	Sex ratio
F1	1	5	46	3	0.06
BC1	3	12	51	23	0.31
BC2	2	3	23	0	0.00
BC3	2	6	12	5	0.29
BC4	3	4	9	4	0.3

Table S6. Sample sizes of individuals used for crosses and asexuality tests in each generation of experiment 5 and sex ratios of asexually produced offspring. *N* fathers is the number of males of the previous generation used to produce the offspring of a given generation. BC stands for backcross.

Name	Parameters	*df*	ΔQAIC
A.1	p_1[constant], f[constant], Φ[constant]	3	7.8
A.2	p_1[constant], f[constant], Φ[time]	4	8.4
A.3	p_1[time], f[constant], Φ[time]	5	10.1
A.4	p_1[constant], f[constant], Φ[step]	4	6.3
A.5	p_1[step], f[constant], Φ[step]	5	8.3
A.6	p_1[gen], f[constant], Φ[step]	8	7.3
A.7	p_1[time], f[time], Φ[time]	6	12.0
A.8	p_1[constant], f[constant], Φ[gen]	7	7.9
A.9	p_1[gen], f[gen], Φ[gen]	15	15.8
B.1	μ [constant], and V[constant]	2	6.2
B.2	μ [time], and V[time]	4	8.8
B.3	μ [step], and V[step]	4	5.8
B.4	μ [step], and V[constant]	3	6.6
B.5	μ [step], and V[time]	4	7.2
B.6	**μ [step], and V[time^2]**	**5**	**0**
B.7	μ [time], and V[time^2]	5	4.0
B.8	μ [constant], and V[time^2]	4	7.4
B.9	μ [step], and V[gen]	7	4.0
B.10	μ [time^2], and V[time^2]	6	2.9
B.11	**μ [time^3], and V[time^2]**	**7**	**1.8**
B.12	μ [gen], and V[time^2]	8	3.6

Table S7. Likelihood models fitted to the data on the proportion of asexually produced males in experiment 5 and their QAIC. Models of category A are the monogenic models. In these models, we suppose two categories of individuals with different rates of rare male production. these two categories are in proportion Φ and 1- Φ . The rates of rare male production in each categories are fitted using p_1 and f parameters (sup. mat. 6). [constant] indicates that the parameter is assumed to be constant across generations; [time] indicates that the parameter is fitted with an intercept and a linear effect of the number of generation; [step] indicates that the parameter is assumed to take two values, one for generation 1 and another for all subsequent generations; [gen] indicates that the parameter is fitted at different values for each generation. Models of category B are the polygenic models. Different constraints on parameter values were investigated in each case to determine if the mean (μ) and variance (V) of the sex-ratio changed across generations. The codes are the same than for the monogenic models, with, in addition, [time^2] and [time^3] referring to a quadratic and cubic effect of the number of generations on the corresponding parameter, respectively. Model B.6 (in bold) is the best model. Model B.11 (also in bold) fits the data only slightly worse, with ΔQAIC < 2 with respect to B.6. Its interpretation is however similar, with a cubic variation of μ with a qualitatively similar shape as in model B.6.

Exp. no.	Exp. name	Principle	Main results
1	LOH in sex-asex hybrids	Estimate LOH at microsatellite markers in asexually reproducing sex-asex F1 hybrids.	Recombination occurs. Its rate differs depending on markers and origin of the rare male.
2	Reproductive mode of F1 hybrid females	Determination of the reproductive mode of female sex-asex F1 hybrids using isolation tests and paternity testing of progeny from paired females.	Most if not all F1 females can reproduce asexually. Some F1 females can be mixed (i.e., reproduce both sexually and asexually).
3	Contagious asexuality *via* hybrid F1 females	Test of asexual reproduction in sexually produced BC1 offspring, produced by mating between F1 hybrid females with mixed reproduction mode and *Akaz* males.	Mixed F1 females can transmit asexuality to their sexually produced daughters.
4	Rare sex in *Ap2n* females	Mass-cross between *Ap2n* females and *Akaz* sexual males; paternity testing on male offspring.	*Ap2n* females can rarely undergo sexual reproduction, likely through normal meiosis.
5	Estimating recombination rate in automicts	Production of several generations of sex-asex hybrids and backcrosses to *Akaz*, while maintaining asexuality genes; use the rate of rare male production at each generation as a proxy for recombination rate.	Introgression of the *Akaz* genome into *Ap2n* increases the recombination rate. Differences likely caused by selection against recombination in *Ap2n.*

Table S8. Summary of the five experiments conducted in this study and their main results.

Tables S9 and S10 are available **here**.

Chapter 2

Questioning the preeminence of clonality among parthenogenetic animals

The current state of the manuscript is not finished. We are still discussing the organization of the content with the authors.

Questioning the preeminence of clonality among parthenogenetic animals

Loreleï Boyer[1]*, Cécile Molinier[1]*, Thomas Lenormand[1], Christoph R. Haag[1]

[1] CEFE, Univ Montpellier, CNRS, Univ Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France

*Authors contributed equally to this work meaning that they carried out and synthesized the bibliographic work as well as wrote the manuscript.

Abstract

Clonality is a form of asexual reproduction defined as the identity between parents and offspring, barring new mutations. Even though non-clonal forms of asexuality exist, clonality is considered the most common asexual reproductive mode. Likewise, theoretical models of the evolution of asexuality generally assume that it equates clonality. Yet, recent evidence has revealed non-clonal forms of reproduction in several species formerly believed to be clonal, and new theoretical results highlight potentially striking evolutionary differences between clonal and non-clonal asexuals. In fact, the problem may be more global and involve many more taxa. We argue that, for methodological and conceptual reasons, there has been a strong confirmation bias favoring clonality. In this paper, we present the different paths through which clonality can emerge in animals. We review the commonly used evidence for clonality and point out potential confusing factors and perception biases. We find that although many asexuals seem clonal, a large part of them is not strictly clonal. These small discrepancies nevertheless may have important impact. Selection for more clonal reproduction and the possible absence of mitotic parthenogens indicate that, over an evolutionary timescale, clonal species may have been non-clonal. Taken together, these conclusions do not support a preeminent role of clonality in the evolution of asexuality. We thus call for a broader inclusion of non-clonal reproductive modes for a more realistic view of asexuality.

Keywords: Parthenogenesis, apomixis, automixis, cytology, genetics, loss of heterozygosity

Introduction

Asexuality is an uncommon reproductive mode in eukaryotes, where offspring are produced by a single individual without reduction and fusion of gametes. Populations of asexual organisms are often thought to have little or no evolutionary potential due to a lack of genetic diversity. However, absence of genetic diversity is expected only under a specific mode of asexual reproduction: clonality. Clonality is defined by the absence of any genetic differences between parent and offspring, barring mutations. It has been known for long that in asexuals that reproduce through the germline (termed parthenogenesis in animals) non-clonal asexuality exists. Still, clonality is considered the most common Asexual Reproductive Mode (ARM) in eukaryotes in most empirical reviews (Suomalainen 1950; De Meeûs et al. 2007; Sköld et al. 2009).

As a consequence, the vast majority of theoretical models on the evolutionary maintenance of sex contrasts sexuality with clonality, excluding other ARMs. In these models, the switch from sex to asexuality is simple, as if it was caused by a unique mutation (Crow and Kimura 1965; Maynard Smith 1978; Barton and Charlesworth 1998; Otto and Lenormand 2002; Otto 2009; Levitis et al. 2017). More generally, asexuals are also considered clonal in most models examining the consequences of asexuality on population genetics (Crow and Kimura 1965; Maynard Smith 1968; West et al. 1999; Hartfield and Keightley 2012), phylogeny (Birky 1996; Janko 2014), and genetic diversity (Kondrashov 1993; Mark Welch and Meselson 2000). Asexuality is also simplified as clonality when studying: the link between asexuality and polyploidy (Saura et al. 1993), why asexuals tend to have a wider geographic distribution than sexuals (geographic parthenogenesis, Haag and Ebert 2004; Hörandl 2009; Vrijenhoek and Parker 2009) and what role asexuality plays in the success of agronomic pests (Hoffmann et al. 2008).

However, since the emergence of sexual reproduction is tightly linked with the evolution of eukaryotes (Hawes 1963; Otto and Lenormand 2002; Speijer et al. 2015; Lenormand et al. 2016), every parthenogenetic eukaryote species necessarily evolved from sexual reproduction. To summarize, there are four broad categories of transitions from sex to asexuality based on genetic consequences: First, several modifications of meiosis produce unreduced daughter cells, thus resulting in asexuality. These meiosis modifications can cause loss of heterozygosity (LOH), in which case they are non-clonal. 1) Some of these modifications, such as gamete duplication, terminal fusion or suppression of the second meiotic division, generally lead to high LOH and thus cannot be clonal. Others cause intermediary LOH, and can lead to clonality if the mechanism responsible for LOH is suppressed. This is the case for 2) central fusion or suppression of the first meiotic division, where LOH is caused by recombination and 3) premeiotic doubling, where LOH is caused by non-sister pairing (see Box 1). Lastly, asexuality could evolve through a switch from meiosis to 4) mitosis, which is always clonal because it does not cause LOH. Although many asexuals are considered mitotic, this last possibility does not appear to be the most parsimonious route of evolution to asexuality (reviewed in Lenormand et al., 2016).

Hence, most routes toward the evolution of asexuality are through diverse modifications of meiosis, of which only few can lead to clonality. Still, numerous model species are considered clonal based on interpretations of early studies which are rarely questioned (Suomalainen 1950; Bell 1982). These initial sources can however be limited technically or conceptually, leading to errors in ARM identification. When clonality was questioned, it sometimes led to debates (e.g., in diploid Artemia: Nougué et al., 2015, aphids and *Daphnia pulex*: Blackman 1979). Yet, clonality has recently been disproved in several famously clonal species (Bdelloids: Simion et al. 2021; Tran Van et al. 2021, Timema: Schwander 2021), highlighting the fact that both methods and concepts used in identification remain unclear. This, together with the tendency of considering clonality as the "default" ARM, suggests that the perception of asexuality is biased toward clonality.

Considering the diversity of ARMs is important and has major implications. Indeed, the few existing theoretical models incorporating non-clonal asexuality found striking differences in consequences and expectations compared to clonality. Because LOH reveals deleterious alleles, it leads to genetic load. Thus, ARMs with low LOH rates are expected to be more frequent than those with high LOH rates. This can be due to selection for lineages with low LOH or to gradual evolution toward lower LOH, perhaps down to clonality (Engelstädter 2008; Archetti 2010). Therefore, as the asexuals found in nature are the most successful, we can wonder how prevalent clonality has been over time.

In this review, we assessed among parthenogenetic animals whether clonality is as prevalent as reported, how strong the bias toward clonality is, and thus whether the evolution of asexuality is mainly shaped by clonality. It was not our intention to review systematically the vast existing body of literature on asexuals, but we wished to provide the reader with examples that cover a wide diversity of taxa and reflect the different methods used for the identification of ARMs. To this end, we collected parthenogenetic species based on several reviews on asexuality in animals (Suomalainen 1950; Bell 1982; Rabeling and Kronauer 2013; Vershinina and Kuznetsova 2016; van der Kooi et al. 2017; Gokhman and Kuznetsova 2018). Among those, we selected species in which an ARM was identified based on any piece of evidence, including both obligate and facultative asexuals. We specifically investigated whether the species was identified as clonal in the literature, what evidence was used and whether changes or debates over this ARM occurred. We analyzed hundreds of papers from 1940 to this day.

We structured our review by the different ways clonality can arise (see above). We further clarified erroneous methods and misconceptions that have been used as proof of clonality. On this basis, we discuss whether it is still legitimate to presume that the great majority of asexuals are clonal, and consider the implications with respect to the importance of clonality in the evolution.

Main text

Asexual reproductive modes with high LOH

First, we focus on two main mechanisms of parthenogenesis via modified meiosis that generate high LOH. Gamete duplication restores ploidy by duplication of a reduced set of chromosomes after the second meiotic division, thus resulting in complete LOH from parent to offspring. Second, "terminal fusion-like" parthenogenesis (refusion of products of the second meiotic division or suppression of the second meiotic division, see Box 1) leads to complete LOH except in the recombinant parts of chromosomes.

LOH is associated with high potential genetic load. Because these ARMs lead to high LOH, they are expected to be rare (Archetti 2010). Indeed, they are mainly found in tychoparthenogenetic species, which are otherwise sexual species with exceptional events of asexual reproduction. Tychoparthenogenesis with high LOH is found in certain reptiles (*e.g., Thamnophis sp.*, *Varanus sp.* and *Ophiophagus sp.:* Lenk *et al.*, 2005; Watts *et al.*, 2006; Reynolds *et al.*, 2012; Card *et al.*, 2021) and sharks (Chapman et al. 2007; Dudgeon et al. 2017). Nevertheless, ARMs with high LOH are in fact also found in species where asexuality represents a significant part of the life cycle, such as in *Meloidogyne sp.* (Triantaphyllou 1966; van Der Beek et al. 1998), oribatid mites (terminal fusion-like parthenogenesis: Palmer and Norton, 1992), tardigrades (terminal fusion-like parthenogenesis: Ammermann, 1967; Bertolani, 1981; Rebecchi *et al.*, 2003), termites (gamete duplication and terminal fusion-like parthenogenesis: Matsuura, Fujimoto and Goka, 2004; Fournier *et al.*, 2016) and stick insects (gamete duplication: *Bacillus rossius*, Pijnacker, 1969). Thus, these ARMs are not as exceptional as expected. Still, most of these species are not obligate asexuals. Perhaps, the sexual part of their life cycles can compensate the costs of high LOH.

Parthenogenesis with high LOH has sometimes been wrongly inferred based on cytological evidence, due to inverted meiosis. Taberly (1987) concluded to terminal fusion-like parthenogenesis in the oribatid mite *Platynothrus peltifer*, but a genetic study did not concur (Palmer and Norton 1992). It was later proposed that this species had inverted meiosis (Wrensch et al. 1994) and that its ARM was actually equivalent to the central fusion-like parthenogenesis (see following section). Central fusion with inverted meiosis was also suspected in *Archegozoetes longisetosus* (Laumann et al. 2008) after cytological observations contrasted with previous genetic results. Except for inverted meiosis, the interpretation of cytological observations is generally straightforward for this type of ARM because of the important meiosis modifications associated (extra doubling, refusion, suppression of the equational division). For instance, in annelids, Christensen (1960) showed that several species reproduce by suppression of the second meiotic division. Therefore, there does not seem to be a bias toward clonality when cytologically identifying ARMs with high LOH.

The observation of LOH by comparison of genetic markers in asexual females and their offspring is strong evidence against clonality (Pearcy et al. 2006; Engelstädter 2008). It is expected under gamete duplication (complete LOH) and terminal fusion-like parthenogenesis (although heterozygosity can be retained in telomeric markers due to recombination). Thus, identifying such ARMs appears relatively straightforward with this method using a few genetic

markers. However, in these ARMs, heterozygosity is lost over the whole genome in one or a few generations. Therefore, it should be difficult to find informative, *i.e.,* heterozygous markers, after an asexual reproduction event. Once heterozygosity is lost, there would be generally no differences between parents and offspring, and no further LOH could be detected. Nevertheless, this method has proven efficient to identify these ARMs. For instance, using parent-offspring comparison, gamete duplication was identified in termites (Fournier et al. 2016; Hellemans et al. 2019) and terminal fusion in termites (Matsuura et al. 2004; Vargo et al. 2012; Yamamoto and Matsuura 2012; Luchetti et al. 2013) and reptiles (Lenk et al. 2005; Reynolds et al. 2012; Card et al. 2021). The reason why heterozygous markers were found in these species might be that in facultative or cyclical parthenogenesis, sex occurs at least occasionally, which is sufficient to restore heterozygosity.

Gamete duplication and terminal fusion-like parthenogenesis cause high LOH. Despite the high cost they generate, these ARMs are in fact found in numerous species. This could be explained by their life cycles which include sexual events. Non-obligate parthenogenesis also explains why LOH is often easily detected in these species. Still, these ARMs can appear close to clonality between successive asexual generations, as heterozygosity is not restored. This however cannot lead to clonality because recombination could happen, and any new mutation appearing in a generation would likely go through LOH in the next, with important fitness consequences for deleterious mutations.

Central fusion-like parthenogenesis

In modified meiosis where the first division is suppressed or the products of the first division fuse (regrouped under “central fusion-like” parthenogenesis), clonality is attained if paired homologous chromosomes do not recombine or in absence of pairing (Suomalainen et al. 1980, see Box 1). If the first division is suppressed, absence of pairing cytologically resembles mitosis (see next section). However, pairing of homologous chromosomes and recombination can occur. For example, in tardigrades, both pairing and recombination were found in three species (Bertolani and Buonagurelli 1975; Rebecchi et al. 2003). This can be the case also if meiosis I is partial or aborted (Bacci et al. 1961; Cognetti 1961, 1962; Scali et al. 2003). If recombination occurs, heterozygosity is lost from the location of crossing-over to the telomeres, or to the next location of crossing-over. This means that LOH is more likely to happen far from the centromere.

LOH, because it is costly, should not happen frequently in central fusion-like parthenogenesis (Engelstädter 2008; Archetti 2010). Accordingly, reduced LOH was found in several species with these ARMs (diploid *Artemia parthenogenetica*: Chapter 1 of this thesis; *Wasmannia auropunctata*: Rey et al. 2011; Cape honey bees: Goudie et al. 2012; Oldroyd et al. 2021), while to our knowledge there are no obligate parthenogens with this ARM and high LOH rates. LOH reduction can be due to low recombination rates, localization of crossing-overs near the telomeric region of chromosomes (as in *Oenothera sp.*, Ranganath 2008), or simply because recombinants do not survive (as in the Cape honey bee: Baudry et al. 2004; Goudie et al. 2012). Due to low LOH rates, species with these ARMs can be erroneously identified as clonal.

Deciphering whether pairing occurs can be especially challenging in older observations, as it was the case for the tardigrade Ramazzottius oberhaeuseri (Rebecchi and Bertolani 1988). Similarly, the first cytological study of the Amazon molly (Poecilia formosa) falsely concluded to clonality because pairing was undetectable (Rasch and Balsano 1974, rectified by Rasch et al. 1982). Meiosis in which Prophase I is elusive or asynchronous among chromosomes (Bishop 1994; Golubovskaya et al. 2002) can also complexify the detection of pairing. Polyploid asexual Artemia are usually described as clonal. However, Rode et al. (*in press*, this paper is available in the Appendix of the thesis) reinterpreted previous cytological observations, where the number of chromosomes observed decreased succinctly before the division. They concluded that meiosis I is aborted and a brief pairing occurs before meiosis II, which could lead to recombination (and thus non-clonal asexuality). Additionally, detecting if recombination happens can also be difficult. For instance, recombination was not observed in a tardigrade, possibly because it had late pairing (Ammermann 1967). Therefore, certainty that pairing is absent is not guaranteed and mistakes can be made. Furthermore, in the case where pairing is reported, because recombination can be rare, potentially many observations are needed to be certain of clonality. These difficulties cause mistakes in identification that lead to a bias toward clonality.

Genetic methods can provide simpler evidence as they can easily give information on several reproductive events, but these methods rely on the occurrence of informative markers. The probability to observe parent-offspring LOH is increased in genetic markers the further they are from the centromere (Pearcy et al. 2006; Fougeyrollas et al. 2015). It is thus important to account for the chromosomal position of the markers used. Specifically, using centromeric markers for parent-offspring comparison could lead to deduce wrongly clonality. For example, in the parasitoid wasp *Trichogramma cacoeciae*, no LOH was found in one microsatellite marker in an iso-female line, which was interpreted as clonality. One alternative interpretation from the authors is that this species reproduces through central fusion-like parthenogenesis and that the marker may be located in the centromeric region, and thus be unlikely to lose heterozygosity. This could well be the case because this marker was highly heterozygous in wild populations among markers with variable rates of heterozygosity (Vavre et al. 2004). Thus, centromeric markers will be informative but will not show LOH. A further difficulty in obtaining informative markers, might be the development of markers in distal regions of chromosomes as they are constituted of highly repetitive elements (Blackburn 1991; Sohn and Nam 2018).

Variation in heterozygosity is an expected consequence of central fusion-like parthenogenesis with recombination. Due to the pattern of LOH along chromosomes, heterozygosity excess should be lower with increasing distance from the centromere (see Box 1). Based on this expectation, central fusion-like parthenogenesis was inferred in diploid *Artemia*, where F_{IS} was consistently variable among populations (Nougué et al. 2015). Similarly, both central and terminal fusion were detected in *Daphnia magna* thanks to chromosomal patterns of heterozygosity (Svendsen et al. 2015). Heterozygosity patterns on chromosomes are also affected by active selection against LOH at specific regions where it is particularly costly. For example, in the Cape honey bee worker line known as the Clone (although they reproduce by central fusion-like parthenogenesis), there are large regions of

retained heterozygosity, notably around the sex locus, which is lethal if homozygous (Goudie et al. 2012). Under central fusion-like parthenogenesis, we thus expect that there are highly heterozygous and highly homozygous genomic regions. This can also explain why LOH may go undetected in central fusion-like parthenogenesis: In wild populations, markers that are the most likely to lose heterozygosity are probably already homozygous, and thus non-informative in parent-offspring comparisons. Hence, the remaining heterozygous markers are those with the lowest probability of LOH, either because they are centromeric or because they are linked to a recessive deleterious allele. For instance, in *Daphnia magna*, Dukić et al. (2019) did not find any LOH events in iso-female lines, although they used markers evenly distributed along the chromosomes. It is possible that markers where LOH could occur had already lost heterozygosity. In diploid *Artemia parthenogenetica*, no LOH occurred in iso-female populations after tens of generations (Nougué et al. 2015). LOH was later observed due to interpopulation crosses that restored heterozygosity along the chromosome, and especially in regions that would have already lost heterozygosity (Chapter 1). This approach could be used at a larger scale to improve detectability of recombination in central fusion-like parthenogenesis. To conclude, the pattern of LOH along chromosomes in central fusion-like parthenogenesis is recognizable. However, it can bias the identification of ARMs toward clonality, as heterozygous (thus informative) markers will be mainly found in parts of the chromosomes that behave the most clonally.

Rare or localized LOH can thus go undetected, and it is unclear how many markers and parent-offspring comparisons with absence of LOH are sufficient to infer clonality confidently. In the Cape honey bee, although central fusion-like parthenogenesis was identified cytologically (Verma and Ruttner 1983), Moritz and Haberl (1994) observed no LOH in parent-offspring comparison based on DNA fingerprinting on 12 markers. However, subsequent studies based on 101 (Baudry et al. 2004) and 6 microsatellite markers (Goudie et al. 2012) reported several LOH events. Moreover, rare occurrences of LOH can look like, and are often interpreted as, other processes such as mutation, mitotic recombination or gene conversion (Tiedemann et al. 2005; Malysheva et al. 2007). We stress that gene conversion and recombination originate from the same molecular mechanism, so that they cannot be considered as completely distinct mechanisms (Keeney 2001). With the advancements of genomics, it is now easier to identify clonality by genotyping a large number of markers distributed over the length of chromosomes with known physical positions, including the telomeric region (Loxdale and Lushai 2003). However, these methods depend on the quality and resolution of genetic tools (map, assembly) which can be difficult to produce in asexuals. For instance, the first genome assembly for the Bdelloid rotifer *Adineta vaga*, suggested clonality (Flot et al. 2013), but a new assembly allowed the detection of LOH in this species (Simion et al. 2021).

In central fusion-like parthenogenesis, recombination, which can be observed cytologically, causes LOH. This generates a pattern of heterozygosity, which can complicate the detection of LOH using genetic markers. Regions that are the most likely to lose heterozygosity tend to be already homozygous and thus non-informative, so that the only informative regions are those that are the least likely to lose heterozygosity. When recombination is rare, detecting LOH is even more difficult and necessitates multiple observations. Genomics could provide a solution to these problems, although it may be complex

in non-model species. If recombination is totally suppressed, this ARM is clonal. This can be achieved by suppression of recombination or by suppression of pairing. The latter, in the case where the first meiotic division is suppressed, will result in a modified meiosis very similar to mitosis.

Mitosis

Parthenogenesis through mitosis undoubtedly leads to clonality. Historically, it was assumed that asexuality exclusively arises by mitosis, however this has been clearly refuted. Although many asexuals are still considered mitotic (Levitis et al. 2017), there is actually no conclusive evidence for parthenogenesis through mitosis in any animal system (Archetti 2010). Still, parthenogenetic mechanisms with one equational division (equivalent to suppression of the first division or mitosis, Box 1) have been associated with mitosis even when pairing was observed, although pairing is a meiotic process (see previous section). For example, *Daphnia pulex* was described as "apomictic" (see Box 1), which was interpreted as mitotic reproduction, although several cytological observations described the first steps of meiosis including pairing (Ojima 1954; Bacci et al. 1961; Zaffagnini and Sabelli 1972). Later on, modified meiosis with pairing was again reported in this species (Hiruta et al. 2010), indicating central fusion-like parthenogenesis with no or very low levels of recombination. Therefore, mitotic parthenogenesis is still thought to be common due to conceptual or vocabulary biases, although now there is no clear indication that mitotic parthenogenesis exists in animals.

Some animals indeed reproduce through one equational division with no pairing (several species of *Meloidogyne*: Marais et al. 1991; Van Der Beek et al. 1998; Janssen et al. 2017; tardigrades: Bertolani 1971, 1973; Bertolani et al. 1987; Rebecchi and Bertolani 1988, 1999; Rebecchi 1991; Guidetti et al. 2019; gastropodes: Mattox 1937; Dougherty 1989; Hemiptera: Nokkala et al. 2008, 2017; one species of Psocoptera: Nokkala and Golub 2006). In these cases, mitotic and central fusion-like parthenogenesis are undistinguishable based on cytological observations. However, numerous cytological remnants can be observed, indicating the underlying cellular process is meiotic rather than mitotic. For instance, in *Dendrobaena octaedra*, no pairing is generally reported (Omodeo 1955; Hongell and Terhivuo 1989) but Casellato and Rodighiero (1972) observed some pairing in one sample. The extruding of a polar body (Acarida: Heinemann and Hughes 1969; *Diploscapter pachys*: Fradin et al. 2017, *Daphnia*: Zaffagnini 1987), the observation of lampbrush chromosomes, and the elaboration of nucleolar ribonucleoproteins (*Poecilia formosa*: Monaco et al. 1984) are other cytological features normally found in meiotic oogenesis. Similarly, the activity of genes specific to meiosis, as was reported in a nematode (Fradin et al. 2017), provides indication on the meiotic mechanism even if some meiotic genes are missing. These traces of meiotic processes constitute evidence against mitosis. Moreover, in gynogenetic animals, oogenesis is triggered by sperm, whose function is to resume arrested meiosis (which always occurs during meiotic divisions specifically to prevent any mitotic cleavage, Lenormand et al. 2016). This means that gynogenetic parthenogenesis is, too, unlikely to be mitotic. Indications and clues on the underlying reproductive mechanism in possibly mitotic species, when found, all indicate modified meiosis rather than mitosis.

Once believed to be the primary ARM, mitosis is still considered a very common parthenogenetic mode. However, it can be confused cytologically with modified meiosis where the first division is suppressed (central fusion-like parthenogenesis). In these cases, small remnants of the original mechanism detected cytologically or with molecular biology are often found, and they always indicate meiosis. Whether mitotic parthenogenesis actually exists in animals is therefore still an open question.

Premeiotic doubling

In premeiotic doubling, chromosomes go through an extra replication, generally before meiosis I. During meiosis I, pairing occurs either between chromosomes originating from the same replication (sister pairing) or homologous chromosomes (non-sister pairing). Non-sister pairing can lead to LOH because it allows sister chromosomes to segregate together (with a probability of 50 %). Recombination between homologous chromosomes reverses the effect by canceling or causing LOH in the recombinant part (Archetti 2010). Sister pairing results in complete retention of heterozygosity, regardless of segregation and recombination (Uzzell 1970). Therefore, premeiotic doubling with exclusive sister pairing is clonal (see Box 1).

A general assumption is that sister chromosomes always pair, because they are more similar (Macgregor and Uzzell 1964; Uzzell and Goldblatt 1967) or, since this ARM often emerges from hybridization, because pairing of homeologous chromosomes (from the two parental species) is impossible (see following section). That is why this ARM is often associated with clonality, without necessarily confirming that sister pairing actually occurs. However, even though sister pairing is expected to be more frequent due to the costs of LOH, exclusive sister pairing could cause mechanistic problems regarding DSB repair (Archetti 2010). Thus, the tendency to consider species with premeiotic doubling as clonal may be erroneous.

Many species with premeiotic doubling were identified as clonal with no evidence, because deciphering sister vs. non-sister pairing was originally near impossible. An exception might be the grasshopper *Warramaba virgo*, in which one chromosome pair was structurally heterozygous, making it possible to decipher which type of pairing occurred. As there was consistent sister pairing of the chromosome, this species was identified as clonal (White et al. 1963). More recently developed cytological tools such as genomic and fluorescence in situ hybridization (GISH and FISH) allow to directly decipher between sister and non-sister pairing by marking each set of sister chromosomes. Using this method, non-sister pairing was detected in the hybrid salamander *Ambystoma laterale X A. jeffersonianum* (Bi and Bogart 2006; Lutes et al. 2010). However, it cannot always apply to every chromosome of a species. For instance, sister-pairing was found in several chromosomes and interpreted as clonality in the lizard *Apidoscelis* (Lutes et al. 2010; Newton et al. 2016) and the fish *Misgurnus anguillicaudatus* (Kuroda et al. 2018). Still, it is unclear whether all chromosomes should behave the same, and hence whether the type of pairing should be identified for each chromosome to conclude on the clonality of a species. The existence of random pairing was proposed (Archetti 2010) and it was suggested that both sister and non-sister pairing occurred in *A. laterale X A. jeffersonianum* (Bogart 2003). Furthermore, non-sister pairing perhaps happens rarely in species for which only sister-pairing has been found, which would not result in strict clonality. To conclude, in

premeiotic doubling, clonality is often inferred with no cytological evidence for sister-pairing or based on few observations of some chromosomes. These conclusions might not be correct, and could constitute an important bias toward clonality.

Chromosome-specific or rare events of non-sister pairing can be more easily detected by parent-offspring genotype comparisons over several regions of the genome. If both sister and non-sister pairing occur, marker positions impact the detectability of LOH, which is maximal if markers are distributed in each pair of chromosomes. Few studies have performed parent-offspring genetic comparisons in species with premeiotic doubling. No parent-offspring differences were detected with DNA fingerprints and microsatellites in *Misgurnus anguillicaudatus,* although marker positions were unknown (Momotani et al. 2002; Itono et al. 2006). More often, because asexuals with this ARM tend to be hybrids, genotyping of specific markers that should be heterozygous based on parental species genotypes are used (Heppich et al. 1982; Dawley et al. 1987; Bogart and Klemens 1997; Tiedemann et al. 2005). However, this method is less reliable as for instance homozygosity at these alleles could be due to either mutation or recombination (Honeycutt and Wilkinson 1989). Although they could provide helpful evidence, genetic methods have only scarcely been employed in the study of this ARM, perhaps stemming from the fact that the possibility of non-clonal premeiotic doubling is not well known. Therefore, the prevalence of clonality in this ARM is still unclear.

Lastly, while it is often little discussed, the exact timing of doubling relative to the process of meiosis I can have dramatically different genetic consequences. Indeed, if doubling is not premeiotic and occurs after chromosome pairing, recombination will cause LOH as in central fusion-like parthenogenesis (see corresponding section). Such a mechanism is rare, but was observed cytologically in stick insects (Scali et al. 1995; Marescalchi and Scali 2001).

In conclusion, due to conceptual bias, most parthenogens reproducing through premeiotic doubling are considered clonal, based on limited evidence. Theoretical and empirical evidence indicate that premeiotic doubling might not be clonal in many cases, although we lack sufficient information for most species with this ARM.

Erroneous methods and misconceptions

Expectations under strict clonality

Phylogenetics and population genetics expectations have been used extensively as evidence for clonality. Under strict and obligate clonality, because other mechanisms never or very rarely intervene, mutation accumulation and genetic drift should generate heterozygosity at all polymorphic loci (Balloux et al. 2003; De Meeûs and Balloux 2005; De Meeûs et al. 2007). Hence, high heterozygosity is often used as evidence for clonality. In *Campeloma decisum*, Johnson (1992) suggested that populations with high heterozygosity rates should reproduce clonally whereas the ones showing fixed homozygosity should reproduce by a non-clonal ARM. An extension of this expectation is the Meselson effect, which causes entire homologous regions to diverge completely in the long term. This specifically tests whether the mutation rate is higher than the LOH rate (Mark Welch and Meselson 2000). Thus, by comparing intra- and inter-population haplotypes divergence, it has been used as evidence for clonality. Another expectation under strict clonality is complete linkage between the

mitochondrial and nuclear genomes shortly after the emergence of clonality. This means that the mitochondrial and nuclear genomes evolve at the same pace (*i.e.,* diversity is produced by mutations only). This should lead to the congruence of mitochondrial and nuclear phylogenies; which was used as evidence for clonality in *Heterocypris incongruens* populations (Chaplin and Hebert 1997).

Under clonality, high heterozygosity is a long-term expectation because it is generated by mutations. It is thus particularly sensitive to confounding effects and restriction by other rare mechanisms. Several confounding factors may cause the expected high heterozygosity and even the Meselson effect, including in non-clonal parthenogens. These are hybridization, polyploidy and gene duplication (Ceplitis 2003; Simon et al. 2003; Mark Welch et al. 2009; Hollister et al. 2019). For instance, the Meselson effect was incorrectly inferred in the Bdelloid rotifers in which Mark Welch and Meselson (2000) detected high allelic divergence. In fact, Mark Welch et al. (2008) later discovered this taxon is paleotetraploid, so that the high divergence measured was actually between anciently but not presently homologous chromosomes. Furthermore, even under clonal reproduction, biological processes such as gene conversion and mitotic recombination, if they happen more frequently than mutations, may prevent sufficient mutation accumulation from generating high heterozygosity. Such a high rate of gene conversion and recombination relative to the rate of mutation accumulation was reported in the obligate asexual *Daphnia pulex* (Tucker et al. 2013). In putatively clonal species, low heterozygosity was found in several species and explained by gene conversion (*Darwinula stevensoni*; Tran Van et al. 2021) or due to rapid sexual-asexual transition and population expansion (*Heterocypris incongruens*; Rossi et al. 2006). This can counter the Meselson effect (Hartfield 2016), even in fissiparous (thus truly mitotic) Nemerta (Ament-Velásquez et al. 2016). Similarly, mito-nuclear incongruence was found in several asexuals reported as clonal, which was explained by accumulation of mutations in a recently derived mitochondrial haplotype (Lorenzo-Carballa et al. 2012) or rare sex events (Schön et al. 2000). Finally, both heterozygosity and mito-nuclear congruence are extreme expectations that are not reliable to assess clonality.

The above predictions under clonality still constitute interesting tools to investigate the consequences of clonality in the long term. A promising direction is to study how ARMs affect different parts of the genome. For instance, specific patterns of F_{IS} along the chromosome are expected under central fusion-like parthenogenesis (see corresponding section). Heterogeneous patterns can also be indicative of a more or less clonal history within the genome: for instance, in one oribatid mite, the Meselson effect was found, but with various intensities relative to certain regions of the genome, that may reflect different histories of LOH rates (Brandt et al. 2021). Thus, using whole genome sequencing, it is possible to find specific regions that have been clonal for a long time in ancient asexuals, although this does not mean that the species has been reproducing clonally for a long time.

Other indirect methods used to test for clonality come from experimentation. The absence of response to artificial selection on an iso-female line has been interpreted as clonality (this was widely used in aphids, reviewed in Blackman 1979). The certainty of this method depends on the number of generations observed and on the genetic basis for the observed trait. Moreover, response to selection is simply a proxy for the renewal of diversity, which is not a good indicator

for clonality, as other reproductive modes generate low diversity, such as selfing or sex in an inbred population. Eventually in aphids, even when variation was observed, it was interpreted as epigenetics processes (Blackman 1979). Secondly, success in grafting tissue of an asexual female on her offspring was also occasionally used to demonstrate clonality (Maslin 1967; Cuellar and Smart 1977; Dawley et al. 1987; Goddard and Dawley 1990; Cordes and Walker 2003, 2006). However, it is not reliable to detect strict clonality as we know that non-clonal grafts can be accepted. All the above-mentioned methodologies were initially developed to differentiate asexual from sexual modes of reproduction. It is clear that their designs were built under the assumption that asexuality was obligate and equivalent to clonality. However, they are not accurate enough to discriminate among all the possible ARMs, nor to be used as evidence for clonality.

Inference of clonality based on erroneous assumptions

In addition to these predictions, clonality is sometimes inferred based on certain features of the parthenogenetic species. Such features are hybridization and polyploidy, which are assumed to cause clonality. These characteristics generally co-occur with asexuality but no clear causality has been established between them so far. The "Balance hypothesis" proposes that hybridization between sexual species with a specific divergence level could directly induce (mitotic) clonality by breaking down meiosis (Moritz et al. 1989). However, there is no evidence of this phenomenon, and the mechanistic process that would be underlying it is unclear. Another assumption is that hybrid origin results in incorrect pairing or segregation that can only be resolved by skipping meiosis, hence reproducing clonally through mitosis (Nokkala et al. 2008). For the same reasons, high structural heterozygosity of homologous chromosomes and other structural anomalies are also thought to lead to clonality (aphids: Normark 1999; Darwinulid ostracods: Tétart 1978; Schön et al. 1998. Anisopolyploid asexuals (with uneven ploidy, *e.g.,* 3n, 5n) are, likewise, strongly assumed to only reproduce clonally (Suomalainen 1950; Bell 1982; Rasch et al. 1982), even though Stalker (1956) advocated that meiosis could occur in triploids. To a lesser extent, even in polyploids with an even number of chromosomes, segregation issues due to entanglement with several pairs have been demonstrated (Lloyd and Bomblies 2016). Many triploids and hybrids indeed appear clonal (*Potamopyrgus antipodarum*: Phillips and Lambert 1989; the tardigrades *Ramazzottius oberhaeuseri*: Rebecchi and Bertolani 1988; *Macrobiotus hufelandi*: Bertolani 1973; *Paramacrobiotus richtersi*: Bertolani 1971; Guidetti et al. 2019). However, polyploid and hybrid asexuals are not all clonal (premeiotic doubling with non-sister pairing: *Ambystoma sp.*, Bi and Bogart 2006; central fusion-like parthenogenesis with recombination: *Carassius auratus*, Zhang et al. 1992 and *Bacillus atticus*, Scali et al. 2003). Furthermore, the detection of LOH in non-clonal polyploids may be particularly difficult, because their LOH rate could be low to null (as the processes normally leading to LOH could still result in a heterozygous genotype). Thus, such species may be identified mistakenly as clonal. The same could happen in non-clonal hybrids because genetic incompatibilities, revealed by LOH events, could lead to the death of recombinants, resulting in an underestimation of LOH rate. Therefore, many more supposedly clonal polyploids and asexuals could be in fact non-clonal (as suggested for polyploid *Artemia* in Rode et al. *in press*). Perhaps, these meiosis modifications even provide an escape to the pairing issues stemming from polyploidy and hybridization by avoiding or cancelling segregation.

Lastly, an assumption that biases the identification of asexuals toward clonality is that ARMs are thought to be shared within taxa. Thus, it is often suggested that a species' ARM is the same as that found in a related species (usually, clonality). For example, clonality was suggested for all non-marine ostracods (Butlin et al. 1998) and oribatid mites (Laumann et al. 2008), based on evidence for only a few species. Clonality was extended in the same manner from *Daphnia magna* (inferred from stability in three allozymes, (Hebert and Ward 1972) to *Daphnia pulex* (Blackman 1979). Similarly, in asexual Lepidoptera, clonality is often inferred on the basis that females in this taxon tend to be achiasmatic (Lokki et al. 1975). However, this was questioned by the finding of female recombination in related species (Elzinga et al. 2013). Perhaps the most extreme example of this "taxonomic group effect" is in vertebrates, in which clonality is considered ubiquitous (Cuellar 1974), which has led to infer clonality falsely in several vertebrates, which turned out to be non-clonal (*Poecilia formosa*: Rasch et al. 1982, *Darevskia armeniaca*: Kupriyanova et al. 2021, *Carassius auratus langsdorfi*: Zhang et al. 1992). Hence, parthenogenesis seems to arise in diverse ways within taxa, and may be a much less conserved trait than has been suggested (chapter 3 of this thesis). In fact, within a given phylum or order, several ARMs may be present. For instance, in termites (Matsuura et al. 2004; Fougeyrollas et al. 2015; Fournier et al. 2016), tardigrades (Ammermann 1967; Bertolani 1981; Rebecchi and Bertolani 1988; Rebecchi et al. 2003; Bergmann et al. 2018) and oribatid mites (Peacock and Weidmann 1961; Taberly 1987; Palmer and Norton 1992; Laumann et al. 2008), a diversity of clonal and non-clonal ARMs was identified. ARM variation is also observed within the same genera (*Meloidogyne sp.*: Castagnone-Sereno et al. 2013, subspecies (*Carassius auratus gibelio and C. auratus langsdorfii*: Emelyanova O.V. 1984; Yamashita et al. 1993; Zhang et al. 2015), and species (*Meloidogyne hapla*: Triantaphyllou 1966). A single individual can reproduce through different modes with facultative parthenogenesis (*Daphnia*: Decaestecker et al. 2009) and rare sex (*Timema*: Schwander 2021, *Artemia*: Chapter 1, *Solenobia triquetrella*: Seiler 1966). Moreover, variation in ARM was reported within single genotype lineages of *Daphnia magna* (Svendsen et al. 2015). This suggests intra-individual variation in ARM could be found. Given the extent of diversity in asexual reproduction even at a small taxonomic scale, it is not reliable to infer ARMs based on what was found in related species or populations, as this may lead to interpretation bias. Questioning the ARM of species presumed clonal may reveal the existence of more diversity and numerous non-clonal asexuals.

Clonality has been inferred many times by testing for expectations that were either too strict and prone to confounding factors, or not accurate enough. Clonality has also been suggested for many species based on incorrect assumptions related to their characteristics or the ARMs of related species. All these means to identify clonality are unreliable and cannot be used as evidence. This means that a great part of supposedly clonal asexuals may in fact reproduce through non-clonal ARMs (Figure 1).

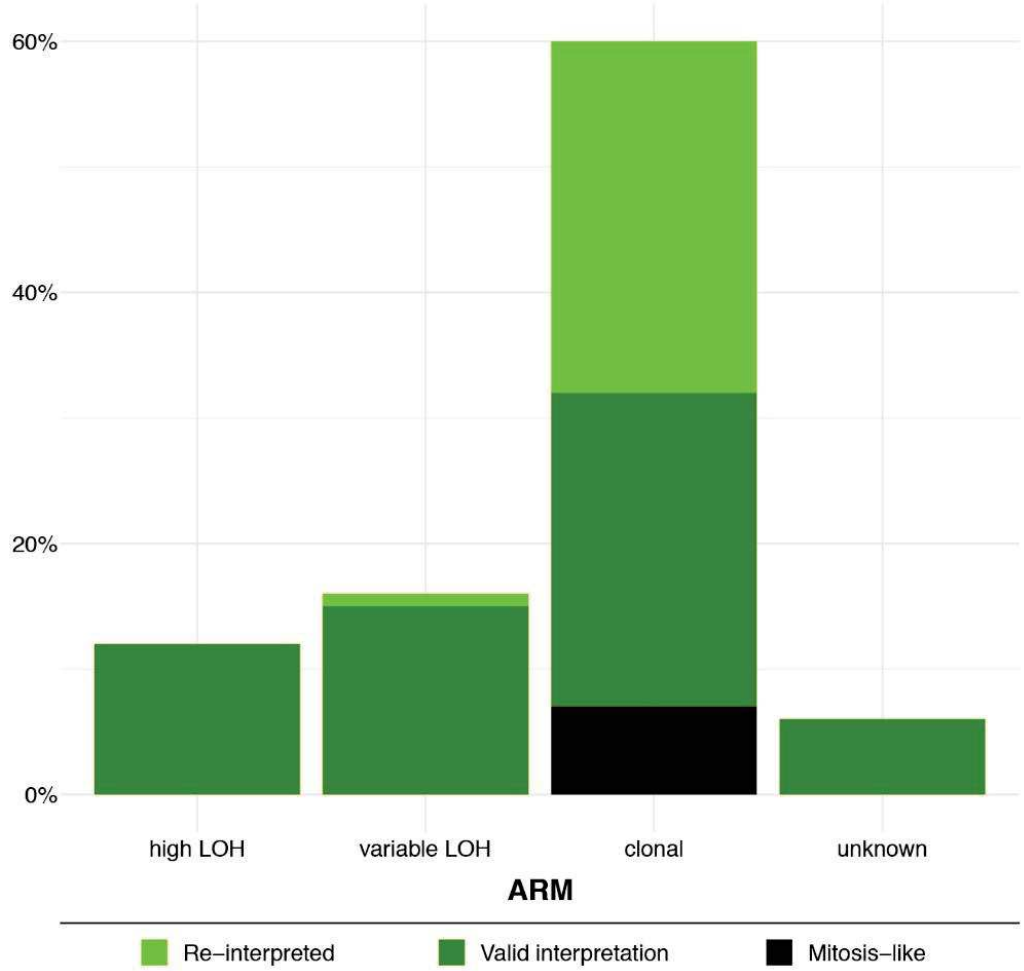


Figure 1. Distribution of the 90 species investigated according to asexual reproductive modes (high LOH ARM, variable LOH non-clonal ARM, clonal, or unknown) based on the literature. Note that four species were each subdivided into two groups in which different ARMs were identified. Light green represents species for which we re-interpreted the ARM, either because the evidence proposed was not conclusive or because the interpretation was erroneous. Dark green represents species with conclusive evidence interpreted correctly, for the identified ARM. In black are species for which there is cytological evidence that pairing does not occur before equational division, suggesting mitosis. Note that this figure is based on a subset of investigated species (supplementary table), to be completed.

Prevalence of clonality

Among our sample of asexual animals investigated (90 species), the ARM of some species is still unknown according to the literature (6.4 %, Figure 1). Over all the other species (for which an ARM is reported), those that are presented as clonal represent about 68.2 % (Figure 1; "clonal"). However, as we discussed over the previous sections, it is not always possible to conclude with certainty that a species is clonal, due to inconclusive evidence or bias in interpretation. Thus, when only accounting for conclusive evidence, interpreted correctly (*i.e.,* following our indications), the proportion of clonal species falls to 54.2 %. According to predictions (Archetti 2010), we actually found that parthenogenetic animals reproduce mainly through ARMs with genetic consequences other than complete LOH, which can be equivalent

or close to clonality (79.7 %, Figure 1; “variable LOH” + “clonal”). This still means that the number of species reproducing through gamete duplication or terminal fusion is surprisingly high considering the high LOH associated with these ARMs. As suggested in the first section, this could be explained by the fact that most of these species are not obligate asexuals. Finally, over all species included, asexuals with no evidence against reproduction through mitosis are in fact very rare (7.4 %), meaning that the great majority of asexual animals reproduce through modified meiosis.

Discussion

In this review, we investigated how common clonality is in parthenogens according to the literature, and what evidence was put forward to determine this ARM. We found many non-clonal species among those we reviewed, in spite of the belief that parthenogenesis is mainly clonal. Furthermore, we found that evidence for clonality was lacking in many species identified as such. This is partially due to technical limitations and misinterpretations leading to incorrect conclusions. Access to modern techniques, such as improved cytology marking and genomics, allows to get rid of many limitations, especially when combining cytology with genetics. Additionally, many conceptual biases, such as the misconception that polyploids and hybrids cannot go through meiosis or that premeiotic doubling is always clonal, further shift the perception of asexuals toward clonality. This shows that a common framework for identifying ARMs, accounting for the diversity in mechanisms and genetic consequences, is necessary. Because of the perception bias caused by these technical and conceptual limitations, it is possible that in addition to the known non-clonal parthenogens, an important part of so-called clonal species is in fact non-clonal too.

Consistently with the high potential costs of LOH, we found that, even though strict clonality is not as prevalent as generally thought, most non-clonal parthenogenetic animals are somewhat close to clonality: they have a low, but non-zero LOH rate. Can we thus approximate parthenogenesis as equivalent to clonality? Deviations from clonality, even if they are small, can have a great impact on the evolution of asexuals (Engelstädter 2008; Archetti 2010). This is because under clonality, it is expected that the major evolutionary force is mutation accumulation. However, recombination, even if rare, can be more frequent than mutations. If recombination is localized, it will generate clustered clonal genomic regions. Additionally, other rare events such as gene conversion and mitotic recombination may occur in otherwise strictly clonal species. Similarly, whether these events happen often enough to disturb evolutionary and genetic expectations under clonality depends on their frequency compared to the mutation rate (Engelstädter 2017). This means that, even when very rare, these events can have significant impacts on the evolution of asexuals.

Moreover, there is evidence indicating that non-clonal asexuals can evolve to become closer to clonality. For instance, fusion of meiotic products can be random (fusion of products of meiosis I or II, Stalker 1954; Asher 1970; Svendsen et al. 2015). However, in many obligate asexuals, cytological mechanisms favor central fusion, which generates less LOH and is thus closer to clonality (Murdy and Carson 1959; Verma and Ruttner 1983; Suomalainen et al. 1987). Such mechanisms could be the result of selection, as LOH is costly. Similarly, the timing

of premeiotic doubling could evolve to favor clonal-like reproduction. In cases where recombination causes LOH, the recombination rate tends to be low, and several cases of reduced recombination compared to related sexual species are known (see central fusion-like parthenogenesis section). Such reduction may result from the selection of lineages with the lowest LOH or progressive reduction of recombination within lineage. Additionally, effective LOH can be further reduced by elimination of recombinants for deleterious alleles. To conclude, evolution toward low LOH is likely to take place in non-clonal asexuals. Because parthenogenesis evolved from sexual species, mostly through meiosis modifications, mechanisms that avoid LOH may not be present at the emergence. This suggests that potentially many clonal or almost clonal species were not close to clonality in the past.

This can be extended to clonal species with an ARM suggestive of mitosis (*i.e.,* one equational division with no pairing, see mitosis section). Contrary to the general belief that many asexuals arise in one step by mitosis (Levitis et al. 2017), only a small part of asexual animals could concur with this origin. Moreover, it is possible that these so-called "mitotic parthenogens" in fact evolved through meiosis modifications, as traces of meiosis persist in some of them. Their ARM might be the result of a longer-term evolution toward clonality, at the beginning of which they were probably not clonal, and during which pairing was suppressed. There is so far no evidence for parthenogenesis through a complete mitotic process in animals.

Transitions from sex to asexuality happen mainly, and perhaps exclusively, through non-clonal meiosis modifications. Therefore, although clonality is frequently observed, non-clonal ARMs likely play an important part in the evolution of parthenogenesis. This means that the majority of theoretical models, which make the assumption that clonal asexuality arises spontaneously, may well be too simplistic. To tackle the challenging evolutionary questions regarding asexuality, it becomes crucial to include non-clonal ARMs, especially those that are close to clonality. In particular, models studying the emergence of asexual populations competing with sexuals need to take into account that asexuality does not likely emerge as clonal, and that there are multiple evolutionary pathways toward clonality.

Conclusion

In this review, we presented evidence that clonal asexuals do not represent a large majority. There are potentially many more non-clonal asexuals because of the strong perception bias toward clonality. Although most parthenogens are clonal or close to clonality, it might not have been the case throughout their evolutionary history. Finally, most and possibly all parthenogens evolved through meiosis modifications, and not by switching to mitosis. Therefore, the incorporation of non-clonal ARMs in theoretical models for the origin and consequences of asexuality is essential. A more accurate vision of asexuality could participate in resolving long-lasting evolutionary questions, such as the rarity of asexuality and the frequent association between asexuality, hybridization and polyploidy. The possibly intricate effects of these ARM on evolutionary, genetical, demography and ecological expectations could allow completely new theories to develop and flourish, enriching the vast field of reproductive systems evolution.

BOX 1: The vocabulary associated with ARMs is very broad and definitions have evolved over time, to the extent that publications now often need to clarify and define the terms chosen. The word "apomixis" was for a long time synonymous with clonality, whereas today one can distinguish between "mitotic apomixis" and "meiotic apomixis"; where reproduction is not necessarily clonal (Archetti 2010). Similarly, automixis was defined as involving meiosis and was therefore considered equivalent to non-clonal asexuality. However, we now know that some types of automixis can generate the same genetic consequences as clonality. Consequently, the terms originally developed for cytologically observed ARMs have evolved to accommodate the associated genetic consequences. This has led to some misunderstandings in the identification of ARMs. Indeed, suppression of the first meiotic division with absence of bivalents is clonal and may be indistinguishable from mitosis, which is why this mode of reproduction is often referred to as "mitotic division", "ameiotic parthenogenesis" or "apomixis".

To differentiate between mitosis- and meiosis-derived parthenogenesis, we propose the following terms:
-Dimeiotic: Two independent cells engage in meiosis to produce offspring. The cells originate from the same (autofecundation) or different (allofecundation) parents. In this case, they are usually of different mating types.
-Monomeiotic: A single cell engages in meiosis to produce offspring (the meiosis is necessarily modified to maintain ploidy). Different modifications exist: premeiotic doubling, central fusion like, terminal fusion like and postmeiotic doubling
-Ameiotic: No meiosis is engaged to produce offspring (*i.e.,* mitosis only).

Offspring origin	**Category**	**Cytological mechanism**	**Synonym**	**Genetic consequence**	**Genetically equivalent to clonality**
Monomeiotic	High LOH (loss of heterozygosity) ARM	One of the four haploid meiotic products or gametes (N) duplicate	Gamete duplication, post meiotic replication, post meiotic doubling.	Total LOH	Never
		Fusion of products of the second meiotic division	Terminal fusion-like parthenogenesis, terminal fusion, fusion of sister nuclei	Total LOH at the centromere. Recombination causes heterozygosity retention, so that it is more likely with distance from the centromere	Never
		Suppression of second meiotic division	Terminal fusion-like parthenogenesis		Never
	Central fusion-like	Fusion of products of the first division	Central fusion-like parthenogenesis, central fusion, fusion of non-sister nuclei	Total heterozygosity at the centromere. Recombination causes LOH, so that it is more likely with distance from the centromere	Only if no recombination
		Suppression of first meiotic division	Central fusion-like parthenogenesis, apomixis, meiotic apomixis		Only if no recombination
	Premeiotic doubling	Duplication of the chromosome number normally before meiosis	Premeiotic doubling, endoreduplication, endomitosis	Duplication before Prophase I: Sister-pairing leads to retention of heterozygosity, non-sister pairing leads to LOH. Duplication after Prophase I: Recombination causes LOH.	Duplication before Prophase I: Only if exclusive sister pairing. Duplication after Prophase I: Only if no recombination.
Ameiotic	Mitosis	One equational division, equivalent to the second meiotic division of meiosis	Mitotic division, apomixis, mitotic apomixis	Total retention of heterozygosity (except for mitotic recombination)	Always

Glossary

Meiotic division: Two meiotic divisions take place during meiosis: a reductional one called meiosis I and an equational one; meiosis II

Crossover: Also called recombination or chiasmata, take place during Prophase I (meiosis I). Portions of paired chromosomes are exchanged. They can be observed cytologically.

Synapsis: Pairing of chromosomes during Prophase I. Also called tetrad, bivalent or diplochromosomes.

Gynogenesis: Parthenogenesis in which the embryo contains only maternal chromosomes due to the failure of the sperm to fuse with the egg nucleus.

Acknowledgements

The authors are grateful to Anne Gorgeon and the Library platform at CEFE for acquiring papers. This work was funded by the Grant ANR-17-CE02-0016-01, GENASEX, from the French National Research Agency. The authors declare no conflicts of interest.

Author contributions

TL, CH, CM and LB conceived the study. CM and LB conducted bibliographic research.

References

Ament-Velásquez, S. L., E. Figuet, M. Ballenghien, E. E. Zattara, J. L. Norenburg, F. A. Fernández-Álvarez, J. Bierne, N. Bierne, and N. Galtier. 2016. Population genomics of sexual and asexual lineages in fissiparous ribbon worms (Lineus, Nemertea): hybridization, polyploidy and the Meselson effect. Mol. Ecol. 25:3356–3369. Blackwell Publishing Ltd.

Ammermann, D. 1967. The cytology of parthenogenesis in the tardigrade *Hypsibius dujardini*. Chromosoma 23:203–20313.

Archetti, M. 2010. Complementation, genetic conflict, and the evolution of sex and recombination. J. Hered. 101:S21–S33. Oxford Academic.

Asher, J. H. 1970. Parthenogenesis and genetic variability. II. One-locus models for various diploid populations. Genetics 66:369–391. Oxford University Press.

Bacci, G., G. Cognetti, and A. M. Vaccari. 1961. Endomeiosis and sex determination in *Daphnia pulex*. Experientia 17:505–506. Birkhäuser-Verlag.

Balloux, F., L. Lehmann, and T. De Meeûs. 2003. The population genetics of clonal and partially clonal diploids. Genetics 164:1635–1644. Oxford Academic.

Barton, N. H., and B. Charlesworth. 1998. Why sex and recombination? Science. 281:1986–1990.

Baudry, E., P. Kryger, M. Allsopp, N. Koeniger, D. Vautrin, F. Mougel, J. M. Cornuet, and M. Solignac. 2004. Whole-genome scan in thelytokous-laying workers of the Cape Honeybee (*Apis mellifera capensis*): Central fusion, reduced recombination rates and centromere mapping using half-tetrad analysis. Genetics 167:243–252.

Bell, G. 1982. The Masterpiece of Nature. University of California Press, Berkeley.

Bergmann, P., M. Laumann, R. A. Norton, and M. Heethoff. 2018. Cytological evidence for automictic thelytoky in parthenogenetic oribatid mites (Acari, Oribatida): Synaptonemal complexes confirm meiosis in *Archegozetes longisetosus*. Acarologia 58:342–356.

Bertolani, R. 1971. Partenogenesi geografica triploide in un Tardigrado (*Macrobiotus richtersi*). Rend. Sc. fis. mat. e nat., Lincei 50:487–489.

Bertolani, R. 1973. Presenza di un biotipo partenogenetico e suo effetto sul rapporto-sessi in *Macrobiotus hufelandi* (Tardigrada). Rend. Sc. fis. mat. e nat., Lincei 54:469–473.

Bertolani, R. 1981. The Taxonomic Position of some Eutardigrades. Bolletino di Zool. 48:197–203.

Bertolani, R., and G. P. Buonagurelli. 1975. Osservazioni cariologiche sulla partenogenesi meiotica di *Macrobiotus dispar* (Tardigrada). Atti della Accad. Naz. dei Lincei. Rend. Ser. 8 53:782–786.

Bertolani, R., S. Garagna, G. C. Manicardi, and C. A. Redi. 1987. *Macrobiotus pseudohufelandi* Iharos as a model for cytotaxonomic study in populations of eutardigrades (Tardigrada). Experientia 43:210–213. Birkhäuser-Verlag.

Bi, K., and J. P. Bogart. 2006. Identification of intergenomic recombinations in unisexual salamanders of the genus *Ambystoma* by genomic in situ hybridization (GISH). Cytogenet. Genome Res. 112:307–312.

Birky, C. W. 1996. Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. Genetics 144:427–437.

Bishop, D. K. 1994. RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell 79:1081–1092.

Blackburn, E. H. 1991. Structure and function of telomeres. Nature 350:569–573.

Blackman, R. L. 1979. Stability and variation in aphid clonal lineages. Biol. J. Linn. Soc. 11:259–277.

Bogart, J. P. 2003. Genetics and Systematics of Hybrid Species. Pp. 109–134 *in* Reproductive Biology and Phylogeny of Urodela. CRC Press.

Bogart, J. P., and M. W. Klemens. 1997. Hybrids and genetic interactions of mole salamanders (*Ambystoma jeffersonianum* and *A. laterale*) (Amphibia, Caudata) in New York and New England. Am. Museum Novit. 3218:1–78.

Brandt, A., P. T. Van, C. Bluhm, Y. Anselmetti, Z. Dumas, E. Figuet, C. M. François, N. Galtier, B. Heimburger, K. S. Jaron, M. Labédan, M. Maraun, D. J. Parker, M. Robinson-Rechavi, I. Schaefer, P. Simion, S. Scheu, T. Schwander, and J. Bast. 2021. Haplotype divergence supports long-term asexuality in the oribatid mite Oppiella nova. Proc. Natl. Acad. Sci. 118. Cold Spring Harbor Laboratory.

Butlin, R., I. Schön, and K. Martens. 1998. Asexual reproduction in nonmarine ostracods. Heredity. 81:473–480.

Card, D. C., F. J. Vonk, S. Smalbrugge, N. R. Casewell, W. Wüster, T. A. Castoe, G. W. Schuett, and W. Booth. 2021. Genome-wide data implicate terminal fusion automixis in king cobra facultative parthenogenesis. Sci. Rep. 11:1–9. Nature Publishing Group.

Casellato, S., and R. Rodighiero. 1972. Karyology of lumbricidae. III° contribution. Caryologia 25:513–524.

Castagnone-Sereno, P., E. G. J. Danchin, L. Perfus-Barbeoch, and P. Abad. 2013. Diversity and evolution of root-knot nematodes, genus meloidogyne: New insights from the genomic era. Annu. Rev. Phytopathol. 51:203–220.

Ceplitis, A. 2003. Coalescence times and the Meselson effect in asexual eukaryotes. Genet. Res. 82:183–190.

Chaplin, J. A., and P. D. N. Hebert. 1997. *Cyprinotus incongruens* (Ostracoda): An ancient asexual? Mol. Ecol. 6:155–168.

Chapman, D. D., M. S. Shivji, E. Louis, J. Sommer, H. Fletcher, and P. A. Prodöhl. 2007. Virgin birth in a hammerhead shark. Biol. Lett. 3:425–427. Royal Society.

Christensen, B. 1960. A comparative cytological investigation of the reproductive cycle of an amphimictic diploid and a parthenogenetic triploid form of *Lumbricillus lineatus* (O.F.M.) (Oligochaeta, Enchytraeidae). Chromosoma 11:365–379. Springer-Verlag.

Cognetti, G. 1961. Endomeiosis in parthenogenetic lines of Aphids. Experientia 17:168–169.

Cognetti, G. 1962. La partenogenesi negli afidi. Bolletino di Zool. 29:129–147.

Cordes, J. E., and J. M. Walker. 2006. Evolutionary and systematic implications of skin histocompatibility among parthenogenetic teiid lizards: Three color pattern classes of *Aspidoscelis dixoni* and one of *Aspidoscelis tesselata*. Copeia 14–26.

Cordes, J. E., and J. M. Walker. 2003. Skin histocompatibility between syntopic pattern classes C and D of parthenogenetic Cnemidophorus tesselatus in New Mexico. J. Herpetol. 37:185–188.

Crow, J. F., and M. Kimura. 1965. Evolution in Sexual and Asexual Populations. Am. Nat. 99:439–450. Science Press.

Cuellar, O. 1974. On the Origin of Parthenogenesis in Vertebrates: The Cytogenetic Factors. Am. Nat. 108:625–648. University of Chicago Press.

Cuellar, O., and C. Smart. 1977. Analysis of histoincompatibility in a natural population of the bisexual whiptail lizard <i>Cnemidophorus tigris<\i>. Transplantation 24:127–133.

Dawley, R. M., R. J. Schultz, and K. A. Goddard. 1987. Clonal reproduction and polyploidy in unisexual hybrids of Phoxinus eos and Phoxinus neogaeus (Pisces; Cyprinidae). Copeia 1987:275.

De Meeûs, T., and F. Balloux. 2005. F-statistics of clonal diploids structured in numerous demes. Mol. Ecol. 14:2695–2702.

De Meeûs, T., F. Prugnolle, and P. Agnew. 2007. Asexual reproduction: Genetics and evolutionary aspects. Cell. Mol. Life Sci. 64:1355–1372.

Decaestecker, E., L. De Meester, and J. Mergeay. 2009. Cyclical parthenogenesis in *Daphnia*: Sexual versus asexual reproduction. Lost Sex Evol. Biol. Parthenogenes. 9789048127:295–316. Springer Netherlands.

Dougherty, B. J. 1989. A karyotypic study of the origin of parthenogenesis in *Campeloma* (Gastropoda:Viviparidae).

Dudgeon, C. L., L. Coulton, R. Bone, J. R. Ovenden, and S. Thomas. 2017. Switch from sexual to parthenogenetic reproduction in a zebra shark. Sci. Rep. 7:1–8. Nature Publishing Group.

Dukić, M., D. Berner, C. R. Haag, and D. Ebert. 2019. How clonal are clones? A quest for loss of heterozygosity during asexual reproduction in *Daphnia magna*. J. Evol. Biol. 32:619–628. Blackwell Publishing Ltd.

Elzinga, J. A., J. Jokela, and L. N. S. Shama. 2013. Large variation in mitochondrial DNA of sexual and parthenogenetic *Dahlica triquetrella* (Lepidoptera: Psychidae) shows multiple origins of parthenogenesis. BMC Evol. Biol. 13.

Emelyanova O.V. 1984. A cytological study of maturation and fertilization processes in hybrids between the crucian carp and the common carp. Tzitologya (Moscow).

Engelstädter, J. 2017. Asexual but not clonal: Evolutionary processes in automictic populations. Genetics 206:993–1009. Genetics.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–1150. John Wiley & Sons, Ltd.

Flot, J. F., B. Hespeels, X. Li, B. Noel, I. Arkhipova, E. G. J. Danchin, A. Hejnol, B. Henrissat, R. Koszul, J. M. Aury, V. Barbe, R. M. Barthélémy, J. Bast, G. A. Bazykin, O. Chabrol, A. Couloux, M. Da Rocha, C. Da Silva, E. Gladyshev, P. Gouret, O. Hallatschek, B. Hecox-Lea, K. Labadie, B. Lejeune, O. Piskurek, J. Poulain, F. Rodriguez, J. F. Ryan, O. A. Vakhrusheva, E. Wajnberg, B. Wirth, I. Yushenova, M. Kellis, A. S. Kondrashov, D. B. Mark Welch, P. Pontarotti, J. Weissenbach, P. Wincker, O. Jaillon, and K. Van Doninck. 2013. Genomic evidence for ameiotic evolution in the bdelloid rotifer *Adineta vaga*. Nature 500:453–457.

Fougeyrollas, R., K. Dolejšová, D. Sillam-Dussés, V. Roy, C. Poteaux, R. Hanus, and Y. Roisin. 2015. Asexual queen succession in the higher termite *Embiratermes neotenicus*. Proc. R. Soc. B Biol. Sci. 282. Royal Society of London.

Fournier, D., S. Hellemans, R. Hanus, and Y. Roisin. 2016. Facultative asexual reproduction and genetic diversity of populations in the humivorous termite *Cavitermes tuberosus*. Proc. R. Soc. B Biol. Sci. 283. Royal Society of London.

Fradin, H., K. Kiontke, C. Zegar, M. Gutwein, J. Lucas, M. Kovtun, D. L. Corcoran, L. R. Baugh, D. H. A. Fitch, F. Piano, and K. C. Gunsalus. 2017. Genome Architecture and Evolution of a Unichromosomal Asexual Nematode. Curr. Biol. 27:2928–2939.

Goddard, K. A., and R. M. Dawley. 1990. Clonal Inheritance of a Diploid Nuclear Genome by a Hybrid Freshwater Minnow (*Phoxinus eos-neogaeus*, Pisces: Cyprinidae). Evolution. 44:1052.

Gokhman, V. E., and V. G. Kuznetsova. 2018. Parthenogenesis in Hexapoda: holometabolous insects. J. Zool. Syst. Evol. Res. 56:23–34.

Golubovskaya, I. N., L. C. Harper, W. P. Pawlowski, D. Schichnes, and W. Z. Cande. 2002. The pam1 gene is required for meiotic bouquet formation and efficient homologous synapsis in maize (Zea mays L.). Genetics 162:1979–1993.

Goudie, F., M. H. Allsopp, M. Beekman, P. R. Oxley, J. Lim, and B. P. Oldroyd. 2012. Maintenance and loss of heterozygosity in a thelytokous lineage of honey bees (*Apis mellifera capensis*). Evolution. 66:1897–1906.

Guidetti, R., M. Cesari, R. Bertolani, T. Altiero, and L. Rebecchi. 2019. High diversity in species, reproductive modes and distribution within the *Paramacrobiotus richtersi* complex (Eutardigrada, Macrobiotidae). Zool. Lett. 5:1–28. BioMed Central.

Haag, C. R., and D. Ebert. 2004. A new hypothesis to explain geographic parthenogenesis. Ann. Zool. Fennici 41:539–544.

Hartfield, M. 2016. Evolutionary genetic consequences of facultative sex and outcrossing. J. Evol. Biol. 29:5–22.

Hartfield, M., and P. D. Keightley. 2012. Current hypotheses for the evolution of sex and recombination. Integr. Zool. 7:192–209.

Hawes, R. S. J. 1963. The Emergence of Asexuality in Protozoa. Q. Rev. Biol. 38:234–242.

Hebert, P. D., and R. D. Ward. 1972. Inheritance during parthenogenesis in *Daphnia magna*. Genetics 71:639–642.

Heinemann, R. L., and R. D. Hughes. 1969. The cytological basis for reproductive variability in the Anoetidae (Sarcoptiformes: Acari). Chromosoma 28:346–356.

Hellemans, S., K. Dolejšová, J. Křivánek, D. Fournier, R. Hanus, and Y. Roisin. 2019. Widespread occurrence of asexual reproduction in higher termites of the Termes group (Termitidae: Termitinae). BMC Evol. Biol. 19:1–14.

Heppich, S., H. G. Tunner, and J. Greilhuber. 1982. Premeiotic chromosome doubling after genome elimination during spermatogenesis of the species hybrid *Rana esculenta*. Theor. Appl. Genet. 61:101–104. Springer-Verlag.

Hiruta, C., C. Nishida, and S. Tochinai. 2010. Abortive meiosis in the oogenesis of parthenogenetic *Daphnia pulex*. Chromosom. Res. 18:833–840.

Hoffmann, A. A., K. T. Reynolds, M. A. Nash, and A. R. Weeks. 2008. A high incidence of parthenogenesis in agricultural pests. Proc. R. Soc. B Biol. Sci. 275:2473–2481. Royal Society.

Hollister, J. D., S. Greiner, M. T. J. Johnson, and S. I. Wright. 2019. Hybridization and a loss of sex shape genome-wide diversity and the origin of species in the evening primroses (Oenothera, Onagraceae). New Phytol. 224:1372–1380. Blackwell Publishing Ltd.

Honeycutt, R. L., and P. Wilkinson. 1989. Electrophoretic Variation in the Parthenogenetic Grasshopper *Warramaba virgo* and its Sexual Relatives. Evolution. 43:1027.

Hongell, K., and J. Terhivuo. 1989. Chromosomal status of the parthenogenetic earthworm *Dendrobaena octaedra* (Sav.) (Oligochaeta: Lumbricidae) in southern Finland. Hereditas 110:179–182.

Hörandl, E. 2009. Geographical parthenogenesis: Opportunities for asexuality. Pp. 161–186 *in* Lost Sex: The Evolutionary Biology of Parthenogenesis. Springer Netherlands.

Itono, M., K. Morishima, T. Fujimoto, E. Bando, E. Yamaha, and K. Arai. 2006. Premeiotic endomitosis produces diploid eggs in the natural clone loach, *Misgurnus anguillicaudatus* (Teleostei: Cobitidae). J. Exp. Zool. Part A Comp. Exp. Biol. 305:513–523.

Janko, K. 2014. Let us not be unfair to asexuals: Their ephemerality may be explained by neutral models without invoking any evolutionary constraints of asexuality. Evolution. 68:569–576. John Wiley & Sons, Ltd.

Janssen, T., G. Karssen, O. Topalović, D. Coyne, and W. Bert. 2017. Integrative taxonomy of root-knot nematodes reveals multiple independent origins of mitotic parthenogenesis. PLoS One 12. Public Library of Science.

Johnson, S. G. 1992. Spontaneous and hybrid origins of parthenogenesis in *Campeloma decisum* (Freshwater prosobranch snail). Heredity (Edinb). 68:253–261.

Keeney, S. 2001. Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. 52:1–53. Academic Press Inc.

Kondrashov, A. S. 1993. Classification of hypotheses on the advantage of amphimixis. J. Hered. 84:372–387. Oxford Academic.

Kupriyanova, L. A., L. D. Safronova, V. B. Sycheva, F. D. Danielyan, and V. G. Petrosyan. 2021. Oogenesis (Prophase 1 of Meiosis) and Mitotic Chromosomes of Parthenogenetic Species *Darevskia armeniaca* (Family Lacertidae). Biol. Bull. 48:274–280. Springer.

Kuroda, M., T. Fujimoto, M. Murakami, E. Yamaha, and K. Arai. 2018. Clonal reproduction assured by sister chromosome pairing in dojo loach, a teleost fish. Chromosom. Res. 26:243–253.

Laumann, M., P. Bergmann, and M. Heethoff. 2008. Some remarks on the cytogenetics of oribatid mites. Soil Org. 80.

Lenk, P., B. Eidenmueller, H. Staudter, R. Wicker, and M. Wink. 2005. A parthenogenetic *Varanus*. Amphib. Reptil. 26:507–514.

Lenormand, T., J. Engelstädter, S. E. Johnston, E. Wijnker, and C. R. Haag. 2016. Evolutionary mysteries in meiosis. Philos. Trans. R. Soc. B Biol. Sci. 371. Royal Society of London.

Levitis, D. A., K. Zimmerman, and A. Pringle. 2017. Is meiosis a fundamental cause of inviability among sexual and asexual plants and animals? Proc. R. Soc. B Biol. Sci. 284. The Royal Society.

Lloyd, A., and K. Bomblies. 2016. Meiosis in autopolyploid and allopolyploid *Arabidopsis*. Curr. Opin. Plant Biol. 30:116–122. Elsevier Ltd.

Lokki, J., E. Suomalainen, A. Saura, and P. Lankinen. 1975. Genetic polymorphism and evolution in parthenogenetic animals. II. Diploid and polyploid *Solenobia triquetrella* (Lepidoptera: Psychidae). Genetics 79:513–525.

Lorenzo-Carballa, M. O., H. Hadrys, A. Cordero-Rivera, and J. A. Andrés. 2012. Population genetic structure of sexual and parthenogenetic damselflies inferred from mitochondrial and nuclear markers. Heredity. 108:386–395. Nature Publishing Group.

Loxdale, H. D., and G. Lushai. 2003. Rapid changes in clonal lines: The death of a "sacred cow." Biol. J. Linn. Soc. 79:3–16. Oxford Academic.

Luchetti, A., V. Scicchitano, and B. Mantovani. 2013. Origin and evolution of the Italian subterranean termite *Reticulitermes lucifugus* (Blattodea, Termitoidae, Rhinotermitidae). Bull. Entomol. Res. 103:734–741. Cambridge University Press.

Lutes, A. A., W. B. Neaves, D. P. Baumann, W. Wiegraebe, and P. Baumann. 2010. Sister chromosome pairing maintains heterozygosity in parthenogenetic lizards. Nature 464:283–286. Nature Publishing Group.

Macgregor, H. C., and T. M. Uzzell. 1964. Gynogenesis in salamanders related to *Ambystoma jeffersonianum*. Science. 143:1043–1045.

Malysheva, D. N., O. N. Tokarskaya, V. G. Petrosyan, F. D. Danielyan, I. S. Darevsky, and A. P. Ryskov. 2007. Genomic variation in parthenogenetic lizard Darevskia armeniaca: Evidence from DNA fingerprinting data. J. Hered. 98:173–178. Oxford Academic.

Marais, M., J. C. De, and W. Kruger. 1991. The cytogenetics of some South African root-knot nematodes (Heteroderidae: Nematoda). Phytophylactica 23:265–272.

Marescalchi, O., and V. Scali. 2001. New DAPI and fish findings on egg maturation processes in related hybridogenetic and parthenogenetic *Bacillus* hybrids (insecta, phasmatodea). Mol. Reprod. Dev. 60:270–276. John Wiley & Sons, Ltd.

Mark Welch, D. B., J. L. Mark Welch, and M. Meselson. 2008. Proceedings of the National Academy of Sciences. Natl. Acad Sci. 105:5145–5149.

Mark Welch, D. B., C. Ricci, and M. Meselson. 2009. Bdelloid rotifers: Progress in understanding the success of an evolutionary scandal. Pp. 259–279 *in* Lost Sex: The Evolutionary Biology of Parthenogenesis. Springer Netherlands.

Mark Welch, D., and M. Meselson. 2000. Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. Science. 288:1211–1215.

Maslin, T. P. 1967. Skin grafting in the bisexual teiid lizard *Cnemidophorus sexlineatus* and in the unisexual *C. tesselatus*. J. Exp. Zool. 166:137–149.

Matsuura, K., M. Fujimoto, and K. Goka. 2004. Sexual and asexual colony foundation and the mechanism of facultative parthenogenesis in the termite *Reticulitermes speratus* (Isoptera, Rhinotermitidae). Insectes Soc. 51:325–332.

Mattox, N. T. 1937. Oogenesis of campeloma rufum, a parthenogenetic snail. Zeitschrift für Zellforsch. und Mikroskopische Anat. 27:455–464.

Maynard Smith, J. 1968. Evolution in Sexual and Asexual Populations. Am. Nat. 102:469–473. University of Chicago Press.

Maynard Smith, J. 1978. The evolution of sex. Cambridge: Cambridge University Press.

Momotani, S., K. Morishima, Q. Zhang, and K. Arai. 2002. Genetic analyses of the progeny of triploid gynogens induced from unreduced eggs of triploid (diploid female x tetraploid male) loach. Aquaculture 204:311–322.

Monaco, P. J., E. M. Rasch, and J. S. Balsano. 1984. Apomictic Reproduction in the Amazon Molly, *Poecilia formosa*, and Its Triploid Hybrids. Evol. Genet. Fishes 311–328. Springer US.

Moritz, C., W. M. Brown, L. D. Densmore, J. W. Wright, D. Vyas, S. Donnellan, M. Adams, and P. Baverstock. 1989. Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekkonidae). Evol. Ecol. unisexual Vertebr. 87–112.

Moritz, R. F. A., and M. Haberl. 1994. Lack of meiotic recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (The cape honeybee). Heredity. 73:98–102.

Murdy, W. H., and H. L. Carson. 1959. Parthenogenesis in *Drosophila mangabeirai* Malog. Am. Nat. 93:355–363. University of Chicago Press.

Newton, A. A., R. R. Schnittker, Z. Yu, S. S. Munday, D. P. Baumann, W. B. Neaves, and P. Baumann. 2016. Widespread failure to complete meiosis does not impair fecundity in parthenogenetic whiptail lizards. Dev. 143:4486–4494. Company of Biologists Ltd.

Nokkala, S., and N. V. Golub. 2006. Automictic and apomictic parthenogenesis in psocids (Insecta: Psocoptera). Folia Biol. (Praha). 54:19–22.

Nokkala, S., V. G. Kuznetsova, and C. Nokkala. 2017. Characteristics of parthenogenesis in *Cacopsylla ledi* (Flor, 1861) (Hemiptera, Sternorryncha, Psylloidea): Cytological and molecular approaches. Comp. Cytogenet. 11:807–817. Pensoft Publishers.

Nokkala, S., A. Maryańska-Nadachowska, and V. G. Kuznetsova. 2008. First evidence of polyploidy in Psylloidea (Homoptera, Sternorrhyncha): A parthenogenetic population of *Cacopsylla myrtilli* (W. Wagner, 1947) from northeast Finland is apomictic and triploid. Genetica 133:201–205.

Normark, B. B. 1999. Evolution in a putatively ancient asexual aphid lineage: Recombination and rapid karyotype change. Evolution. 53:1458–1469.

Nougué, O., N. O. Rode, R. Jabbour-zahab, A. Ségard, L. M. Chevin, C. R. Haag, and T. Lenormand. 2015. Automixis in *Artemia*: Solving a century-old controversy. J. Evol. Biol. 28:2337–2348.

Ojima, Y. 1954. Some Cytological Observations on Parthenogenesis in *Daphnia pulex* (de Geer). J. Fac. Sci. Hokkaido Univ. Ser. VI. Zool. 12:230–235.

Oldroyd, B. P., B. Yagound, M. H. Allsopp, M. J. Holmes, G. Buchmann, A. Zayed, and M. Beekman. 2021. Adaptive, caste-specific changes to recombination rates in a thelytokous honeybee population. Proc. R. Soc. B Biol. Sci. 288. Royal Society Publishing.

Omodeo, P. 1955. Cariologia dei Lumbricidae II Contributo. Caryologia 8:135–178.

Otto, S. P. 2009. The evolutionary enigma of sex. Am. Nat. 174. The University of Chicago Press.

Otto, S. P., and T. Lenormand. 2002. Resolving the paradox of sex and recombination. Nat. Rev. Genet. 3:252–261. Nature Publishing Group.

Palmer, S. C., and R. A. Norton. 1992. Genetic diversity in thelytokous oribatid mites (Acari; Acariformes: Desmonomata). Biochem. Syst. Ecol. 20:219–231. Pergamon.

Peacock, A. D., and U. Weidmann. 1961. Recent work on the cytology of animal parthenogenesis. Przegl. zool 5:101–122.

Pearcy, M., O. Hardy, and S. Aron. 2006. Thelytokous parthenogenesis and its consequences on inbreeding in an ant. Heredity. 96:377–382.

Phillips, N. R., and D. M. Lambert. 1989. Genetics of *Potamopyrgus antipodarum* (Gastropoda: Prosobranchia): evidence for reproductive modes. New Zeal. J. Zool. 16:435–445.

Pijnacker, L. P. 1969. Automictic parthenogenesis in the stick insect *Bacillus rossius* Rossi (Cheleutoptera, phasmidae). Genetica 40:393–399. Martinus Nijhoff, The Hague/Kluwer Academic Publishers.

Rabeling, C., and D. J. C. Kronauer. 2013. Thelytokous parthenogenesis in eusocial hymenoptera. Annu. Rev. Entomol. 58:273–292.

Ranganath, R. M. 2008. Meiotic chromosome pairing and recombination take refuge in the telomeres. Nat. Rev. Genet. 9:318–318.

Rasch, E., and J. Balsano. 1974. Mechanism for meiotic restitution during oogenesis in a triploïd, unisexual fish. J. Cell Biol. Vol. 63.

Rasch, E. M., P. J. Monaco, and J. S. Balsano. 1982. Cytophotometric and autoradiographic evidence for functional apomixis in a gynogenetic fish, *Poecilia formosa* and its related, triploid unisexuals. Histochemistry 73:515–533.

Rebecchi, L. 1991. Karyological analysis on *Mactobiotus pseudohufelandi* (Tardigrada, macrobiotidae) and a new finding of a tetraploid population. Caryologia 44:301–307.

Rebecchi, L., and R. Bertolani. 1988. New cases of parthenogenesis and polyploidy in the genus *Ramazzottius* (tardigrada, hypsibiidae) and a hypothesis concerning their origin. Int. J. Invertebr. Reprod. Dev. 14:187–196.

Rebecchi, L., and R. Bertolani. 1999. Spermatozoon Morphology of Three Species of Hypsibiidae (Tardigrada, Eutardigrada) and Phylogenetic Evaluation. Zool. Anz. 238:319–328.

Rebecchi, L., V. Rossi, T. Altiero, R. Bertolani, and P. Menozzi. 2003. Reproductive modes and genetic polymorphism in the tardigrade *Richtersius coronifer* (Eutardigrada, Macrobiotidae). Invertebr. Biol. 122:19–27. John Wiley & Sons, Ltd.

Rey, O., A. Loiseau, B. Facon, J. Foucaud, J. Orivel, J. M. Cornuet, S. Robert, G. Dobigny, J. H. C. Delabie, C. D. S. F. Mariano, and A. Estoup. 2011. Meiotic recombination dramatically decreased in thelytokous queens of the little fire ant and their sexually produced workers. Mol. Biol. Evol. 28:2591–2601.

Reynolds, R. G., W. Booth, G. W. Schuett, B. M. Fitzpatrick, and G. M. Burghardt. 2012. Successive virgin births of viable male progeny in the checkered gartersnake, *Thamnophis marcianus*. Biol. J. Linn. Soc. 107:566–572. Oxford Academic.

Rode, N. O., R. Jabbour-Zahab, L. Boyer, É. Flaven, F. Hontoria, G. Van Stappen, F. Dufresne, C. Haag, and T. Lenormand. *in press*. The origin of asexual brine shrimps. Am. Nat.

Rossi, V., G. Benassi, S. Leonardi, A. Piotti, and P. Menozzi. 2006. Clonal diversity of *Heterocypris incongruens* (Crustacea: Ostracoda) in Northern Italian ricefields. Arch. fur Hydrobiol. 166:225–240.

Saura, A., J. Lokki, and E. Suomalainen. 1993. Origin of Polyploidy in Parthenogenetic Weevils. J. Theor. Biol. 163:449–456.

Scali, V., M. Passamonti, O. Marescalchi, and B. Mantovani. 2003. Linkage between sexual and asexual lineages: Genome evolution in Bacillus stick insects. Biol. J. Linn. Soc. 79:137–150.

Scali, V., F. Tinti, B. Mantovani, and O. Marescalchi. 1995. Mate recognition and gamete cytology features allow hybrid species production and evolution in *Bacillus* stick insects. Bolletino di Zool. 62:59–70. Informa UK Limited.

Schön, I., R. K. Butlin, H. I. Griffiths, and K. Martens. 1998. Slow molecular evolution in an ancient asexual ostracod. Proc. R. Soc. B Biol. Sci. 265:235–242.

Schön, I., A. Gandolfi, E. Di Masso, V. Rossi, H. I. Griffiths, K. Martens, and R. K. Butlin. 2000. Persistence of asexuality through mixed reproduction in *Eucypris virens* (Crustacea, Ostracoda). Heredity. 84:161–169.

T. Schwander, "Consequences of parthenogenesis on stick insect genomes." Keynote at the online ESEB satellite meeting: Genomic signatures and consequences of sex and asexuality. https://worm-lab.eu/sex-asex-genomics-satellite#rec323795419, 2021, June 16.

Seiler, J. 1966. Die Zytologie der Bastarde aus der Kreuzung parthenogenetischer Weibchen von *Solenobia triquetrella* F. R. mit Männchen der bisexuellen Rasse - I. Mitteilung. Chromosoma 19:405–438. Springer-Verlag.

Simion, P., J. Narayan, A. Houtain, A. Derzelle, L. Baudry, E. Nicolas, R. Arora, M. Cariou, C. Cruaud, F. R. Gaudray, C. Gilbert, N. Guiglielmoni, B. Hespeels, D. K. Kozlowski, K. Labadie, A. Limasset, M. Lliros, M. Marbouty, M. Terwagne, J. Virgo, R. Cordaux, E. G. Danchin, B. Hallet, R. Koszul, T. Lenormand, J.-F. Flot, and K. Van Doninck. 2021. Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer Adineta vaga. Sci. Adv. 7.

Simon, J. C., F. Delmotte, C. Rispe, and T. Crease. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. Biol. J. Linn. Soc. 79:151–163.

Sköld, H. N., M. Obst, M. Sköld, and B. Åkesson. 2009. Stem cells in asexual reproduction of marine invertebrates. Pp. 105–137 *in* Stem Cells in Marine Organisms. Springer Netherlands, Dordrecht.

Sohn, J. Il, and J. W. Nam. 2018. The present and future of de novo whole-genome assembly. Brief. Bioinform. 19:23–40. Oxford University Press.

Speijer, D., J. Lukeš, and M. Eliáš. 2015. Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. Proc. Natl. Acad. Sci. U. S. A. 112:8827–8834.

Stalker, H. D. 1956. A case of polyploïdy in Diptera. Proc. Natl. Acad. Sci. 42:194–199.

Stalker, H. D. 1954. Parthenogenesis in *Drosophila*. Genetics 39:4–34. Oxford University Press.

Suomalainen, E. 1950. Parthenogenesis in Animals. Adv. Genet. 3:193–253.

Suomalainen, E., A. Saura, and J. Lokki. 1987. Cytology and evolution in parthenogenesis. CRC Press.

Suomalainen, E., A. Saura, J. Lokki, and T. Teeri. 1980. Genetic polymorphism and evolution in parthenogenetic animals. Theor. Appl. Genet. 1980 573 57:129–132. Springer.

Svendsen, N., C. M. O. Reisser, M. Dukić, V. Thuillier, A. Ségard, C. Liautard-Haag, D. Fasel, E. Hürlimann, T. Lenormand, Y. Galimov, and C. R. Haag. 2015. Uncovering cryptic asexuality in *Daphnia magna* by RAD sequencing. Genetics 201:1143–1155.

Taberly, G. 1987. Researches on the thelytokous parthenogenesis of two species of Oribatid mites - Trhypochthonius tectorum (Berlese) and *Platynothrus peltifer* (Koch). I. Acarologia 28:187–198.

Tétart, J. 1978. Les garnitures chromosomiques des Ostracodes d'eau douce. Trav. Lab. Hydrobiol. Univ. Gren:69-70: 113-140.

Tiedemann, R., K. Moll, K. B. Paulus, and I. Schlupp. 2005. New microsatellite loci confirm hybrid origin, parthenogenetic inheritance, and mitotic gene conversion in the gynogenetic Amazon molly (*Poecilia formosa*). Mol. Ecol. Notes 5:586–589. John Wiley & Sons, Ltd.

Tran Van, P., Y. Anselmetti, J. Bast, Z. Dumas, N. Galtier, K. S. Jaron, K. Martens, D. J. Parker, M. Robinson-Rechavi, T. Schwander, P. Simion, and I. Schön. 2021. First annotated draft genomes of nonmarine ostracods (Ostracoda, Crustacea) with different reproductive modes. G3 (Bethesda). 11.

Triantaphyllou, A. C. 1966. Polyploidy and reproductive patterns in the root-knot nematode *Meloidogyne hapla*. J. Morphol. 118:403–413.

Tucker, A. E., M. S. Ackerman, B. D. Eads, S. Xu, and M. Lynch. 2013. Population-genomic insights into the evolutionary origin and fate of obligately asexual *Daphnia pulex*. Proc. Natl. Acad. Sci. U. S. A. 110:15740–15745.

Uzzell, T. 1970. Meiotic Mechanisms of Naturally Occurring Unisexual Vertebrates. Am. Nat. 104:433–445. University of Chicago Press.

Uzzell, T. M., and S. M. Goldblatt. 1967. Serum proteins of salamanders of *Ambystoma jeffersonianum* complex, and the origin of the triploid species of this group. Evolution. 21:345–354. Wiley.

van Der Beek, J. G., J. A. Los, and L. P. Pijnacker. 1998. Cytology of parthenogenesis of five Meloidogyne species. Fundam. Appl. Nematol. 21:393–399.

van der Kooi, C. J., C. Matthey-Doret, and T. Schwander. 2017. Evolution and comparative ecology of parthenogenesis in haplodiploid arthropods. Evol. Lett. 1:304–316. Blackwell Publishing Ltd.

Vargo, E. L., P. E. Labadie, and K. Matsuura. 2012. Asexual queen succession in the subterranean termite *Reticulitermes virginicus*. Proc. R. Soc. B Biol. Sci. 279:813–819. Royal Society.

Vavre, F., J. H. De Jong, and R. Stouthamer. 2004. Cytogenetic mechanism and genetic consequences of thelytoky in the wasp *Trichogramma cacoeciae*. Heredity (Edinb). 93:592–596. Nature Publishing Group.

Verma, S., and F. Ruttner. 1983. Cytological analysis of the thelytokous parthenogenesis in the Cape honeybee (*Apis mellifera capensis* Escholtz). Apidologie 14:41–57.

Vershinina, A. O., and V. G. Kuznetsova. 2016. Parthenogenesis in Hexapoda: Entognatha and non-holometabolous insects. J. Zool. Syst. Evol. Res. 54:257–268. Blackwell Publishing Ltd.

Vrijenhoek, R. C., and E. D. Parker. 2009. Geographical parthenogenesis: General purpose genotypes and frozen niche variation. Pp. 99–131 *in* Lost Sex: The Evolutionary Biology of Parthenogenesis. Springer Netherlands.

Watts, P. C., K. R. Buley, S. Sanderson, W. Boardman, C. Ciofi, and R. Gibson. 2006. Parthenogenesis in Komodo dragons. Nature 444:1021–1022. Nature Publishing Group.

West, S. A., C. M. Lively, and A. F. Read. 1999. A pluralist approach to sex and recombination. J. Evol. Biol. 12:1003–1012.

White, M. J. D., J. Cheney, and K. H. L. Key. 1963. A parthenogenetic species of grasshopper with complex structural heterozygosity (Orthoptera: Acridoidea). Aust. J. Zool. 11:1–19.

Wrensch, D. L., J. B. Kethley, and R. A. Norton. 1994. Cytogenetics of Holokinetic Chromosomes and Inverted Meiosis: Keys to the Evolutionary Success of Mites, with Generalizations on Eukaryotes. Pp. 282–343 *in* Mites. Springer, Boston, MA.

Yamamoto, Y., and K. Matsuura. 2012. Genetic influence on caste determination underlying the asexual queen succession system in a termite. Behav. Ecol. Sociobiol. 66:39–46.

Yamashita, M., J. Jiang, H. Onozato, T. Nakanishi, and Y. Nagahama. 1993. A Tripolar Spindle Formed at Meiosis I Assures the Retention of the Original Ploidy in the Gynogenetic Triploid Crucian Carp, Ginbuna *Carassius auratus langsdorfii* fish oocytes/gynogenesis/meiosis/spindle formation/histone H1 kinase. Dev. Growth Differ. 35:631–636.

Zaffagnini, F. 1987. Reproduction in *Daphnia*. Mem. dell'Istituto Ital. di Idrobiol. 45:245–284.

Zaffagnini, F., and B. Sabelli. 1972. Karyologic observations on the maturation of the summer and winter Eggs of *Daphnia pulex* and *Daphnia middendorffiana*. Chromosoma 36:193–203.

Zhang, F., T. Oshiro, and F. Takashima. 1992. Chromosome synapsis and recombination during meiotic division in gynogenetic triploid ginbuna, *Carassius auratus langsdorfii*. Japanese J. Ichthyol. 39:151–155.

Zhang, J., M. Sun, L. Zhou, Z. Li, Z. Liu, X. Y. Li, X. L. Liu, W. Liu, and J. F. Gui. 2015. Meiosis completion and various sperm responses lead to unisexual and sexual reproduction modes in one clone of polyploid *Carassius gibelio*. Sci. Rep. 5:1–14. Nature Publishing Group.

Supplementary data

Supplementary table: The subset of species studied on which the figure is based. Each species is classed according to the asexual reproductive modes classed into the following categories: "high LOH ARM", "variable LOH", "clonal", or "unknown") based on the literature. Note that four species were each subdivided into two in which different ARMs were identified. For each species we assessed whether the attributed category from the literature is conclusive (there is conclusive evidence to support the authors' interpretation, whether their conclusion is in agreement in light of the methodological or conceptual reasons developed in the review, and finally. When the class of ARM is clonal, we specified whether or not there is any evidence against mitosis as the underlying cytological process.

Species	Class of ARM	Conclusive evidence	Agreement with interpretation	No evidence against mitosis	References
Meloidogyne hapla race B	clonal	yes	yes	yes	[1], [2]
Meloidogyne africana	clonal	yes	yes	yes	[3]

Meloidogyne ardenensis	clonal	yes	yes	yes	[3]
Meloidogyne javanica	clonal	yes	yes	yes	[2]–[5]
Campeloma rufum	clonal	yes	yes	yes	[6]
Histiostoma feroniarum	clonal	yes	yes	yes	[7]
Poecilia formosa	clonal	yes	yes	yes	[8]
Epiphanes senta	clonal	yes	yes	NA	[9], [10], reviewed in [11]
Asplanchna intermedia	clonal	yes	yes	NA	[9], [12], [13], reviewed in [11]
Asplanchna amphora	clonal	yes	yes	NA	[9], [14], reviewed in [11]
Asplanchna priodonta	clonal	yes	yes	NA	[9], [15], [16], reviewed in [11]
Meloidogyne arenaria	clonal	yes	yes	NA	[5]
Meloidogyne incognita	clonal	yes	yes	NA	[5], [17]
Meloidogyne partityla	clonal	yes	yes	NA	[5]
Diplocaspter pachys	clonal	yes	yes	NA	[18]
Potamopyrgus jenkinsi = P. antipodarum	clonal	yes	no	NA	[19]–[22]
Melanoides tuberculata	clonal	yes	yes	NA	[23]
Campeloma parthenum	clonal	yes	no	NA	[24]
Campeloma decisum	clonal	no	no	NA	[25]
Dendrobaena octaedra	clonal	yes	no	NA	[26]–[30]
Ramazzottius oberhaeuseri	clonal	yes	yes	NA	[31]
Macrobiotus hufelandi	clonal	yes	yes	NA	[32]
Macrobiotus richtersi=Paramacrobiotus fairbanski	clonal	yes	yes	NA	[33]
Platynothrus peltifer	clonal	no	no	NA	[34]
Trhypochthonious tectorum	clonal	no	no	NA	[34]
Archegozs longisetosus	clonal	yes	yes	NA	[35]
AIIonothrus gigandcus	clonal	yes	yes	NA	[34]
Darwinulidae	clonal	no	NA	NA	[36]
Eucypris virens	clonal	yes	yes	NA	[37]
Daphnia pulex	clonal	yes	no	NA	[38]–[42]
Daphnia magna	clonal	yes	yes	NA	[43]
Warramaba virgo = Moraba virgo	clonal	yes	no	NA	[44], [45]
Bacillus whitei	clonal	yes	no	NA	[46], [47]
Carausius morosus	clonal	yes	yes	NA	[48]
Brevicoryne brassicae	clonal	no	no	NA	[49], [50]
Myzus persicae	clonal	yes	yes	NA	[51]–[53]
Acyrthosiphon pisum	clonal	no	no	NA	[51]
Myzus antirrhinii	clonal	yes	yes	NA	[54]
"Sitobion near fragariae"	clonal	yes	yes	NA	[52]
Sitobion avenae	clonal	yes	yes	NA	[52], [55], [56]
Amphorophora tuberculata	clonal	yes	yes	NA	[57]
Sitobion miscanthi	clonal	yes	yes	NA	[52], [58]
Aphis fabae	clonal	no	no	NA	[51]
Cathormiocerus aristatus	clonal	yes	no	NA	[59], [60]
Eusomus ovulum	clonal	no	NA	NA	[61]
Liophloeus tesselatus	clonal	no	NA	NA	[61]
Aramigus tessellatus	clonal	no	NA	NA	[62]
Otiorrhynchus ligustici	clonal	no	NA	NA	[61]
Otiorrhynchus raucus	clonal	yes	no	NA	[60], [61]
Otiorrhynchus ovatus	clonal	yes	no	NA	[61], [63]
Otiorrhynchus tristis	clonal	no	NA	NA	[61]
Polydrosus inustus	clonal	yes	no	NA	[60], [61]
Strophosoma	clonal	yes	no	NA	[59], [60]
Trophiphorus micans	clonal	no	NA	NA	[61]

Trichogramma cacoeciae	clonal	yes	no	NA	[64]
Chrosomus eos-neogaeus	clonal	no	NA	NA	[65]
Pelophylax esculentus = Rana esculenta	clonal	yes	yes	NA	[66]
Lacerta unisexualis = Darevskia unisexualis	clonal	yes	yes	NA	[67]
Apidoscelis tesselata	clonal	yes	no	NA	[68]
Bacillus lynceorum	clonal	yes	no	NA	[46], [47]
Adineta vaga	variable LOH	yes	yes	NA	[69]
Campeloma decisum	variable LOH	no	no	NA	[25]
Dactylobiotus parthenogeneticus	variable LOH	yes	yes	NA	[70]
Richtersius coronifer	variable LOH	yes	yes	NA	[71]
Artemia parthenogenetica (diploids)	variable LOH	yes	yes	NA	[72], [73]
Artemia parthenogenetica (polyploids)	variable LOH	yes	yes	NA	[74], [75]
Bacillus atticus	variable LOH	yes	yes	NA	[76]
Timema sp.	variable LOH	yes	yes	NA	[77]
Reticulitermes virginicus	variable LOH	yes	yes	NA	[78]
Reticulitermes lucifugus	variable LOH	yes	yes	NA	[79]
Embiratermes neotenicus	variable LOH	yes	yes	NA	[80]
Drosophila mangabeirai	variable LOH	yes	yes	NA	[81]
Apis mellifera capensis	variable LOH	yes	yes	NA	[82], [83]
Carassius auratus langsdorfi	variable LOH	yes	yes	NA	[84]
Ambystoma laterale x A. jeffersonianum	variable LOH	yes	yes	NA	[85]
Lacerta saxicola = Darevskia armeniaca	variable LOH	yes	yes	NA	[67]
Meloidogyne hapla race A	high LOH	yes	yes	NA	[1], [2]
Meloidogyne fallax	high LOH	yes	yes	NA	[2]
Meloidogyne floridensis	high LOH	yes	yes	NA	[86]
Dactylobiotus dispar = Macrobiotus dispar	high LOH	yes	yes	NA	[87]
Hypsibius dujardini	high LOH	yes	yes	NA	[88]
Cheyletus eruditus	high LOH	yes	yes	NA	[89]
Bacillus rossius	high LOH	yes	yes	NA	[90]
Reticulitermes speratus	high LOH	yes	yes	NA	[91]
Cavitermes tuberosus	high LOH	yes	yes	NA	[92]
Palmitermes impostor	high LOH	yes	yes	NA	[92]
Spinitermes trispinosus	high LOH	yes	yes	NA	[92]
Inquilinitermes inquilinus.	high LOH	yes	yes	NA	[92]
Meloidogyne chitwoodi	unknown	NA	NA	NA	[5]
Ramazzottius oberhaeuseri	unknown	NA	NA	NA	[93]
Brevipalpus obovatus	unknown	NA	NA	NA	[94]
Heminothrus ornatissimus	unknown	NA	NA	NA	[95]
Solenobia triquetrella =Dahlica triquetrella	unknown	NA	NA	NA	[96]
Ochthiphila polystigma	unknown	NA	NA	NA	[97]

References of the supplementary data

[1] A. C. Triantaphyllou, "Polyploidy and reproductive patterns in the root-knot nematode Meloidogyne hapla," *J. Morphol.*, vol. 118, no. 3, pp. 403–413, 1966.

[2] J. G. Van Der Beek, J. A. Los, and L. P. Pijnacker, "Cytology of parthenogenesis of five Meloidogyne species," *Fundam. Appl. Nematol.*, vol. 21, no. 4, pp. 393–399, 1998.

[3] T. Janssen, G. Karssen, O. Topalović, D. Coyne, and W. Bert, "Integrative taxonomy of root-knot nematodes reveals multiple independent origins of mitotic parthenogenesis," *PLoS One*, vol. 12, no. 3, p. e0172190, Mar. 2017.

[4] A. C. Triantaphyllou, "Oögenesis in the root-knot nematode meloidogyne javanica," *Nematologica*, vol. 7, no. 2, pp. 105–113, 1962.
[5] M. Marais, J. C. De, and W. Kruger, "The cytogenetics of some South African root-knot nematodes (Heteroderidae: Nematoda)," 1991.
[6] N. T. Mattox, "Oogenesis of campeloma rufum, a parthenogenetic snail," *Zeitschrift für Zellforsch. und Mikroskopische Anat.*, vol. 27, no. 4, pp. 455–464, Dec. 1937.
[7] R. L. Heinemann and R. D. Hughes, "The cytological basis for reproductive variability in the Anoetidae (Sarcoptiformes: Acari)," *Chromosoma*, vol. 28, no. 3, pp. 346–356, Sep. 1969.
[8] M. Rasch, P. J. Monaco, and J. S. Balsano, "Histochemistry Cytophotometric and Autoradiographic Evidence for Functional Apomixis in a Gynogenetic Fish, Poeciliaformosa and Its Related, Triploid Unisexuals," *Histochemistry*, vol. 73, pp. 515–533, 1982.
[9] M. Serra and T. W. Snell, "Sex loss in monogonont rotifers," in *Lost Sex: The Evolutionary Biology of Parthenogenesis*, Springer, Ed. Dordrecht, 2009, pp. 281–294.
[10] A. F. Shull, "Chromosomes and the Life Cycle of Hydatina Senta," *Biol. Bull.*, vol. 41, no. 1, pp. 55–61, 1921.
[11] C. W. Birky and J. J. Gilbert, "Parthenogenesis in rotifers: The control of sexual and asexual reproduction," *Integr. Comp. Biol.*, vol. 11, no. 2, pp. 245–266, 1971.
[12] D. D. Whitney, "The chromosome cycle in the rotifer, Asplanchna intermedia.," *Anat. Rec*, vol. 29, p. 107, 1924.
[13] A. Tauson, "Die reifungsprozesse der parthenogenetischen eier von Asplanchna intermedia Huds," *Zeitschrift für Zellforsch. und Mikroskopische Anat.*, vol. 1, no. 1, pp. 57–84, 1924.
[14] D. D. Whitney, "The chromosome cycle in the rotifer asplanchna amphora," *J. Morphol.*, vol. 47, pp. 415–433, 1929.
[15] O. Storch, "Parthenogenese und Eireifung der heterogonen Rädertiere.," *Zeitschr. f. ind. Abst.-u. Vererbgsl.*, vol. 30, pp. 309–312, 1923.
[16] O. Storch, "Die Eizellen der heterogonen Rädertiere: nebst allgemeinen Erörterungen über die Cytologie des Sexualvorganges und der Parthenogenese," *Zool. Jahrb. Abt.*, vol. 45, pp. 309–404, 1924.
[17] A. C. Triantaphyllou, "Oogenesis and the Chromosomes of the Parthenogenetic Root-knot Nematode Meloidogyne incognita ~," 1981.
[18] H. Fradin *et al.*, "Genome Architecture and Evolution of a Unichromosomal Asexual Nematode," *Curr. Biol.*, vol. 27, no. 19, pp. 2928-2939.e6, Oct. 2017.
[19] N. R. Phillips and D. M. Lambert, "Genetics of potamopyrgus antipodarum (Gastropoda: Prosobranchia): Evidence for reproductive modes1," *New Zeal. J. Zool.*, vol. 16, no. 3, pp. 435–445, 1989.
[20] L. Hauser, G. R. Carvalho, R. N. Hughes, and R. E. Carter, "Clonal structure of the introduced freshwater snail Potamopyrgus antipodarum (Prosobranchia: Hydrobiidae), as revealed by DNA fingerprinting," *Proc. R. Soc. B Biol. Sci.*, vol. 249, no. 1, pp. 19–25, 1992.
[21] D. Weetman, L. Hauser, and G. R. Carvalho, "Reconstruction of microsatellite mutation history reveals a strong and consistent deletion bias in invasive clonal snails, Potamopyrgus antipodarum," *Genetics*, vol. 162, no. 2, pp. 813–822, 2002.
[22] D. Weetman, L. Hauser, and G. R. Carvalho, "Heterogeneous evolution of microsatellites revealed by reconstruction of recent mutation history in an invasive apomictic snail, Potamopyrgus antipodarum," *Genetica*, vol. 127, no. 1–3, pp. 285–293, 2006.
[23] S. Samadi *et al.*, "Density and variability of dinucleotide microsatellites in the parthenogenetic polyploid snail Melanoides tuberculata," *Mol. Ecol.*, vol. 7, no. 9, pp. 1233–1236, 1998.
[24] B. J. Dougherty, "A karyotypic study of the origin of parthenogenesis in Campeloma (Gastropoda:Viviparidae)," 1989.
[25] S. G. Johnson, "Spontaneous and hybrid origins of parthenogenesis in campeloma decisum (Freshwater prosobranch snail)," *Heredity (Edinb).*, vol. 68, no. 3, pp. 253–261, 1992.
[26] M. Mustonen, J. Haimi, J. Kesäniemi, H. Högmander, and K. E. Knott, "Variation in gene expression within clones of the earthworm Dendrobaena octaedra," *PLoS One*, vol. 12, no. 4, pp. 1–16, 2017.

[27] P. Omodeo, “Cariologia dei Lumbricidae II Contributo,” *Caryologia*, vol. 8, no. 1, pp. 135–178, Jan. 1955.
[28] K. Hongell and J. Terhivuo, “Chromosomal status of the parthenogenetic earthworm Dendrobaena octaedra (Sav.) (Oligochaeta: Lumbricidae) in southern Finland,” *Hereditas*, vol. 110, no. 2, pp. 179–182, 1989.
[29] S. Casellato and R. Rodighiero, “Karyology of lumbricidae. III° contribution,” *Caryologia*, vol. 25, no. 4, pp. 513–524, Jan. 1972.
[30] V. Simonsen and M. Holmstrup, “Deviation from apomictic reproduction in Dendrobaena octaedra?,” *Hereditas*, vol. 145, no. 4, pp. 212–214, 2008.
[31] L. Rebecchi and R. Bertolani, “Spermatozoon Morphology of Three Species of Hypsibiidae (Tardigrada, Eutardigrada) and Phylogenetic Evaluation,” *Zool. Anz.*, vol. 238, pp. 319–328, 1999.
[32] R. Bertolani, “Presenza di un biotipo partenogenetico e suo effetto sul rapporto-sessi in Macrobiotus hufelandi (Tardigrada),” *Rend. Sc. fis. mat. e nat., Lincei*, vol. 54, pp. 469–473, 1973.
[33] R. Guidetti, M. Cesari, R. Bertolani, T. Altiero, and L. Rebecchi, “High diversity in species, reproductive modes and distribution within the Paramacrobiotus richtersi complex (Eutardigrada, Macrobiotidae),” *Zool. Lett. 2019 51*, vol. 5, no. 1, pp. 1–28, Jan. 2019.
[34] S. C. Palmer and R. A. Norton, “Genetic diversity in thelytokous oribatid mites (Acari; Acariformes: Desmonomata),” *Biochem. Syst. Ecol.*, vol. 20, no. 3, pp. 219–231, 1992.
[35] P. Bergmann, M. Laumann, R. A. Norton, and M. Heethoff, “Cytological evidence for automictic thelytoky in parthenogenetic oribatid mites (Acari, Oribatida): Synaptonemal complexes confirm meiosis in archegozetes longisetosus,” *Acarologia*, vol. 58, no. 2, pp. 342–356, 2018.
[36] I. Schön and K. Martens, “No slave to sex,” *Proc. R. Soc. B Biol. Sci.*, vol. 270, no. 1517, pp. 827–833, 2003.
[37] V. Rossi, A. Piotti, A. Baltanás, G. Benassi, and P. Menozzi, “Genetic diversity and mixed reproduction in Eucypris virens (Crustacea: Ostracoda),” *Fundam. Appl. Limnol.*, vol. 172, no. 2, pp. 147–159, 2008.
[38] P. D. N. Hebert and T. Crease, “Clonal diversity in populations of Daphnia pulex reproducing by obligate parthenogenesis,” *Heredity (Edinb).*, vol. 51, no. 1, pp. 353–369, 1983.
[39] C. Hiruta, C. Nishida, and S. Tochinai, “Abortive meiosis in the oogenesis of parthenogenetic Daphnia pulex,” *Chromosom. Res.*, vol. 18, no. 7, pp. 833–840, 2010.
[40] N. Keith *et al.*, “High mutational rates of large-scale duplication and deletion in Daphnia pulex,” *Genome Res.*, vol. 26, no. 1, pp. 60–69, Jan. 2016.
[41] A. R. Omilian, M. E. A. Cristescu, J. L. Dudycha, and M. Lynch, “Ameiotic recombination in asexual lineages of Daphnia,” *Proc. Natl. Acad. Sci.*, vol. 103, no. 49, pp. 18638–18643, Dec. 2006.
[42] A. E. Tucker, M. S. Ackerman, B. D. Eads, S. Xu, and M. Lynch, “Population-genomic insights into the evolutionary origin and fate of obligately asexual Daphnia pulex,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, no. 39, pp. 15740–15745, 2013.
[43] P. D. Hebert and R. D. Ward, “Inheritance during parthenogenesis in Daphnia magna.,” *Genetics*, vol. 71, no. 4, pp. 639–642, 1972.
[44] M. Kearney, M. J. Blacket, J. L. Strasburg, and C. Moritz, “Waves of parthenogenesis in the desert: Evidence for the parallel loss of sex in a grasshopper and a gecko from Australia,” *Mol. Ecol.*, vol. 15, no. 7, pp. 1743–1748, Jun. 2006.
[45] M. J. D. White, J. Cheney, and K. H. L. Key, “A parthenogenetic species of grasshopper with complex structural heterozygosity (Orthoptera: Acridoidea),” *Aust. J. Zool.*, vol. 11, no. 1, pp. 1–19, 1963.
[46] V. Scali, M. Passamonti, O. Marescalchi, and B. Mantovani, “Linkage between sexual and asexual lineages: Genome evolution in Bacillus stick insects,” in *Biological Journal of the Linnean Society*, 2003, vol. 79, no. 1, pp. 137–150.
[47] V. Scali, F. Tinti, B. Mantovani, and O. Marescalchi, “Bacillus stick insects,” *Ital. J. Zool.*, vol. 62, no. 1, pp. 59–70, Jan. 1995.

[48] L. P. Pijnacker and J. Harbott, “Structural heterozygosity and aneuploidy in the parthenogenetic stick insect Carausius morosus Br. (Phasmatodea: Phasmatidae),” *Chromosoma*, vol. 76, no. 2, pp. 165–174, 1980.

[49] L. Ruiz-Montoya, J. Núñez-Farfán, and J. Vargas, “Host-associated genetic structure of Mexican populations of the cabbage aphid Brevicoryne brassicae L. (Homoptera: Aphididae),” *Heredity (Edinb).*, vol. 91, no. 4, pp. 415–421, 2003.

[50] G. Cognetti, “La partenogenesi negli afidi,” *Bolletino di Zool.*, vol. 29, no. 2, pp. 129–147, Jan. 1962.

[51] R. L. Blackman, “Early development of the parthenogenetic egg in three species of aphids (homoptera : Aphididae),” *Int. J. Insect Morphol. Embryol.*, vol. 7, no. 1, pp. 33–44, Jan. 1978.

[52] D. F. Hales, A. C. C. Wilson, M. A. Sloane, J. C. Simon, J. F. Legallic, and P. Sunnucks, “Lack of detectable genetic recombination on the X chromosome during the parthenogenetic production of female and male aphids,” *Genet. Res.*, vol. 79, no. 3, pp. 203–209, 2002.

[53] B. Fenton, G. Malloch, M. Navajas, J. Hillier, and A. N. E. Birch, “Clonal composition of the peach-potato aphid Myzus persicae (Homoptera: Aphididae) in France and Scotland: Comparative analysis with IGS fingerprinting and microsatellite markers,” *Ann. Appl. Biol.*, vol. 142, no. 3, pp. 255–267, 2003.

[54] D. Hales, A. C. Wilson, J. M. Spence, and R. L. Blackman, “Confirmation that Myzus antirrhinii (Macchiati) (Hemiptera: Aphididae) occurs in Australia, using morphometrics, microsatellite typing and analysis of novel karyotypes by fluorescence in situ hybridisation,” *Aust. J. Entomol.*, vol. 39, no. 3, pp. 123–129, Jul. 2000.

[55] L. Haack, J. C. Simon, J. P. Gauthier, M. Plantegenest, and C. A. Dedryver, “Evidence for predominant clones in a cyclically parthenogenetic organism provided by combined demographic and genetic analyses,” *Mol. Ecol.*, vol. 9, no. 12, pp. 2055–2066, Dec. 2000.

[56] N. J. Miller, “Population Structure and Gene Flow in a Host Alternating Aphid, Pemphigus Bursarius,” University of Birmingham, 2000.

[57] R. L. Blackman and D. F. Hales, “Behaviour of the X chromosomes during growth and maturation of parthenogenetic eggs of Amphorophora tuberculata (Homoptera, Aphididae), in relation to sex determination,” *Chromosoma*, vol. 94, no. 1, pp. 59–64, 1986.

[58] H. D. Loxdale, S. Vorwerk, and A. Forneck, “The unstable ‘clone’: Evidence from monitoring AFLP-based mutations for short-term clonal genetic variation in two asexual lineages of the grain aphid, Sitobion avenae (F.),” *Bull. Entomol. Res.*, vol. 103, no. 1, pp. 111–118, 2013.

[59] V. E. Gokhman and V. G. Kuznetsova, “Parthenogenesis in Hexapoda: holometabolous insects,” *J. Zool. Syst. Evol. Res.*, vol. 56, no. 1, pp. 23–34, Feb. 2018.

[60] D. Lachowska, M. Rożek, and M. Holecová, “New data on the cytology of parthenogenetic weevils (Coleoptera, Curculionidae),” *Genetica*, vol. 134, no. 2, pp. 235–242, 2008.

[61] V. Y. Nazarenko and S. Y. Morozov-Leonov, “Clonal Structure of Some Weevil Species (Coleoptera, Curculionidae) from Central Ukraine,” *Vestn. Zool.*, vol. 52, no. 4, pp. 313–322, 2018.

[62] B. B. Normark and A. A. Lanteri, “Incongruence between Morphological and Mitochondrial-DNA Characters Suggests Hybrid Origins of Parthenogenetic Weevil Lineages (Genus Aramigus),” *Syst. Biol.*, vol. 47, no. 3, pp. 475–494, 1998.

[63] M. Rozek, D. Lachowska, M. Holecovà, and Ł. Kajtoch, “Karyology of parthenogenetic weevils (Coleoptera, Curculionidae): Do meiotic prophase stages occur?,” *Micron*, vol. 40, no. 8, pp. 881–885, 2009.

[64] F. Vavre, J. H. De Jong, and R. Stouthamer, “Cytogenetic mechanism and genetic consequences of thelytoky in the wasp Trichogramma cacoeciae,” *Heredity (Edinb).*, vol. 93, no. 6, pp. 592–596, 2004.

[65] J. Lafond, P. Hénault, C. Leung, and B. Angers, “Unexpected oogenic pathways for the triploid fish chrosomus eos-neogaeus,” *J. Hered.*, vol. 110, no. 3, pp. 370–377, 2019.

[66] M. Doležálková, A. Sember, F. Marec, P. Ráb, J. Plötner, and L. Choleva, “Is premeiotic genome elimination an exclusive mechanism for hemiclonal reproduction in hybrid males of the genus Pelophylax?,” *BMC Genet.*, vol. 17, no. 1, p. 100, Jul. 2016.

[67] L. A. Kupriyanova, L. D. Safronova, V. B. Sycheva, F. D. Danielyan, and V. G. Petrosyan, "Oogenesis (Prophase 1 of Meiosis) and Mitotic Chromosomes of Parthenogenetic Species Darevskia armeniaca (Family Lacertidae)," *Biol. Bull. 2021 483*, vol. 48, no. 3, pp. 274–280, Jun. 2021.

[68] A. A. Lutes, W. B. Neaves, D. P. Baumann, W. Wiegraebe, and P. Baumann, "Sister chromosome pairing maintains heterozygosity in parthenogenetic lizards," *Nature*, vol. 464, no. 7286, pp. 283–286, Mar. 2010.

[69] P. Simion *et al.*, "Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer Adineta vaga," *Sci. Adv.*, vol. 7, no. 41, p. eabg4216, 2021.

[70] R. Bertolani, "Evolution of the reproductive mechanisms in tardigrades - A review," *Zool. Anz.*, vol. 240, no. 3–4, pp. 247–252, 2001.

[71] L. Rebecchi, V. Rossi, T. Altiero, R. Bertolani, and P. Menozzi, "Reproductive modes and genetic polymorphism in the tardigrade Richtersius coronifer (Eutardigrada, Macrobiotidae)," *Invertebr. Biol.*, vol. 122, no. 1, pp. 19–27, Mar. 2003.

[72] L. Boyer, R. Jabbour-Zahab, M. Mosna, C. R. Haag, and T. Lenormand, "Not so clonal asexuals: Unraveling the secret sex life of Artemia parthenogenetica," *Evol. Lett.*, vol. 5, no. 2, pp. 164–174, Apr. 2021. (Chapter 1 of this thesis)

[73] O. Nougué *et al.*, "Automixis in Artemia: Solving a century-old controversy," *J. Evol. Biol.*, vol. 28, no. 12, pp. 2337–2348, 2015.

[74] N. O. Rode *et al.*, *in press* "The origin of asexual brine shrimps," *Am. Nat.*

[75] E. Goldschmidt, "Fluctuation in chromosome number in Artemia salina," *J. Morphol.*, vol. 91, pp. 111–133, 1952.

[76] O. Marescalchi and V. Scali, "Automictic parthenogenesis in the diploid-triploid stick insect bacillus atticus and its flexibility leading to heterospecific diploid hybrids," *Invertebr. Reprod. Dev.*, vol. 43, no. 2, pp. 163–172, 2003.

[77] T. Schwander, "Consequences of parthenogenesis on stick insect genomes." Keynote at the online ESEB satellite meeting: Genomic signatures and consequences of sex and asexuality. https://worm-lab.eu/sex-asex-genomics-satellite#rec323795419, 2021, June 16.

[78] E. L. Vargo, P. E. Labadie, and K. Matsuura, "Asexual queen succession in the subterranean termite Reticulitermes virginicus," *royalsocietypublishing.org*, vol. 279, no. 1729, pp. 813–819, 2012.

[79] A. Luchetti, V. Scicchitano, and B. Mantovani, "Origin and evolution of the Italian subterranean termite Reticulitermes lucifugus (Blattodea, Termitoidae, Rhinotermitidae)," *Bull. Entomol. Res.*, vol. 103, no. 6, pp. 734–741, 2013.

[80] R. Fougeyrollas *et al.*, "Asexual queen succession in the higher termite Embiratermes neotenicus," *Proc. R. Soc. B Biol. Sci.*, vol. 282, no. 1809, Jun. 2015.

[81] W. H. Murdy and H. L. Carson, "Parthenogenesis in Drosophila mangabeirai Malog.," *Am. Nat.*, vol. 93, no. 873, pp. 355–363, Nov. 1959.

[82] S. Verma and F. Ruttner, "Cytological analysis of the thelytokous parthenogenesis in the Cape honeybee (Apis mellifera capensis Escholtz).," *Apidologie*, vol. 14, no. 1, pp. 41–57, 1983.

[83] E. Baudry *et al.*, "Whole-genome scan in thelytokous-laying workers of the Cape Honeybee (Apis mellifera capensis): Central fusion, reduced recombination rates and centromere mapping using half-tetrad analysis," *Genetics*, vol. 167, no. 1, pp. 243–252, 2004.

[84] F. Zhang, T. Oshiro, and F. Takashima, "Chromosome synapsis and recombination during meiotic division in gynogenetic triploid ginbuna, Carassius auratus langsdorfii," *Japanese J. Ichthyol.*, vol. 39, no. 2, pp. 151–155, 1992.

[85] K. Bi and J. P. Bogart, "Identification of intergenomic recombinations in unisexual salamanders of the genus Ambystoma by genomic in situ hybridization (GISH)," *Cytogenet. Genome Res.*, vol. 112, no. 3–4, pp. 307–312, 2006.

[86] Z. A. Handoo *et al.*, "Morphological, Molecular, and Differential-Host Characterization of Meloidogyne floridensis n. sp. (Nematoda: Meloidogynidae), a Root-Knot Nematode Parasitizing Peach in Florida," 2004.

[87] R. Bertolani and G. P. Buonagurelli, “Osservazioni cariologiche sulla partenogenesi meiotica di Macrobiotus dispar (Tardigrada),” *Atti della Accad. Naz. dei Lincei. Rend. Ser. 8*, vol. 53, pp. 782–786, 1975.

[88] D. Ammermann, “[The cytology of parthenogenesis in the tardigrade Hypsibius dujardini].,” *Chromosoma*, vol. 23, no. 2, pp. 203–20313, 1967.

[89] J. H. Oliver Jr, “Cytogenetics of mites and ticks,” *Annu. Rev. Entomol.*, vol. 22, no. 1, pp. 407–429, 1977.

[90] L. P. Pijnacker, “Automictic parthenogenesis in the stick insect Bacillus rossius Rossi (Cheleutoptera, phasmidae),” *Genetica*, vol. 40, no. 1, pp. 393–399, Dec. 1969.

[91] K. Matsuura, M. Fujimoto, and K. Goka, “Sexual and asexual colony foundation and the mechanism of facultative parthenogenesis in the termite Reticulitermes speratus (Isoptera, Rhinotermitidae),” *Insectes Soc.*, vol. 51, no. 4, pp. 325–332, 2004.

[92] D. Fournier, S. Hellemans, R. Hanus, and Y. Roisin, “Facultative asexual reproduction and genetic diversity of populations in the humivorous termite Cavitermes tuberosus,” *Proc. R. Soc. B Biol. Sci.*, vol. 283, no. 1832, Jun. 2016.

[93] L. Rebecchi and R. Bertolani, “New cases of parthenogenesis and polyploidy in the genus ramazzottius (tardigrada, hypsibiidae) and a hypothesis concerning their origin,” *Int. J. Invertebr. Reprod. Dev.*, vol. 14, no. 2–3, pp. 187–196, 1988.

[94] L. P. Pijnacker, M. A. Ferwerda, H. R. Bolland, and W. Helle, “Haploid female parthenogenesis in the false spider mite Brevipalpus obovatus (Acari: Tenuipalpidae),” *Genetica*, vol. 51, no. 3, pp. 211–214, Jul. 1980.

[95] S. C. Palmer and R. A. Norton, “Genetic diversity in thelytokous oribatid mites (Acari; Acariformes: Desmonomata),” *Biochem. Syst. Ecol.*, vol. 20, no. 3, pp. 219–231, Apr. 1992.

[96] J. A. Elzinga, J. Jokela, and L. N. S. Shama, “Large variation in mitochondrial DNA of sexual and parthenogenetic Dahlica triquetrella (Lepidoptera: Psychidae) shows multiple origins of parthenogenesis,” *BMC Evol. Biol.*, vol. 13, no. 1, 2013.

[97] H. D. Stalker, “a Case of Polyploidy in Diptera,” *Proc. Natl. Acad. Sci.*, vol. 42, no. 4, pp. 194–199, 1956.

Chapter 3

Asexual production of males by ZW recombination in *Artemia parthenogenetica*

The manuscript has been submitted.

Asexual production of males by ZW recombination in *Artemia parthenogenetica*

Loreleï Boyer[1*], Roula Jabbour-Zahab[1], Pauline Joncour[2], Sylvain Glémin[2], Christoph R. Haag[1], Thomas Lenormand[1]

1 CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

2 CNRS, ECOBIO (Ecosystèmes, biodiversité, évolution), University of Rennes 1, UMR 6553, Rennes, France

* corresponding author

Abstract

In some asexual species, parthenogenetic females occasionally produce males, which may strongly affect the evolution and maintenance of asexuality if they cross with related sexuals and transmit genes causing asexuality to their offspring ("contagious parthenogenesis"). How these males arise in the first place has remained enigmatic, especially in species with sex chromosomes. Here, we test the hypothesis that rare, asexually produced males of the crustacean *Artemia parthenogenetica* are produced by recombination between the Z and W sex chromosomes during non-clonal parthenogenesis, resulting in ZZ males through loss of heterozygosity at the sex determination locus. We used RAD-sequencing to compare asexual mothers with their male and female offspring. Markers on several sex-chromosome scaffolds indeed lost heterozygosity in all male but no female offspring, suggesting that they correspond to the sex-determining region. Other sex-chromosome scaffolds lost heterozygosity in only a part of the male offspring, consistent with recombination occurring at a variable location. Alternative hypotheses for the production of these males (such as partial or total hemizygosity of the Z) could be excluded. Rare males are thus produced because recombination is not entirely suppressed during parthenogenesis in *A. parthenogenetica*. This finding may contribute to explaining the maintenance of recombination in these asexuals.

Keywords: Recombination; Loss of heterozygosity; Sex determination; Contagious parthenogenesis

Introduction

Parthenogenesis usually results in all-female offspring, but many obligate parthenogens are still able to occasionally produce males, such as aphids (Blackman 1972; Rispe et al. 1999; Simon et al. 1999), *Daphnia* (Innes and Hebert 1988), darwinulid ostracods (Smith et al. 2006), nematodes (Snyder et al. 2006), parasitoid wasps (Sandrock and Vorburger 2011), *Potamopyrgus* snails (Neiman et al. 2012) and thrips (van der Kooi and Schwander 2014a). Even though these "rare males" have been reported for a long time, their evolutionary significance remains elusive and controversial (Lynch 1984; Maccari et al. 2013; Engelstädter 2017; Abatzopoulos 2018). If these asexually produced males are non-functional, which apparently is the case in several groups (reviewed in van der Kooi and Schwander 2014b), they can be considered genetic or developmental accidents. The production of non-functional males is costly for parthenogenetic lineages, with the cost depending on the rate at which they are produced (Lynch 1984; Engelstädter 2008; Engelstädter et al. 2011; Neiman et al. 2012). In contrast, if rare males are functional, their evolutionary significance depends on the availability of sexually reproducing females (from related sexual species or lineages, or from their own lineage, if asexuality in females is facultative), with which they can successfully cross and produce fertile offspring. When rare males successfully reproduce with sexual females, they can potentially transmit the gene(s) controlling for asexual reproduction to their offspring, thus generating new parthenogenetic lineages (Hebert and Crease 1983; Simon et al. 2003). Yet whether or not these crosses are successful in generating new asexual lineages and at which rate successful crosses may occur will depend on multiple factors, including the mechanism by which rare males are produced in the first place. Indeed, these mechanisms can be purely accidental or more systematic, the latter generating a regular production of rare males which could be maintained and selected upon. Selection on rare male production could be mediated by selection on the rate of contagion (e.g, in cases where recently derived asexuals enjoy a large fitness advantage). The maintenance of male production may also have important genomic consequences, in particular by contributing to the maintenance of recombination, in cases where the mechanism of male production is based on recombination. Hence, deciphering the mechanism by which males are produced (whether it is accidental or not and whether it is based on recombination) is an important prerequisite to be able to evaluate their possible evolutionary significance.

The possible mechanisms of rare male production strongly depend on the sex determination system occurring in the ancestral sexual species from which parthenogenetic lineages are derived. There are many possibilities, but we opt to classifying them based on genetic patterns that can directly be observed in rare males compared to their parthenogenetic mothers. We distinguish five broad patterns (Fig. 1). Under **Pattern 1**, there is no systematic association between alleles found heterozygous in the mother and the genotype of male offspring. This may occur for instance if males result from accidental phenotypic or genetic "errors" (van der Kooi and Schwander 2014b). For instance, a mutation or transposable element insertion in or near the sex-determining locus may perturb female sex determination of an embryo and result in the production of a male. Similarly, a fortuitous environmental or hormonal variation may induce male development. This occurs for instance with residual

environmental sex determination in asexuals derived from species with environmental sex determination (Hebert and Crease 1983; Innes et al. 2000). **Pattern 2** corresponds to the loss of an entire sex chromosome. This may result in male production in species, in which the ancestral sex-determination system is ZZ/ZW, XX/XO, haplodiploidy with a complementary locus (Complementary sex determination, CSD), or depends on X/autosome balance. Mechanistically, the loss of an entire chromosome can occur for instance through non-disjunction. This has been observed in obligately parthenogenetic aphids, where XX mothers produce XO sons (Wilson et al. 1997). Here, it is important to underline that the production of XO male is the "regular" system (Blackman and Hales 1986) in related species of aphids where parthenogenesis alternates with sexual reproduction (cyclical parthenogenesis). **Pattern 3** corresponds to the loss of a part of a sex chromosome (the part that includes the sex-determination locus). This may occur under the same sex determination systems as pattern 2, but the likely mechanism are large-scale deletion or complex chromosomal rearrangement, rather than non-disjunction. This has been observed for instance in stick insects (Pijnacker and Ferwerda 1980). **Pattern 4** corresponds to a complete loss of heterozygosity (LOH) through autozygosity on the sex chromosome pair. Note that we distinguish here LOH leading to autozygosity from hemizygosity (patterns 2 and 3). Pattern 4 may apply to species in which the ancestral sex-determination system is ZZ/ZW, or CSD (Engelstädter 2008). Possible mechanisms include parthenogenesis based on different modifications of meiosis (Archetti 2010; Lenormand et al. 2016). Under these modes of parthenogenesis, all chromosomes (sex chromosomes, as well as autosomes) become homozygous in a single generation. This occurs for instance in rare cases of parthenogenesis in the king cobra, which lead to male offspring, due to terminal fusion (Card et al. 2021). Rare parthenogenesis in heterogametic females leading to the production of males is found in several other species: Komodo dragons where it is associated with complete LOH (Watts et al. 2006), turkey (Olsen and Marsden 1954), and silkworm where induced gamete duplication results in all male offspring (Strunnikov 1995). **Pattern 5** corresponds to partial LOH on the sex chromosome pair. As in pattern 4, it may apply to species in which the ancestral sex-determination system is ZZ/ZW or CSD. Possible mechanisms include modes of parthenogenesis which, in the presence of recombination, lead to partial LOH (Archetti 2010; Svendsen et al. 2015; Lenormand et al. 2016). Under all these mechanisms, the production of rare males occurs due to recombination. For instance, under central fusion or suppression of meiosis I (Archetti 2010), rare males can be produced by LOH at the sex-determination locus through a recombination event between the centromere and this sex-determining locus. This mechanism apparently explains rare male production in CSD species, as in the Cape honeybee (Goudie et al. 2012) and perhaps in *Cataglyphis* ants (Doums et al. 2013). It has also been proposed for ZW *Artemia parthenogenetica* (Browne and Hoopes 1990; Abreu-Grobois and Beardmore 2001; Nougué et al. 2015; Boyer et al. 2021; Rode et al. *in press*) on which we focus in this paper.

Artemia parthenogenetica (hereafter *Ap*) is an obligate asexual species, in which functional males are asexually produced at low rates (Maccari et al. 2013). These rare males are capable of contagious parthenogenesis (Maccari et al. 2014), through mating with females of a closely related sexual species (*A. sp. Kazakhstan*, hereafter *Akaz*, or *A. urmiana*). Moreover, *Ap* females have been shown to rarely engage in sex in the laboratory, which suggests that

crosses within asexual populations, through rare males and rare sex in females, might also possible (Boyer et al. 2021). In any case, contagious parthenogenesis was found to contribute to the evolution and diversification of the *Ap* clade (Rode et al. *in press*). Interestingly, the small rate of rare male production seems to consistently differ among *Ap* lineages, perhaps depending on their age (Maccari et al. 2013). Additionally, repeated "contagious" backcrosses of *Ap* to *Akaz* result in an increase in rare male production (Boyer et al. 2021). These observations suggest that rare male production is a genetically controlled trait in *Ap* (MacDonald and Browne 1987; Maccari et al. 2013; Boyer et al. 2021).

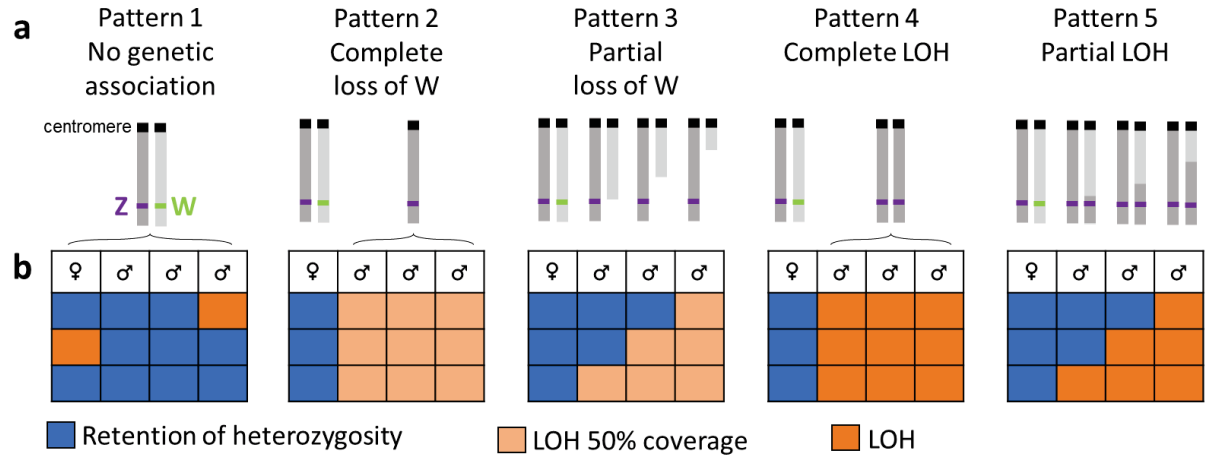


Figure 1. Possible genomic patterns that can be observed in rare males, according to different hypotheses of rare male production. The figure shows the different cases adapted for ZW species, as in *Ap*. (**a**) The ZW chromosome pair is represented with the centromere (black square) and sex-determination locus (purple/green). (**b**) Expectations of LOH patterns on male and female sex chromosome scaffolds (see methods). Each column of the small tables represent individuals (one female and three males). Each line represents a scaffold ordered on the chromosome (top being closer to the centromere). Blue cells represent scaffolds for which maternal heterozygosity was maintained. Dark orange cells represent scaffolds that went through LOH. Light orange cells represent scaffolds that went through LOH and a reduction of 50% of their coverage compared to the scaffolds that retained heterozygosity.

In the genus *Artemia*, females are heterogametic ZW, while males are ZZ (Bowen 1963; Stefani 1963). *Ap* are not clonal, but reproduce through a modified meiosis in which the first meiotic division is suppressed but some recombination still occurs (Nougué et al. 2015; Boyer et al. 2021). This mode of parthenogenesis is genetically equivalent to central fusion automixis and leads, as mentioned above, to LOH in the recombinant part of chromosomes, distal (with respect to the centromere) from the position of the crossing-over. Historically, the production of rare male was suggested to occur through the terminal fusion of meiotic products (Stefani 1964), which would lead to complete LOH (pattern 4). Given the more recent findings about the mode of parthenogenesis in *Ap*, rare males may rather be produced by recombination leading to partial LOH (pattern 5) and therefore ZZ offspring at the sex determining locus (Nougué et al. 2015; Boyer et al. 2021; Rode et al. *in press*). However, the sex determination locus and its chromosomal location are unknown, and it is also entirely possible that males are produced by mechanisms not corresponding to this simple scenario. In fact, almost any of the

other mechanisms discussed above is possible. Only few can be discarded based on available data. For instance, terminal fusion without recombination is unlikely, as males retain at least some heterozygosity (Abreu-Grobois and Beardmore 2001; Maccari et al. 2013).

In this study, we investigated the mechanism of rare male production by assessing patterns of LOH on the sex chromosomes between mothers and their male and female offspring using RAD-sequencing data. For each of the possible mechanisms for ZW-females, we generated expectations for the patterns 1-5 mentioned above (Fig. 1) for LOH and coverage (to account for possible hemizygosity rather than homozygosity) in rare males, and we then compared observed patterns to these expectations. We also searched for candidate scaffolds for the sex locus in *Artemia* by selecting scaffolds that lost heterozygosity in all male but no female offspring. We discuss the implications of our findings regarding the evolutionary significance of rare males in parthenogenetic species and the maintenance of recombination in parthenogenetic *Artemia*.

Methods

Sex chromosomes in *Artemia*

Generally, the sex chromosomes of *Artemia* are not well characterized. Genetic maps, including Z and W chromosomes, have been obtained in *A. franciscana* (de Vos et al. 2013) and *Akaz* (Haag et al. 2017). Genomic studies have confirmed early cytogenetic observations (Barigozzi 1975), suggesting that the ZW system in *Af* is moderately young and contains a pseudo-autosomal region (de Vos et al. 2013). Two non-recombining parts (Huylmans et al. 2019) show different degree of Z-W divergence. However, the relative size of these regions is unknown. We also do not know whether the sex chromosomes are acrocentric or metacentric (both types are found in *Artemia* chromosomes, Accioly et al., 2014), and the degree of heteromorphy is unclear. Stefani (1963) reported low heteromorphy of sex chromosomes in *A. salina*, W being possibly slightly longer than Z. On the contrary, in *A. franciscana*, high heteromorphy was reported (W being the smallest chromosome, Parraguez et al., 2009), but no heteromorphy was found in another study (Accioly et al. 2014). The position of the sex-determining locus relative to the pseudo-autosomal part of ZW is unknown.

Sample acquisition and sequencing

The individuals used in this study belong to new parthenogenetic lineages generated by crossing rare *Ap* males with *Akaz* females (Boyer et al. 2021). Since meiosis modification in *Ap* involves suppression of meiosis I with some recombination (Nougué et al. 2015; Boyer et al. 2021), we expect genomic regions far from the centromere to accumulate homozygosity, and therefore to contain no informative markers for LOH in wild lineages (Boyer et al. 2021). Using novel, experimentally generated lineages, rather than individuals sampled from nature, allowed us to circumvent this problem and to investigate LOH in heterozygous markers distributed throughout the genome. Moreover, novel asexual lines generated by hybridization produce rare males at a higher rate than natural *Ap* (6.4 % vs. 0.4 %, Maccari et al. 2013; Boyer et al. 2021). Regarding their sex chromosomes, these new asexual lineages are expected to have a Z-

chromosome inherited from *Ap* and a W-chromosome from *Akaz*. Specifically, this study thus investigated whether rare male production in these novel asexual lineages occurs through LOH on sex chromosomes due to recombination between pseudo-autosomal regions of W and Z.

The crosses and rearing conditions have been previously described in detail (Boyer et al. 2021). Shortly, laboratory-maintained *Ap* populations were regularly inspected for rare males. Three rare males were found in a culture of a population sample originally obtained from Aigues-Mortes, France (population PAM7, Nougué et al. 2015) and one from a culture obtained by hatching cysts from lake Urmia, Iran (collection F. Amat, Instituto de Acuicultura de Torre de la Sal, Spain). These four rare males were crossed with five sexual females from *Akaz* (hatched from cycsts, Artemia Reference Center cyst number: ARC1039). The crosses resulted in multiple lineages, which were maintained for several generations of parthenogenetic reproduction. When these lineages produced rare males, we kept the mother, the son, and, when possible, a daughter, for genomic analysis. Across all lineages, nine sons and three of their sisters were used in the analyses, together with all of their mothers (21 individuals in total, fig. S1).

To minimize contamination with non-*Artemia* DNA from microorganisms, individual *Artemia* were washed in two successive baths (10 minutes each) prior to sampling: (1) a 0.05 % sodium hypochlorite solution and (2) sterile salt water (to remove the hypochlorite). Subsequently, the digestive tracks were removed by dissection (to further avoid contamination with gut bacteria), as well as the ovisacs of females (to avoid mixing the DNA of the females with offspring DNA). The resulting tissue was shortly washed in deionized water to remove salt, dried on absorbent paper, and stored in 96% ethanol at -20°C until DNA extraction.

Genomic DNA was extracted using the DNEasy Blood & Tissue kit (Qiagen). Each sample was homogenized in 180 µl of ATL buffer, incubated for 8h at 56°C (700 rpm shaking speed) after addition of 20 µl Proteinase K, and then incubated (5 minutes at room temperature) with 4 µl RNase A. The subsequent extraction steps were carried out according to the manufacturer's protocol. Library construction and RAD-sequencing were carried out by the Montpellier GenomiX platform (MGX, Montpellier, France). Library construction followed the protocol of Baird et al. (2008), with the restriction enzyme *Pst*I and twelve PCR cycles after ligation of P2 adapters. Sequencing was conducted on an Illumina NovaSeq 6000 flow cell, resulting in 725 million sequences (paired-end, 150 bp).

Demultiplexing and filtering

We used process-radtags (Stacks v.2.59, Catchen et al. 2013) for demultiplexing, to correct barcodes with one mismatch, and to filter reads with low quality (<0.1 % of reads) or no RAD-tags (11.5 % of reads). We obtained 279 million pairs, with 13 million sequences per individual, on average. Using Trimmomatic v.0.39 (Bolger et al. 2014), we removed cut sites and trimmed all sequences to 139bp. We further retained only reads with quality scores >20 (average) and >15 (all 4 bp windows). This filtering resulted in 10.4% of pairs being removed (because either one or both sequences did not pass), and 250 million pairs being retained.

SNP calling and VCF filtering

For SNP-calling, we used the de novo/reference hybrid approach as described in Paris et al. (2017) and Rochette and Catchen (2017) in Stacks. We ran "ustacks" on each individual separately, grouping reads that differed by two nucleotides or less into "loci" (parameters: M = 2, m = 3, N = 2). This resulted in an average of 347'564 loci per individual with a mean depth of 23.4 x. We then used "cstacks" to create a catalog of loci across the nine mothers, allowing for a maximum of two differences between individuals for the same locus (parameter: n = 2). This resulted in a catalog of 753'124 loci. The loci of each individual (including offspring) were then compared to the catalog using "sstacks". On average, 340'601 loci per individual matched with catalog loci. In the next step, we assembled the loci with their paired-end reads using tsv2bam. SNPs and genotypes were called using "gstacks" with default parameters (model: marukilow, parameters: var_alpha = 0.01, gt_alpha = 0.05) and removed PCR duplicates. The latter step resulted in a loss of 78 % of read pairs (PCR or optical duplicates). We retained 36.5 million read pairs, resulting in a total of 733'964 loci with an average depth of 5.7 x, on which genotypes were called.

The catalog loci were mapped to the first-generation draft reference genome of *Akaz*, using bwa mem v.0.7.17 (Li and Durbin 2009), and sorted with samtools v.1.13 (Li et al. 2009). Details of this assembly are given in Appendix 1. We integrated the genomic positions of the loci into the Stacks pipeline using stacks-integrate-alignment with default parameters (mapping quality >20, alignment coverage >60 % and identity percentage >60 %). A large proportion (95 %) of loci mapped to the reference genome, but 32 % of these were removed due to insufficient mapping quality or alignment coverage. The 494'551 remaining loci were filtered using "populations", keeping only SNPs that were present in more than 13 individuals and removing duplicate loci (separate catalog loci with identical genomic positions). The resulting VCF, containing 359'187 SNPs on 73'402 scaffolds was filtered further, using vcftools v.0.1.17 (Danecek et al. 2011). Given the average depth of 5.7 x, we only kept genotypes based on 2 to 13 reads, thus excluding genotypes with a depth of 1 (which contain no information on whether they are homozygous or heterozygous) as well as genotypes based on more than two times the average depth (because of the elevated risk that these loci included collapsed paralogs). In total, 5.7 million genotypes (on average 271'000 per individual) were retained for the analysis. Fig. S2 shows the depth distribution in the data before filtering.

The sequencing of our library resulted in a high number of duplicate sequences, which were removed as explained above. As a consequence, individual genotype calls were based on a relatively low sequencing depth compared to what is typical for RAD-sequencing data (Rochette and Catchen 2017). Nonetheless, our downstream analysis, investigating LOH between mothers and offspring, shows that even low-coverage RAD-sequencing data can produce highly robust results if the uncertainty of the genotype and the information content of the different loci is adequately taken into account throughout the analysis. Specifically, our analysis is based on genotype likelihoods, rather than fixed genotype calls. These genotype likelihoods were obtained from Stacks. Additionally, we combined likelihood information from all SNPs (minimum = 2) on a given scaffold and used filters to remove data containing little or ambiguous information and to reduce noise. These approaches allowed us to conduct a quantitative analysis of LOH, by propagating uncertainty throughout the analysis rather than

overconfidently relying on genotype calls. Similar methods are increasingly used also elsewhere in analysis of genomic data (Korneliussen et al. 2014; Rastas 2017).

Data analysis: LOH in male and female offspring

The analysis was carried out using the package vcfR v.1.12.0 (Knaus and Grünwald 2017) in R v.4.1.1 (R Core Team 2021) and genotype likelihoods (GL, actually log-likelihoods; Maruki and Lynch 2015; Rochette et al. 2019) obtained as a part of the vcf output in Stacks. Based on the genotype likelihoods (GL), we calculated the probabilities for a given individual (mother or offspring) to be homozygous, P_{hom}, or heterozygous, P_{het}, at a given SNP site. To obtain these probabilities (which have the advantage to sum up to 1), we used exp(GL) to convert the log-likelihoods to likelihoods. Then, we obtained P_{het} by dividing the likelihood of the heterozygous genotypes by the sum of the likelihoods of all three possible genotypes (because there were two alleles at each site, and thus three genotypes: homozygous for the reference allele, for the alternative allele or heterozygous) and P_{hom} from $1 - P_{het}$.

For each offspring, we restricted our analysis to SNPs for which P_{het} of the mother was > 0.5. In total, we carried out 766'228 SNP comparisons between mothers and offspring (representing 231'238 SNP localizations on 35'202 scaffolds). For each SNP comparison, we calculated P_{LOH} (the probability that LOH occurred) by multiplying the P_{het} of the mother with the P_{hom} of the offspring. To remove ambiguous SNP comparisons, we removed those with P_{LOH} between 0.2 and 0.8. For reads containing more than one SNP, we then combined the information by calculating the average P_{LOH} across all SNPs on the read. For the calculation of the average, each SNP was weighted by the P_{het} of the mother to give more weight to SNPs that were identified as heterozygous in the mother with higher confidence. To remove instances where the different SNPs on the same read gave conflicting information, we removed comparisons with an average P_{LOH} between 0.2 and 0.8 as for single ambiguous SNPs. Given that recombination is expected to be rare and that the scaffolds of our draft genome are relatively short (9'638 bp, on average), we then proceeded in the same way, combining the information of all loci (individual SNPs or combined SNPs per read) within scaffolds and retaining only scaffolds with at least two loci. The resulting average P_{LOH} per scaffold was again weighted by the per-locus average P_{het} of the mother, and ambiguous scaffolds (i.e., those with average P_{LOH} between 0.2 and 0.8) were removed. In total, we obtained 125'663 per-scaffold average P_{LOH} estimates, representing 25'032 different scaffolds, whose combined lengths represent 53% of the total assembly length.

Identifying autosomal and ZW scaffolds

We used three approaches to identify scaffolds on autosomes vs. sex chromosomes. Together, they resulted in the identification of 96 scaffolds on the sex chromosomes and 1'998 autosomal scaffolds (after the removal of 17 scaffolds with conflicting information, i.e., being assigned to sex chromosomes by one method and to autosomes by another). Given a haploid chromosome number of 21 (Barigozzi 1974), this gives an average of about 100 scaffolds identified per autosome. Fig. S3 shows the number of scaffolds identified for Z and autosomes by the different methods.

First, we used a re-analysis of the raw data from the *Akaz* genetic map (Haag et al. 2017), to integrate the map with the genome assembly. We generated a vcf, using the *de novo*/reference hybrid approach in Stacks described above and filtered loci and individuals as in Haag et al. (2017). We established a preliminary correspondence between our markers and those of the Haag et al. (2017) map, based on segregation patterns among the offspring and used R/qtl (Broman et al. 2003) functions ripple and dropone to further order markers along the linkage groups and to remove markers whose segregation patterns did not fit the linkage group. We then combined markers within scaffolds, removing scaffolds with inconsistent markers (i.e., with different markers mapping do different linkage groups), as well as scaffolds mapping to different linkage groups between male and female maps. In total, this procedure resulted in the identification of 22 Z-linked and 360 autosomal scaffolds.

Second, we assessed orthology between transcripts identified by RNA-sequencing of four *Akaz* males and four *Akaz* females with Z-linked genes and autosomal genes in *A. franciscana* (Huylmans et al. 2019). For RNA-sequencing, live *Akaz* individuals were washed for 2 minutes in sterile, deionized water and shortly dried on absorbent paper before they were flash-frozen in liquid nitrogen and stored at -80°C. RNA extraction was performed using the "NucleoSpin RNA Set for NucleoZOL" kit (Macherey-Nagel), following the manufacturer's instructions. RNA-sequencing was performed by Genewiz (Leipzig, Germany). The RNAseq reads were mapped to the *Akaz* reference genome (with repeat regions masked using RepeatMasker, Smit et al. 2013) using hisat2 (Kim et al. 2019). Protein coding sequences were identified with Augustus (Stanke et al. 2006), and their orthology to genes on *A. franciscana* scaffolds identified as autosomal or Z-linked (Huylmans et al. 2019) was assessed with OrthoFinder (Emms and Kelly 2015). In cases where groups of orthologs were identified (rather than one-to-one orthologs), *Akaz* and *Af* sequences were aligned using MACSE (Ranwez et al. 2011), and synonymous distance dS was estimated using codeML in PAML (Yang 1997). Only ortholog pairs with the lowest dS were retained for further analysis. In total these procedures allowed identification of 21 Z-linked and 1'670 autosomal *Akaz* scaffolds. *A. franciscana* has an estimated divergence of 32 MY from *Ap* and *Akaz* (Baxevanis et al. 2006). In this analysis, we made the assumption of synteny between *A. franciscana* and *Akaz*, based on the fact that only a single chromosomal rearrangement is known in *Artemia* (Barigozzi 1974). However, to assess the robustness of our results to this assumption, we performed the downstream analyses both including and excluding scaffolds that were identified as sex-linked by this method only.

Third, we used the RNA-sequencing data (see previous paragraph) to identify putative sex-linked SNPs that were consistently homozygous in *Akaz* males (putatively ZZ) and consistently heterozygous (putatively ZW) in *Akaz* females. Reads were mapped to the *Akaz* reference genome with the STAR aligner (Dobin and Gingeras 2015) and duplicates were removed with Picard RemoveDuplicates (Broad Institute). Variants were called with GATK HaplotypeCaller (van der Auwera and O'Connor 2020). Only variants genotyped in all individuals and with a minimal coverage of 10 were retained for this analysis. We selected loci with a minor allele frequency of less than 0.1 (homozygous-tendency) in all males and of more than 0.1 (heterozygous-tendency) in all females. We then identified scaffolds where the frequency of such loci was higher than expected by chance. To do so, we first computed the probability p_{zw}, that a SNP could show by chance a ZW pattern (meaning being homozygous in

all males and heterozygous in all females) given the average heterozygosity in males and females (across 200,635 identified SNP with a minimum of 10x depth in every individual). For each scaffold, we observed the number of SNPs showing a ZW pattern and we then tested whether their proportion exceeded p_{zw} (using a simple binomial test). Finally, we applied a Bonferroni correction across scaffolds for multiple testing. We used this correction for its high stringency. This method allowed us to identify 58 sex-linked scaffolds.

LOH on sex chromosomes and autosomes

After filtering (see above), we retained a P_{LOH} estimate for 77 of the 96 sex-linked scaffolds in at least one mother-offspring pair (521 mother-offspring comparisons in total). Considering $P_{LOH} > 0.8$ as LOH and $P_{LOH} < 0.2$ as heterozygosity retention (i.e., no LOH), we constructed a map of the scaffolds along the sex chromosome by ordering scaffolds according to LOH frequency across mother-offspring pairs. We further arranged the map manually by grouping scaffolds with LOH. To distinguish whether LOH events were due to homozygosity or hemizygosity (see Fig. 1), we compared the depth of the scaffolds that lost heterozygosity with the depth of the scaffolds that retained maternal heterozygosity. To compute this, we first scaled each scaffold depth by the average individual scaffold depth (to correct for small variation of sequencing effort among individuals). Then, we computed the mean scaled depth for ZW scaffold that lost and did not lose heterozygosity.

We investigated whether males and females were produced by meiosis showing different recombination patterns on autosomes. To do so, we compared autosomal LOH rates for male and female offspring. Among the 1'998 autosomal scaffolds, 1'393 were retained for the analysis (9'072 P_{LOH} estimates for individual mother-offspring comparisons in total). To analyse the data, we fitted likelihood models as described in Appendix 2. Because patterns of autosomal LOH seemed variable among individuals, we fitted a model allowing for inter-individual variation in rates of autosomal LOH. Specifically, we assumed that individual rates of autosomal LOH followed a Beta distribution. Because autosomal LOH rate was particularly variable in males, we then tested whether these distributions differed between males and females using likelihood ratio tests.

Candidate scaffolds linked to the sex-determining locus

To assess which candidate scaffolds are likely most closely linked to the sex-determining locus, we identified scaffolds for which all male offspring (for which data were available) lost heterozygosity ($P_{LOH} > 0.8$) while all female offspring retained heterozygosity ($P_{LOH} < 0.2$). Here, we used all scaffolds, regardless of whether we were able to assign them to the sex chromosomes, autosomes, or not. After filtering, a total of 15'337 scaffolds had a P_{LOH} estimate for at least one male and one female offspring. But among those that fitted the expected pattern (LOH in all male, no LOH female offspring), data were available for only 38% of offspring, on average. We therefore also added cases where the mother genotype was missing or of too low quality, as long as at least one offspring was clearly heterozygous, as this unambiguously indicates heterozygosity retention (and therefore indicates that the mother was heterozygous as well). We used the same quality filters as above (P_{LOH} lower than 0.2), and obtained 5'631 additional genotypes on 202 scaffolds, which were used to check whether these scaffolds still

matched the expected patterns. Two scaffolds were removed from the final candidate list because they were assigned, by above-mentioned approaches, to autosomes.

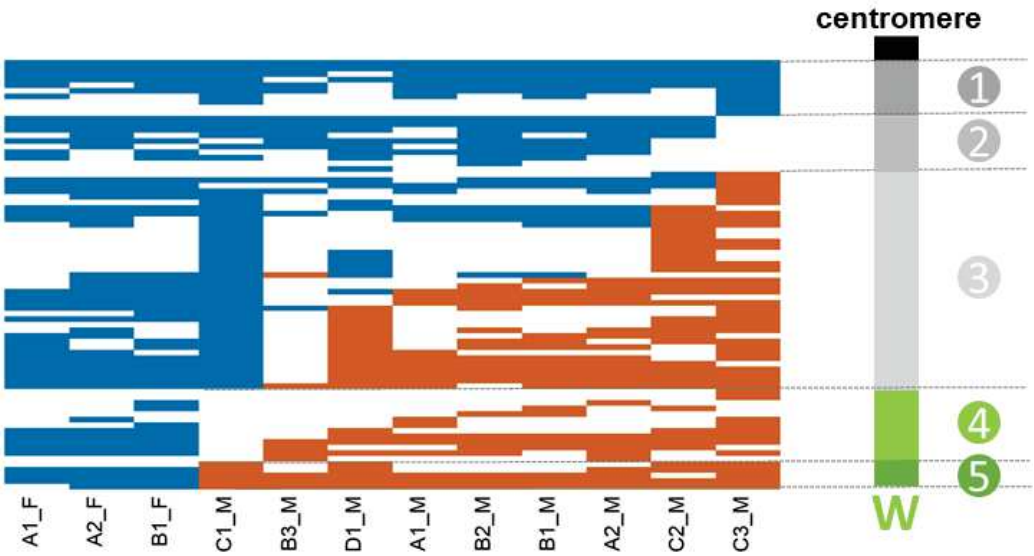


Figure 2. LOH on the 77 sex chromosome scaffolds. Each column represents an offspring, first the three female offspring then the nine male offspring, and each line represents a scaffold. Blue indicates heterozygosity retention ($P_{LOH} < 0.2$), orange indicates LOH ($P_{LOH} > 0.8$), while white indicates that information is not available for a given scaffold in a given offspring. Scaffolds are ordered from top to bottom according to increasing P_{LOH} frequency across offspring, and offspring are ordered from left to right according to increasing P_{LOH} frequency across scaffolds. On the right, a possible reconstruction of the W according to the results. (1) Non recombining region near the centromere (13% of the scaffolds / 17.1% of bp sequenced). This region can be extended to region (2), although information is missing for some scaffolds where recombination could have taken place (the whole region would then be 26% of the scaffolds / 30.2% of bp sequenced). (3) Pseudo-autosomal region where some recombination is observed (51% of the scaffolds / 49.1% of bp sequenced. (5) Non-recombining region around the sex-determination locus (6% of the scaffolds / 8.0% of bp sequenced). This region can be extended to region (4), although information is missing for some scaffolds where recombination could have taken place (the whole region would then be 23% of the scaffolds / 20.7% of bp sequenced).

Results

LOH on sex chromosomes

Patterns of LOH in male and female offspring

The expected patterns of LOH on sex chromosome scaffolds according to the different hypotheses are represented in Fig. 1b. None of the female offspring lost heterozygosity on any of the 77 sex-chromosome scaffolds. In contrast, all male offspring lost heterozygosity in some of these scaffolds (Fig. 2). LOH on 18 scaffolds (23 % of all scaffolds) was shared among all males, that is all genotyped males lost heterozygosity on these scaffolds, whereas no LOH in any of the genotyped males was observed on 20 scaffolds (26%). For the remaining scaffolds,

LOH was variable among males (Fig. 2). With a single exception (one scaffold in male B3 for which we cannot exclude genotyping error or erroneous sex chromosome assignment), the LOH patterns of all scaffolds were consistent with a single crossover having occurred at a variable location between W and Z during the production of every male offspring. Crossovers results in retention of heterozygosity for all scaffolds between the centromere (putative location: top of Fig. 2) and the crossover location, while leading to LOH for all scaffolds between the crossover location and the telomere (putative location: bottom of Fig. 2). The scaffolds in Fig. 2 are therefore likely ordered approximately as they are on the sex chromosomes. The scaffolds for which LOH was consistently observed in all males contain the inferred location of the sex-determining locus (putatively becoming ZZ in all males while remaining WZ in all female offspring). The ratio of the mean scaled depth of scaffolds that lost heterozygosity relative to the depth of scaffolds that maintained heterozygosity was 1.12 ± 0.03, so not consistent with hemizygosity (expected ratio = 1/2). Excluding scaffolds assigned by orthology with *Af* (i.e., only including those identified by the genetic map and the RNAseq studies) reduced the data set to 68 ZW scaffolds but had no qualitative nor major quantitative effect on the results (Fig. S4).

Candidate scaffolds likely linked to the sex-determining locus

When considering all scaffolds with LOH data, that is, including those that could not be assigned to either sex chromosomes or autosomes, and adding the information of scaffolds that were not genotyped in the mother, we identified 58 scaffolds potentially linked to the sex-determining locus. However, two were assigned to the autosomes and were removed. Three of the remaining scaffolds were assigned to the sex chromosomes, and we could not infer the assignation of the others. Given the low number of offspring, these may contain a number of false positives, potentially even including autosomal scaffolds. Nonetheless, with the additional data considered (see methods), an LOH estimate was available for almost 80 % of offspring, on average for a given scaffold.

LOH on autosomes

The distribution of autosomal LOH among offspring differed between males and females (likelihood ratio test, $P = 0.002$). Autosomal LOH was significantly larger (and more variable) in males than in females. More specifically, in males, LOH rate followed a beta distribution with an estimated mean of 0.07 and variance of 0.01 ($a = 0.67$; $b = 8.41$). In females, LOH rate followed a beta distribution with an estimated mean of 4.10^{-3} and a near-zero variance of 10^{-9} ($a = 17.6$; $b = 4.0 \times 10^{6}$; Fig. 3). Excluding scaffolds assigned by orthology with *A. franciscana* (i.e., only including those identified by the genetic map) reduced the data set to 260 autosomal scaffolds but had no qualitative nor major quantitative effect on the results (Fig. S5).

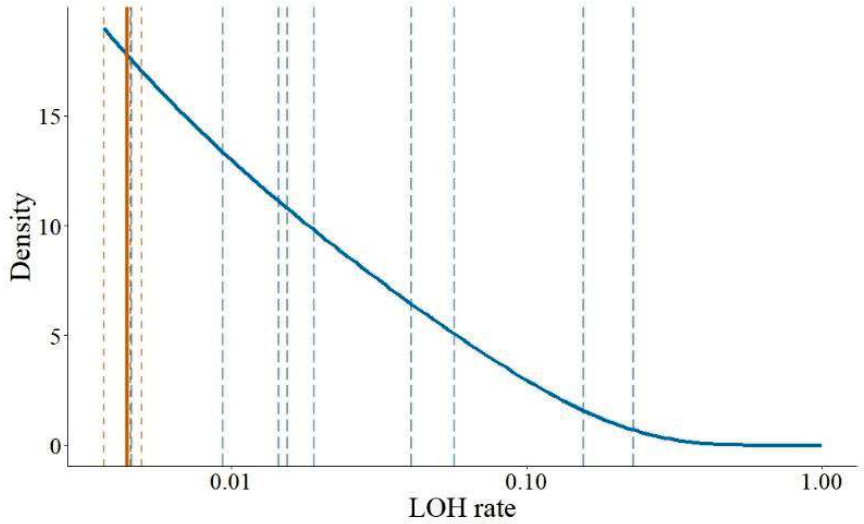


Figure 3. LOH rate on autosomes in male (blue) and female (orange) offspring. Dashed lines represent LOH rates in autosomal scaffolds for individual offspring, and solid lines represent the estimated distributions from our best model. Note the log-scale of the x-axis.

Discussion

Rare males are produced asexually by recombination on the sex chromosomes

We found LOH patterns on sex chromosome scaffolds exactly as expected under the hypothesis that rare males result from LOH events due to recombination breakpoints occurring at a variable location between the centromere of the sex chromosome pair and the sex-determining region (pattern 5 in Fig. 1). We found a systematic genetic signature of sex chromosome LOH in all males and absence thereof in all females. This observation excludes environmental or other errors in sex differentiation as well as localized mutation or TE insertion near or at the sex-determination locus as the main explanation for the production of rare males. LOH in males concerned only a part of the sex chromosome scaffolds, while others retained heterozygosity in all males. This observation rules out non-disjunction that would lead to a complete loss of the W (i.e., ZO males) as well as modified meiosis mechanisms (e.g., terminal fusion without recombination) resulting in homozygosity over the entire chromosome. Note that terminal fusion leads to systematic LOH at all scaffolds (no recombination) or at the centromere-proximal scaffolds (with recombination) on all autosomes as well. This mechanism (suggested by Stefani, 1964, for *Ap*) can therefore be excluded according to our results (no systematic LOH on autosomes, neither in males nor in females). Finally, sequencing depth of sex chromosome scaffolds that lost heterozygosity was similar (even slightly higher) than the depth of scaffolds that retained heterozygosity. This strongly suggests that LOH is due to homozygosity rather than hemizygosity and therefore caused by recombination during modified meiosis rather than by partial W deletion.

Given these findings, we can conclude with high confidence that the nine males in our analysis were produced by LOH at the sex-determining locus, caused by ZW crossover events

that occurred during modified meiosis in their mother. This mechanism is in line with our knowledge of the asexual reproductive mode of *Ap* (Nougué et al. 2015; Boyer et al. 2021), and has been previously suggested as a possible mechanism for rare male production (Browne and Hoopes 1990; Nougué et al. 2015; Boyer et al. 2021). Recombination on sex chromosomes can lead to ZZ or WW offspring, not for the entire chromosome, but at least for the region relevant for sex-determination. While WW individuals are probably non-viable, ZZ individuals develop as rare males.

Rare males were therefore produced by recombination, but interestingly, it also appears that they were often produced by meiosis showing particularly high recombination rates overall, i.e. even on autosomes (compared to the meiosis leading to female offspring). In autosomal scaffolds, the distribution of LOH rate differed between male and female offspring. Females had a consistently very low rate of LOH while males displayed very variable LOH (up to 16%). Males and females might be the result of reproductive events with different recombination rates. However, since there is a high variation in male offspring LOH, it seems more likely that this difference is actually the result of continuous intra-mother variation in recombination rate, and that males simply happen to be produced by (modified) meiosis having a higher than average number of crossing overs on all chromosomes, including the sex chromosomes. The difference observed between male offspring with the most LOH and female offspring could also be partly due to population or cross variation: Although the females are controls as they are the sisters of males in our analysis, we do not have data for the sisters of the males that happen to show the highest LOH.

While *Ap* females reproduce mainly through modified meiosis, they can rarely reproduce sexually, likely through a normal meiosis (Boyer et al. 2021). In contrast, rare males produced by these *Ap* females seem to mainly or always undergo normal meiosis as no evidence for unreduced sperm (i.e., no evidence for triploidy in their offspring) was found (Boyer et al. 2021). This suggests that both normal and modified meiosis pathways exist in *Ap* and that the frequency of the expression of one or the other pathway is sex-specific.

Finally, our proof that rare males are produced by recombination validates the use of the rate of rare male production as a good proxy to measure residual recombination rate in asexual lineages (Nougué et al. 2015; Boyer et al. 2021). Of course, this is only a measure of the recombination rate on the sex chromosome pair, and it may not necessarily reflect global recombination rate in the genome. However, it may be a less biased estimate for wild populations, as the history of past LOH precludes to estimate LOH rates in an unbiased manner using genetic markers (since the loci away from the centromere, which are the most likely to undergo LOH are unlikely to be heterozygous in the first place).

Explaining the heterogeneity of rare male production among lineages

Different *Ap* lineages produce rare males at different rates (Maccari et al. 2013). According to our results, this can be explained by these lineages having different recombination rates. Under modified meiosis where meiosis I is suppressed, recombination leads to LOH. Similarly to inbreeding, LOH leads to lower fitness, as it reveals recessive deleterious mutations

(loss-of-complementation, Archetti 2004). Hence, it is likely that there is selection for a lower recombination rate within asexual lineages (Engelstädter 2017). This was suggested in the Cape honeybee, which also reproduce by a modified meiosis where recombination causes LOH (Goudie et al. 2012). Reduction of the recombination rate would result in a lower rate of male production. In *Ap*, this scenario is consistent with the observation that rare male production increases in asexual females obtained from successive backcrosses to the sexual species *Akaz* (therefore introgressing sexual recombination determinants, Boyer et al. 2021). Now that there is conclusive evidence for rare male production by recombination, we can firmly interpret this earlier result as evidence for selection against recombination in asexual lineages compared to the sexual species. In some lineages, in which seemingly no rare males are produced (Maccari et al. 2013), recombination might have been lost altogether (or equivalently the position of crossing-over might have evolved to be telomeric, and thus not cause LOH). Similarly, in *Ap* polyploids, no male has ever been observed. To explain this observation, it is often assumed that these polyploids reproduce by clonal apomixis. This is however unlikely, as they are derived from crosses involving asexual diploids (Rode et al. *in press*). They are therefore likely to share the same meiosis modification as the diploids. The absence of rare males in these lineages may rather result from the preferential pairing of Z with Z and W with W, drastically limiting the opportunity for recombination between Z and W. Furthermore, in tetraploids and pentaploids, two subsequent LOH would be required to produce ZZZZ or ZZZZZ males, which further reduces the likelihood of occurrence of rare males (Rode et al. *in press*).

Long term evolution of recombination in *Ap* lineages

If LOH is costly, why then is non-zero recombination and with it rare male production maintained in most *Ap* lineages? One possibility is that most lineages are relatively recent, and recent lineages maintain some recombination. This could be because they were recently generated by a sexual event involving a rare male produced by recombination or because of introgression of recombination alleles if the new lineage was created by crossing with a female from a sexual species (Boyer et al. 2021). Consistent with this idea is the observation that the highest rates of rare male production occur in *Ap* lineages from Central Asia (Maccari et al. 2013), that is, from the distribution range of the sexual species, where repeated crosses are most likely to occur. Yet, phylogenetic evidence suggests that crosses with sexual species leading to contagious asexuality occurred only rarely in the evolutionary history of extant diploid *Ap* (Rode et al. *in press*). The generation of new asexual lineages may thus more frequently involve rare males and rare sexual events in *Ap* females species (Boyer et al. 2021). Such within-*Ap* crosses would be more difficult to detect with phylogenetic evidence. Another possibility is that rare within-*Ap* crosses or contagious parthenogenesis might sometimes lead to new asexual lineages with fewer or masked deleterious mutations, thus “rescuing” old asexual lineages from long-term decrease in fitness. However, whether the possibility to generate new asexual lineages via rare males indeed can lead to selection for maintaining non-zero recombination requires further study. It should notably involve a study of the costs of LOH through unmasking recessive deleterious mutations and the possibility that selection pressures may differ for the asexuality-determining region(s) vs. the rest of the genome.

Structure of the Z chromosomes

The fact that males are produced by recombination means that portions of the W and Z chromosomes are indeed pseudo-autosomal, and that this pseudo-autosomal region (PAR) is located between the centromere and the sex-determining locus. Moreover, the observed heterogeneity of male LOH on the sex chromosomes suggests that crossovers may occur at many different locations and thus that the PAR is relatively large (51-81 % of the sex chromosome scaffolds underwent LOH in at least one male while retaining heterozygosity in at least one other male, suggesting that all these scaffolds are located in the PAR). The non-recombining region near the centromere contains 13%-26 % of all sex chromosome scaffolds with P_{LOH} data and the non-recombining region containing the sex-determining locus 6% - 23 % of all sex chromosome scaffolds with P_{LOH} data. Note that it is possible that a part of the PAR is on a second chromosome arm or located terminally after the sex-determining region. However, if present, these regions are likely small (or have a short genetic distance) as otherwise we should have observed sex chromosome LOH in females as well. Fig. 2 represents these findings and a possible structure of the W.

We identified 56 scaffolds potentially associated with the sex-determining locus, but of these, only three scaffolds could be assigned to the sex chromosome pair. Better genomic resources for *Ap* might allow narrowing this list. Nonetheless, these scaffolds constitute an important first step towards identifying the sex-determining locus in *Artemia*.

Conclusion

In this study, we compared RAD-sequencing data between asexual *Artemia* females and their male and female offspring. We demonstrated that rare ZZ males are produced by recombination between W and Z sex chromosomes, as a result of a non-clonal asexual reproductive mode. The data also allowed us to infer the likely structure of the sex chromosomes, the localization of the sex-determining locus, and a list of candidate scaffolds associated with the sex-determining locus. This study shows that the consequences of non-clonal asexuality, even occurring through rare events, can be significant. Rare males potentially are major actors in the long-term evolution of *Artemia*. Knowing how rare males are produced in parthenogens, when combined with reliable genomics resources, can provide essential insight into their evolutionary significance, and the consequences of contagious parthenogenesis.

Acknowledgements

The authors are grateful to G. Van Stappen for providing the *Akaz* sample. We thank E. Ortega, Q. Rougemont and E. Beyne for helpful advice with the bioinformatics analysis. We thank M.-P. Dubois and The Genomics, Molecular Ecology, and Experimental Evolution platform (GEMEX) at CEFE. RAD-sequencing library construction and sequencing was performed by the MGX platform (Montpellier, France). RNA-sequencing was conducted by the Genewiz company, (Leipzig, Germany). We are thankful to the GenOuest bioinformatics core facility for providing computing infrastructure for part of this study. This work was funded by the Grant ANR-17-CE02-0016-01, GENASEX, from the French National Research Agency.

Author contributions

T.L. and C.H. acquired funding and supervised the study. T.L., L.B., and C.H. conceived and planned the study. R.Z. and L.B. obtained the individuals and conducted the extractions. L.B., P.J., S.G., and T.L. analyzed the data. L.B., T.L., and C.H. wrote the original manuscript and produced figures. L.B., T.L., C.H., and S.G. revised the manuscript.

References

Abatzopoulos, T. J. 2018. The repeated emergence of asexuality , the hidden genomes and the role of parthenogenetic rare males in the brine shrimp Artemia. J. Biol. Res. 1–5. BioMed Central.

Abreu-Grobois, F., and J. Beardmore. 2001. The generation of males by diploid parthenogenetic *Artemia* cannot occur in the way Stefani suggested. P. 1 *in* A. Maeda-Martinez, B. Timms, D. Rogers, G. Murugan, and A. Abreu-Grobois, eds. Proceedings of the 4th International Large Branchiopod Symposium. La Paz, Baja California, Mexico.

Accioly, I. V., I. M. C. Cunha, J. C. M. Tavares, and W. F. Molina. 2014. Chromosome Banding in Crustacea. I. Karyotype, Ag-NORs, C Banding and Treatment with EcoRI, PstI and KpnI Restriction Endonucleases in *Artemia franciscana*. Biota Amaz. 4:15–19.

Archetti, M. 2010. Complementation, genetic conflict, and the evolution of sex and recombination. J. Hered. 101:1–13.

Archetti, M. 2004. Recombination and loss of complementation: A more than two-fold cost for parthenogenesis. J. Evol. Biol. 17:1084–1097.

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. A. Lewis, E. U. Selker, W. A. Cresko, and E. A. Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3:1–7.

Barigozzi, C. 1974. Artemia: a survey of its significance in genetic proplems. P. *in* T. Dobzhansky, M. Hecht, and W. Steere, eds. Evolutionary Biology. Springer, Boston, MA.

Baxevanis, A. D., I. Kappas, and T. J. Abatzopoulos. 2006. Molecular phylogenetics and asexuality in the brine shrimp *Artemia*. Mol. Phylogenet. Evol. 40:724–738.

Blackman, R. L. 1972. The inheritance of life-cycle differences in *Myzus persicae* (Sulz.) (Hem., Aphididae). Bull. Entomol. Res. 62:281–294.

Blackman, R. L., and D. F. Hales. 1986. Behaviour of the X chromosomes during growth and maturation of parthenogenetic eggs of *Amphorophora tuberculata* (Homoptera, Aphididae), in relation to sex determination. Chromosoma 94:59–64.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Bowen, S. T. 1963. The Genetics of *Artemia Salina*. II. White Eye, a Sex-Linked Mutation. Biol. Bull. 124:17–23.

Boyer, L., J.-Z. R., M. Mosna, C. R. Haag, T. Lenormand, R. Zahab, M. Mosna, C. R. Haag, and T. Lenormand. 2021. Not So Clonal Asexuals: Unraveling The Secret Sex Life Of Artemia parthenogenetica. Evol. Lett. 5:164–174.

Broman, K. W., H. Wu, Ś. Sen, and G. A. Churchill. 2003. R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890.

Browne, R. A., and C. W. Hoopes. 1990. Genotype Diversity and Selection in Asexual Brine Shrimp (Artemia). Evolution 44:1035–1051.

Card, D. C., F. J. Vonk, S. Smalbrugge, N. R. Casewell, W. Wüster, T. A. Castoe, G. W. Schuett, and W. Booth. 2021. Genome-wide data implicate terminal fusion automixis in king cobra facultative parthenogenesis. Sci. Rep. 11:1–9.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. Stacks: An analysis tool set for population genomics. Mol. Ecol. 22:3124–3140.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, and R. Durbin. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158.

de Vos, S., P. Bossier, G. van Stappen, I. Vercauteren, P. Sorgeloos, and M. Vuylsteke. 2013. A first AFLP-Based Genetic Linkage Map for Brine Shrimp *Artemia franciscana* and Its Application in Mapping the Sex Locus. PLoS One 8:1–10.

Dobin, A., and T. R. Gingeras. 2015. Mapping RNA-seq reads with STAR. Curr. Protoc. Bioinform. 51.

Doums, C., C. Ruel, J. Clémencet, P. Fédérici, L. Cournault, and S. Aron. 2013. Fertile diploid males in the ant *Cataglyphis cursor*: A potential cost of thelytoky? Behav. Ecol. Sociobiol. 67:1983–1993.

Emms, D. M., and S. Kelly. 2015. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol. 16:1–14. Genome Biology.

Engelstädter, J. 2017. Asexual but Not Clonal : Evolutionary Processes in Automictic Populations. Genetics 206:993–1009.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–1150.

Engelstädter, J., C. Sandrock, and C. Vorburger. 2011. Contagious parthenogenesis, automixis, and a sex determination meltdown. Evolution 65:501–511.

Goudie, F., M. H. Allsopp, M. Beekman, P. R. Oxley, J. Lim, and B. P. Oldroyd. 2012. Maintenance and loss of heterozygosity in a thelytokous lineage of honey bees (*Apis mellifera capensis*). Evolution 66:1897–1906.

Haag, C. C. R., L. Theodosiou, R. Jabbour-Zahab, T. Lenormand, R. Zahab, and T. Lenormand. 2017. Low recombination rates in sexual species and sex-asex transitions. Philos. Trans. R. Soc. London 372:20160461.

Hebert, P. D. N., and T. Crease. 1983. Clonal diversity in populations of *Daphnia Pulex* reproducing by obligate parthenogenesis. Heredity 51:353–369.

Huylmans, A. K., M. A. Toups, A. MacOn, W. J. Gammerdinger, and B. Vicoso. 2019. Sex-biased gene expression and dosage compensation on the artemia franciscana Z-chromosome. Genome Biol. Evol. 11:1033–1044.

Innes, D. J., C. J. Fox, and G. L. Winsor. 2000. Avoiding the cost of males in obligately asexual *Daphnia pulex* (Leydig). Proc. R. Soc. B Biol. Sci. 267:991–997.

Innes, D. J., and P. D. N. Hebert. 1988. The Origin and Genetic Basis of Obligate Parthenogenesis in *Daphnia pulex*. Evolution 42:1024–1035.

Kim, D., J. M. Paggi, C. Park, C. Bennett, and S. L. Salzberg. 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. 37:907–915.

Knaus, B. J., and N. J. Grünwald. 2017. vcfr: a package to manipulate and visualize variant call format data in R. Mol. Ecol. Resour. 17:44–53.

Korneliussen, T. S., A. Albrechtsen, and R. Nielsen. 2014. ANGSD: Analysis of Next Generation Sequencing Data. BMC Bioinformatics 15:1–13.

Lenormand, T., J. Engelstädter, S. E. Johnston, E. Wijnker, and C. R. Haag. 2016. Evolutionary mysteries in meiosis. Philos. Trans. R. Soc. B Biol. Sci. 371.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

Lynch, M. 1984. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. Q. Rev. Biol. 59:257–290.

Maccari, M., F. Amat, F. Hontoria, and A. Gómez. 2014. Laboratory generation of new parthenogenetic lineages supports contagious parthenogenesis in *Artemia*. PeerJ 2:e439.

Maccari, M., A. Gómez, F. Hontoria, and F. Amat. 2013. Functional rare males in diploid parthenogenetic *Artemia*. J. Evol. Biol. 26:1934–1948.

MacDonald, G. H., and R. A. Browne. 1987. Inheritance and reproductive role of rare males in a parthenogenetic population of the brine shrimp, *Artemia parthenogenetica*. Genetica 75:47–53.

Maruki, T., and M. Lynch. 2015. Genotype-frequency estimation from high-throughput sequencing data. Genetics 201:473–486.

Neiman, M., K. Larkin, A. R. Thompson, and P. Wilton. 2012. Male offspring production by asexual *Potamopyrgus antipodarum*, a New Zealand snail. Heredity 109:57–62.

Nougué, O., N. O. Rode, R. Jabbour-zahab, A. Ségard, L. M. Chevin, C. R. Haag, and T. Lenormand. 2015. Automixis in Artemia: Solving a century-old controversy. J. Evol. Biol. 28:2337–2348.

Olsen, M., and S. Marsden. 1954. Natural parthenogenesis in turkey eggs. Science 120:545–546.

Paris, J. R., J. R. Stevens, and J. M. Catchen. 2017. Lost in parameter space: a road map for stacks. Methods Ecol. Evol. 8:1360–1373.

Parraguez, M., G. Gajardo, and J. A. Beardmore. 2009. The new world artemia species *A. franciscana* and *A. persimilis* are highly differentiated for chromosome size and heterochromatin content. Hereditas 146:93–103.

Pijnacker, L. P., and M. A. Ferwerda. 1980. Sex chromosomes and origin of males and sex mosaics of the parthenogenetic stick insect *Carausius morosus* Br. Chromosoma 79:105–114.

Ranwez, V., S. Harispe, F. Delsuc, and E. J. P. Douzery. 2011. MACSE: Multiple alignment of coding SEquences accounting for frameshifts and stop codons. PLoS One 6.

Rastas, P. 2017. Lep-MAP3: Robust linkage mapping even for low-coverage whole genome sequencing data. Bioinformatics 33:3726–3732.

Rispe, C., J. Bonhomme, and J. C. Simon. 1999. Extreme life-cycle and sex ratio variation among sexually produced clones of the aphid *Rhopalosiphum padi* (Homoptera: Aphididae). Oikos 254–264.

Rochette, N. C., and J. M. Catchen. 2017. Deriving genotypes from RAD-seq short-read data using Stacks. Nat. Protoc. 12:2640–2659. Nature Publishing Group.

Rochette, N. C., A. G. Rivera-Colón, and J. M. Catchen. 2019. Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol. Ecol. 28:4737–4754.

Rode, N. O., R. Jabbour-Zahab, L. Boyer, É. Flaven, F. Hontoria, G. Van Stappen, F. Dufresne, C. Haag, and T. Lenormand. *in press*. The origin of asexual brine shrimps. Am. Nat.

Sandrock, C., and C. Vorburger. 2011. Single-Locus recessive inheritance of asexual reproduction in a parasitoid wasp. Curr. Biol. 21:433–437.

Simon, J. C., F. Delmotte, C. Rispe, and T. Crease. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. Biol. J. Linn. Soc. 79:151–163.

Simon, J. C., N. Leterme, and A. Latorre. 1999. Molecular markers linked to breeding system differences in segregating and natural populations of the cereal aphid *Rhopalosiphum padi* L. Mol. Ecol. 8:965–973.

Smit, A., R. Hubley, and P. Green. 2013. RepeatMasker. WA: Institute for Systems Biolog, Seattle.

Smith, R. J., T. Kamiya, and D. J. Horne. 2006. Living males of the "ancient asexual" Darwinulidae (Ostracoda: Crustacea). Proc. R. Soc. B Biol. Sci. 273:1569–1578.

Snyder, D. W., C. H. Opperman, and D. McK. Bird. 2006. A Method for Generating *Meloidogyne incognita* Males. J. Nematol. 38:192–194.

Stanke, M., O. Schöffmann, B. Morgenstern, and S. Waack. 2006. Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. BMC Bioinformatics 7:1–11.

Stefani, R. 1963. Il centromero non localizzato in *Artemia salina* Leach. Rend. Accad. Naz. Lincei Cl. Sci. fis. mat. nat 35:375–378.

Stefani, R. 1964. The origin of males in parthenogenetic populations of *Artemia salina*. Riv. Biol. 57:147.

Strunnikov, V. 1995. Control over reproduction, sex, and heterosis of the silkworm. Harwood Academic Publishers., Luxembourg.

Svendsen, N., C. M. O. Reisser, M. Dukić, V. Thuillier, A. Ségard, C. Liautard-Haag, D. Fasel, E. Hürlimann, T. Lenormand, Y. Galimov, and C. R. Haag. 2015. Uncovering cryptic asexuality in Daphnia magna by RAD-sequencing. Genetics 201:1143–1155. Genetics.

R Core Team 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

van der Auwera, G. A., and B. D. O'Connor. 2020. Genomics in the Cloud: Using Docker, GATK, and WDL in Terra. 1st ed.

van der Kooi, C. J., and T. Schwander. 2014a. Evolution of asexuality via different mechanisms in grass thrips (thysanoptera: Aptinothrips). Evolution 68:1883–1893.

van der Kooi, C. J., and T. Schwander. 2014b. On the fate of sexual traits under asexuality. Biol. Rev. 89:805–819. John Wiley & Sons, Ltd (10.1111).

Watts, P. C., K. R. Buley, S. Sanderson, W. Boardman, C. Ciofi, and R. Gibson. 2006. Parthenogenesis in Komodo dragons. Nature 444:1021–1022.

Wilson, A. C. C., P. Sunnucks, and D. F. Hales. 1997. Random loss of X chromosome at male determination in an aphid, Sitobion near fragariae, detected using an X-linked polymorphic microsatellite marker. Genet. Res. 69:233–236.

Yang, Z. 1997. Paml: A program package for phylogenetic analysis by maximum likelihood. Bioinformatics 13:555–556.

Supplementary materials

1. Assembly

MinION assembly : We first produced a *de novo* assembly of MinION reads with wtdbg2 (Ruan and Li 2020). Reads that were not used were merged with the obtained contigs. 10X Illumina reads were mapped to this MinION assembly with bwa mem. The resulting bam files were used to correct the MinION assembly with Pilon (Walker et al. 2014).

10X assembly : We then used 10X Illumina reads to produce a second *de novo* assembly with Supernova (Weisenfeld et al. 2018). Two pseudohaplotypes were created with the "mkoutput" command. 10X reads were then mapped against the first pseudohaplotype with bowtie2 (Langmead et al. 2009). The resulting bam file was used to remove duplicated contigs and cut overlapping parts of the supernova pseudohaplotype with "Purge_haplotigs". Finally, we used Links (Warren et al. 2015) to link the contigs of this purged assembly with the MinION corrected contigs to get our final reference assembly (Tab S1).

Measure	Result	N
Sum	1247144234	129396
N50	27463	11676
N60	19082	17114
N70	12065	25372
N80	7247	38763
N90	3605	62956
N100	102	129396

Table S1. Characteristics of the assembly. N represents the number of scaffolds included in the calculation.

BUSCO **:** We used BUSCO (Manni et al. 2021) to check for completeness by comparing to the arthropod database. This analysis is summarized in Tab S2.

BUSCOs	Result	Percentage
Complete BUSCOs	491	48.5%
Complete and single-copy BUSCOs	476	47.0%
Complete and duplicated BUSCOs	15	1.5%
Fragmented BUSCOs	156	15.4%
Missing BUSCOs	366	36.1%
Total BUSCO groups searched	1013	

Table S2. Results of the BUSCO analysis.

2. Analysis of autosomal LOH

We analysed the LOH data using likelihood. We note q the proportion of autosomal scaffolds losing heterozygosity. We assume that q values are Beta distributed with parameters a and b, and that these parameters can vary between males and females. We note n_i and m_i the observed number of scaffolds that loose or retain heterozygosity for individual i. We note **n** and **m** the vector of all n_i and m_i and $\boldsymbol{\theta}$ the vector of parameters to be estimated. The likelihood of the data can then be written

$$L(\mathbf{m}, \mathbf{p}|\boldsymbol{\theta}) = \prod_i \int_0^1 \beta(a, b; x) B(n_i + m_i, x; n_i) dx, \qquad (1)$$

where $\beta(a,\ b\,; x)$ denotes the probability to draw x in a Beta distribution with parameters a and b and where $B(n, x; k)$ denotes the probability to draw k success among n trials with a probability of success x (i.e. in a binomial distribution with parameters n and x). We estimated a and b parameters independently for males and females and, in another model where a and b were constrained to be identical between males and females. We tested whether male and female LOH differed by comparing these models with a likelihood ratio test. These tests were done using Mathematica v.9 (Wolfram Research 2012).

Supplementary figures

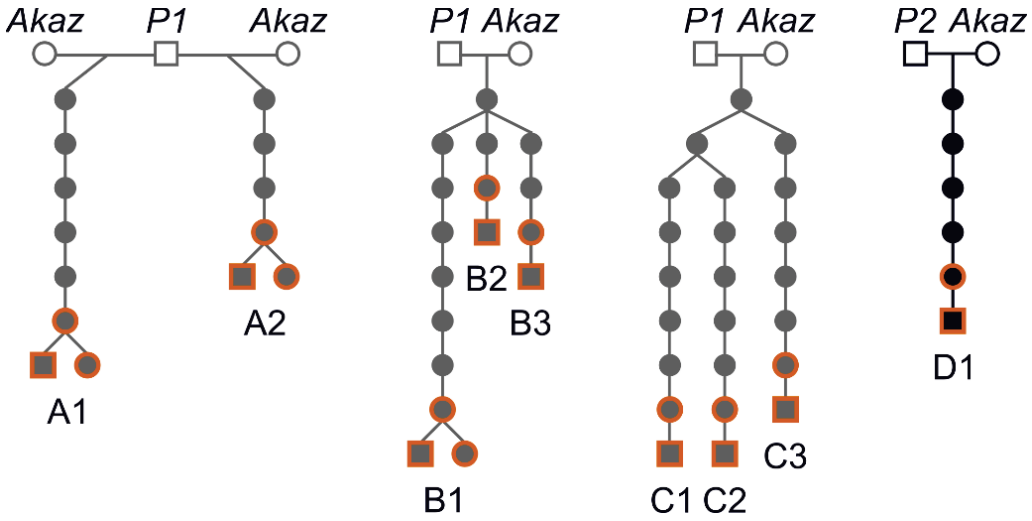


Figure S1. Origin of the individuals studied. Circles represent female individuals and squares represent male individuals. Open circles and squares represent the sexual females and rare males used to generate new asexual lineages. Lineages in grey were produced by rare males originating from the P1 (Aigues-Mortes) population, while the lineage in black was produced by a rare male from the P2 (Lake Urmia) population. The individuals included in the study are highlighted in orange.

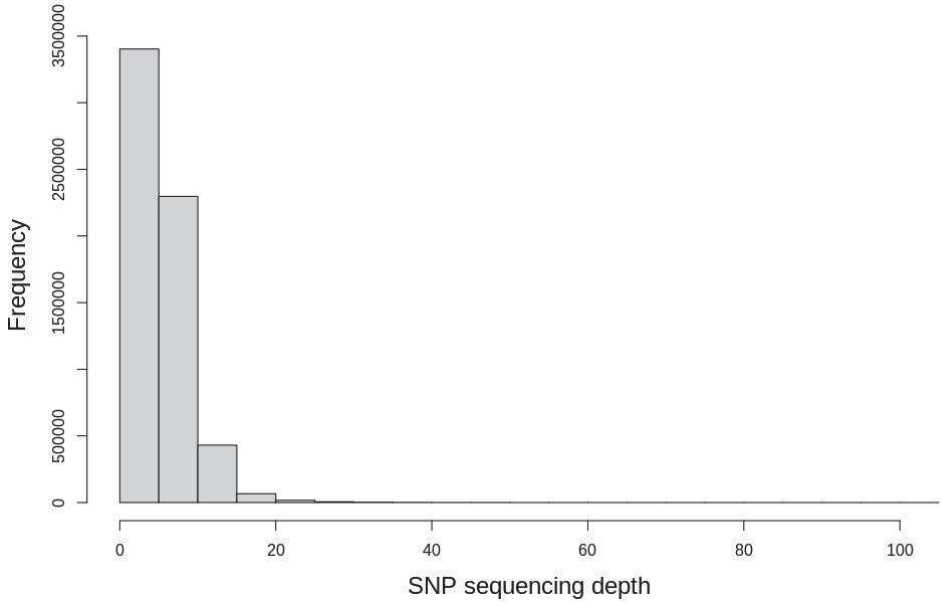


Figure S2. Distribution of sequencing depth per SNP.

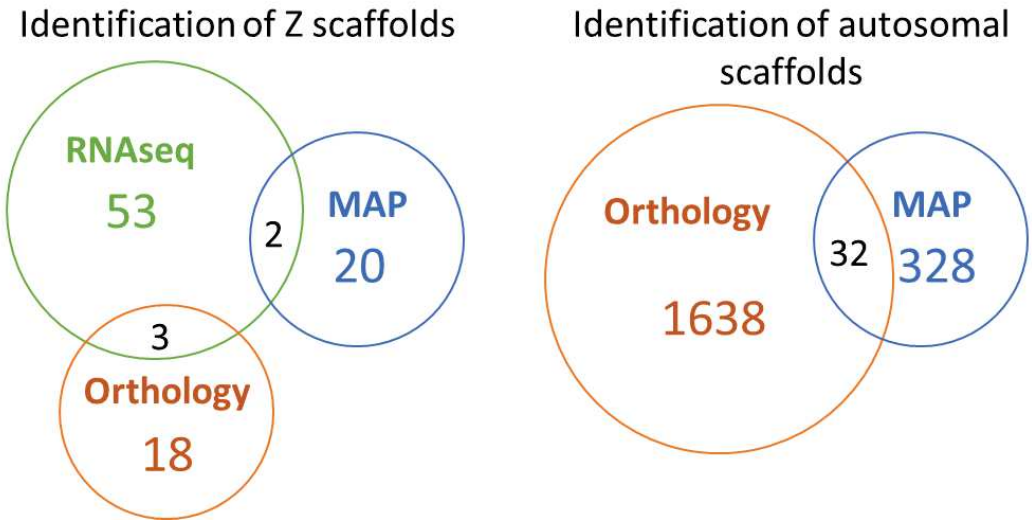


Figure S3. Identification of Z and autosomal scaffolds by the different methods. RNAseq refers to the comparison of SNP heterozygosity between males and females. MAP refers to the assignation of scaffolds to the linkage map of *Akaz*. Orthology refers to the assignation of scaffolds based on the autosomal/Z assignation made by Huylmans et al. 2019.

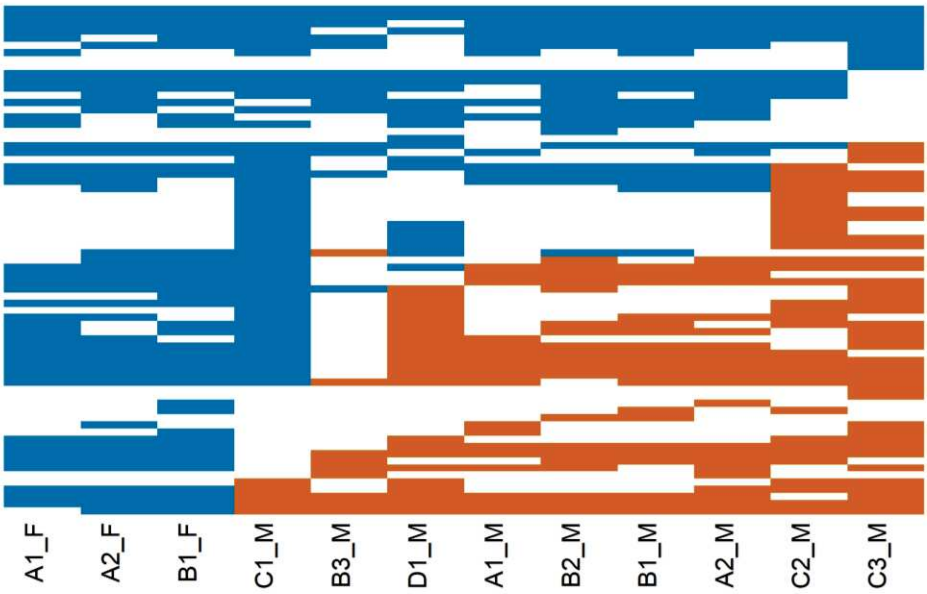


Figure S4. LOH on the sex chromosome scaffolds after removal of scaffolds identified by orthology with *A. franciscana*. Figure construction is the same as Figure 2 (main text). The removal of the 6 scaffolds did not affect the pattern observed on the figure.

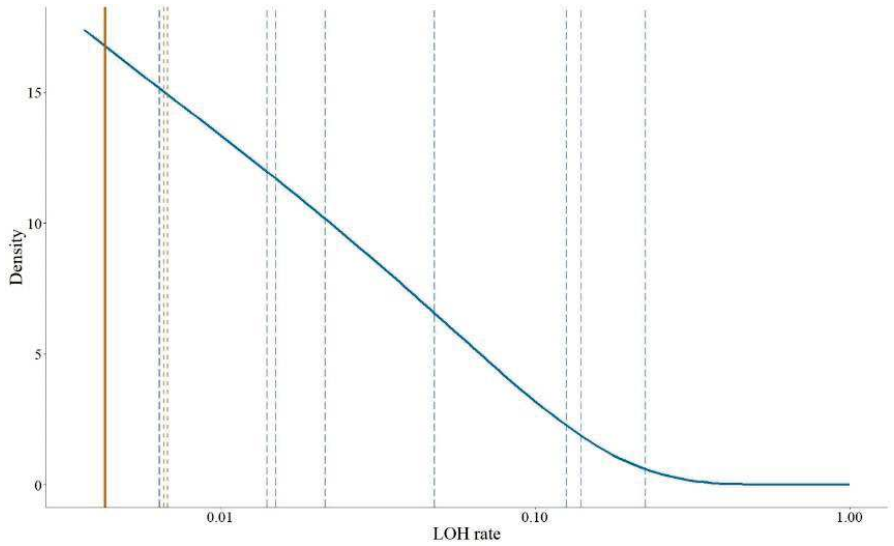


Figure S5. LOH rate on 260 autosomal scaffolds in male (blue) and female (orange) offspring after removal of scaffolds identified by orthology with *A. franciscana*. Dashed lines represent LOH rates in autosomal scaffolds for individual offspring, and solid lines represent the estimated distributions from our best model. One of the females presented an LOH rate of 0, and is thus not represented on this log-scaled figure. The scaffold removal largely did not affect the results. The best model of autosomal LOH distribution among offspring also displayed a difference between males and females (likelihood ratio test, $P = 0.03$). In males, LOH rate followed a beta distribution of parameters a=0.80 and b=11.2. In females, LOH rate followed a beta distribution with an estimated mean of 4.10^{-3} and a near-zero variance of 10^{-9}.

Supplementary references

Langmead, B., C. Trapnell, M. Pop, and S. L. Salzberg. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10.

Manni, M., M. R. Berkeley, M. Seppey, F. A. Simão, and E. M. Zdobnov. 2021. BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. Mol. Biol. Evol. 38:4647–4654.

Ruan, J., and H. Li. 2020. Fast and accurate long-read assembly with wtdbg2. Nat. Methods 17:155–158.

Walker, B. J., T. Abeel, T. Shea, M. Priest, A. Abouelliel, S. Sakthikumar, C. A. Cuomo, Q. Zeng, J. Wortman, S. K. Young, and A. M. Earl. 2014. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9.

Warren, R. L., C. Yang, B. P. Vandervalk, B. Behsaz, A. Lagman, S. J. M. Jones, and I. Birol. 2015. LINKS: Scalable, alignment-free scaffolding of draft genomes with long reads. Gigascience 4. GigaScience.

Weisenfeld, N. I., V. Kumar, P. Shah, D. M. Church, and D. B. Jaffe. 2018. Direct determination of diploid genome sequences. Genome Res. 28:757–767.

Wolfram Research, I. 2012. Mathematica 9.0. Wolfram Research, Inc., Champaign, IL.

Discussion

I. How does the transition from sex to asexuality explain the distribution of clonal and non-clonal asexuals?

a. Non-clonal asexuals are more common than previously thought

In chapter 2, I reported a bias toward clonality in the description of asexual reproductive modes distribution in the literature (in animals). This bias is mainly caused by (i) misconceptions about the genetics or cytology of asexuals, such as the belief that polyploid asexuals can only reproduce clonally, (ii) biological limitations, for instance LOH tends to be low or localized in non-clonal asexuals sampled *in natura*. For a better description and understanding of the distribution of asexuals, it is necessary to work within a common framework taking into account the diversity of possible asexual reproductive modes. Moreover, observing LOH in *Ap2n* sex-asex hybrids was made possible by crossing sexual and asexual *Artemia* (see chapter 1). Such new method may be used to identify many more non-clonal asexual species (for species producing rare males). Another way to identify non-clonal asexuals is to use genomics (Svendsen et al. 2015; Simion et al. 2021). To this end, it would be necessary to better understand the impact of non-clonal asexuality on the genome, in order to provide robust population genetics expectations to which natural asexual populations could be compared (i.e., distribution of F_{IS} under central fusion automixis, Nougué et al. 2015).

A substantial part of asexual eukaryotes is not clonal, and potentially many clonal species used to be non-clonal (see chapter 2). LOH thus plays an important part in the evolution of asexuals, and in particular in early stages of transition, when it may be much higher. Understanding the effect of LOH on fitness according to different asexual reproductive modes, and how these can evolve, is therefore crucial to explain the distribution of asexuals in nature (Archetti 2010; Engelstädter 2017).

b. LOH: A barrier to the evolution of asexuality

LOH has immediate, potentially high costs because it unmasks recessive deleterious mutations. An emerging asexual lineage with a high LOH rate on large portions of the chromosome is thus unlikely to establish and invade a sexual population where a lineage with no LOH would be successful (Archetti 2004). This constitutes a strong barrier to the evolution of asexuality from sex in eukaryotes (Engelstädter 2008). How widespread this barrier is depends on the frequency of each type of meiosis modification that generate asexual reproduction. Given the prevalence of non-clonal asexuality in nature, it is possible that the majority of emerging asexual lineages display high LOH, and most of them quickly go extinct. Factors inherited from parental species may also determine the success of emerging asexual lineages. For instance, a lineage emerging spontaneously from a parental species with high homozygosity will not undergo as much LOH. Additionally, under central fusion-like parthenogenesis, an asexual lineage will be more successful if it evolved from a sexual species with a lower recombination rate (Haag et al. 2017).

The costs of LOH should result in selection for lower LOH rates, which would make emerging asexual lineages more successful (Nougué et al. 2015; Engelstädter 2017). However, evolving toward a lower LOH rate is only possible under some of the existing non-clonal asexual reproductive modes. Under central fusion-like parthenogenesis, LOH can be reduced through a decreased recombination rate or a shift of crossing-over localization toward the telomeric region of chromosomes. Indeed, we observed a reduced LOH rate in *Ap2n* compared to *Akaz* (see chapter 1), as is also the case in other asexuals with central fusion-like parthenogenesis (Rey et al. 2011; Goudie et al. 2012). Still, LOH is not completely suppressed since I observed it in sex-asex hybrid lineages. Rare males, which are generated by recombination and LOH (see chapter 3), are also still produced by natural populations in many *Ap* lineages. Low, but non-zero LOH rate in non-clonal asexuals seems to be a common characteristic (see chapter 2), although the reason behind that is unclear. Perhaps, there is an optimal LOH or recombination rate that asexual lineages evolve toward, which is often non-zero. Alternatively, the optimal LOH rate may be zero, but at very low rates selection would be too weak to completely suppress LOH. It is also possible that LOH has other consequences, such as male production, which through contagious asexuality could result in non-zero LOH (see chapter 3 and part IIIa).

II. Asexual genome evolution

a. What is the effect of low LOH?

Under strict clonality, there are large genetic associations and selection is weaker, so that mutation and drift should strongly affect the evolution of an asexual lineage's genome (see introduction, Muller 1964; Gerrish and Lenski 1998). Clonal asexuals shoud thus have a lower adaptability. Under non-clonal asexuality, LOH events allow the purging of deleterious mutations, and break up genetic association (when there is recombination), so that selection can be stronger. On the long term, to have this countering effect, non-clonal asexual should have LOH rates higher than the mutation rate. This means that even a very low LOH rate could be very impactful on the evolution of an asexual lineage (for instance, it can prevent the haploidization of expression, Fyon and Lenormand 2018). Interestingly, even in "clonal" asexuals (i.e., without LOH), gene conversion and mitotic recombination can still occur, sometimes at rates higher than the mutation rate (Tucker et al. 2013), also questioning the definition of clonality. Therefore, asexual genomes can behave more or less clonally depending on mutation, recombination and LOH rates.

b. Genomic regions are more or less clonal

Patterns of LOH can also generate contrasting levels of "clonality" within the same genome (Brandt et al. 2021). Under central fusion-like parthenogenesis, the probability of LOH varies along chromosomes, from null at the centromere to high at the telomeres (maximum=2/3, Svendsen et al. 2015). This expected pattern of varying LOH, and thus heterozygosity within the genome allowed the identification of central fusion-like parthenogenesis in *Artemia* (Nougué et al. 2015). If the LOH probability increases in a monotonous fashion between these two extremes, we could expect that the region from the centromere to the point where LOH

probability is higher than the mutation rate behaves clonally. From that point to the telomeres, clonal divergence on homologues and accumulation of deleterious mutations would be opposed by LOH. However, the increase in LOH is not necessarily monotonous along the chromosomes. For instance, lethal alleles of two loci located close to each other could be each on a different homologous chromosome. In this case, any recombination event in this region would result in homozygosity for either of the lethal alleles (see figure 1). Thus, no recombinant offspring would survive, so that no purging of these lethal alleles would be possible. Therefore, clonality (i.e., absence of LOH), and thus heterozygosity, can be maintained locally (Engelstädter 2017). In the Cape honeybee, several regions where heterozygosity is maintained were identified, and could bear such deleterious alleles (Goudie et al. 2013). In particular, heterozygosity is maintained around the csd locus, which in case of LOH results in diploid males, a lethal phenotype (Goudie et al. 2013). The suppression of recombination due to these lethal alleles can remain local if more than one crossing-over by chromosome can occur. In this case, two successive crossing-overs between the centromere and the lethal alleles would allow LOH in this region while maintaining heterozygosity in the lethal alleles. However, if the recombination rate is low enough that no more than one crossing-over event by chromosome can occur, this could extend recombination suppression to a larger region. In this case, any crossing-over between the centromeres and the lethal alleles would have lethal consequences. Thus, a potentially large non-recombining, functionally clonal region could develop on non-clonal asexual chromosomes.

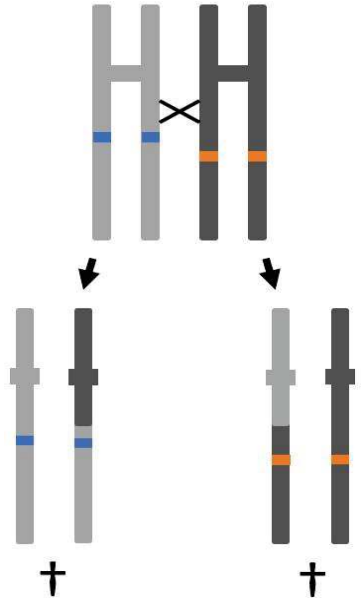


Figure 1. Recombination in a chromosome bearing two recessive lethal mutations under central fusion-like parthenogenesis. The light grey homologous chromosome bears the blue recessive lethal mutation, while the dark grey homologous chromosome bears the orange recessive lethal muation. The black cross represents a crossing-over event occurring between the centromere and the blue mutation (which is closest to the centromere). The possible recombinant offspring are represented below. Recombinant offspring are either homozygous for the blue lethal allele or for the orange lethal allele. Neither can survive.

III. The role of rare sex

a. Genetic conflict and male production

At each asexual contagion event, the asexuality gene(s) is(are) necessarily transmitted for the offspring to reproduce asexually. On the other hand, only half of the genome of the asexual parent lineage are transmitted to the newly-generated lineage. Due to this difference in

transmission rate, contagious asexuality does not affect in the same way the asexuality gene(s) and the rest of the asexual genome. Asexuality gene(s) can "escape" from a declining lineage suffering from the long-term costs of asexuality through contagion. The portion of the rest of the genome transmitted to new lineages would however be divided by two at each contagion event. Therefore, asexuality gene(s) may have a much higher longevity than the rest of a lineage's genome. Identifying asexuality genes in *Artemia* would be useful to estimate the true age of asexuality in this genus (Rode et al., *in press*). This could be achieved by using contagious asexuality to cross and backcross *Ap2n* with *Akaz*. We could then identify regions of the genomes inherited by all asexual offspring of these crosses, which would likely be associated with the asexuality gene(s). Comparing the divergence from *Akaz* of this region and the rest of the genome would thus indicate whether the asexuality gene(s) are in fact older (Tucker et al. 2013, Rode et al., *in press*).

Finding that asexuality gene(s) is(are) older than the rest of the genome would demonstrate that the lineages observed *in natura* originate from contagious asexuality, while older lineages went extinct. This would mean that this mechanism plays an important role in maintaining asexuality in the long term. In this case, asexuality gene(s) could be associated with factors allowing contagious asexuality (see chapter 3). In the case of *Ap2n*, as males are produced by recombination on the sex chromosomes, this might explain why recombination, and thus LOH, is maintained (see chapter 3). Still, if recombination was suppressed together with male production, contagion could still occur via females rarely reproducing sexually (see chapter 1). An explanation for why recombination is maintained might be that contagious events, through introgression of sexual genes, generate lineages with higher recombination rate (see chapter 1), and thus more frequent male production and contagion events. An interesting point is that rare male production (and thus recombination rate) varies among *Ap2n* populations, where Central Asian populations have a male production of around 1% and the other populations of <5‰ (Maccari et al. 2013). This could be due to their recent origin and proximity with related sexual species that can be involved in contagious asexuality.

b. Does inter-population sex have a significant impact?

We found that *Ap2n* females were capable of rare sex and that a part of sex-asex hybrid females had a mixed reproduction (see chapter 1). This suggests that, additionally to genes conferring the capacity to reproduce asexually, there are genes controlling the frequency of asexual vs. sexual reproduction. The transition from sex to asexuality often involves facultative asexuality (Simon et al. 2003). In light of these recent findings, it is thus possible that in *Artemia*, asexuality first emerged as facultative, and not near-obligate as it is in lineages found in nature. In any case, the *Ap2n-Kaz* mitotype, to which all populations studied in this PhD belong, is estimated to be a backcross of an *Ap2n* lineage on the sexual species *Akaz* (Rode et al., *in press*). Based on the phenotypes of the sex-asex hybrids, at least part of the offspring generated by such cross should have been facultative asexuals with a higher rate of sexual reproduction. The reason why we only observe very rare sexual reproduction in natural asexual populations is unclear: perhaps by chance the hybrid lineage that succeeded happened to be almost obligate, or mixed lineages could not be maintained, maybe due to frequent crossing with *Ap2n*. If near-obligate asexuality evolved secondarily in *Artemia*, then the gene(s)

controlling for this phenotype should be younger than the asexuality gene(s). Otherwise, if the two regions have a similar age, it would mean that both traits appeared simultaneously, or that near-obligate asexuality evolved very shortly after the capacity to reproduce asexually.

c. How frequent is sex in asexuals?

In *Artemia*, even though rare males can be produced at a rate up to 1.7% (Maccari et al. 2013), and even more in sex-asex hybrids and backcrosses (see chapter 1), only two contagion events are reported for the entire evolutionary history of the genus (producing *Ap2n-Kaz* and *Ap4n*, Rode et al., *in press*). However, it is possible that such events occur much more frequently but that only a few generate successful lineages that can spread. It would be interesting to study populations that are likely in sympatry with *Akaz*, *Aurm* or *Aunk* (see Rode et al., *in press* for suggestions of where these populations might be). In particular, if these populations are found, we could investigate whether contagious asexuality occurs locally. This could be done by simply looking for sex-asex hybrids, or by observing whether the diversity of these asexual populations is higher than in populations not sympatric with Central Asian sexual species (this was reported in *Daphnia*: Hebert et al. 1989 and planarians: Pongratz et al. 1998). If these crosses are rarely successful, this could explain why only few contagion events are reported through population genetics studies.

In a natural population of *Ap2n*, we found a very low rate of rare sex in females: ~2‰. In this case, even in a population with a high rate of rare male production (1%), the probability that the two events co-occur, allowing sex between asexuals, would be 2.10^{-5} which is extremely low (see chapter 1). Even then, the two individuals would have to mate successfully and produce viable offspring. Moreover, sex within the same lineage may result in very high inbreeding depression and are thus not likely to succeed. Still, intra-asexual crosses may have happened in *Artemia*, including between the two *Ap2n* mitotypes (Rode et al., *in press*). Perhaps, such events are more likely to happen during the emergence of asexual lineages, especially through contagion, since crosses with the sexual population would increase male production and the rate of sexual reproduction. Therefore, the sex events that shaped the *Artemia* genus may have happened in a relatively short period of time while the frequency of sexual reproduction vs. asexual reproduction was still high enough (see chapter 1). It is possibly the case for the two *Ap2n* mitotypes as they both originate from crosses with *Akaz*, but probably not for the *Ap4n* lineage, as it seemingly results from a contagion event on *Asin*, which has a different geographical distribution (Rode et al., *in press*).

Conclusion

During this PhD, I found that non-clonal asexuality is more common than previously thought, which may play an important part in the evolution of reproduction in eukaryotes as a barrier to asexuality. Using *Artemia* as a case study, I demonstrated that *Ap2n* have a reproductive mode genetically equivalent to central fusion automixis with recombination, and thus LOH. I found that rare males are produced through this reproductive mode, as a result of recombination on the sex chromosomes. This could indicate that recombination and thus LOH is maintained in this system through contagious asexuality and male production. I also found

that asexual females could rarely engage in sex, and that sex-asex hybrids can be facultative asexuals. This questions whether *Artemia* asexuals emerged as obligate or facultative, and how contagious asexuality affects the evolution of asexuals. To better understand the evolutionary history of *Artemia* and the forces shaping eukaryote asexuals evolution, an exciting path would be to identify the gene(s) of asexuality in this genus. This would inform us on whether the transition to asexuality occurred in one or several mutational steps, and comparing this region to the rest of the genome will indicate how old asexuality really is in *Artemia*. Comparing its age to the age of the genes controlling for near-obligate asexuality would also indicate how much facultative asexuality has been involved in the evolution of asexual *Artemia*. Additionally, localizing the potential parental species of *Ap2n* and studying sympatric populations could provide important information such as the rate of contagion and other sex events, their effects on reproductive mode and fitness. Another important question is the effect of non-clonal asexuality on genome structure, as more or less clonal regions may develop due to recessive deleterious mutations. This could be investigated using models simulating the evolution of chromosomes under non-clonal asexuality. In conclusion, by accounting for non-clonal and non-obligate asexuality, especially during early stages of transition from sex, we should understand better the distribution of asexuals in eukaryotes, and their evolutionary history.

References

Archetti, M. 2010. Complementation, genetic conflict, and the evolution of sex and recombination. J. Hered. 101:1–13.

Archetti, M. 2004. Recombination and loss of complementation: A more than two-fold cost for parthenogenesis. J. Evol. Biol. 17:1084–1097.

Brandt, A., P. T. Van, C. Bluhm, Y. Anselmetti, Z. Dumas, E. Figuet, C. M. François, N. Galtier, B. Heimburger, K. S. Jaron, M. Labédan, M. Maraun, D. J. Parker, M. Robinson-Rechavi, I. Schaefer, P. Simion, S. Scheu, T. Schwander, and J. Bast. 2021. Haplotype divergence supports long-term asexuality in the oribatid mite *Oppiella nova*. Proc. Natl. Acad. Sci. 118.

Engelstädter, J. 2017. Asexual but Not Clonal : Evolutionary Processes in Automictic Populations. Genetics 206:993–1009.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–1150.

Fyon, F., and T. Lenormand. 2018. Cis-regulator runaway and divergence in asexuals. Evolution 72:426–439.

Gerrish, P. J., and R. E. Lenski. 1998. The fate of competing beneficial mutations in an asexual population. Genetica 102:127–144.

Goudie, F., M. H. Allsopp, M. Beekman, P. R. Oxley, J. Lim, and B. P. Oldroyd. 2012. Maintenance and loss of heterozygosity in a thelytokous lineage of honey bees (*Apis mellifera capensis*). Evolution 66:1897–1906.

Goudie, F., M. H. Allsopp, and B. P. Oldroyd. 2013. Selection on overdominant genes maintains heterozygosity along multiple chromosomes in a clonal lineage of honey bee. Evolution 68:125–136.

Haag, C. R., L. Theodosiou, R. Zahab, and T. Lenormand. 2017. Low recombination rates in sexual species and sex-asex transitions. Philos. Trans. R. Soc. B Biol. Sci. 372:1–11.

Hebert, P. D., M. J. Beaton, S. S. Schwartz, and D. J. Stanton. 1989. Polyphyletic Origins of Asexuality in *Daphnia pulex*. I. Breeding-System Variation and Levels of Clonal Diversity. Evolution 43:1004–1015.

Maccari, M., A. Gómez, F. Hontoria, and F. Amat. 2013. Functional rare males in diploid parthenogenetic *Artemia*. J. Evol. Biol. 26:1934–1948.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. Mol. Mech. Mutagen. 1:2–9.

Nougué, O., N. O. Rode, R. Jabbour-zahab, A. Ségard, L. M. Chevin, C. R. Haag, and T. Lenormand. 2015. Automixis in Artemia: Solving a century-old controversy. J. Evol. Biol. 28:2337–2348.

Pongratz, N., T. F. Sharbel, L. W. Beukeboom, and N. K. Michiels. 1998. Allozyme variability in sexual and parthenogenetic freshwater planarians: Evidence for polyphyletic origin of parthenogenetic lineages through hybridization with coexisting sexuals. Heredity 81:38–47.

Rey, O., A. Loiseau, B. Facon, J. Foucaud, J. Orivel, J. M. Cornuet, S. Robert, G. Dobigny, J. H. C. Delabie, C. D. S. F. Mariano, and A. Estoup. 2011. Meiotic recombination dramatically decreased in thelytokous queens of the little fire ant and their sexually produced workers. Mol. Biol. Evol. 28:2591–2601.

Rode, N. O., R. Jabbour-Zahab, L. Boyer, É. Flaven, F. Hontoria, G. Van Stappen, F. Dufresne, C. Haag, and T. Lenormand. (*in press*). The origin of asexual brine shrimps. Am. Nat.

Simion, P., J. Narayan, A. Houtain, A. Derzelle, L. Baudry, E. Nicolas, R. Arora, M. Cariou, C. Cruaud, F. R. Gaudray, C. Gilbert, N. Guiglielmoni, B. Hespeels, D. K. Kozlowski, K. Labadie, A. Limasset, M. Lliros, M. Marbouty, M. Terwagne, J. Virgo, R. Cordaux, E. G. Danchin, B. Hallet, R. Koszul, T. Lenormand, J.-F. Flot, and K. Van Doninck. 2021. Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer *Adineta vaga*. Sci. Adv. 7.

Simon, J., F. Delmotte, C. Rispe, and T. Crease. 2003. Phylogenetic relationships between parthenogens and their sexual relatives : the possible routes to parthenogenesis in animals. Biol. J. Linn. Soc. 79:151–163.

Svendsen, N., C. M. O. Reisser, M. Dukić, V. Thuillier, A. Ségard, C. Liautard-Haag, D. Fasel, E. Hürlimann, T. Lenormand, Y. Galimov, and C. R. Haag. 2015. Uncovering cryptic asexuality in *Daphnia magna* by RAD sequencing. Genetics 201:1143–1155.

Tucker, A. E., M. S. Ackerman, B. D. Eads, S. Xu, and M. Lynch. 2013. Population-genomic insights into the evolutionary origin and fate of obligately asexual *Daphnia pulex*. Proc. Natl. Acad. Sci. U. S. A. 110:15740–15745.

Summary of the thesis in French

Résumé étendu de la thèse en français

Causes et conséquences évolutives de l'asexualité non-clonale chez *Artemia*

La reproduction sexuée est un caractère ancestral chez les eucaryotes, chez lesquels la plupart des espèces se reproduisent exclusivement par voie sexuée (Simon et al. 2003). La parthénogenèse est un type de reproduction asexuée (donc sans fécondation) par la voie germinale, via la production de gamètes non réduits. Ce mode de reproduction a évolué plusieurs fois indépendamment des eucaryotes sexués, mais il est rare et les lignées asexuées sont généralement jeunes (Schön et al. 2009). Pour expliquer la distribution de la parthénogenèse chez les eucaryotes, il est nécessaire de comprendre comment les espèces à reproduction asexuée peuvent évoluer et perdurer. Les coûts et les avantages de la reproduction asexuée par rapport à la reproduction sexuée sont difficiles à démêler, tant sur le plan théorique qu'empirique.

L'asexualité est souvent associée à la clonalité, c'est-à-dire à la production d'une progéniture identique au parent (à l'exception des mutations). Par conséquent, la majorité des espèces parthénogénétiques seraient clonales (Suomalainen 1950), et les théories sur l'évolution des asexués se concentrent principalement sur l'émergence les conséquences de la clonalité. Selon la théorie la plus connue, les sexués devraient payer le "double coût des mâles " (Maynard Smith 1978), c'est-à-dire que, à chaque génération, le nombre d'individus capables de se reproduire chez les sexués (les femelles) est réduit de moitié par rapport aux asexués. La rareté des asexués serait donc un paradoxe, qui suggère que l'asexualité a un coût encore plus élevé. En effet, les espèces parthénogénétiques à reproduction clonale souffrent d'une accumulation de mutations délétères (Muller 1932) et d'un fort déséquilibre de liaison (Gerrish and Lenski 1998).

Cependant, une diversité de modes de reproduction parthénogénétiques non-clonaux existent dans la nature. En particulier, il est peu probable que les asexués eucaryotes aient évolué, en une seule étape, de la méiose complète (c'est-à-dire le sexe, caractère ancestral des eucaryotes) à une reproduction clonale proche de ou équivalente à la mitose. Il est en fait plus probable que la transition de la reproduction sexuée vers la parthénogenèse implique des modifications de la méiose, dont une partie seulement a des conséquences clonales. Les modes de reproduction asexués non clonaux sont caractérisés par des coûts différents (Suomalainen et al. 1987), notamment liés à la possibilité ou non de recombiner. La principale conséquence de ces modes de reproduction est la perte d'hétérozygotie (LOH, pour "*loss of heterozygosity*") qui peut révéler des allèles récessifs délétères dans la population (Archetti 2004). Cela pourrait jouer un rôle important dans l'évolution de la parthénogenèse en rendant les premières étapes de transition de la reproduction sexuée vers la reproduction asexuée coûteuses. Ainsi, étudier l'impact des modes de reproduction asexués non-clonaux sur le génome devrait aider à comprendre comment les lignées parthénogénétiques non-clonales répondent aux forces évolutives.

Ces questions s'appliquent aux espèces à parthénogenèse obligatoire, qui n'alternent pas reproduction sexuée et asexuée. Cependant, de nombreux asexués "obligatoires" sont en fait capables de sexe rare. Même si les évènements de sexe ne sont pas fréquents, ils peuvent avoir d'importantes conséquences sur l'évolution des asexués (D'Souza and Michiels 2010). De plus, cela pose la question de la fréquence de la reproduction sexuée au moment où ces lignées parthénogénétiques ont émergé à partir d'espèces sexuées.

Les Artémies sont des petits crustacés adaptés aux milieux hypersalins. Le genre *Artemia* comprend des espèces sexuées et des lignées asexuées avec différents niveaux de ploïdie, toutes décrites comme *Artemia parthenogenetica*. Les *Artemia* parthénogénétiques diploïdes (*Ap2n*) ont un mode de reproduction asexuée par méiose modifiée : l'automixie par fusion centrale (Nougué et al. 2015). Dans ce mode de reproduction, la méiose I est supprimée, ce qui donne une descendance diploïde. Cependant, l'appariement des chromosomes homologues est maintenu, par conséquent l'hétérozygotie parentale est conservée sauf si les chromosomes recombinent. La recombinaison génère du LOH chez les descendants recombinants, depuis l'emplacement du crossing-over jusqu'à l'extrémité du chromosome. Ainsi, la probabilité de perdre l'hétérozygotie est nulle au centromère et augmente à mesure que le locus considéré se rapproche de la région télomérique. Les *Ap2n* produisent également rarement des mâles. Ces mâles rares sont capables de se croiser avec les femelles d'espèces d'*Artemia* sexuées apparentées, et de transmettre l'asexualité à leur progéniture (Maccari et al. 2014). Ce processus, appelé asexualité contagieuse, permet la génération de nouvelles lignées asexuées, qui pourraient apporter de la variation et régénérer l'hétérozygotie dans les génomes asexués.

Au cours de ce doctorat j'ai étudié le mode de reproduction des *Ap2n*, ce qu'il peut indiquer sur l'origine de ces lignées, et comment il peut affecter son évolution. L'asexualité contagieuse dans ce système contribue également à cette question et offre en outre la possibilité de croiser des sexués et des asexués. J'ai également étudié la prévalence et les conséquences génétiques de l'asexualité non clonale chez les animaux. Enfin, j'ai évalué si les mâles rares chez les *Ap2n* étaient produits par recombinaison (et donc LOH).

Dans le premier chapitre, j'ai étudié la vie sexuelle cryptique des *Ap2n*, chez qui aucun LOH n'avait été rapporté. J'ai pu générer des hybrides sexués-asexués en laboratoire en utilisant l'asexualité contagieuse. Toutes les femelles hybrides étaient capables de reproduction asexuée, ce qui signifie que le(s) gène(s) d'asexualité est(sont) dominant(s) chez *Artemia*. Grâce à ces hybrides, j'ai pu montrer que la recombinaison se produit dans les lignées asexuées, provoquant du LOH. Cela confirme que les *Ap2n* se reproduisent *via* automixie par fusion centrale. Ces différences ne peuvent généralement pas être observées chez les asexués échantillonnés dans la nature, car une fois l'hétérozygotie perdue, les évènements de recombinaison suivants ne laissent pas de trace. De plus, en utilisant des tests de paternité, j'ai pu montrer qu'une partie des femelles hybrides sont à la fois capable de se reproduire sexuellement et asexuellement. Elles peuvent également transmettre l'asexualité à leurs descendants produits sexuellement, c'est-à-dire qu'elles sont capables d'asexualité contagieuse. Enfin, j'ai pu montrer avec une expérience de croisement de masse que les femelles asexuées de la nature se reproduisent également rarement de manière sexuée (avec un taux de ~2‰). Dans l'ensemble, j'ai trouvé que la plupart des faits précédemment connus sur l'asexualité des Artémies n'étaient pas exacts.

Plus généralement, ces résultats suggèrent que les preuves de reproduction strictement clonale des espèces asexuées doivent être reconsidérées. De plus, le sexe rare et les conséquences de l'asexualité non clonale, comme le flux génétique au sein des asexués, doivent être plus largement pris en compte dans des modèles plus réalistes pour le maintien du sexe et la persistance des lignées asexuées.

Dans le deuxième chapitre, j'ai étudié la distribution de différents modes de reproduction asexués clonaux et non-clonaux chez les animaux. En plus de la découverte de l'automixie par fusion centrale chez les *Ap2n*, des preuves récentes ont révélé des formes non clonales de reproduction asexuée chez plusieurs espèces auparavant considérées comme clonales (Simion et al. 2021, Schwander 2021). Cela pourrait indiquer que l'asexualité non-clonale est plus commune et concerne beaucoup plus de taxa que ce que l'on pensait précédemment. Dans ce cas, il y aurait un fort biais en faveur de la clonalité. J'ai étudié les preuves de clonalité couramment utilisées et souligné les facteurs de confusion et les biais de perception potentiels. Bien que de nombreux asexués paraissent se reproduire clonalement, j'ai trouvé qu'une grande partie d'entre eux n'est pas strictement clonale. Il y a donc, pour des raisons méthodologiques et conceptuelles, un biais vers la clonalité dans la perception des asexués. Les divergences entre asexués clonaux et non-clonaux, si elles sont souvent mineures, peuvent néanmoins avoir un impact important. De plus, la sélection pour une reproduction plus proche de la clonalité et l'absence possible d'espèces asexuées se reproduisant par la mitose indiquent que, sur une échelle de temps évolutive, même les espèces clonales ont pu être non clonales par le passé. Dans l'ensemble, ces conclusions suggèrent que la parthénogénèse non-clonale joue un rôle plus important que prédit dans l'évolution de l'asexualité. Cela appelle donc à une inclusion plus large des modes de reproduction non-clonaux dans les modèles théoriques d'évolution de l'asexualité.

Dans le troisième chapitre, j'ai étudié comment les mâles rares sont produits par les *Ap2n*. On connaît mal le rôle évolutif des mâles parfois produits par des espèces asexuées. S'ils sont capables d'asexualité contagieuse, ils peuvent largement influencer l'évolution de l'asexualité et son maintien. Cependant, s'ils ne peuvent pas se reproduire, ils ne peuvent représenter qu'un coût de reproduction (Engelstädter 2008). La manière dont ces mâles sont générés est mal connue, en particulier chez les espèces possédant des chromosomes sexuels. Comprendre le mécanisme de production de mâles chez les asexués peut donner des indications sur leur rôle et leur devenir évolutif. Cela peut déterminer s'ils résultent d'une erreur génétique ou de développement ou d'un mécanisme génétique bien défini, et quels facteurs peuvent réguler leur production. J'ai testé l'hypothèse selon laquelle les mâles rares produits par certaines lignées d'*Ap2n* sont le résultat d'une recombinaison sur les chromosomes sexuels ZW, générant une perte d'hétérozygotie et donc une descendance ZZ. J'ai utilisé des données de RAD-seq pour comparer les femelles asexuées à leurs descendants mâles et femelles. Les descendants mâles ont tous perdu l'hétérozygotie sur une portion des chromosomes sexuels, alors que les descendantes femelles ont toutes conservé l'hétérozygotie sue ces chromosomes. Les mâles rares sont donc effectivement produits par recombinaison sur le ZW pendant la méiose chez leur mère asexuée. Grâce à ces données, j'ai obtenu des indications supplémentaires sur la structure des chromosomes sexuels, l'emplacement du locus de détermination du sexe, et la distribution du taux de recombinaison au sein des femelles asexuées individuelles.

Globalement, ces résultats démontrent que des mâles rares sont produits parce que la recombinaison n'est pas entièrement supprimée dans les lignées asexuées. Ce processus d'asexualité contagieuse par l'intermédiaire de mâles rares peut être maintenu par la sélection parmi les lignées asexuées, lorsque la fitness des asexués nouvellement formés est initialement élevée mais diminue avec le temps (par exemple, par l'accumulation de mutations délétères). Les lignées asexuées les plus anciennes pourraient donc être remplacées à successivement par des lignées plus récentes.

Le LOH chez les asexués non clonaux constitue une forte barrière à l'évolution de l'asexualité à partir du sexe chez les eucaryotes (Engelstädter 2008). Étant donné la prévalence de l'asexualité non clonale dans la nature, il est possible que la majorité des lignées asexuées émergentes présentent un LOH élevé et que la plupart d'entre elles s'éteignent rapidement. Des facteurs hérités des espèces parentales peuvent également déterminer le succès des lignées asexuées émergentes. Par exemple, dans le cas de l'automixie par fusion centrale, une lignée asexuée aura plus de succès si elle a évolué à partir d'une espèce sexuée ayant un taux de recombinaison plus faible (Haag et al. 2017). Les coûts du LOH devraient entraîner une sélection pour des taux de LOH plus faibles (Nougué et al. 2015; Engelstädter 2017), bien que l'évolution vers un taux de LOH plus faible ne soit possible que dans certains des modes de reproduction asexués non clonaux existants. Dans le cas de la l'automixie par fusion centrale, le LOH peut être réduit par une diminution du taux de recombinaison ou un déplacement de la localisation des crossing-over vers la région télomérique des chromosomes.

Dans le cadre de l'asexualité non clonale, les événements de LOH permettent de purger les mutations délétères et de briser les associations génétiques (lorsqu'il y a recombinaison), de sorte que la sélection peut être plus forte. Comme les conséquences de la clonalité sur la génétique des populations sont fortement affectées par les mutations et la dérive, elles peuvent être contrées par des taux de LOH supérieurs au taux de mutation. Ainsi, même un taux de LOH très faible peut avoir un impact important sur l'évolution d'une lignée asexuée. Par conséquent, les génomes asexués peuvent se comporter de manière plus ou moins clonale en fonction des taux de mutation, de recombinaison et de LOH. Les patrons de LOH peuvent également générer des niveaux contrastés de "clonalité" au sein d'un même génome (Brandt et al. 2021). Sous automixie par fusion centrale, la probabilité de LOH varie le long des chromosomes, de nulle au centromère à élevée aux télomères (maximum=2/3, Svendsen et al. 2015). De plus, la recombinaison réelle observée dans la descendance peut également être affectée par des allèles récessifs létaux, qui pourraient maintenir l'hétérozygotie localement (Engelstädter 2017). Par conséquent, une région non recombinante, fonctionnellement clonale, pourrait se développer dans les chromosomes asexués non clonaux.

Grâce à l'asexualité contagieuse, Le ou les gènes d'asexualité pourraient "s'échapper" d'une une lignée en déclin souffrant des coûts à long terme de l'asexualité. La portion du reste du génome transmise aux nouvelles lignées serait cependant divisée par deux à chaque événement de contagion. Par conséquent, le ou les gènes d'asexualité peuvent avoir une longévité bien supérieure à celle du reste du génome d'une lignée. L'identification des gènes d'asexualité chez Artemia serait utile pour estimer l'âge réel de l'asexualité dans ce genre (Rode et al., *in press*). Si le ou les gènes d'asexualité sont plus anciens que le reste du génome, cela

démontrerait que les lignées observées dans la nature sont issues de l'asexualité contagieuse, alors que les lignées plus anciennes se sont éteintes. Cela signifierait que ce mécanisme joue un rôle important dans le maintien de l'asexualité à long terme. Dans le cas des Artémies asexuées, comme les mâles sont produits par recombinaison sur les chromosomes sexuels, cela pourrait expliquer pourquoi la recombinaison, et donc le LOH, sont maintenus.

La transition du sexe vers l'asexualité implique souvent l'asexualité facultative (Simon et al. 2003). Puisque les Artémies asexuées et les hybrides sexe-asex peuvent se reproduire sexuellement, il est donc possible que chez *Artemia*, l'asexualité soit d'abord apparue comme facultative, et non comme quasi-obligatoire comme c'est le cas dans les lignées trouvées dans la nature. La raison pour laquelle nous n'observons que de très rares évènements de reproduction sexuée dans les populations naturelles asexuées n'est pas claire. Si l'asexualité quasi-obligatoire a évolué secondairement chez *Artemia*, alors le ou les gènes contrôlant ce phénotype devraient être plus jeunes que le ou les gènes d'asexualité.

Au cours de cette thèse, j'ai découvert que l'asexualité non clonale est plus courante qu'on ne le pensait, ce qui pourrait jouer un rôle important dans l'évolution de la reproduction chez les eucaryotes en tant que barrière à l'asexualité. En utilisant les Artémies asexuées comme étude de cas, j'ai démontré qu'elles ont un mode de reproduction génétiquement équivalent à l'automixie par fusion centrale, avec recombinaison, et donc LOH. J'ai constaté que les mâles sont produits par ce mode de reproduction, en conséquence de la recombinaison sur les chromosomes sexuels. Cela pourrait indiquer que la recombinaison et donc le LOH sont maintenus dans ce système par l'asexualité contagieuse et la production de mâles. En conclusion, en tenant compte de l'asexualité non-clonale et non-obligatoire, en particulier pendant les premiers stades de transition depuis la reproduction sexuée, nous devrions mieux comprendre la distribution des asexués chez les eucaryotes, ainsi que leur histoire évolutive.

Références

Archetti, M. 2004. Recombination and loss of complementation: A more than two-fold cost for parthenogenesis. J. Evol. Biol. 17:1084–1097.

Brandt, A., P. T. Van, C. Bluhm, Y. Anselmetti, Z. Dumas, E. Figuet, C. M. François, N. Galtier, B. Heimburger, K. S. Jaron, M. Labédan, M. Maraun, D. J. Parker, M. Robinson-Rechavi, I. Schaefer, P. Simion, S. Scheu, T. Schwander, and J. Bast. 2021. Haplotype divergence supports long-term asexuality in the oribatid mite *Oppiella nova*. Proc. Natl. Acad. Sci. 118.

D'Souza, T. G., and N. K. Michiels. 2010. The costs and benefits of occasional sex: Theoretical predictions and a case study. J. Hered. 101:1–8.

Engelstädter, J. 2017. Asexual but Not Clonal : Evolutionary Processes in Automictic Populations. Genetics 206:993–1009.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–1150.

Gerrish, P. J., and R. E. Lenski. 1998. The fate of competing beneficial mutations in an asexual population. Genetica 102:127–144.

Haag, C. R., L. Theodosiou, R. Zahab, and T. Lenormand. 2017. Low recombination rates in sexual species and sex-asex transitions. Philos. Trans. R. Soc. B Biol. Sci. 372:1–11.

Maccari, M., F. Amat, F. Hontoria, and A. Gómez. 2014. Laboratory generation of new parthenogenetic lineages supports contagious parthenogenesis in *Artemia*. PeerJ 2:e439.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge Univ. Press.

Muller, H. J. 1932. Some genetic aspects of sex. Am. Nat.

Nougué, O., N. O. Rode, R. Jabbour-zahab, A. Ségard, L. M. Chevin, C. R. Haag, and T. Lenormand. 2015. Automixis in Artemia: Solving a century-old controversy. J. Evol. Biol. 28:2337–2348.

Rode, N. O., R. Jabbour-Zahab, L. Boyer, É. Flaven, F. Hontoria, G. Van Stappen, F. Dufresne, C. Haag, and T. Lenormand. (*in press*). The origin of asexual brine shrimps. Am. Nat.

Schön, I., K. Martens, and P. Van Dijk. 2009. Lost Sex, The Evolutionary Biology of Parthenogenesis.

Simion, P., J. Narayan, A. Houtain, A. Derzelle, L. Baudry, E. Nicolas, R. Arora, M. Cariou, C. Cruaud, F. R. Gaudray, C. Gilbert, N. Guiglielmoni, B. Hespeels, D. K. Kozlowski, K. Labadie, A. Limasset, M. Lliros, M. Marbouty, M. Terwagne, J. Virgo, R. Cordaux, E. G. Danchin, B. Hallet, R. Koszul, T. Lenormand, J.-F. Flot, and K. Van Doninck. 2021. Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer *Adineta vaga*. Sci. Adv. 7.

Simon, J. C., F. Delmotte, C. Rispe, and T. Crease. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. Biol. J. Linn. Soc. 79:151–163.

Suomalainen, E. 1950. Parthenogenesis in Animals. Adv. Genet. 3:193–253.

Suomalainen, E., A. Saura, and J. Lokki. 1987. Cytology and evolution in parthenogenesis. CRC Press.

Svendsen, N., C. M. O. Reisser, M. Dukić, V. Thuillier, A. Ségard, C. Liautard-Haag, D. Fasel, E. Hürlimann, T. Lenormand, Y. Galimov, and C. R. Haag. 2015. Uncovering cryptic asexuality in *Daphnia magna* by RAD sequencing. Genetics 201:1143–1155.

Acknowledgements

Remerciements

Acknowledgements / Remerciements

Tout d'abord, je suis reconnaissante envers les luttes féministes et sociales grâce auxquelles j'ai pu étudier et faire une thèse, et envers toutes les personnes qui se battent pour rendre et maintenir les études accessibles à toutes et tous. J'ai énormément appris pendant ces trois ans et quelques, grâce à toutes les personnes dont les noms figurent dans cette section.

Merci à Thomas et Christoph de m'avoir encadrée et transmis votre vision de la science. I am grateful to the jury of my PhD: I. Schön, T. Schwander, S. Coelho and N. Bierne, for kindly accepting to evaluate this work. I would also like to thank the members of my thesis committees for their time and advice: K. Van Doninck, S. Coelho, P. Nosil, A. Estoup and P-O. Cheptou. Merci à Roula de ton investissement qui a permis la génération de ces données, mais aussi de m'avoir formée à l'expérimental et la biologie moléculaire, le tout avec une patience et une bienveillance sans limite. Merci à Cécile Molinier pour ton énergie et le temps passé à discuter de la reproduction des Daphnies et des Artémies. Merci à Nicolas Rode pour ses conseils et son optimisme. Merci aux personnes avec qui j'ai pu travailler et échanger de manière enrichissante dans le cadre de l'ANR GENASEX: Sylvain Glémin, Denis Roze, et Pauline Joncour. Merci à Marie-Pierre Dubois de m'avoir accueillie sur la plateforme GEMEX. Merci également aux plateformes GenSeq et MGX.

Merci aux personnes qui m'ont conseillée et aidée dans mes analyses de génomique : Pauline Joncour, Quentin Rougemont, Emmanuelle Beyne. Merci en particulier à Enrique Ortega de m'avoir accompagnée depuis le début, avec une grande générosité (et un humour douteux). Merci à Jeanne Tonnabel pour tes conseils et tes encouragements. Merci à Patrice David pour son rôle de directeur de filière doctorale. Merci aux étudiants que j'ai eu la chance d'encadrer pendant leur stage : Fabian Dourguin et Marie Riffis, j'ai beaucoup appris à vos côtés ! Je voudrais aussi remercier Christophe Thébaud, dont les conseils m'ont menée jusqu'à cette thèse. Merci à l'université de Montpellier de m'avoir permis d'effectuer une expérience d'enseignement. Merci aux étudiants et aux enseignants-chercheurs que j'ai pu côtoyer et merci en particulier à Patricia Cucchi de m'avoir beaucoup appris et transmis ta passion.

Merci à l'équipe GEE du CEFE de m'avoir accueillie pendant ces trois ans. Merci à mes collègues de bureau pour leur compagnie et les discussions passionnantes : Cécile, Pierre, Martijn, Christelle, Méril, Antoine, Paul. Merci aux non-permanents (et à Tim) qui ont constitué l'essentiel de ma vie sociale pendant cette période : Léa, Kévin, Mike, Nathalie, Riccardo, Solange, Jeanne, Fanny, Stanislas, et tant d'autres. Merci à Méril de ne jamais nous laisser perdre foi en nous. Merci à PAC pour le chocolat. Merci à Daniel, Claire, Bruno et Linda pour les activités extra-thèse.

Merci à ma famille pour leur soutien et de leur affection. Merci à ma maman pour m'avoir toujours encouragée dans cette voie et soutenue à plus d'un titre. Merci à Bily d'être toujours là pour rire et pour parler. Merci à Jade pour le covoiturage et (surtout) les longues discussions. Merci à tous les amis de Toulouse avec qui j'ai pu continuer à vivre de super moments malgré l'éloignement : Vanessa, Florian, Thomas, Rémi, Seyrane et tous les autres. Merci à François de m'avoir aidé à trouver la confiance à force d'heures passées au téléphone jusqu'à tard dans la nuit. Merci bien sûr à Cécile pour ton amitié (malgré ta première impression ;)) et pour avoir servi de crash test en traversant toutes les étapes de la thèse juste avant moi !

Merci enfin à Sergeï, Ours, Odin, Papy (c'est bien une seule personne) d'être présent chaque jour, et de me donner une force immense.

Annex

The origin of asexual brine shrimps

This manuscript is in press. I participated in it by doing bibliographic research and producing a figure on the major findings regarding the origin of asexual Artemia lineages (Figure 1).

The origin of asexual brine shrimps

Nicolas Olivier Rode[1,2,*], Roula Jabbour-Zahab[1], Loreleï Boyer[1], Élodie Flaven[1], Francisco Hontoria[3], Gilbert Van Stappen[4], France Dufresne[5], Christoph Haag[1], Thomas Lenormand[1,*]

[1] CEFE - UMR 5175 1919 route de Mende, 34293 Montpellier cedex 5, France

[2] CBGP, Univ Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France

[3] Instituto de Acuicultura de Torre de la Sal, IATS-CSIC, 12595 Ribera de Cabanes, Castellón, Spain

[4] Laboratory of Aquaculture & Artemia Reference Center, Ghent University, B-9000 Gent, Belgium

[5] Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski UQAR, Rimouski, Canada

[*] E-mails: nicolas.rode@inrae.fr, thomas.lenormand@cefe.cnrs.fr

Abstract

Determining how and how often asexual lineages emerge within sexual species is central to our understanding of sex-asex transitions and the long-term maintenance of sex. Asexuality can arise “by transmission” from an existing asexual lineage to a new one, through different types of crosses. The occurrence of these crosses, cryptic sex, variation in ploidy and recombination within asexuals greatly complicates the study of sex-asex transitions, as they preclude the use of standard phylogenetic methods and genetic distance metrics. In this study we show how to overcome these challenges by developing new approaches to investigate the origin of the various asexual lineages of the brine shrimp *Artemia parthenogenetica*. We use a large sample of asexuals, including all known polyploids, and their sexual relatives. We combine flow cytometry with mitochondrial and nuclear DNA data. We develop new genetic distance measures and methods to compare various scenarios describing the origin of the different lineages. We find that all diploid and polyploid *A. parthenogenetica* likely arose within the last 80,000 years through successive and nested hybridization events that involved backcrosses with different sexual species. All *A. parthenogenetica* have the same common ancestor and therefore likely carry the same asexuality gene(s) and reproduce by automixis. These findings radically change our view of sex-asex transitions in this group, and show the importance of considering asexuality “by transmission” scenarios. The methods developed are applicable to many other asexual taxa.

Key words: Polyploidy, Automixis, Hybridization, Contagious Asexuality, Parthenogenesis, Genetic distance

Introduction

Understanding why sexual reproduction is so widespread among eukaryotes, despite the well-known costs of sex (Maynard Smith 1978; Otto and Lenormand 2002; Meirmans et al. 2012), requires an understanding of how and how often sex-asex transitions can occur (Delmotte et al. 2001; Simon et al. 2003; Archetti 2004; Lenormand et al. 2016; Engelstädter 2017; Haag et al. 2017; Boyer et al. 2021). Here we refer to asexual reproduction as reproduction without "syngamy" (i.e. without the fusion of male and female gametes, but with the possibility of recombination). Most theoretical models on the origin and frequency of sex-asex transitions adopt a simplistic view and assume that an asexual clone can emerge immediately from a sexual ancestor. Yet, although it is true that extant parthenogenetic species are derived from sexual ancestors, most of these transitions likely occured in several steps and include non-clonal modes of asexual reproduction, which can impact the fitness of asexual lineages at both short and long evolutionary timescales (Asher 1970; Suomalainen et al. 1987; Archetti 2004, 2010; Engelstädter 2017). Historically, cytologists distinguished automictic and apomictic asexuals based on the presence or absence of "mixis" (i.e. fusion of meiotic products). From a genetic standpoint, apomixis refers to clonal reproduction, which is functionally equivalent to mitosis. Cytologic and genetic definitions are contradictory because mixis is not always required for non-clonal reproduction, e.g. when either meiosis I or II is aborted (Asher 1970). Here, we use the genetic definition, and refer to automixis as any modification of meiosis that leads to non-clonal inheritance (i.e., maintenance of non-zero meiotic recombination).

In this study, we focus on sex-asex transitions in animals, aiming at a better understanding of both the origin of parthenogenetic lineages and their genomic evolution. We distinguish among four different types of origin of asexuality. Asexuality can arise either spontaneously (e.g., by mutation, "spontaneous origin", Simon et al. 2003), through the presence of endosymbionts ("symbiotic origin"; Simon et al 2003), through hybridization between two different sexual species ("hybrid origin", Cuellar 1987; Moritz et al. 1989; Simon et al. 2003; Kearney 2005), or through transmission from an existing asexual lineage to a new one (hereafter "origin by transmission"). Such transmission events may occur through rare males produced by asexual lineages that may transmit asexuality genes by mating with related sexual females ("contagious asexuality", Hebert and Crease 1983; Simon et al. 2003; Paland et al. 2005; Jaquiéry et al. 2014). Yet, transmission through crosses between asexual females and sexual males are also possible: either asexual females may rarely produce reduced eggs by meiosis and undergo rare sex, or their unreduced eggs may be fertilized, leading to an elevated ploidy level in the new lineage (e.g., production of a triploid lineage through the fertilization, by a haploid sperm, of an unreduced diploid egg produced by an asexual female).

Because of this diversity of possible scenarios, many simple methods fail to provide a robust approach to elucidate how different asexual lineages emerge and how they relate to each other. We can identify five major hurdles that need to be addressed for a comprehensive understanding of sex-asex transitions in animals. We emphasize that most of these hurdles also undermine our understanding of sex-asex transitions in other eukaryotes, although they may involve other specific issues (e.g. van Dijk 2009; Lee et al. 2010).

First, although traditional phylogenetic methods can be used to study the maternal origin of asexual lineages using mitochondrial markers, they can be misleading in many cases. Technically, the presence of nuclear mitochondrial pseudogenes ("numts", Lopez et al. 1994) can result in incorrect inferences regarding the age of asexual ineages (Bi and Bogart 2010). More fundamentally, classical phylogenetic methods based on nuclear markers might work when asexuality arises spontaneously and when recombination is absent, but they can be very misleading otherwise. Phylogenetic trees cannot depict the potentially reticulated history of asexual lineages, when the origin of asexual lineages involve crosses and/or when recombination is present. A better approach consists in using the discordances between mitochondrial and nuclear markers to reveal the history of hybrid crosses (Schurko et al. 2009). Finally, with asexuality “by transmission”, the age of asexual lineages becomes an ambiguous concept. Indeed, different parts of the genome of these asexuals may have experienced asexuality for very different periods of time (Tucker et al. 2013).

The second hurdle to understanding sex-asex transitions is that recombination may persist in asexual lineages. While the absence of recombination in apomicts maintains heterozygosity levels across generations (except for mutation, gene conversion, and mitotic recombination events), the presence of recombination under automixis can result in a loss of heterozygosity (LOH) between generations. When the rate of LOH is heterogeneous across genomic regions along the chromosome (ranging from 0% to 100%, depending on the distance of the region from the centromere (Nougué et al. 2015*b*; Svendsen et al. 2015; Boyer et al. 2021)), different genomic regions will coalesce at different points in time, providing different phylogenetic signals. Hence, there are considerable uncertainties in the patterns of molecular variation to be expected within and among asexual genomes, as classic genetic distance metrics do not account for heterogeneity in LOH among markers. In addition, LOH could have different fitness consequences, depending on the mode of asexuality (Archetti 2004, 2010; Engelstädter 2017) or on the sex-determination system of the ancestral sexual species (Engelstädter 2008). For instance, LOH in ZW asexual females could produce low fitness ZZ and WW offspring, reducing the rate of transition to asexuality in ZW sexual species.

The third hurdle to understanding sex-asex transitions is that asexuality is often associated with polyploidy, at least in animals (Moritz et al. 1989; Dufresne and Hebert 1994; Otto and Whitton 2000), so that studying the origin of asexuality (e.g. allo- or autopolyploid origin) requires the use of specific genetic distance metrics that are defined across different ploidy levels (Clark & Jasienuk 2011). In addition, both the lack of dosage information (i.e. exact number of each allele) or the variation in nuclear DNA content among individuals from the same ploidy level can make the discrimination among ploidy levels difficult (e.g. Neiman et al. 2011). Finally, elucidating the role of polyploidy in sex-asex transitions is difficult. Most studies fail to reveal whether polyploidy is a cause, a consequence, or even just a correlate of asexuality in animals (Neiman et al. 2014).

The fourth hurdle to understanding sex-asex transitions is the potential occurrence of rare sex events in asexual taxa, in other circumstances than those of contagious asexuality, mentioned above. Meiosis might sometimes occur normally in asexual females (e.g. De Meester et al. 2004; Rey et al. 2013; Boyer et al. 2021). A haploid egg produced through regular meioses

can be fertilized by a haploid sperm from a rare male of the same or a different asexual lineage or from a male from related sexual species. These rare events of cryptic sex may be difficult to detect in the field, especially if divergence between parents is low or if sampling is incomplete.

A fifth hurdle to understanding sex-asex transitions is the technical and methodological difficulty of identifying and exhaustively sampling the closest extant sexual species of asexual lineages, as they often have very different geographical distributions (Kearney 2005). In addition, the closest sexual populations might be extinct or might themselves result from the hybridization between divergent sexual populations, so that many different sex-asex transition scenarios need to be considered. Overall, except in species where asexuality is directly caused by endosymbionts, ruling out a potential hybrid origin of asexuality and demonstrating that asexuality arose spontaneously remains very difficult, due to this sampling challenge.

These biological and methodological challenges might exist for virtually all asexual taxa, and failing to address them consistenly and jointly might provide an incomplete picture of the origin and evolution of asexual lineages. Some of these issues have recently been addressed in several systems; for instance the description of automixis in Daphnia (Svendsen et al. 2015), the identification asexuality genes in various asexual arthropods (Sandrock and Vorburger 2011; Tucker et al. 2013; Yagound et al. 2020), or the occurrence of recombination in ancient asexual such as bdelloid rotifers (Simion et al. 2020).

In this study, we address all the hurdles that compromise our understanding sex-asex transitions in *Artemia parthenogenetica*, a group of asexual crustaceans that has been studied for decades and is emblematic of the multiple challenges encountered when studying the origin asexual species. This group includes diploid and polyploid asexual lineages that are found worldwide except on the American continent (Bowen et al. 1978; Browne 1992). The distribution of asexual lineages within the genus is exceptionally asymmetric, as they are all more closely related to Old-world sexual species: *A. sinica*, *A. tibetiana*, *A. urmiana* and an uncharacterized species from Kazakhstan, hereafter named *A.* sp kaz (Asem et al. 2016; see Table 1 for the nomenclature and abbreviations of the different taxa). The geographical distribution of both diploid and polyploid asexuals is much larger than that of these sexual species. *Artemia* resting stages can be dispersed by waterbirds (Sánchez et al. 2012) or humans (Rode et al. 2013) over large geographical distances.

Although the origin of asexual *Artemia* lineages has been extensively studied since Bowen *et al*. (1978), previous studies have failed to jointly address the five hurdles to understanding sex-asex transitions mentioned above. In particular the paternal origins of asexual lineages have never been investigated so that neither a potential hybrid nor a contagious origin of asexuality have been tested. Fig. 1 presents a synthesis of the major conclusions of landmark papers. Briefly, asexuality in *Artemia* was first thought to have a single origin (Bowen and Sterling 1978; Abreu-Grobois and Beardmore 1982; Beardmore and Abreu-Grobois 1983; Abreu-Grobois 1987), such that the species was considered as an ‘ancient asexual scandal’ (Judson and Normark 1996). When both nuclear and mitochondrial markers and more sexual species (*Aurm*, *Atib* and *Asin*) were considered, the evidence pointed to multiple and much more recent origins of asexuality (Baxevanis et al. 2006; Muñoz et al. 2010; Maniatsi et al. 2011; Eimanifar et al. 2015; Asem et al. 2016). These phylogenetic investigations reported that *A.*

parthenogenetica was likely to be a polyphyletic group (Baxevanis et al. 2006; Maniatsi et al. 2011). Based on mitochondrial data, *Ap2n* falls into two distinct maternal lineages, *Ap2n-kaz* and *Ap2n-urm,* whose mitochondrial haplotypes are closer to those of *Akaz* (mt-2n*k*) and *Aurm* (mt-2n*u*) respectively (Muñoz et al. 2010). A third *Ap2n* mitochondrial lineage has also been recently described (Maccari et al. 2013*a*). Based on mitochondrial and nuclear data, *Ap3n* are thought to maternally derive either from *Aurm* (Maniatsi et al. 2011) or from *Ap2n* (Asem et al. 2016). *Ap4n* and *Ap5n* are thought to have emerged successively: the former from an *Asin* female and the latter from an *Ap4n* female (Maniatsi et al. 2011; Asem et al. 2016).

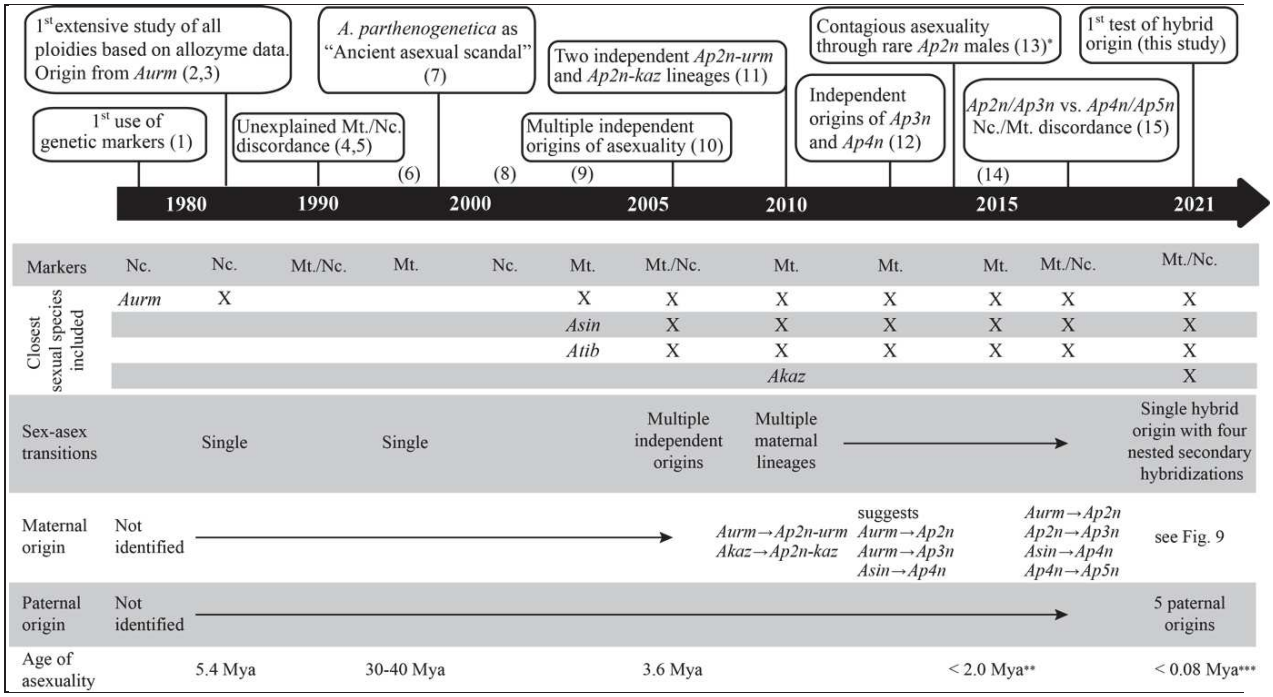


Figure 1. Time line of the major findings regarding the origin of diploid and polyploid asexual *Artemia* lineages. References : 1 : (Bowen and Sterling 1978); 2 : (Abreu-Grobois and Beardmore 1982) ; 3 : (Beardmore and Abreu-Grobois 1983); 4 : (Browne and Hoopes 1990); 5 : (Browne and Bowen 1991); 6: (Perez et al. 1994) ; 7 : (Judson and Normark 1996); 8 : (Nascetti et al. 2003) ; 9 : (Bossier et al. 2004) ; 10 : (Baxevanis et al. 2006) ; 11 : (Muñoz et al. 2010) ; 12 : (Maniatsi et al. 2011) ; 13 : (Maccari et al. 2013*a*) ; 14 : (Eimanifar et al. 2015); 15 : (Asem et al. 2016). Mya: millions year ago.

*: F1 hybrids between *Ap2n* and *Aurm* observed by (1) but no contagion observed before (13).

**: Based on *Aurm*/*Ap2n-kaz* divergence.

***: Age of the most recent common ancestor of all extent asexual lineages (asexuality gene(s) may be older).

The reproductive mode of *A. parthenogenetica* has also been intensely investigated for over a century. Cytogenetic studies in diploids have yielded contradictory results by reporting almost all known forms of automixis (Narbel-Hofstetter 1964; Nougué et al. 2015*b*), while most cytogenetic studies in polyploids have reported that they reproduce through apomixis (Barigozzi 1974). Recent genetic studies showed that *Ap2n* reproduces through a mechanism genetically equivalent to central fusion automixis (Nougué et al. 2015*b*; Boyer et al. 2021),

which leads to loss of heterozygosity in centromere-distal chromosomal regions due to recombination (Svendsen et al. 2015). *A. parthenogenetica* has a ZW sex-determination system and produces males at low frequency (Stefani 1964). These rare males are thought to arise through rare recombination events between the Z and W chromosomes in *Ap2n* females, which results in a LOH at the sex locus and the production of ZZ males (Browne and Hoopes 1990; Abreu-Grobois and Beardmore 2001; Boyer et al. 2021). Last, for a long time, rare males have been thought to be useless and irrelevant to *Ap2n* reproduction (MacDonald and Browne 1987; Simon et al. 2003), but experiments showed they can transmit asexuality when crossed with *Aurm* and *Akaz* sexual females (Maccari et al. 2014; Boyer et al. 2021). Moreover, recent experiments have shown that some *Ap2n* females can engage very rarely in sex in the laboratory, with normal meiosis and recombination (Boyer et al. 2021). Hence contagious asexuality and/or sexual reproduction may occur at small rates within *Ap2n* asexuals *in natura*.

In addition, previous studies also suffered from a number of limitations. The first limitation is the lack of reliable nuclear markers available across asexual and sexual taxa. The most informative dataset (23 allozyme markers) dates back to the 80's, but shows a limited resolution with few alleles per marker (Abreu-Grobois and Beardmore 1982). Similarly, nuclear sequences (ITS1, Na^+/K^+ ATPase, etc.) show limited diversity across asexual and sexual taxa (Baxevanis et al. 2006; Asem et al. 2016). Microsatellite markers described in Muñoz *et al.* (2008) present null alleles and fail to amplify in some sexual species (Maccari et al. 2013*b*). Mitochondrial data also present limitations due to the potential co-amplification of nuclear pseudogenes (Wang et al. 2008). Indeed, several studies have reported difficulties in amplifying COI sequences using universal primers or primers designed for the distantly related *A. franscicana* (Wang et al. 2008) and *A. salina* (Asem et al. 2016). Mitochondrial-nuclear discordance represents crucial evidence for hybridization scenarios, yet many studies did not combine nuclear and mitochondrial data (Fig. 1) and relied only on mitochondrial data for taxonomic identification (Sainz-Escudero et al. 2021). Furthermore, in many studies ploidy of all samples is not directly assessed or is based on existing literature regarding populations previously sampled in the same locality (e.g. Baxevanis et al. 2006). However, a same locality may host different (sexual and/or asexual) populations whose occurrence varies spatially or temporally (e.g. Agh et al. 2007). Importantly, ploidy cannot be assessed based on the number of alleles at each locus genetic markers as *Artemia* individuals from each ploidy level often exhibit only two alleles at each locus (Nougué et al. 2015*a*); whether this lack of variation is the result of LOH or of some other mechanisms has never been studied. Finally, no previous study has included all known sexual species and all ploidy levels. A failure to include the most closely related sexual species may result in asexuality appearing more ancient than it actually is (e.g. Perez et al. 1994). Similar biases due to limited sampling are frequent in studies of the age and origin of asexual taxa (Tucker et al. 2013).

In this paper, we investigate potential origins "by transmission" of diploid and polyploid asexual lineages, considering all new experimental information regarding the reproductive mode of *A. parthenogenetica* (Maccari et al. 2014; Nougué et al. 2015*b*; Boyer et al. 2021). We test the hypothesis that diploid and polyploid asexual lineages may represent a mixture of different "hybrids" resulting from several events of contagion and secondary backcrosses with different sexual species. We build a series of tailored population genetic methods to test whether

asexual *Artemia* of various ploidies have a hybrid origin and we attempt to identify the corresponding parental species. We also investigate whether secondary crosses or contagion events can be identified. To this end, we conduct the first study that includes an exhaustive sampling of asexual lineages (*Ap2n-kaz*, *Ap2n-urm*, *Ap3n*, *Ap4n*, *Ap5n*) and major sexual relatives (*Aurm, Akaz*, *Atib*, *Asin*) and that combines nuclear and mitochondrial data with ploidy data (based on flow cytometry). Finally, we also test for the presence of cryptic sex in *Ap2n*. In the absence of sexual reproduction, we can indeed expect *Ap2n* individuals with different mitochondrial haplotypes to be characterized by different and specific nuclear backgrounds. In contrast, in the presence of cryptic sex, we expect a discordance between mitochondrial haplotypes and nuclear backgrounds (hereafter "mito-nuclear discordance"). We find that, after a single hybrid origin of one diploid asexual lineage, all other asexuals emerged through a series of four nested hybridization events involving several sexual species. Overall, this new approach changes our view of sex-asex transitions in *Artemia*. It may prove to be a valuable tool to investigate sex-asex transitions in other taxa, especially when classical phylogenetic approaches are not appropriate.

Species name	Abbreviation	mt abbreviation
A. sinica	Asin	mt-sin
A. urmiana	Aurm	mt-urm
A. tibetiana	Atib	mt-tib
A. sp. Kazakhstan	Akaz	mt-kaz
Diploid A. parthenogenetica (2n) with Aurm-type mitochondria	Ap2n-urm	mt-2nu
Diploid A. parthenogenetica (2n) with Akaz-type mitochondria	Ap2n-kaz	mt-2nk
Triploid A. parthenogenetica (3n)	Ap3n	mt-3n
Tetraploid A. parthenogenetica (4n)	Ap4n	mt-4n
Pentaploid A. parthenogenetica (5n)	Ap5n	mt-5n

Table 1. List of species names and abbreviations

Methods

Samples

Based on the existing literature (Abreu-Grobois and Beardmore 1982; Muñoz et al. 2010; Maniatsi et al. 2011; Maccari et al. 2013*a*), we chose 37 populations from Eurasia and Africa, including both asexual strains (described as diploid, triploid, tetraploid and pentaploid) and the four closest sexual species (Fig. 2). We obtained samples from cyst bank collections and wild-collected adults (Fig. 2; Table S4). Cysts were hatched and individuals maintained following

protocols described in (Rode et al. 2011). It has recently been shown that at least some *Ap2n* females can reproduce sexually in the laboratory at a rate of ~2‰ in the presence of males (Boyer et al. 2021). However, for simplicity (and because the capacity to undergo rare sexual reproduction *in natura* is unknown), we only categorized individuals are sexuals vs. asexuals and did not consider facultative asexuality. The reproductive mode of each population was verified based on the presence or absence of males among adults. When at least one male was present, we separated asexual from sexual females according to morphological characters (Maccari et al. 2013*b*). All five populations with at least one male consisted of a mixture of asexual females with females from different sexual species (*A. franciscana*: AIM, BOL, SAG, *A. sinica*: DON, or *A. salina*: BDP).

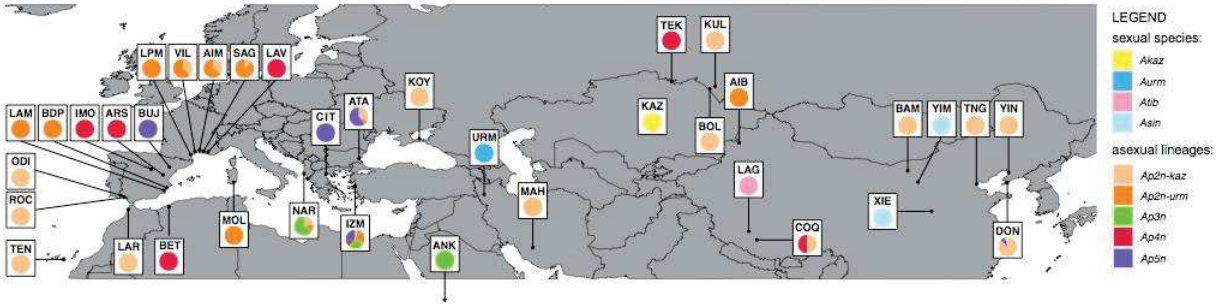


Figure 2. Geographic distribution of the sexual and asexual *Artemia* samples genotyped in this study. Each color corresponds to a mitogroup. AIB: Aibi Lake (China); AIM: Aigues-Mortes (France); ANK: Ankiembe (Madagascar); ARS: Arcos de la salinas (Spain); ATA1/ ATA2: Atanasovko Lake (Bulgaria); BAM: Unknown, Bameng area (China); BDP: Bras del port (Spain); BET: Bethioua (Algeria); BOL: Bolshoye Yarovoe (Russia); BUJ: Bujaraloz (Spain); CIT: Citros (Greece); COQ: Co Qen (China); DON: Dongjiagou (China); IMO: Imón (Spain); IZM: Izmir (Turkey); KAZ: Unknown location (Kazakhstan); KOY: Koyashskoye (Ukraine); KUL: Kulundinskoye (Russia); LAG: Lagkor Co (China); LAM: La Mata (Spain); LAR: Larache (Morocco); LAV: Lavalduc (France); LPM: La Palmes (France); MAH: Maharlu Lake (Iran); MOL: Molentargius (Italy); NAR: Narte (Albania); ODI: Odiel (Spain); ROC: N. S. Rocío (Spain); SAG: Salin De Giraud (France); TEK: Teke Lake (Kazakhstan); TEN: Tenefé (Spain); TNG: Tanggu (China); URM: Urmia Lake (Iran); VIL: Sète-Villeroy (France); XIE: Xiechi Lake (China); YIM: Unknown location, Yimeng area (China); YIN: Yingkou (China). Due to the scale of the map, the ANK *Ap3n* population is represented with an arrow pointing towards its location (Ankiembe, Madagascar). Mitochondrial data could not be obtained for KOY (Koyashskoye, Ukraine), which is represented based on the analysis of 17 *Ap2n-kaz* individuals from Maccari et al (2013*a*) that used the same cyst sample.

Ploidy

We characterized the ploidy of putatively diploid and polyploid asexual females, as well as of males and females of each sexual species (147 individuals in total) using flow cytometry as described in (Nougué et al. 2015*b*) and sup. mat. 1. Data from (Nougué et al. 2015*b*) were added to the dataset, resulting in a total sample size of 206 individuals (Table S4). We tested for difference in genome size between asexual and sexual lineages using *t*-tests. All significant *P-values* remained significant after Bonferroni correction, so we present only the uncorrected values for simplicity.

COI genotyping

A fragment of the mitochondrial Cytochrome c Oxidase Subunit I (COI) gene of 336 individuals (Table S4) was amplified using primers 1/2COI_Fol-F and 1/2COI_Fol-R following the protocol of Muñoz et al. (2010). Because these primers turned out to lack specificity and resulted in the amplification of a large number of numts (see below), we amplified the COI sequence of 23 additional individuals using the more specific primers Co1APAR-F(5'- TTTGGAGCTTGAGCAGGAAT-3') and Co1APAR-R(5'- TGCGGGATCAAAGAAAGAAG-3') (see sup. mat. 2). PCR products were purified and directly sequenced (i.e. with no cloning), using an ABI Prism Big Dye Terminator Kit (Applied Biosystems, Warrington, UK) on an ABI PRISM 3130xl sequencer. After removing sequences with indels and sequences from the same individual that were identical, we recovered 359 sequences that were split into two datasets: dataset1, which only included high quality sequences (198 sequences including at least 538 sites in the final alignment and without any ambiguous position), and dataset2, which included all other sequences (161 sequences that were either too short or included one or more ambiguous positions). COI phylogenetic inferences are often biased by the inclusion of numts (Buhay 2009) or chimeric sequences that result from the coamplification by PCR of mitochondrial sequences with contaminating sequences (either numts or mitochondrial sequences from other individuals; e.g. Dubey et al. 2009). We developed a new method to identify potential chimeric sequences based on a dataset composed of known numts sequences (see "chimera detection" section below). To build this dataset, we performed additional cloning and sequencing of PCR fragments amplified either from total DNA or from DNA enriched from mitochondrial DNA (sup. mat. 2, Table S4). Cloning allowed the recovery of 32 numt sequences without indels (which we identified as minority sequences among those obtained from the same individual through cloning, sup. mat. 2) and seven mitochondrial sequences (hereafter dataset3). We built a dataset that combined these 39 sequences (dataset3), our 198 sequences (dataset1) and 748 *Artemia* spp. high quality sequences from GenBank without any indel (sup. mat. 2). All analyses of the final dataset of 985 COI sequences were performed in R 3.6.3 (www.r-project.org).

Analyses of mitochondrial data (1): pseudogene detection

We aligned sequences using MAFFT v7.427 (Katoh et al. 2002) as implemented within the package ips (v0.0.11; Heibl 2008) with default settings. To detect potential numts without indels, we translated sequences into amino acid sequences using the invertebrate mitochondrial DNA genetic code. To detect potential numts, we tested for changes in the polarity of amino acid residues (Kunz et al. 2019). For each sample, we estimated the absolute difference in polarity (i.e. PP1 in Cruciani et al. 2004) between derived and ancestral amino-acid sequences. Based on the observed distribution of this polarity difference across the 985 protein sequences (Fig. S1, sup. mat. 3), we set 0.1 as the threshold in polarity differences above which sequences were labeled as potential numts. This procedure allowed the reliable detection of 65% of the reference numts obtained by cloning (i.e. 21 of the 32 known numts). We also detected 26 additional sequences (including 25 sequences from GenBank) that were labelled as potential numts for the rest of the analyses.

Analyses of mitochondrial data (2): haplotype reference set

Poorly aligned positions were removed using gblocks 0.91b as implemented within the package ips (v0.0.11; Heibl 2008) and collapsed into 230 unique haplotypes using the haplotype function of the package haplotypes (v1.1.2; Aktas 2020).

Analyses of mitochondrial data (3): chimera detection

We designed a quantitative test to detect and exclude potential chimeric sequences. The principle of the method is to determine, for each focal sequence, how many mutations could be "explained" by assuming that this sequence represents a chimera. We compared each haplotype sequence to the remaining 229 sequences in the dataset to identify the most similar one. For each mutation differing between the focal sequence and the most similar one, we provide a score of one if this mutation (i.e. the same SNP) was found in another unrelated sequence of the dataset or zero otherwise. For each of the 230 haplotype sequences, we computed the sum of this score over the different SNPs of each haplotype. We considered that each sequence could have acquired a mutation which happened to be present in an unrelated sequence of the dataset (score =1), but that it could not have acquired two or more of these mutations (score >1). Hence, haplotype sequences with score equal or greater than two were considered as potentially chimeric (see sup. mat. 4 for details). We removed 80 haplotypes (corresponding to 101 samples) that appeared as potential chimeras resulting from the co-amplification of a mitochondrial sequence and either a numt or contamination from other mitochondrial sequences. Overall, we obtained 123 reference non-chimeric haplotypes (corresponding to 884 samples).

Analyses of mitochondrial data (4): haplotype assignation

To study the phylogenetic relationship among major asexual and sexual taxa (*Ap2n-kaz*, *Ap2n-urm*, *Ap3n*, *Ap4n*, *Ap5n*, *Aurm*, *Akaz*, *Atib*, *Asin*), we built a haplotype network based on the 123 reference haplotypes and using the parsimnet function (95% probability of parsimony, Templeton et al. 1992) of the package haplotypes (v1.1.2; Aktas 2020). We found the same tree topology when building a maximum likelihood phylogeny with the phangorn package (v2.5.5 Schliep 2011).

In addition, we assigned the 61 *Ap2n* sequences in dataset2 to either *Ap2n-kaz*, *Ap2n-urm* or other sequences (i.e. numts or chimeras). To do so, we aligned them with the 123 reference haplotype sequences and with the 107 numt and chimeric sequences and estimated pairwise genetic distances as described above. For each sequence in dataset2, the mitochondrial haplotype was assigned based on the identity of the closest reference haplotype(s). Whenever a numt, a potential numt or a chimera was found among the closest sequences, the *Ap2n* mitochondrial haplotype was set as "unknown". We could successfully assign to either *Ap2n-kaz* or *Ap2n-urm* 46 of the *Ap2n* sequences.

Microsatellite genotyping

We genotyped 432 individuals with a panel of 12 microsatellite markers (see Muñoz et al. 2008; Nougué et al. 2015*a* for details regarding markers and amplification protocol). Data from Nougué *et al.* (2015*b*) were added to the dataset, resulting in 489 typed individuals (Table S4,

sup. mat. 5). Standardization was achieved by adding DNA from the same individual onto the different plates. Genotype data at three microsatellite markers (Apdq01TAIL, Apdq02TAIL, Apdq03TAIL) were only used when investigating the relationship among *Ap2n*, but excluded when analyzing the full dataset due to the presence of null alleles in polyploids and sexuals (Maniatsi et al. 2011).

Microsatellite data analyses (1): Lynch distance

The Lynch genetic distance is a genetic distance estimate based on a band sharing index, i.e., one minus the similarity index, equation 1 in Lynch (1990). It is an appropriate metric to broadly compare well-separated sexual and asexual groups with different ploidy levels, such as *Ap2n*, *Ap3n*, *Ap4n, Ap5n,* and the different sexual species (i.e. ignoring the occurrence of LOH in *Ap2n*). We computed the average pairwise nuclear distance between and within lineages using the Lynch genetic distance, as implemented in the *polysat* package (v1.7-4; Clark and Jasieniuk 2011) in R. To visualize distance data, we transformed individual pairwise distances into Principal Coordinate Axes using the 'cmdscale' function in the *stats* v3.6.3 package. To investigate the homogeneity of the reference sexual populations, we investigate whether they show a signal of admixture (Estoup et al. 2016). We examined the variation in allele sizes within individuals from each sexual taxon (*Aurm*, *Akaz*, *Atib*, *Asin*; sup. mat. 6).

Microsatellite data analyses (2): genetic distance for automictic Ap2n

Because it does not account for the possibility of LOH, the Lynch genetic distance is of limited use regarding relationships within *Ap2n*. We therefore present a new genetic distance measure for automicts, accounting for the different possible paths between diploid genotypes (sup. mat. 6, Table S1). We assumed that mutations rate is the same across loci, but that LOH varies across loci proportionally to the average inbreeding coefficient (*Fis*), which can be independently estimated. This new measure weights events according to the relative magnitude of LOH and mutation events (with rates r and μ respectively). This genetic distance is a proxy for the time length of the path between individuals (or averaged across different possible paths, according to their relative probability of occurrence). For instance, with this new distance measure, two individuals with genotype AA and AB are weighed as distant if the locus has a strongly negative *Fis* (low LOH rate), since their difference likely results from a single A to B mutation. However, they are not weighed as distant if the locus has a strongly positive *Fis* (high LOH), as AA can result from an LOH event from an AB parent.

Monophyly of Ap2n-kaz and Ap2n-urm clades

In the absence of sexual reproduction, we expect *Ap2n-kaz* and *Ap2n-urm* individuals to be characterized by different and specific nuclear backgrounds. In contrast, in the presence of sexual reproduction between *Ap2n-kaz* and *Ap2n-urm* individuals, we expect a discordance between mitochondrial haplotypes and nuclear backgrounds (hereafter "mito-nuclear discordance"). Using data from the 12 microsatellite makers, we used our new genetic distance metric to compute pairwise genetic distances among 127 *Ap2n* individuals with known mitochondrial haplotypes. Using a randomization test, we first investigated whether the genetic distance between *Ap2n-kaz* and *Ap2n-urm* lineages was significantly larger than that within *Ap2n-kaz* or *Ap2n-urm* lineages. As this test only considers average nuclear distances between

and within lineages, the difference might be significant even if *Ap2n-kaz* and *Ap2n-urm* clade are not monophyletic (e.g. due to rare events of sexual reproduction between *Ap2n-kaz* and *Ap2n-urm* individuals). Using the pairwise distance matrix computed above, we first built a neighbor-joining (NJ) tree using the *nj* function from the ape package (v5.4-1; Paradis and Schliep 2019) and estimated branch length using non-negative least squares (nnls.tree function from the phangorn package, v2.5.5 Schliep 2011). We computed the 95% confidence interval of this branch length by resampling microsatellite markers to build 1000 bootstrap replicates. Finally, we built NJ trees separately for *Ap2n-kaz* and *Ap2n-urm* and assembled them into a single tree where each lineage is monophyletic. We again estimated the branch length of this tree using non-negative least square and then tested whether the estimated branch length was outside of the 95% confidence interval computed above.

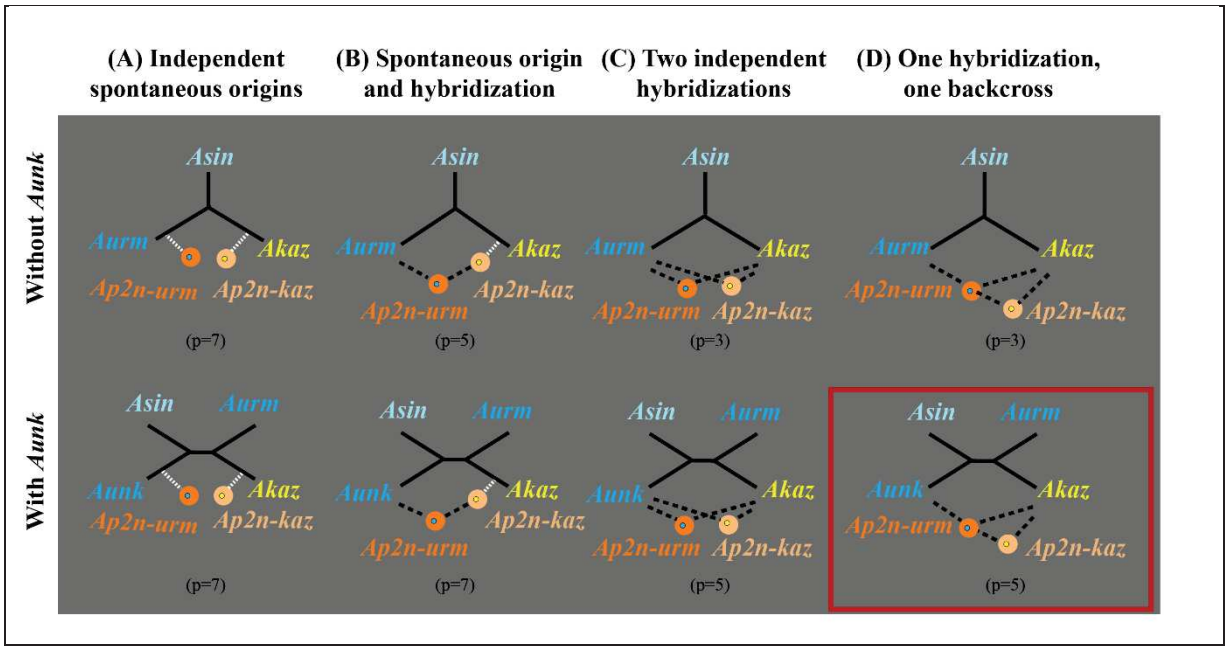


Figure 3. Scenarios for the origin of *Ap2n-kaz* and *Ap2n-urm*. The two lineages are respectively depicted by light and dark orange circles. The color of the dot within each circle represents the maternal lineage of the mitochondrion (blue: mt-2n*u* from an *Aurm* mother, yellow: mt-2n*k* from an *Akaz* mother). White dotted lines and black dashed lines indicate spontaneous and hybrid origins, respectively. In (A) and in the presence of *Aunk*, the nine branch lengths are not identifiable. The branch length leading to *Aunk* cannot be fitted and was therefore dropped. In (B), the scenarios illustrated assumes a spontaneous origin of *Ap2n-kaz* in *Akaz* followed by a cross with *Aurm* or *Aunk*. We also considered the reciprocal scenario (origin in *Aurm* or in *Aunk* and cross with *Akaz*). In (D), the scenarios illustrated shows an origin of *Ap2n-urm* through hybridization followed by a backcross with *Akaz*. The reciprocal scenario with a backcross with *Aurm* or *Aunk* was also considered. Only one topology (where *Asin* is closest to *Aunk*) is represented for all scenarios involving *Aunk* (second row). The two other topologies were also considered (*Asin* closest either to *Akaz* or *Aurm*, respectively). The best model is indicated by the red rectangle and involved one hybridization with an unknown species and backcross on *Akaz* (bottom right). The number of parameters fitted for each model (corresponding to the number of identifiable branch lengths) is given by the number between parentheses below each scenario.

Evolutionary origin of Ap2n-kaz and Ap2n-urm

To investigate the evolutionary relationships between *Ap2n-kaz* and *Ap2n-urm* and the three sexual species (*Aurm*, *Akaz* and *Asin*), we considered different scenarios based on the Lynch genetic distance (Fig. 3). We considered two independent spontaneous origins within *Aurm* and *Akaz* (Fig. 3A), a single spontaneous origin followed by a hybridization event (Fig. 3B), two independent hybridization events (Fig. 3C), and one hybridization and one backross event (Fig. 3D). These four scenarios were considered with or without the presence of an unknown species, denoted *Aunk*, which is assumed to carry mt-*urm*. We did not consider the scenarios where *Aunk* carried mt-*kaz*. Indeed, nuclear data indicated that all *Ap2n* are much closer to *Akaz* than to *Aurm* (see Results), and it is therefore very likely that *Ap2n-kaz* inherited their mitochondria directly from *Akaz*. Because the outgroup *Asin* can have different positions (it can be closest to either *Akaz*, *Aurm* or *Aunk*), each scenario was evaluated assuming all three possible topologies. For each topology, we described each branch length by a parameter. The number of identifiable parameters is given for each scenario in Fig. 3. For scenarios in which *Ap2n* arose spontaneously, a branch length between *Ap2n* and the sexual species was included. When *Ap2n* arose from a hybrid cross, the genetic distance between them and a given sexual species was computed as the averages of the branch lengths between either parent and that sexual species. The model corresponding to each scenario was fitted using least square to the matrix of Lynch genetic distance among *Akaz*, *Aurm*, *Asin*, *Ap2n-kaz* and *Ap2n-urm*. To avoid any confounding effect due to potential cryptic sex between *Ap2n-kaz* and *Ap2n-urm*, we computed this matrix after removing 13 *Ap2n* individuals with discordant mito-nuclear data (see result section). We assumed that genetic distance within each species or within each of two *Ap2n* lineages was negligible compared to the genetic distance among species (i.e. we ignored divergence within each sexual or asexual lineage). Models were compared based on corrected Akaike's information criterion (AICc, Hurvich and Tsai 1989). We computed the difference(ΔAICc) between the AICc of a given model and that of the model with lowest AICc. Models with ΔAICc higher than two were considered as poorly supported (Burnham and Anderson 2002).

Evolutionary origin of Ap3n, Ap4n and Ap5n

We assumed the maternal origin of the ancestor of *Ap3n*, *Ap4n* and *Ap5n* lineages to be known based on mitochondrial data (see results below). For each ploidy level, we compared different scenarios involving different paternal origins (see sup. mat. 7 for details). For each ploidy level and each scenario, we simulated 10,000 synthetic hybrids using a custom script in R. For each hybrid, we first randomly sampled a mother with the observed mitochondrial haplotype in our dataset (i.e. *Ap2n-kaz* for *Ap3n*, *Asin* for *Ap4n* and *Ap4n* for *Ap5n*). Second, to draw a haploid genotype (representing the sperm genotype), we randomly sampled an individual for each paternal origin (i.e. *Akaz*, *Ap2n-kaz*, *Ap2n-urm*, *Aurm*, *Atib* or *Asin*) and randomly sampled one allele of this individual at each locus. We assumed that the 12 microsatellite loci were unlinked, so that probabilities of sampling alleles were independent across loci. We assumed that alternative scenarios involving fertilization by an unreduced sperm were less likely (e.g. origin of *Ap3n* through the fertilization of a reduced *Ap2n-kaz* egg by an unreduced sperm or origin of *Ap4n* through the fertilization of an unreduced *Asin* egg by

an unreduced sperm). We then computed average Lynch genetic distance based on the 100 synthetic hybrids closest to *Ap3n*, *Ap4n* or *Ap5n* individuals in our dataset.

Results

Ploidy characterization

The results from the flow cytometry measurements are summarized in Fig. 4. The ploidy levels detected in each population were in good agreement with those found in previous cytological or genetic studies (Abreu-Grobois and Beardmore 1982; Muñoz et al. 2010; Maccari et al. 2013*a*), except for two *Ap5n* populations previously described as *Ap4n* (BUJ and CIT, Abatzopoulos et al. 1986; Amat et al. 1994; Maniatsi et al. 2011). Interestingly, the genome size of *Ap2n-kaz* was not significantly different from that of *Ap2n-urm* (4.74 pg SD=0.17 vs. 4.65 pg SD=0.20 respectively; t=-1.93, df=50.48, *P-value*=0.059). The *Ap2n-kaz* genome size was significantly lower than *Akaz* (4.92 pg SD=0.14; t=-3.21, df=14.74, *P-value*=0.006), and *Ap2n-urm* genome size was significantly higher than *Aurm* (4.22 pg SD=0.20; t=4.46, df=5.67, *P-value*=0.005). The genome size of *Ap3n* (7.13 pg SD=0.43) was consistent with their ploidy level and was not significantly different from 1.5 times that of *Ap2n-kaz* (t=-0.16, df=37.63, *P-value*=0.88). Although *Ap4n* harbor an *Asin* mitochondrion (Asem et al. 2016), their genome size (10.15 pg SD=0.34) was more than twice that of *Asin* (2 x 4.74=9.49 pg SD=0.24; t=4.44, df=6.38, *P-value*=0.004). In contrast, the size of the genome of *Ap5n* (12.22 pg SD=0.45) that also harbor an *Asin* mitochondria was not significantly different from the value 2.5 times that of *Asin* (t=2.15, df=4.57, *P-value*=0.09). These observations seem inconsistent with the scenario of an origin of *Ap4n* through an endoduplication in *Asin*. Interestingly, the genome size of *Atib* was 57%, 34% and 39% larger than that of *Aurm*, *Akaz* and *Asin* respectively, suggesting an increase in genome size in the lineage leading to *Atib*.

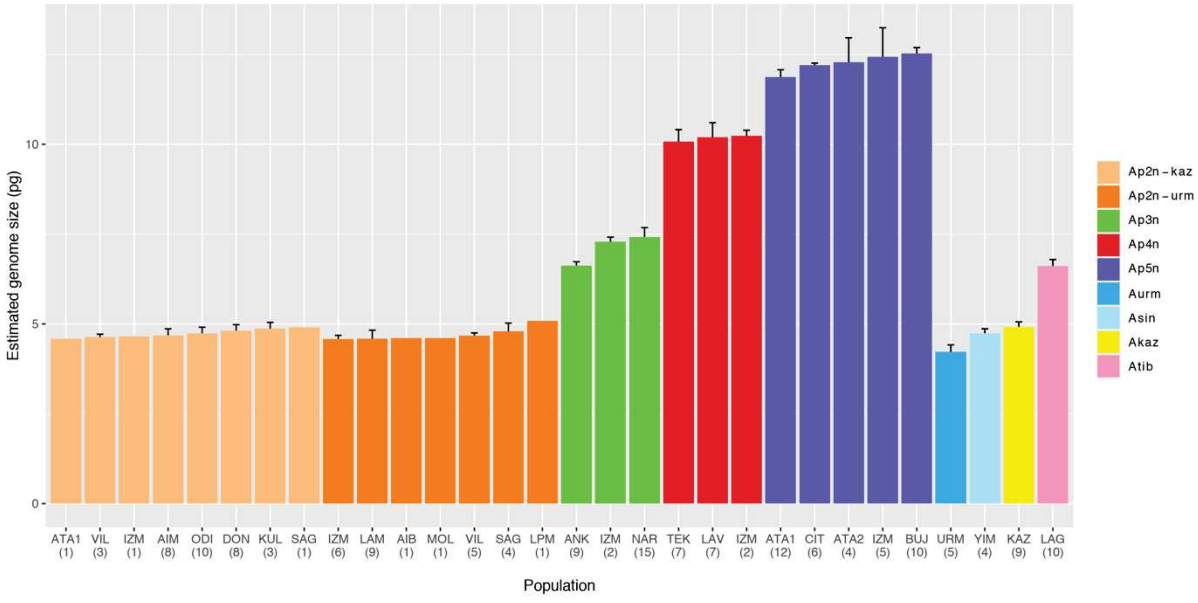


Figure 4. Estimated genome size of *Artemia* sexual and asexual lineages. Genome size in pg of diploid *Ap2n-kaz* and *Ap2n-urm*, triploid, tetraploid and pentaploid asexual lineages and related sexual species (*A. urmiana*, *A. sinica*, *A.* sp Kaz, and *A. tibetiana*). Mean ± SD C-values

are shown. Abbreviations for population labels are provided as in Fig. 2. Number below population labels represent sample sizes. 37 *Ap2n* individuals had unknown mitochondrial haplotypes and are not represented.

COI genotyping

Ap2n, *Ap3n*, *Ap4n* and *Ap5n* were found in 24, 3, 7 and 5 populations respectively (Table S4, Fig. 2). Among the 37 populations sampled, only five (ATA, IZM, NAR, COQ and DON) were composed of individuals with different ploidies. Similarly, among the 24 *Ap2n* populations, 13 comprised only individuals with *Ap2n-kaz* haplotypes, five comprised only individuals with *Ap2n-urm* haplotypes and six (AIM, ATA, VIL, SAG, IZM, NAR) comprised individuals with both *Ap2n-kaz* and *Ap2n-urm* haplotypes (Fig. 2). Consistent with previous studies, *Ap2n-kaz* is the clade with the largest geographic distribution (Fig. 2). When including all sequences found in GenBank, the distribution of *Ap2n-urm* is restricted to the Mediterranean area (Spain, France, Italy and Turkey), around the Black sea (Atanasovko Lake, Bulgaria; Oybuskoye Lake, Ukraine) and in Western China (Aibi Lake, Lagkor Co). Polyploids also have a large geographic distribution (Fig. 2).

Among the 950 sequences from dataset1 and from GenBank, we found 47 sequences that had a large change in the polarity of the amino-acid sequence and 88 sequences that included two or more mutations found in another unrelated sequence of the dataset. These sequences were likely to be numts and chimeras respectively. The remaining 850 COI sequences were collapsed into 123 unique haplotypes of diploid mt-2n*k*, diploid mt-2n*u*, triploid, tetraploid and pentaploid asexual lineages and related sexual species (Fig. 5). In line with Maccari et al. (2013*a*), we found three networks separated by more than 30 mutation steps. The fourth network described in their study corresponded to pseudogenes (GenBank accession: EF615587-8) that differ from other sequences by several non-synonymous mutations that changed polarity. Each asexual lineage was characterized by a majority haplotype found at high frequency in many populations and recently-derived satellite haplotypes found at a lower frequency in a few populations (Fig. 5), as previously observed in *Ap2n* lineages (Muñoz et al. 2010; Maccari et al. 2013*a*). This observation of low haplotypic diversity with a star-like shape is consistent with a recent range expansion of the different asexual lineages, which now have widespread geographical distributions. No haplotype was shared between asexual lineages and either of the sexual species. The majority haplotype of *Ap2n-kaz* differed by six mutations from the closest *Akaz* haplotype (but one haplotype of *Ap2n-kaz* differed from *Akaz* by just one mutation). The majority haplotype of *Ap2n-urm* differed by one mutation from the closest *Aurm* haplotype. The majority hapotype of *Ap3n* were identical to the closest *Ap2n-kaz* haplotype, and *Ap4n* and *Ap5n* had the same majority happlotype, which differed by a single mutation from the closest *Asin* haplotype (Fig 5). We found a 18% divergence between *Aurm* and *Asin* COI haplotypes from reference mitochondrial genomes used in Sainz-Escudero et al. (2021), which corresponds to a divergence time of 6.6 Mya (5.47-7.40 My) in their study. Assuming that COI substitution rate is constant, one mutation in Fig. 5 corresponds to a divergence of 0.019% and represents an approximate age of 0.068 Myr (0.056-0.076 Myr). Using divergence between *Atib* and *Asin* COI haplotypes and the divergence time estimate from Sainz-Escudero et al. (2021) provides a qualitatively similar age estimate of 0.062 Myr (0.051-0.079 Myr). Although these results rely

on strong assumptions (accurate dating of the fossil used for calibration and constant molecular clock), they suggest that all extant asexual lineages potentially emerged more recently than previously thought (i.e. less than 80,000 years ago).

The first network includes haplogroups corresponding to *Ap2n-kaz*, *Ap2n-urm*, *Ap3n*, *Aurm*, *Akaz*, and *Atib*. This result confirms the existence of the two distinct major *Ap2n* lineages, *Ap2n-kaz* and *Ap2n-urm*. The third *Ap2n* lineage described in (Maccari et al. 2013*a*) possibly represents a chimera between two divergent PCR-amplified sequences in that study (Fig. S2). Consistent with Maccari et al. 2013a, the haplogroup mt-tib1 included most *Atib* sequences from Lagkor Co (also known as Gaize lake, Zheng and Sun 2013).

Triploid samples (*Ap3n*), whose assignation is based on flow cytometry and nuclear genotype, were found to be nested within *Ap2n-kaz* (Fig. 5). This suggests that triploids are maternally derived from this diploid lineage. *Ap3n* were characterized by a very low mitochondrial diversity (Table 2) despite their large geographical distribution (Madagascar, Turkey, Albania). When including sequences from other studies (retrieved from NCBI), we found triploids with sequences identical to those of the closely related *Ap2n-kaz* haplotypes and we observed diploids with sequences identical to those of *Ap3n* haplotypes (Fig. 5).

Among sexuals, *Aurm* had a larger haplotypic diversity than *Akaz* and *Atib* from Lagkor Co, which is likely due to a larger sample size, which increases the likelihood of sampling rare alleles (Fig. 5, Table 2).

A second network consisted of one *Atib* sequence from Lagkor Co (AtibLAG2) and sequences from other sexual populations (Haiyan Lake, Jingyu Lake, Nima, Yangnapengco, Qi Xiang Cuo, etc.) from the Qinghai-Tibet plateau, that we refer to as mt-tib2 (Fig. 5). This haplogroup differed by more than 35 mutation steps from mt-tib1 and segregates at a frequency of ~3% in Lagkor Co (1 over 34 *Atib* sequences). No sequence from the mt-tib1 haplogroup was found in populations from Haiyan and Jingyu Lakes. Only a few sequences per population were available from GenBank, so making general conclusions is not possible.

The third network included sequences from *Ap4n*, *Ap5n* and *Asin*. We found that some *Ap4n* and *Ap5n* samples shared the same mitochondrial haplotype (Fig. 5). We found low haplotype diversity among *Ap4n* and *Ap5n* haplotypes (in contrast to Asem et al. 2016; Table 2). The diversity found in *Ap4n* and *Ap5n* by Asem *et al.* (2016) was due to 12 sequences, which were likely to be numts or chimeras according to our analysis. *Ap4n* and *Ap5n* were closest to a haplotype from an *Asin* sample from Lake Dus-Khol (aka Lake Svatikovo, East Siberia, Russia; Naganawa and Mura 2017), which was found to be chimeric between the *Ap4n* mitochondrial haplotype and an *Ap5n* numt (Table S5). This suggests that *Asin* from Lake Dus-Khol, *Ap4n* and *Ap5n* share the same mitochondrial haplotype and at least one numt. Hence, *Ap4n* might have originated in East Siberia from an *Asin* mother. Mitochondrial diversity levels in the *Asin* population from Xiechi Lake (AsinXIE and XIE haplotypes on Fig. 5) were similar to those observed in other sexual species.

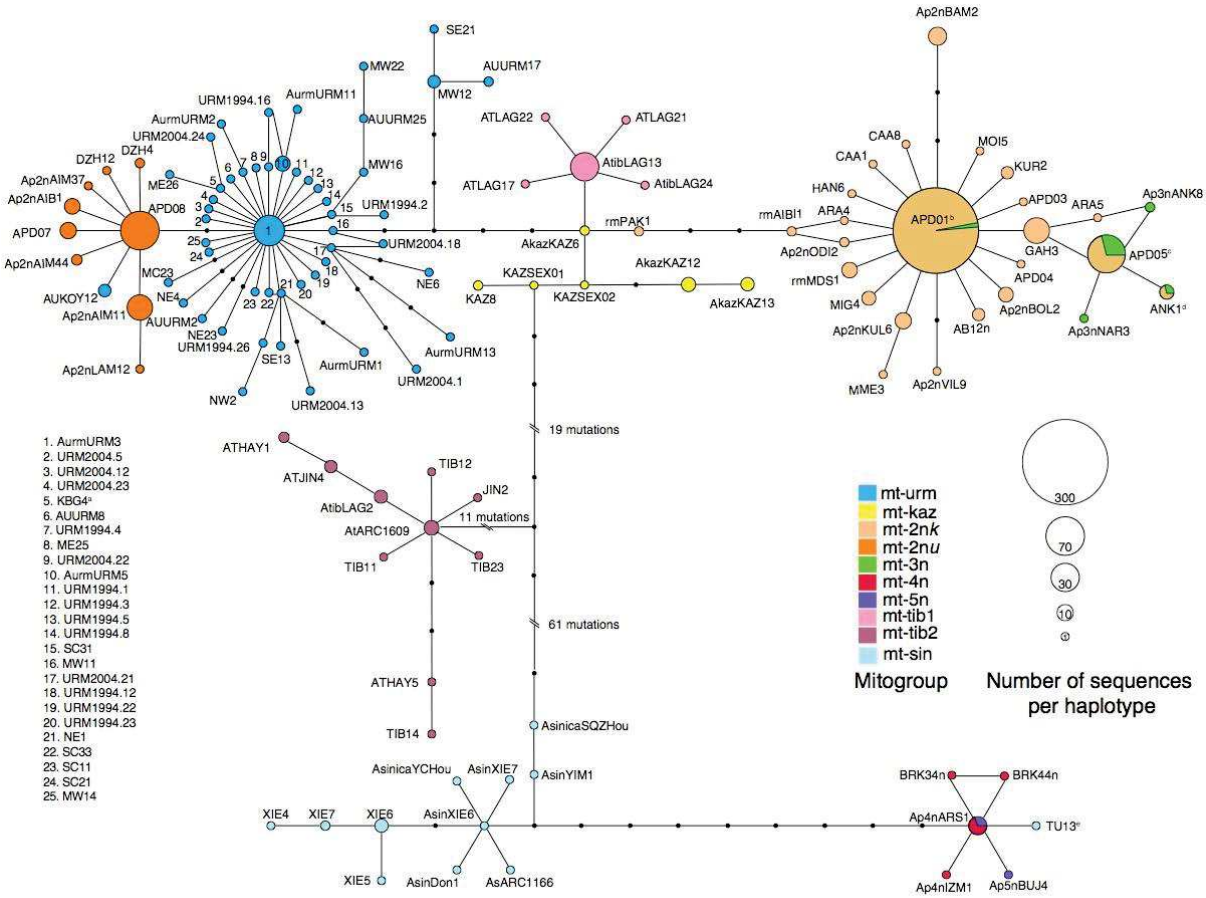


Figure 5. Statistical parsimony network of mitochondrial haplotypes from diploid mt-2n*k*, diploid mt-2n*u*, triploid mt-3n, tetraploid mt-4n and pentaploid mt-5n asexual lineages and related sexual species (mt-urm: *A. urmiana*, mt-sin: *A. sinica*, mt-kaz: *A.* sp Kaz, and mt-tib: *A. tibetiana*). Haplotype including samples with different ploidies are represented with pie charts. Circle diameter is proportional to the relative haplotype frequency among the 850 sequences that were neither numts nor chimeras. Connecting lines indicate single substitutions and small black circles represent putative missing haplotypes. Haplotype codes correspond to those reported in Table S5.

[a]The KBG4 sequence of a cyst from Kara-Bogaz-Gol (Turkmenistan) was molecularly assigned to *Aurm* by Eimanifar et al. 2014.
[b]Sequences from five *Ap3n* individuals (based on cytology; Asem et al. 2016) from Aibi Lake (China) had an APD01 *Ap2n-kaz* haplotype.
[c]Sequences from five *Ap2n* individuals (based on cytology; Asem et al. 2016) from Akkikol Lake (China) had an ANK1 *Ap3n* haplotype.
[d]Sequences from 52 individuals (based on morphology; Muñoz et al. 2010, Maccari et al. 2013a, Eimanifar et al. 2014, Eimanifar et al. 2015) had the same APD05 haplotype as *Ap3n* individuals in our dataset.
[e]The TU13 *Asin* sequences from Siberia was found to be chimeric between the *Ap4nARS1* mitochondrial haplotype and an *Ap5n* numt, it was included to illustrate that *Ap4n* and *Ap5n* likely originated from Siberian *Asin*

	Aurm	*Ap2n-urm*	*Ap2n-kaz*	*Ap3n*	*Akaz*	*Atib* (LAG)	*Atib* (others)*	*Asin*	*Ap4n*	*Ap5n*
Aurm	0.007	.	.	.	.	.	.	.	.	.
Ap2n-urm	0.009	0.004	.	.	.	.	.	.	.	.
Ap2n-kaz	0.026	0.029	0.005	.	.	.	.	.	.	.
Ap3n	0.030	0.033	0.007	0.003	.	.	.	.	.	.
Akaz	0.018	0.021	0.016	0.021	0.005	.	.	.	.	.
Atib (LAG)	0.017	0.020	0.015	0.015	0.007	0.003	.	.	.	.
Atib (others)*	0.075	0.080	0.081	0.083	0.073	0.074	0.006	.	.	.
Asin	0.184	0.188	0.175	0.175	0.180	0.175	0.167	0.006	.	.
Ap4n	0.206	0.210	0.196	0.196	0.202	0.197	0.187	0.021	0.002	.
Ap5n	0.206	0.210	0.197	0.196	0.202	0.197	0.184	0.021	0.002	0.002

Table 2. Mitochondrial (Kimura-2-parameter on COI sequences) genetic distances between and within mitogroups of sexual species and asexual lineages. See Table S5 for the Accession Numbers of the 123 unique haplotype sequences.
* Distances based on *Atib* sequences from other populations than Lagkor Co (LAG) were computed separately.

Microsatellite genotyping

Genetic distances within and among asexual and sexual lineages are represented in Fig. 6 and Table 3. The proportion of total variability explained by the first, second and third axes of the PCoA were to 28.6%, 25.9% and 19.2% respectively (Fig. 6). We observed a larger genetic diversity within both *Ap2n-kaz* and *Ap2n-urm*, than within *Ap3n*, *Ap4n,* or *Ap5n* polyploids. Sharing of multilocus genotypes among populations was rare except for some geographically close populations (*Ap2n*: VIL/AIM, YIN/DON, *Ap5n*: IZM/ATA/CIT). Consistent with mitochondrial data, *Ap2n-kaz* were more closely related with *Akaz* than with *Aurm*. Surprisingly, *Ap2n-urm* were also more closely related to *Akaz* than to *Aurm*. Compared to *Ap2n-kaz* and *Ap2n-urm*, *Ap3n* were more closely related to *Aurm*. Compared to *Ap2n-kaz*, *Ap2n-urm* and *Ap3n*, *Ap4n* and *Ap5n* were more closely related to *Asin*, consistent with mitochondrial data. Variation in microsatellite allele size was smaller in *Akaz* and *Asin* (YIM population) than in *Aurm* and *Atib*, which suggests that the later two populations might be admixed (Fig. S3, sup. mat. 6).

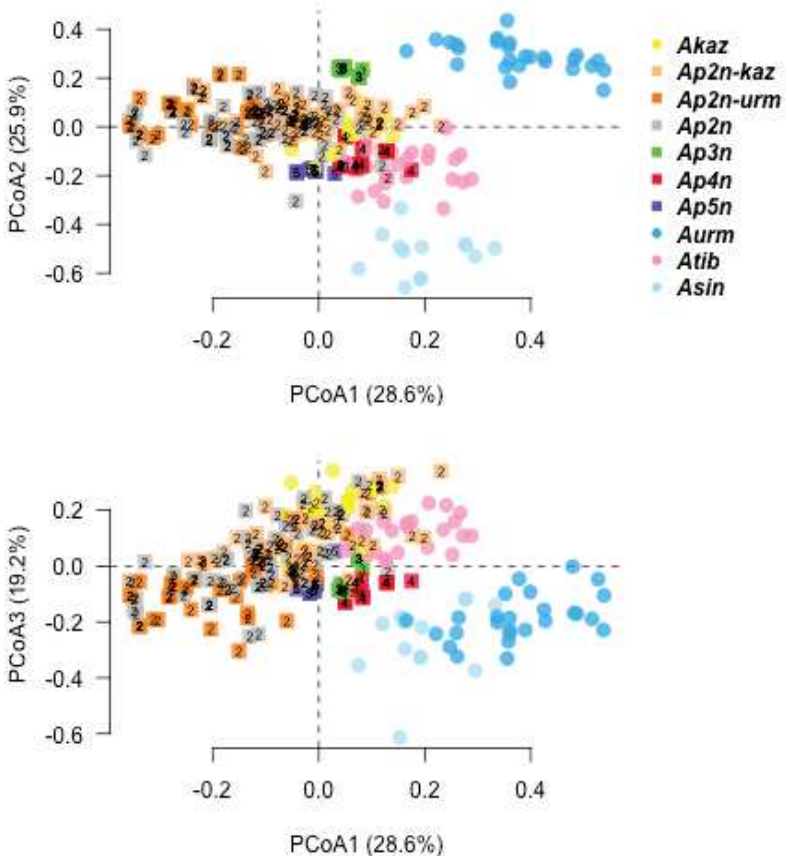


Figure 6. Principal coordinate analyses of asexual lineages and sexual species. The first three principal coordinate axes are shown, with the percentage of variation represented by each axis given between parentheses. Sexual and asexual taxa are represented with filled circles and filled squared, respectively. Numbers within filled squares represent the ploidy level. *Ap2n* individuals with unknown mitochondrial haplotypes are represented in grey

	Aurm	Ap2n-urm	Ap2n-kaz	Ap3n	Akaz	Atib	Asin	Ap4n	Ap5n
Aurm	0.40	.	.	.	.	.	.	.	.
Ap2n-urm	0.71	0.30	.	.	.	.	.	.	.
Ap2n-kaz	0.67	0.35	0.30	.	.	.	.	.	.
Ap3n	0.52	0.42	0.41	0.06	.	.	.	.	.
Akaz	0.69	0.43	0.34	0.47	0.26	.	.	.	.
Atib	0.71	0.53	0.45	0.51	0.41	0.27	.	.	.
Asin	0.90	0.75	0.75	0.83	0.76	0.58	0.18	.	.
Ap4n	0.62	0.41	0.37	0.47	0.40	0.36	0.50	0.12	.
Ap5n	0.70	0.38	0.37	0.49	0.41	0.41	0.47	0.23	0.04

Table 3. Nuclear genetic distances between and within mitogroups of sexual species and asexual lineages. Lynch distance was computed based on the 9 microsatellite markers from Nougué et al (2015a). The 113 Ap2n individuals with unknown mitochondrial haplotypes were excluded for the computation.

Monophyly of Ap2n mitochondrial clades

The NJ tree based on our genetic distance for automicts, which accounts for recombination and null alleles, is represented in Fig. 7. Individuals that cluster together in this tree often had the same mitochondrial haplotype group (*Ap2n-kaz* or *Ap2n-urm*). In other words, nuclear genetic distance between pairs of individuals with mt-2n*k* and mt-2n*u* mitochondrial haplotypes was larger, on average, than that between pairs of individuals with the same mitochondrial haplotype group (Fig. 7; distance d(*Ap2n-kaz-Ap2n-urm*)=9.23; d(*Ap2n-urm-Ap2n-urm*)=8.51; d(*Ap2n-kaz-Ap2n-kaz*)=8.22). This association between mitochondrial haplotype and nuclear background was highly significant: among 10000 randomizations of the dataset, the estimated genetic distance between pairs of individuals with the same mitochondrial haplotype was never lower than observed genetic distances of 8.51 or 8.22 (i.e. $P<0.0001$). We identified 13 individuals with a potential mismatch between mitochondrial haplotype and nuclear background (arrows in Fig. 7). Importantly, the mitochondrial haplotypes of these individuals were neither unique nor shared with sexual species (*Aurm*, *Akaz*), but rather corresponded to major *Ap2n* haplotypes. Moreover, based on the bootstrap analysis, the total length of the NJ tree was not significantly lower than of a tree with forced monophyly of *Ap2n-urm* and *Ap2n-kaz* (i.e., without mito-nuclear discordance, P=0.295). Hence, we could not rule out the hypothesis that the mito-nuclear discordance patterns occurred by chance.

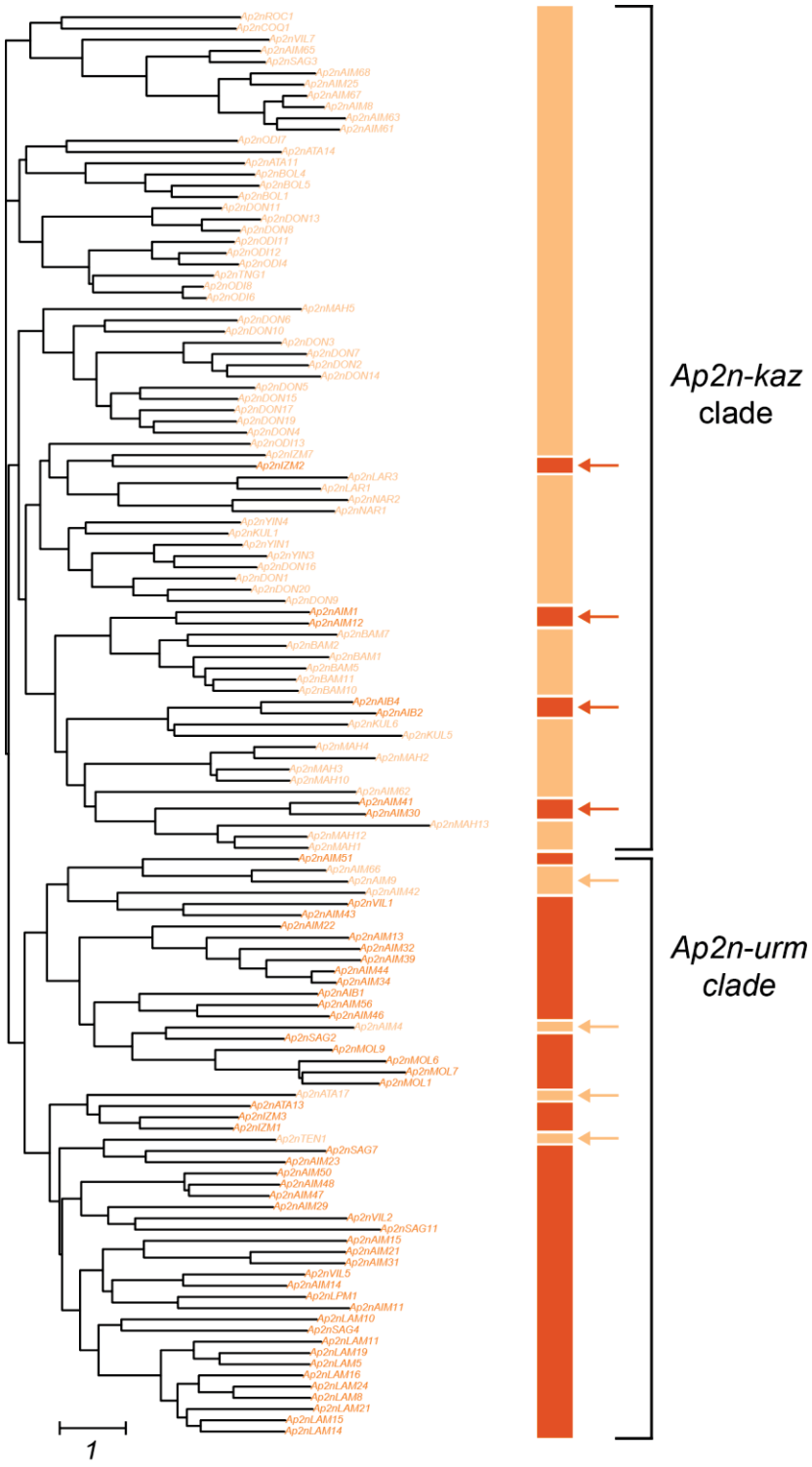
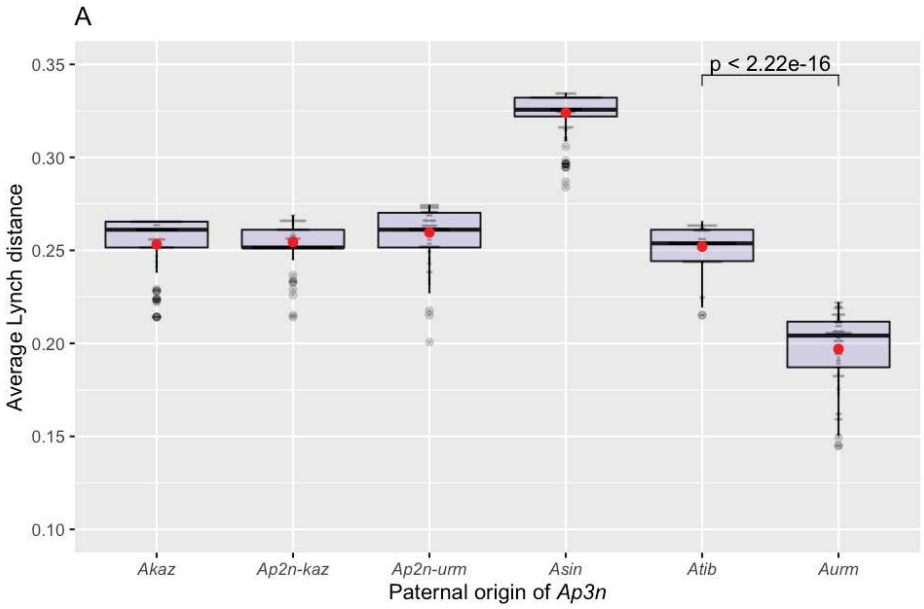
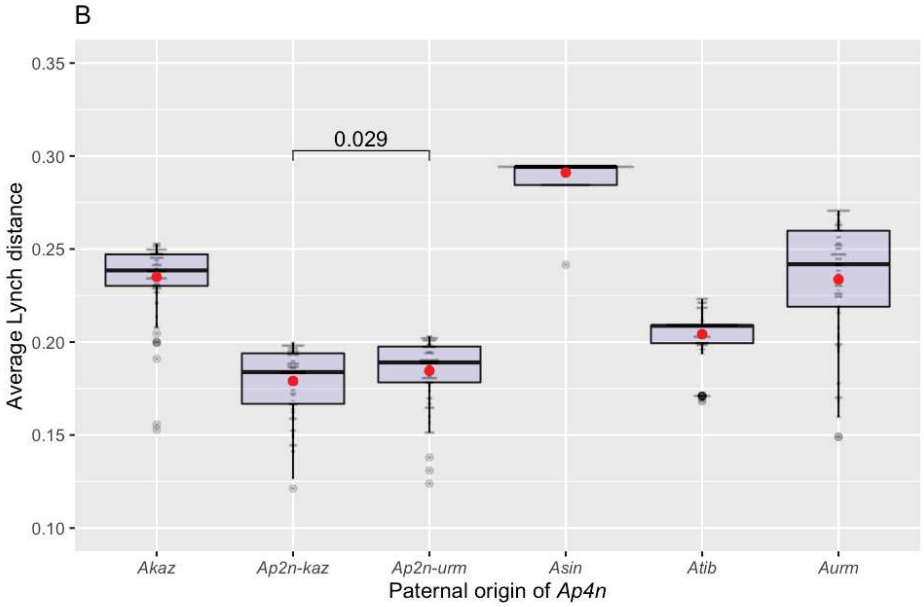


Figure 7. Neighbor-joining tree between pairs of *Ap2n* individuals based on our nuclear genetic distance for automicts. Individual labels are colored based on mitochondrial haplotypes. Square brackets indicate the two asexual clades *Ap2n-urm* and *Ap2n-kaz* based on the match between nuclear and mitochondrial data. Horizontal arrows indicate the few individuals or groups of individuals with mito-nuclear discordances. The association between mitochondrial haplotype and nuclear background is highly significant. Bootstrap values could not be computed due to a low number of markers (note this topology does not fit the data significantly better than the topology where *Ap2n-urm* and *Ap2n-kaz* form monophyletic groups, see Results for details).


A
p < 2.22e-16
Average Lynch distance
0.35
0.30
0.25
0.20
0.15
0.10
Akaz
Ap2n-kaz
Ap2n-urm
Asin
Atib
Aurm
Paternal origin of Ap3n


B
0.029
Average Lynch distance
0.35
0.30
0.25
0.20
0.15
0.10
Akaz
Ap2n-kaz
Ap2n-urm
Asin
Atib
Aurm
Paternal origin of Ap4n

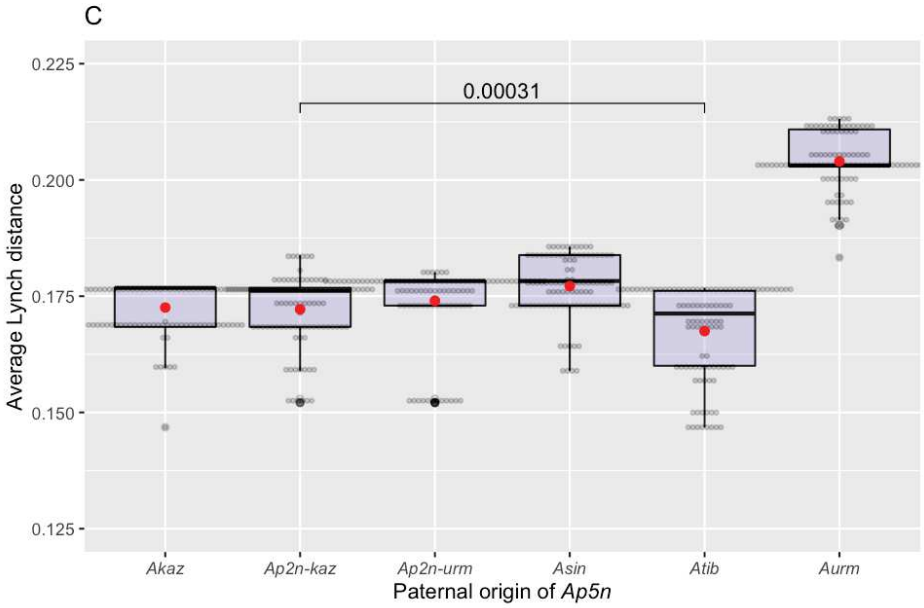

C
0.00031
Average Lynch distance
0.225
0.200
0.175
0.150
0.125
Akaz
Ap2n-kaz
Ap2n-urm
Asin
Atib
Aurm
Paternal origin of Ap5n

Figure 8. Comparison of the different scenarios for the paternal origin of (A) *Ap3n*, (B) *Ap4n* and (C) *Ap5n*. For each ploidy level and each paternal origin, we estimated Lynch genetic distance across 9 microsatellite loci between 10,000 simulated hybrids and real individuals of our dataset with the corresponding ploidy level (*Ap3n*: n=16, *Ap4n*: n=23, *Ap5n*: n=15). The horizontal line and the red dot respectively represent the median and the mean of the 100 synthetic hybrids with the smallest genetic distance. See main text for details.

Evolutionary origin of Ap2n-kaz and Ap2n-urm

To compare the different scenarios for the evolutionary origin of Ap2n-kaz and Ap2n-urm, we used the average pairwise genetic distance between Ap2n-kaz, Ap2n-urm, Akaz, Aurm and Asin (Table 3). The scenario with the emergence of Ap2n lineages through one hybridization and one backcross event (Fig. 3D) had the highest support (Table S2). According to this scenario, Ap2n-urm first arose through a hybridization event between an Akaz male and an Aunk female, carrying a mt-urm. Ap2n-kaz arose through a backcross between an Akaz female and an Ap2n-urm rare male. All other scenarios could be ruled out (ΔAICc > 2.5, Table S2), including scenarios involving a spontaneous origin, two independent hybrid origins, or a different order of events (e.g. *Ap2n-kaz* being the F1 and *Ap2n-urm* the backcross).

Evolutionary origin of Ap3n, Ap4n and Ap5n

For *Ap3n*, average genetic distances inferred from the scenario involving an *Aurm* paternal origin were significantly lower than other genetic distances (P<2e-16, Table S3, Fig. 8A). For *Ap4n*, average genetic distances inferred from the scenario involving an *Ap2n-kaz* paternal origin was slightly better than that with an *Ap2n-urm* paternal origin (P=0.029, Table S3, Fig. 8B). For *Ap5n*, average genetic distances inferred from the scenario involving an *Atib* paternal origin were significantly lower than genetic distances from other scenarios (P<0.0003, Table S3, Fig. 8C).

Discussion

General implications for the study of the origin and evolution of asexuals

Our study proposes new ways to investigate the origin of asexual lineages. We try to address the five hurdles that frequently face such studies, using approaches that can likely be used in other asexual taxa than *A. parthenogenetica*. First, when considering potential hybrid or contagious origins of asexuality, our study showcases that the genealogy of asexual groups might include several origins and/or several nested hybridization events. Hence, the origin of asexuality gene(s) should be clearly distinguished from the origin of asexuals clades that carry these genes. Indeed, we find a single origin of asexuality gene(s) in *A. parthenogenetica*, followed by several nested hybridization with different sexual species. Second, heterogeneity in LOH within and across chromosomes should be carefully considered in automictic asexuals. In particular, with central fusion automicts, which are frequent, heterozygosity can be high close to the centromere, but decreases further away from the centromere. Thus, LOH creates a heterogeneity in coalescence times both among markers along each chromosome and between asexuality gene(s) and the rest of the genome. The methods developed in this study should

improve genetic distance estimates between automictic asexual lineages by explicitly accounting for this heterogeneity. In contrast to a widespread hypothesis regarding the evolution of asexuality in ZW lineages (Engelstädter 2008), we show that this sex-determination system does not represent a major obstacle to the evolution of asexuality, provided that recombination is low enough (or quickly evolved to low levels; Boyer et al. 2021). In addition, in these systems, the production of rare ZZ males may allow for the production of new asexual lineages through contagious asexuality. Third, our results support the hypothesis that polyploidy is often a consequence rather than cause of asexuality (Neiman et al. 2014). In addition, our findings suggest that asexual groups with odd ploidy levels (3n, 5n) can reproduce through automixis. This implies that, despite the uneven number of chromosomes, pairing between some homologous chromosomes does occur, but that this pairing does not prevent the formation of an unreduced egg. Fourth, the occurence of rare sex can greatly impact the genomic evolution of asexual lineages by increasing their diversity. The genomic consequences of these events depend on whether they occur within an asexual lineage, between different asexual lineages or between asexual lineages and related sexual species. Rare sex events within an asexual lineage can only be detected when there is some genetic diversity within asexual populations. Importantly, selection might favor a particular asexual lineage within a population and may locally erase the genetic footprints of rare sex events, increasing the difficulty to detect them. Rare sex between different asexual lineages or between asexual lineages and related sexual species, can be detected through admixture tests or through cyto-nuclear discordances. The new method developed here to test for cyto-nuclear discordances assesses whether groups defined based on mitochondria are monophyletic at the nuclear level. This method should be widely applicable in other systems. Fifth, although our sampling was as exhaustive as possible, our results point to particular geographic locations towards which sampling efforts of sexual relatives should be directed in the future in order to refine the estimates of the age for the origin of asexuality in *Artemia* (see below).

Origins of asexuality in Artemia: overview

The most parsimonious scenario to summarize our main results is presented in Fig. 9 and indicates that that both diploid and polyploid asexual *Artemia* harbor nuclear genomes that are admixed between the nuclear genomes of *Akaz* and one or several of the other sexual species. Additional sampling and more genetic data may reveal that some specific crosses are actually more complex and/or that other scenarios need to be statistically compared, e.g. scenarios involving serial backcrosses.

The relatively low diversity in mitochondrial haplotypes suggests that contagious asexuality is present but rare in *Artemia* (or leads most of the time to unfit hybrids). Despite the relatively frequent occurrence of rare males in *Ap2n* (Maccari et al. 2013*b*), our data suggest that contagious asexuality via rare males has occurred twice, leading to the *Ap2n-kaz* and *Ap4n* lineages with no evidence for further successful events of contagious asexuality. Indeed, the diversity of mitochondrial haplotypes within each of the main asexual clades is limited and these haplotypes are not intermingled with haplotypes found in sexual lineages. Mutation (rather than backcrossing with unsampled sexual populations) thus seems the most

parsimonious explanation for the observed haplotype diversity within each main lineage of asexual *Artemia.*

In addition, each mitochondrial haplotype is only a few mutational steps away from the mitochondria of the different sexual species (*Akaz*, *Aurm*, *Asin*), which suggests that all extant asexual lineages are more recent than previously thought (less than 80,000 years old compared to more than 3 and 0.84 Mya; Perez et al. 1994; Baxevanis et al. 2006; Eimanifar et al. 2015). This estimate represents the age of the most recent common ancestor of all these lineages (i.e. the oldest extant lineage *Ap2n-urm*, from which all other lineages likely arose through subsequent hybridization events). Importantly, the loci that cause asexuality may be much older than our rough estimate of the age of their common ancestor. Indeed, we cannot exclude that all original asexual lineages went extinct, leaving only recently derived ones. This issue can only be solved by comparing the divergence of the region(s) directly associated with asexuality with that of the rest of the genome (Tucker et al. 2013). We discuss the implications of these findings for *A. parthenogenetica* asexuality before discussing each of these events in details.

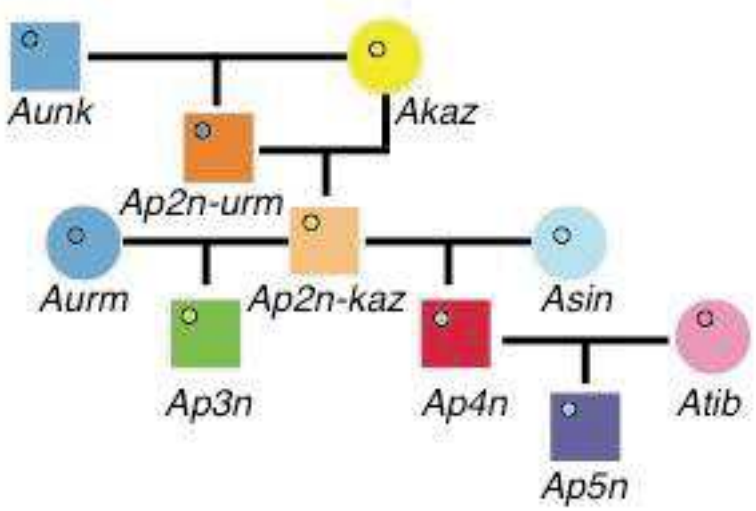


Figure 9. Most likely scenario for the origin of diploids (*Ap2n-kaz* and *Ap2n-urm*) and polyploid (*Ap3n*, *Ap4n*, *Ap5n*) asexual *Artemia* lineages. Briefly, *Ap2n-urm* likely arose through hybridization between a *Aunk* female from an unknown species or population (carrying a mitochondrion close to mt-*urm*) and an *Akaz* male (note that *Ap2n-urm* could also derive from a series of backcrosses with the parental species, and there is currently no data to sort this out without knowing this unknown species/population). *Ap2n-kaz* likely arose as a backcross between an *Ap2n-urm* rare male and an *Akaz* female. *Ap3n* likely arose through the fertilization of *Ap2n-kaz* female by an *Aurm* male. *Ap4n* likely arose through the fertilization of *Asin* female by an *Ap2n-kaz* male, followed by an endoduplication in this diploid offspring or one of its descendants. Finally, *Ap5n* likely resulted from the fertilization of an *Ap4n* female by an *Atib* male. Sexual and asexual taxa are represented by filled circles and filled squares, repectively. The filled circle within each symbol represents the mitochondrial haplotype, which shows the maternal origin of each asexual lineage.

Diploids and polyploids may all be automicts

Our findings strongly suggest that all asexual *Artemia* probably derive from a single original hybrid ancestor. The same asexuality genes are therefore very probably shared among

all, diploid and polyploid *A. parthenogenetica*. We found that the apparent polyphyly of the group (Baxevanis et al. 2006) results from a history of nested crosses between asexuals and sexual relatives.

Automixis in *Ap2n* has been a major source of confusion throughout one century of cytological observations. Recent genetic data have clarified this debate, and support the conclusion that *Ap2n* reproduce by central fusion automixis (Nougué et al. 2015*b*; Boyer et al. 2021). In contrast most of the literature on polyploids is not controversial and claim that they are apomictic (Brauer 1894; Artom 1931; Barigozzi 1944, 1974). Reproduction of polyploids via an automictic process that would involved recombination has been ruled out based on three types of observations. First, polyploids do not seem to produce rare males (Goldschmidt 1952; Metalli and Ballardin 1970; Chang et al. 2017). Second, each ploidy level shows high heterozygosity, but little clonal diversity (Abreu-Grobois and Beardmore 1982; Zhang and King 1992; Maniatsi et al. 2011) . Third, cytological observations have been claimed to refute automixis (due to the failure to observe meiosis, the authors inferred that sister chromatids separate through a mitosis; (Barigozzi 1974)).

Asexuality is likely to have the same genetic determinism in diploid and polyploid asexual lineages, cince they share a common asexual ancestor. If true, this means that the distinction between *Ap2n* automicts and polyploid apomicts may be erroneous. Beyond shared ancestry, several lines of evidence support that polyploids may well, in fact, have the same reproductive mode as diploids.

First, the cytological evidence is not as clear-cut as often reported. This confusion relies on different definitions of automixis by cytologists and geneticists. The former use the fusion of meiotic products as a criterion, while the latter use the genetic consequences of modified meiosis as a criterion (Asher 1970; Nougué et al. 2015*b*; Svendsen et al. 2015). Most cytologists did not consider the possibility that automixis could occur through the abortion of one of the two meiotic steps. In fact, this aborted meiosis has been described by Goldschmidt (1952) in *Ap3n* and *Ap5n* polyploids. She did not observe any fusion of meiotic products, but a brief synapsis and the production of a polar body. Furthermore, she observed that the number of elements (bivalent or univalent) drops during diakinesis and increases afterwards to reach univalent number at metaphase. These observations refute the occurrence of apomixis. They show that meiosis I is aborted at the end of prophase I (with bivalents being separated within the oocyte, but the resulting univalents not being distributed to different different daughter cells and instead re-aligned at the equatorial plate) and jumps directly to metaphase II. Note that in *Ap3n* and *Ap5n*, this brief pairing of homologues can easily occur despite the odd number of chromosomes (one chromosome simply stays unpaired during prophase I), as observed in other animal species (e.g. Christiansen and Reyer 2009). This meiosis modification corresponds to the reproductive mode of *Ap2n* (Nougué et al. 2015*b*; Boyer et al. 2021) and exactly matches “central fusion automixis” as defined by geneticists. According to Asher (1970), the suppression (or abortion) of meiosis I is genetically equivalent to automixis with central fusion as defined cytologically (i.e., where fusion actually occurs), while supression or abortion of meiosis II is cytologically equivalent to automixis with terminal fusion. Other authors refer to the suppression (or abortion) of one of the meiotic divisions as "meiotic apomixis" (e.g. Archetti

2010). However, this term refers both to the suppression of meiosis I and the suppression of meiosis II, whose outcome differ genetically when recombination is present. For this reason, we prefer the term "central fusion automixis" in the large, genetic sense, noting that it includes meiotic apomixis with supression of meiosis I.

Second, if polyploids are automicts, they may occasionally lose heterozygosity because of recombination, as observed in *Ap2n* lineages (Boyer et al. 2021). However, there are good reasons to expect that, in polyploids, homeologous chromosomes (i.e. pairs of chromosomes derived from the two parental species of allopolyploids; Glover et al. 2016) are more divergent than non-homeologous chromosmes (i.e. pairs of chromosomes derived from only one of the two parental species) and that they will pair much less frequently, which likely results in almost no LOH between homeologs. This also likely limits recombination-generated genetic variation in polyploids. In addition, it might also drastically reduce the rate of rare male production in polyploids. In ZZW triploids, Z chromosomes are likely to preferentially pair and recombine (leaving the W unpaired), before all chromosomes realign on the equatorial plate and meiosis II starts. Similarly, in ZZWW tetraploids or ZZZWW pentaploids, W non-homeologous chromosomes are likely to preferentially pair together (and even if Z and W homoeologous chromosomes would pair, two subsequent LOH would be require to produce ZZZZ or ZZZZZ males). Furthermore, as in all polyploids, there is probably a strong selection pressure to reduce the number of cross overs to avoid interlocking cross over events among different pairs of chromatids (Lenormand et al. 2016), which may reduce recombination in polyploids and reinforce this apparent apomictic-like reproduction. As we often observe only two alleles in polyploids, recombination rates between non-homeologous chromosomes might be small but greater than the mutation rate. Hence, the absence of rare males in polyploids is not necessarily an argument against central fusion automixis. Polyploids *A. parthenogenetica* may therefore not be apomicts. They may reproduce by central fusion automixis, but polyploidy and the absence of pairing between homoeologous chromosomes would make this reproductive mode genetically very close to apomixis. This interpretation is open to further tests, but it would explain why polyploids have apomictic-like reproduction, while being derived from automictic lineages as suggested by our study.

Hybrid origin of Ap2n-urm and contagious origin of Ap2n-kaz via an Ap2n-urm rare male

Our best scenario is that *Ap2n-urm* likely arose through hybridization between a female from and *Aunk*, an unknown sexual species with a mitochondrial haplotype close to that of *Aurm.*-It is quite likely that *Aunk* would be related to *Akaz* and *Aurm*, and therefore present in Crimea or in Central Asia, if not extinct. Interestingly, one sexual population from Crimea could be a good candidate. It is currently described as an *Aurm* population (Abatzopoulos et al. 2009; Maccari et al. 2013*a*), but, unexpectedly, its mitochondrial haplotype is closer to that of *Ap2n-urm* than to that of *Aurm* (1 versus 3 mutational steps, respectively, in the network on Fig. 5). Unfortunately, we could not obtain this sample for this study.

According to our best model, the second *Ap2n* group originated through a backcross between a rare male of this first lineage and an *Akaz* female. The genome size of *Ap2n-kaz* is consistent with this scenario. The backcross might have occurred almost immediately after the first occurrence of *Ap2n-urm*, as rare males may be produced at a higher rate in young asexual

lineages (Boyer et al. 2021) and as both crosses rely on the presence of *Akaz*, which likely has a very limited geographical range, at least today. All *Ap2n* lineages seem to branch from these two major groups, and we did not find firm evidence of any further event of contagious asexuality. Such secondary contagion through rare males would indeed capture new mitochondrial haplotypes from sexual species, which would be easily detected. The rmPAK1 sequence in Fig. 4 from Maccari et al. (2013*b*) might be a rare candidate, but without nuclear data, we cannot further investigate this possibility. Overall, the low diversity among *Ap2n* haplotypes suggests that secondary contagion is probably very rare. The restricted geographical distributions of sexuals in Central Asia and around the Crimean peninsula and their spatial or temporal segregation from asexuals (Mura and Nagorskaya 2005; Shadrin and Anufriieva 2012) might indeed limit the chances of contagion.

Recent experimental evidence (Boyer et al. 2021) has shown that *Ap2n-kaz* may occasionally reproduce sexually. Reproduction between *Ap2n-kaz* and *Ap2n-urm* could perhaps explain some mito-nuclear discordance observed in our data (Fig 7). Alternatively, *Ap2n-kaz* and *Ap2n-urm* might form monophyletic clades and these discordances might be due to our limited number of microsatellite markers. Finally, sexual reproduction might preferentially occur within *Ap2n-kaz* and *Ap2n-urm*, as most populations are composed of individuals from a single clade (Fig. 2). Mitochondrial divergence is low within *Ap2n-kaz* and *Ap2n-urm*, so that we could not test and detect mito-nuclear discordance within each clade with our method. Additional mitochondrial and nuclear data will be needed to better test these different hypotheses.

Origin by transmission of Ap3n via an Ap2n-kaz female

All *Ap3n* branch within the *Ap2n-kaz* lineage and show very limited genetic diversity at both the mitochondrial and nuclear levels, despite having a worldwide distribution, from Madagascar to the Mediterranean. This result is in line with that of Maniatsi et al (2011) that found only two different clones across 10 *Ap3n* populations. It contrasts with the large allozyme diversity reported within and among three *Ap3n* populations by Abreu-Grobois and Beardmore (1982). Our samples (NAR, IZM, ANK) belong to a single *Ap3n* lineage that likely arose through a cross between an *Ap2n-kaz* female and an *Aurm* male. When considering samples from previously published studies, we found several mitochondrial haplotypes that are shared between *Ap3n* and *Ap2n-kaz* (Fig. 5 and sup. mat. 9). This might have been caused by improper assignation of ploidy levels of these samples or from the independent origin of several, highly related, but distinct, *Ap3n* lineages. Further sampling, genotyping and careful check of ploidy levels should resolve the matter.

Contagious origin of Ap4n via an Ap2n-kaz rare male

We found that *Ap4n* show very limited genetic diversity, consistent with previous studies (Abreu-Grobois and Beardmore 1982; Maniatsi et al. 2011). In the most likely scenario (Fig. 8), *Ap4n* resulted from a standard hybrid cross between reduced haploid gametes from an *Asin* mother and an *Ap2n-kaz* rare male (other scenarios involving unreduced *Asin* gametes are less likely as they require the combination of multiple rare events). Tetraploidy would have occurred through endoduplication in one of these hybrids (or within its descendants). This scenario is

consistent with the observation that most microsatellite loci have only two alleles in those individuals. The genome size of *Ap4n* is 7% larger than that of *Asin* and *Ap2n-kaz* combined, which suggest an increase in genome size following polyploidization. Multiple origins of *Ap4n* is unlikely, since we would expect to observe the capture of several mitochondrial haplotypes provided the mitochondrial diversity observed in *Asin*. Historically, the hybridization event probably took place in East Siberia as *Ap4n* carry a mitochondrion that is only found in an *Asin* sample from this area (see sup. mat. 9 for a discussion of mitochondrial diversity within *Asin*). Finally, all the *Ap4n* lineages observed in the Mediterranean area would be a subsample of few successful lineages, which reached a worldwide distribution.

Origin by transmission of Ap5n via an Ap4n female

Based on mitochondrial data (Fig. 5), *Ap5n* are derived from an *Ap4n* unreduced gamete fertilized by a male of unknown origin. The microsatellite data agree with this scenario as *Ap5n* genotype are very close to *Ap4n* and suggests an *Atib* paternal origin. However, based on flow cytometry data, the genome size of a hybrid resulting from the fertilization of an *Ap4n* unreduced egg by a reduced *Atib* sperm would be 10.0% larger than the observed genome size of *Ap5n.* This discrepancy may be due to secondary reduction in *Ap5n* genome size or to an incorrect paternal assignment. The second and third best candidates would be *Ap2n-kaz* and *Akaz* respectively and the genome size of hybrids resulting from these crosses would better match that of *Ap5n* (2.4% and 3.1% larger, respectively). Additional nuclear data will indicate whether *Atib* paternal origin is the correct inference.

Implications for the study of the origin of asexual lineages

A more robust dating of the origin of asexuality in *Artemia* and establishing firm scenarios for the origin of the different asexual lineages requires the different sexual species to be more extensively sampled and characterized. *Ap2n-urm* is clearly more closely related to the sexual Crimean population from Lake Koyashskoe than to *Aurm* based on both mitochondrial (Fig. 3) and nuclear data (Abatzopoulos et al. 2009; Sainz-Escudero et al. 2021). This suggests that the Lake Koyashskoe population might be *Aunk*. Additional nuclear genetic data from this and other populations (e.g. Kara-Bogaz-Gol) is required to confirm this hypothesis. Similarly, mitochondrial and nuclear genetic data from sexual populations from the Altai region currently described as *Aurm* (Mura and Nagorskaya 2005; Shadrin and Anufriieva 2012) could help decipher whether one of these populations actually correspond to the *Akaz* population involved in the paternal origin of *Ap2n-urm* and/or the maternal origin of *Ap2n-kaz*. In addition, studying *Asin* populations from Siberia (which are very divergent from *Asin* populations from China; Fig. 5) should shed a light on the maternal origin of *Ap4n*. Finally, studying the diversity of *Atib* populations outside the reference population used to describe this species (Lagkor Co) could help assess the paternal origin of *Ap5n*. Indeed, we confirm that most individuals from Lagkor Co have mt-tib1 mitochondrial haplotypes very close to mt-*kaz* (Fig. 5), whereas all other sexual populations from the Qinghai-Tibet plateau assigned as *Atib* only carry the very divergent mt-tib2 haplotype (as previously found in Maccari et al. (2013*a*)). Our microsatellite data suggests that the Lagkor Co population might be admixed (Fig. S3). Hence, it is possible that the Lagkor Co population has been introgressed by a mitochondrial haplotype closely

related to mt-*kaz* (Maccari et al. 2013*a*). Alternatively, all other populations may represent another undescribed sexual species that can hybridize with *Atib* individuals, resulting in a low frequency of the divergent mt-tib2 haplotype in Lagkor Co. Similarly, our microsatellite data suggest that Aurm might be admixed between two divergent populations (Fig. S3), which is consistent with the observation of two very divergent ITS1 haplotypes that segregate within Aurm (Eimanifar et al. 2014; Sainz-Escudero et al. 2021). Additional genetic data from populations from the Qinghai-Tibet plateau and from Crimea will be required to disentangle among different admixture scenarios and resolve potential mito-nuclear discordances in Atib and *Aurm*.

Taxonomic implications

The taxonomy of asexual lineages is often ambiguous, as clearly exemplified by our case study. Provided that multiple hybridization events can give rise to different asexual lineages, phylogenetically-defined monophyly is a poor criterion of the taxonomic description of asexual lineages. Asexuality also prevents the use of species delimitation criteria based on interfertility between different taxa. Facing these difficulties, it may be more biologically relevant to focus on the common origin of asexual lineages and their common reproductive mode. In our case study, it makes sense to collectively refer to *A. parthenogenetica* as a relevant taxonomic unit, as they probably inherited the same asexuality gene(s) from their common ancestor. The drawback of this taxonomic approach, in *Artemia* and other groups, may be that a single name hides the diversity of hybridization events that led to the different lineages. As a consequence, a minimal convenient way to designate these taxa would be to distinguish the major groups derived from these crosses, if they can be distinguished. For instance, in *A. parthenogenetica*, five major groups may summarize the major sex-asex transitions: *Ap2n-kaz*, *Ap2n-urm*, *Ap3n*, *Ap4n* and *Ap5n*. Finally, taxonomic issues, lineage history and age, may also be better resolved by directly identifying and studying the genomic regions associated with asexuality (Sandrock and Vorburger 2011; Tucker et al. 2013; Yagound et al. 2020). As our case study shows, sex-asex transitions are quite different from the idealized view of an apomictic mutant arising in a sexual species. Ultimately, a better characterization and understanding of sex-asex transitions represents a pivotal step to refine our theories for the long-term maintenance of sexual reproduction and the extant distribution of sexual and asexual taxa.

Acknowledgements

We thank Christ Mahieu (Laboratory of Aquaculture & *Artemia* Reference Center), Paco Amat (Instituto de Acuicultura de Torre de la Sal) and Marta Sanchez (University of Sevilla) for providing samples and Emmanuel Douzery (ISEM, University of Montpellier) and Arnaud Estoup (CBGP, INRAE) for advice on phylogenetic and population genetic methods. We thank Marie-Pierre Dubois and the GEMEX molecular platform of CEFE laboratory, and the GENSEQ platform of the Centre Méditerranéen Environnement Biodiversité (LABEX CEMEB, Montpellier, France) for assistance with molecular laboratory work. We also thank Maria Orive and two anonymous reviewers for their useful comments.

Author contributions

NOR, FD and TL conceived the study. FH and GVS provided samples. NOR and RJZ performed flow cytometry measurements with advice from FD. EF, RJZ and FD performed genotyping. NOR, LB, CH and TL reviewed the existing literature. NOR and TL analyzed the data and wrote the manuscript with inputs from the co-authors. TL managed the project and funding.

Data Accessibility Statement

Supplementary materials are available here. The 234 sequences from dataset1 and dataset3 are deposited in GenBank (accession numbers are provided in Table S5). Microsatellite and flow cytometry data, as well as scripts used for data analysis will be archived on Dryad upon acceptance of the manuscript

References

Abatzopoulos, T. J., F. Amat, A. D. Baxevanis, G. Belmonte, F. Hontoria, S. Maniatsi, S. Moscatello, et al. 2009. Updating Geographic Distribution of *Artemia urmiana* Günther, 1890 (Branchiopoda: Anostraca) in Europe: An Integrated and Interdisciplinary Approach. International Review of Hydrobiology 94:560–579.

Abatzopoulos, T. J., C. D. Kastritsis, and C. D. Triantaphyllidis. 1986. A study of karyotypes and heterochromatic associations in *Artemia* , with special reference to two N. Greek populations. Genetica 71:3–10.

Abreu-Grobois, F. 1987. A review of the genetics of *Artemia*. Pages 61–99 *in* P. Sorgeloos, D. A. Bengtson, W. Declair, and B. Jaspers, eds. Artemia research and its applications. vol 1. Universa Press, Wetteren.

Abreu-Grobois, F. A., and J. A. Beardmore. 1982. Genetic differentiation and speciation in the brine shrimp <i>Artemia<\i>. Pages 345–376 *in* B. C, ed. Mechanisms of Speciation. Alan R. Liss, New York.

Abreu-Grobois, F., and J. Beardmore. 2001. The generation of males by diploid parthenogenetic *Artemia* cannot occur in the way Stefani suggested. Page 1 *in* A. Maeda-Martinez, B. Timms, D. Rogers, G. Murugan, and A. Abreu-Grobois, eds. Proceedings of the 4th International Large Branchiopod Symposium. La Paz, Baja California, Mexico.

Agh, N., T. J. Abatzopoulos, I. Kappas, G. Van Stappen, S. M. Razavi Rouhani, and P. Sorgeloos. 2007. Coexistence of sexual and parthenogenetic Artemia populations in Lake Urmia and neighbouring lagoons. International Review of Hydrobiology 92:48–60.

Aktas, C. 2020. haplotypes: Manipulating DNA Sequences and Estimating Unambiguous Haplotype Network with Statistical Parsimony. R package version 1.1.2.

Amat, F., C. Barata, F. Hontoria, J. C. Navarro, and I. Varó. 1994. Biogeography of the genus *Artemia* (Crustacea, Branchiopoda, Anostraca) in Spain. International Journal of Salt Lake Research 3:175–190.

Archetti, M. 2004. Recombination and loss of complementation: a more than two-fold cost for parthenogenesis. Journal of Evolutionary Biology 17:1084–1097.

Archetti, M. 2010. Complementation, genetic conflict, and the evolution of sex and recombination. Journal of Heredity 101:1–13.

Artom, C. 1931. L'origine e l'evoluzione della partenogenesi attraverso i differenti biotipi di una specie collettiva (*Artemia salina* L.) con speciale riferimento al biotipo diploide partenogenetico di Sète. Tip. del Senato, Roma,.

Asem, A., A. Eimanifar, and S. C. Sun. 2016. Genetic variation and evolutionary origins of parthenogenetic Artemia (Crustacea: Anostraca) with different ploidies. Zoologica Scripta.

Asher, J. H. 1970. Parthenogenesis and genetic variability. II. One-locus models for various diploid populations. Genetics 66:369–391.

Barigozzi, C. 1944. I fenomeni cromosomici delle cellule germinale in *Artemia salina*. Chromosoma 2:549–575.

———. 1974. Artemia: a survey of its significance in genetic proplems. *in* T. Dobzhansky, M. Hecht, and W. Steere, eds. Evolutionary Biology. Springer, Boston, MA.

Baxevanis, A. D., I. Kappas, and T. J. Abatzopoulos. 2006. Molecular phylogenetics and asexuality in the brine shrimp *Artemia*. Molecular Phylogenetics and Evolution 40:724–738.

Beardmore, J., and F. Abreu-Grobois. 1983. Taxonomy and evolution in the brine shrimp Artemia. Pages 153–164 *in* G. Oxford and D. Rollinson, eds. Protein Polymorphism: Adaptive and Taxonomic Significance. Academic Press, New York.

Bi, K., and J. P. Bogart. 2010. Time and time again: Unisexual salamanders (genus *Ambystoma*) are the oldest unisexual vertebrates. BMC Evolutionary Biology 10:238–252.

Bossier, P., W. Xiaomei, F. Catania, S. Dooms, G. Van Stappen, E. Naessens, and P. Sorgeloos. 2004. An RFLP database for authentication of commercial cyst samples of the brine shrimp Artemia spp. (International Study on Artemia LXX). Aquaculture 231:93–112.

Bowen, S. T., and G. Sterling. 1978. Esterase and malate dehydrogenase isozyme polymorphisms in 15 *Artemia* populations. Comparative biochemistry and physiology. B, Comparative biochemistry 61:593–5.

Bowen, T., J. P. Durkin, G. Sterling, and L. S. Clark. 1978. *Artemia* hemoglobins: genetic variation in parthenogenetic and zygogenetic populations. Biol. Bull. 155:273–287.

Boyer, L., R. Zahab, M. Mosna, C. Haag, and T. Lenormand. 2021. Not So Clonal Asexuals: Unraveling The Secret Sex Life Of Artemia parthenogenetica. Evolution Letters 5:164–174.

Brauer, A. 1894. Zur Kenntniss der Reifung des parthenogenetisch sich en- twickelenden Eies von *Artemia salina*. Arch. Mikr. Anat. 43:162–222.

Browne, R. A. 1992. Population-Genetics and Ecology of *Artemia* - Insights Into Parthogenetic Reproduction. Trends in Ecology & Evolution 7:232–237.

Browne, R. A., and S. T. Bowen. 1991. Taxonomy and population genetics of *Artemia*. In Browne, R. A., P. Sorgeloos, C. N. A. Trotman (eds), Artemia Biology 221–235.

Browne, R. A., and C. W. Hoopes. 1990. Genotype diversity and selection in asexual brine shrimp (Artemia). Evolution 44:1035–1051.

Buhay, J. 2009. COI -like sequences are becoming problematic in molecular systematic and DNA barcoding studies. J. Crustac Biol. 29:96–110.

Burnham, K., and D. Anderson. 2002. Model selection and multimodel inference: A practical information-theoretic approach (2nd editio.). Springer-Verlag, New York.

Chang, M., A. Asem, and S. Sun. 2017. The incidence of rare males in seven parthenogenetic Artemia (Crustacea: Anostraca) populations. Turkish Journal of Zoology 41:138–143.

Christiansen, D. G., and H.-U. Reyer. 2009. From clonal to sexual hybrids: genetic recombination via triploids in all-hybrid population of water frogs. Evolution 63:1754–1768.

Clark, L. V., and M. Jasieniuk. 2011. polysat: An R package for polyploid microsatellite analysis. Molecular Ecology Resources 11:562–566.

Cruciani, G., M. Baroni, E. Carosati, M. Clementi, R. Valigi, and S. Clementi. 2004. Peptide studies by means of principal properties of amino acids derived from MIF descriptors. Journal of Chemometrics 18:146–155.

Cuellar, O. 1987. The evolution of parthenogenesis: a historical perspective. Pages 43–97 *in* P. Moens, ed. Meiosis. Academic Press, Orlando.

De Meester, L., A. Gómez, and J. Simon. 2004. Evolutionary and ecological genetics of cyclical parthenogens. Pages 122–134 *in* A. Moya and E. Font, eds. Evolution from molecules to ecosystems. Oxford University Press, Oxford.

Delmotte, F., N. Leterme, J. Bonhomme, C. Rispe, and J. C. Simon. 2001. Multiple routes to asexuality in an aphid species. Proceedings. Biological sciences / The Royal Society 268:2291–9.

Dufresne, F., and P. D. N. Hebert. 1994. Hybridization and origins of polyploidy. Proceedings of the Royal Society of London B 258:141–146.

Eimanifar, A., G. Van Stappen, B. Marden, and M. Wink. 2014. Artemia biodiversity in Asia with the focus on the phylogeography of the introduced American species Artemia franciscana Kellogg, 1906. Molecular Phylogenetics and Evolution 79:392–403.

Eimanifar, A., G. Van Stappen, and M. Wink. 2015. Geographical distribution and evolutionary divergence times of Asian populations of the brine shrimp Artemia (Crustacea, Anostraca). Zoological Journal of the Linnean Society 174:447–458.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–1150.

———. 2017. Asexual but not clonal: Evolutionary processes in automictic populations. Genetics 206:993–1009.

Estoup, A., V. Ravigné, R. Hufbauer, R. Vitalis, M. Gautier, and B. Facon. 2016. Is There a Genetic Paradox of Biological Invasion? Annual Review of Ecology, Evolution, and Systematics 47:51–72.

Glover, N. M., H. Redestig, and C. Dessimoz. 2016. Homoeologs: What are they and how do we infer them? Trends in Plant Science. Elsevier Ltd.

Goldschmidt, E. 1952. Fluctuation in chromosome number in Artemia salina. Journal of Morphology 9:111–134.

Haag, C. C. R., L. Theodosiou, R. Jabbour-Zahab, T. Lenormand, R. Zahab, and T. Lenormand. 2017. Low recombination rates in sexual species and sex-asex transitions. Philosophical Transaction of the Royal Society of London (B) 372:20160461.

Hebert, P. D. N., and T. Crease. 1983. Clonal diversity in populations of Daphnia Pulex reproducing by obligate parthenogenesis. Heredity 51:353–369.

Heibl, C. 2008. PHYLOCH: R language tree plotting tools and interfaces to diverse phylogenetic software packages.

Hurvich, C. M., and C. L. Tsai. 1989. Regression and time series model selection in small samples. Biometrika 76:297–307.

Jaquiéry, J., S. Stoeckel, C. Larose, P. Nouhaud, C. Rispe, L. Mieuzet, J. Bonhomme, et al. 2014. Genetic control of contagious asexuality in the pea aphid. PLoS Genetics 10:1–10.

Judson, O. P., and B. B. Normark. 1996. Ancient asexual scandals. Trends in Ecology and Evolution 11:41–46.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059–3066.

Kearney, M. 2005. Hybridization, glaciation and geographical parthenogenesis. Trends in Ecology & Evolution 20:495–502.

Kunz, D., W. Tay, S. Elfekih, K. Gordon, and P. De Barro. 2019. Take out the rubbish–Removing NUMTs and pseudogenes from the Bemisia tabaci cryptic species mtCOI database. BioRxiv 724765.

Lee, S. C., M. Ni, W. Li, C. Shertz, and J. Heitman. 2010. The evolution of sex: a perspective from the fungal kingdom. Microbiology and molecular biology reviews 74:298–340.

Lenormand, T., J. Engelstädter, S. E. S. E. Johnston, E. Wijnker, and C. R. Haag. 2016. Evolutionary mysteries in meiosis. Philosophical Transactions of the Royal Society B-Biological Sciences 371:20160001.

Lopez, J. V., N. Yuhki, R. Masuda, W. Modi, and S. J. O'Brien. 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. Journal of Molecular Evolution 39:174–190.

Lynch, M. 1990. The similarity index and DNA fingerprinting. Molecular Biology and Evolution 7:478–484.

Maccari, M., F. Amat, and A. Gómez. 2013*a*. Origin and genetic diversity of diploid parthenogenetic *Artemia* in Eurasia. PloS one 8:e83348.

Maccari, M., F. Amat, F. Hontoria, and A. Gómez. 2014. Laboratory generation of new parthenogenetic lineages supports contagious parthenogenesis in *Artemia*. PeerJ 2:e439.

Maccari, M., A. Gómez, F. Hontoria, and F. Amat. 2013*b*. Functional rare males in diploid parthenogenetic *Artemia*. Journal of Evolutionary Biology 26:1934–1948.

MacDonald, G. H., and R. A. Browne. 1987. Inheritance and reproductive role of rare males in a parthenogenetic population of the brine shrimp, *Artemia parthenogenetica*. Genetica 75:47–53.

Maniatsi, S., A. D. Baxevanis, I. Kappas, P. Deligiannidis, A. Triantafyllidis, S. Papakostas, D. Bougiouklis, et al. 2011. Is polyploidy a persevering accident or an adaptive evolutionary pattern? The case of the brine shrimp *Artemia*. Molecular Phylogenetics and Evolution 58:353–364.

Maynard Smith, J. 1978. The evolution of sex.

Meirmans, S., P. G. Meirmans, and L. R. Kirkendall. 2012. The costs of sex: Facing real-world complexities. Quarterly Review of Biology 87:19–40.

Metalli, P., and E. Ballardin. 1970. Radiobiology of artemia: radiation effects and ploidy. Current topics in radiation research quarterly 181–240.

Moritz, C., W. M. Brown, L. D. Densmore, J. W. Wright, D. Vyas, S. Donnellan, M. Adams, et al. 1989. Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekkonidae). Pages 87–112 *in* R. M. Dawley and J. P. Bogart, eds. Evolution and ecology of unisexual vertebrates. New York State Museum, Albany, NY.

Muñoz, J., A. Gómez, A. J. Green, J. Figuerola, F. Amat, and C. Rico. 2010. Evolutionary origin and phylogeography of the diploid obligate parthenogen *Artemia parthenogenetica* (Branchiopoda: Anostraca). PLoS ONE 5.

Muñoz, J., a J. Green, J. Figuerola, F. Amat, and C. Rico. 2008. Characterization of polymorphic microsatellite markers in the brine shrimp *Artemia* (Branchiopoda: Anostraca). Molecular ecology resources 9:547–50.

Mura, G., and L. Nagorskaya. 2005. Notes on the distribution of the genus Artemia in the former USSR countries (Russia and adjacent regions). Journal of Biological Research.

Naganawa, H., and G. Mura. 2017. Two new cryptic species of Artemia (Branchiopoda, Anostraca) from Mongolia and the possibility of invasion and disturbance by the aquaculture industry in East Asia. Crustaceana 90:1679–1698.

Narbel-Hofstetter, M. 1964. Les alterations de la meiose chez les animaux parthenogenetiques. Springer Verlag, Wien.

Nascetti, G., P. Bondanelli, A. Aldinucci, and R. Cimmaruta. 2003. Genetic structure of bisexual and parthenogenetic populations of Artemia from Italian brackish-hypersaline waters. Oceanologica Acta 26:93–100.

Neiman, M., D. Paczesniak, D. M. Soper, A. T. Baldwin, and G. Hehman. 2011. Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. Evolution 65:3202–3216.

Neiman, M., T. F. Sharbel, and T. Schwander. 2014. Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. Journal of evolutionary biology 27:1346–59.

Nougué, O., E. Flaven, R. Jabbour-Zahab, N. O. Rode, M.-P. Dubois, and T. Lenormand. 2015*a*. Characterization of nine new polymorphic microsatellite markers in *Artemia parthenogenetica*. Biochemical Systematics and Ecology 58:59–63.

Nougué, O., N. O. N. O. Rode, R. Jabbour-zahab, A. Ségard, L.-M. Chevin, C. R. Haag, and T. Lenormand. 2015*b*. Automixis in Artemia: Solving a century-old controversy. Journal of Evolutionary Biology 28:2337–2348.

Otto, S. P., and T. Lenormand. 2002. Resolving the paradox of sex and recombination. Nature Review Genetics 3:252–261.

Otto, S. P., and J. Whitton. 2000. Polyploid incidence and evolution. Annual Review of Genetics 34:401–437.

Paland, S., J. K. Colbourne, and M. Lynch. 2005. Evolutionary history of contagious asexuality in Daphnia pulex. Evolution 59:800–813.

Paradis, E., and K. Schliep. 2019. Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528.

Perez, M. L., J. R. Valverde, B. Batuecas, F. Amat, R. Marco, and R. Garesse. 1994. Speciation in the *Artemia* genus: mitochondrial DNA analysis of bisexual and parthenogenetic brine shrimps. Journal of molecular evolution 38:156–68.

Rey, O., B. Facon, J. Foucaud, A. Loiseau, and A. Estoup. 2013. Androgenesis is a maternal trait in the invasive ant *Wasmannia auropunctata*. Proceedings of the Royal Society B: Biological Sciences 280:20131181.

Rode, N. O. N. O., E. J. P. E. J. Lievens, A. Segard, E. Flaven, R. Jabbour-Zahab, T. Lenormand, F. Elodie, et al. 2013. Cryptic microsporidian parasites differentially affect invasive and native *Artemia* spp. International Journal for Parasitology 43:795–803.

Rode, N. O. O., A. Charmantier, and T. Lenormand. 2011. Male-female coevolution in the wild : evidence from a time series in *Artemia franciscana*. Evolution 65:2881–2892.

Sainz-Escudero, L., E. K. López-Estrada, P. C. Rodríguez-Flores, and M. García-París. 2021. Settling taxonomic and nomenclatural problems in brine shrimps, *Artemia* (Crustacea: Branchiopoda: Anostraca), by integrating mitogenomics, marker discordances and nomenclature rules. PeerJ 9.

Sánchez, M. I., F. Hortas, J. Figuerola, and A. J. Green. 2012. Comparing the potential for dispersal via waterbirds of a native and an invasive brine shrimp. Freshwater Biology 57:1896–1903.

Sandrock, C., and C. Vorburger. 2011. Single-Locus recessive inheritance of asexual reproduction in a parasitoid wasp. Current Biology 21:433–437.

Schliep, K. P. 2011. phangorn: phylogenetic analysis in R. Bioinformatics 27:592–593.

Schurko, A. M., M. Neiman, and J. M. Logsdon. 2009. Signs of sex: what we know and how we know it. Trends in Ecology & Evolution 24:208–217.

Shadrin, N., and E. Anufriieva. 2012. Review of the biogeography of Artemia Leach, 1819 (Crustacea: Anostraca) in Russia. International Journal of Artemia Biology 2:51–61.

Simion, P., J. Narayan, A. Houtain, A. Derzelle, L. Baudry, E. Nicolas, M. Cariou, et al. 2020. Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer Adineta vaga. bioRxiv 2020.06.16.155473.

Simon, J., F. Delmotte, C. Rispe, and T. Crease. 2003. Phylogenetic relationships between parthenogens and their sexual relatives : the possible routes to parthenogenesis in animals. Biological Journal of the Linnean Society 79:151–163.

Suomalainen, E., A. Saura, and J. Lokki. 1987. Cytology and Evolution in Parthenogenesis. CRC Press, Boca Raton FL.

Svendsen, N., C. M. O. Reisser, M. Dukić, V. Thuillier, A. Ségard, C. Liautard-Haag, D. Fasel, et al. 2015. Uncovering cryptic asexuality in *Daphnia magna* by RAD-sequencing. Genetics 201:1143–1155.

Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.

Tucker, A. E., M. S. Ackerman, B. D. Eads, S. Xu, and M. Lynch. 2013. Population-genomic insights into the evolutionary origin and fate of obligately asexual *Daphnia pulex*. Proceedings of the National Academy of Sciences of the United States of America 110:15740–5.

van Dijk, P. J. 2009. Apomixis: Basics for non-botanists. Pages 47–62 *in* I. Schön, K. Martens, and P. J. van Dijk, eds. Lost sex. The evolutionary biology of parthenogenesis. Springer Science, Dordrecht, The Netherlands.

Wang, W., Q. Luo, H. Guo, P. Bossier, G. Van Stappen, P. Sorgeloos, N. Xin, et al. 2008. Phylogenetic Analysis of Brine Shrimp (Artemia) in China Using DNA Barcoding. Genomics, Proteomics and Bioinformatics 6:155–162.

Yagound, B., K. A. Dogantzis, A. Zayed, J. Lim, P. Broekhuyse, E. J. Remnant, M. Beekman, et al. 2020. A Single Gene Causes Thelytokous Parthenogenesis, the Defining Feature of the Cape Honeybee Apis mellifera capensis. Current Biology 30:2248-2259.e6.

Zhang, L., and C. E. King. 1992. Genetic variation in sympatric populations of diploid and polyploid brine shrimp (*Artemia parthenogenetica*). Genetica 85:211–221.

Zheng, B., and S. Sun. 2013. Review of the biogeography of Artemia Leach, 1819 (Crustacea: Anostraca) in China. International Journal of Artemia Biology 3:20–50.