Eukaryotic Sex:

Its Evolutionary Origins and Maintenance over Time and Space

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SUMMARY

Sex is ubiquitous in eukaryotes. This means it is to be found all across the phylogenetic tree and in all sorts of habitats – although not necessarily at the same frequency nor in the same form. Understanding the delicate balance between the costs and the benefits of sex in all its incarnations is still a major challenge in evolutionary biology. During this doctoral work I tried to paint a full picture of eukaryotic sex by studying in turn its origins, its maintenance, and some of its consequences for sexual organisms.

Focusing first on the deep evolutionary origins of sex, I modelled a new hypothesis regarding the origin of cell-cell fusion in early eukaryotes, a necessary first step towards sex. I showed that if those early eukaryotes already depended on mitochondria, and if the genomes of those mitochondria were accumulating deleterious mutations, then cellular fusion could have evolved as an emergency response to restore vital mitochondrial functions. This represents only a small step in the complicated chain of events that occurred during eukaryogenesis.

Moving on to the present day, I reviewed the literature on the geographic distribution of related sexual and asexual forms (geographic parthenogenesis). I found that well-known broad scale trends, such as asexuals occupying higher latitudes and larger ranges, tend to obscure in the literature finer-scale patterns, that might hold interesting insight into the ecological selection pressures maintaining sex. Hypotheses to explain geographic patterns are many, but overall experimental validation is still largely lacking. For this review I drew together examples from different sorts of parthenogenesis, from selfing, and from vegetative reproduction, arguing that they all fit within the framework of geographic parthenogenesis and that this diversity should be leveraged to better understand what characteristics of sex are being selected or counter-selected in different habitats.

One such usual characteristic of sex is the necessity to find a sexual partner. By building and analysing a spatially-explicit, individual-based model, I showed that asexuals readily take over the front of an expansion wave when they are less affected by reproductive failure than sexuals in the absence of conspecifics. This produces patterns similar to those considered typical of geographic parthenogenesis, and therefore emphasizes the importance of considering the recolonization history when trying to identify selection pressures responsible for the loss of sex in previously glaciated areas.

Finally, after exploring what selects for sex, I ended with what sex selects for. To do that I studied the case of sperm competition in small animals for which sperm size matters more than sperm number. Though still in its early stage, this work suggests that the optimal allocation between sperm size and number depends on a male's phenotypic condition and its competitive environment, and that males of intermediate condition might be the first ones to invest in sperm size when competition risk increases.

This thesis ends with a discuss of the diversity of sexual and asexual systems found in eukaryotes, with a focus on the additional traits and functions that became associated with one or the other mode of reproduction over evolutionary times. I suggest that those functions make the costs and benefits of sex highly taxon-specific, and that the benefits of recombination, the most defining feature of sex, might be most fruitfully studied in facultatively sexual unicellular eukaryotes, or perhaps even viruses and bacteria.

ACKNOWLEDGEMENTS

I've always loved acknowledgement sections. What a great opportunity to craft something personal, light-hearted, perhaps poetic, filled with fun nods to friends, family and colleagues, everyone who mattered and helped throughout the PhD years. I was looking forward to writing mine.

But my plans for this acknowledgement section had to be changed - like many other much more important plans I was making for my life. They have to be changed in order to take reality into account. I do not think there is a high chance that the world in 20 years looks like the world I was imagining when I started this PhD. We are rapidly changing the climate of our planet and destroying its biosphere, pushing it ever nearer to unpredictable catastrophic tipping points, already seeing millions suffer, predicting billions more to suffer - and collectively, what are we doing? We are looking away.

I am terrified about the future.

So to the countless precious people who surround me that I respect, value, love, and who throughout this PhD have helped me with your presence, and inspired me with your actions, thank you. I hope you will know who you are, and I will not name you.

Instead, I dedicate this work to everyone fighting environmental battles, especially people taking part in the Gilets Jaunes movement in France and in the Extinction Rebellion worldwide. Brave women and men who are willing to openly and peacefully disobey the law in their desperation to ensure a liveable future for all of us. Without them, this piece of scientific work would be utterly pointless. For who will care about the evolutionary history of Eukaryotes when faced with mass starvation? Who will pass down fundamental knowledge in a world of global wars? Where will knowledge itself go, when there is no-one left to *know*?

If I made dramatic changes to my life plans, it is because the most important thing in the world right now is also the most important thing there ever was: working to decrease the probability of taking humankind and most inhabitants of this incredibly beautiful planet on a fast track to an absolute disaster of our own making.

How long will we wait? How much will we risk? There is so terribly much to love, and so terribly much to lose. This fight needs all our effort. So all my effort I will put in.

NOTE ON PUBLISHING ETHICS

It is not an easy thing to publish according to one's ethics. Even non-academic readers will easily be able to relate: anyone who has ever stood puzzled in a Swiss supermarket will understand the state of mind I am referring to - should I buy these organic apples wrapped in plastic, or these plastic-free apples covered in layers of synthetic pesticides? Similarly, I sat wondering whether one should prioritize making their publications open access, or boycotting big publishing groups. The balance I found, and was spoiled enough to be able to follow painlessly so far, was to try and publish in society-owned journals. They are not all open-access, but at least part of the profits go to fund seminars, conferences, student grants, and other nice things that benefit the science community and not only the shareholders of the publishing company. To address the problem of paywalls locking science away from the citizens it belongs to (like my Mum), until we collectively build a healthier publishing system, I believe it is forgivable to spread the word about the work of a certain brave Alexandra Elbakyan.

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CHAPTER I General Introduction: the problem with sex

In 1982, sex was branded the Queen of problems in evolutionary biology (Bell, 1982). To this day, this statement is routinely used to introduce papers investigating its costs, benefits, various incarnations, phylogenetic distribution. But what is really meant by sex, who does it and how, and why is its existence considered such a paradox? The answers are not as obvious as it seems once one delves into the diversity of eukaryotic life, away from the model organisms that have shaped the thinking of biologists over the centuries.

To refine my understanding of the problems posed by sex, during the years that led to this thesis I tried to approach it under a multiplicity of angles, doing my best to fight my inherent taxonomic chauvinism and reading about as broad a variety of organisms as possible. Consistent with this goal, I drew for this introduction at least one example from each of the seven eukaryotic realms illustrated in Fig. 1 but one (can you find which is missing?).

I start here by giving a working definition of sex, followed by a non-exhaustive overview of the hypotheses about its origins and continued presence in eukaryotes, and why they all seem to be partially unsatisfactory. The next four chapters are constituted by work I have done on three aspects of sex: its origins, its maintenance, and some of the bizarre evolutionary consequences it can have on organisms that engage in it. Finally, the discussion tries to draw from this diversity of perspectives in order to assess to what extent sex still represents a problem for theory, and try to go beyond what is still the standard narrative surrounding it.

Sex: can we agree on what it is?

THIS THINGS EUKARYOTES DO...

To reach the most fundamental definition of sex, the same mental layers generally need to be peeled off one by one by lay people and unspecialized biologists alike.

To get it out of the way: sex is not about copulation, as becomes painfully obvious as soon as one remembers the land plant (Fig. 1, Archaeplastids) sitting on one's desk. Sexuals partners, indeed, need not even meet. Insect-pollinated plants enrol help from a third party to be the physical link between two partners (Lankinen & Karlsson Green, 2015), while their wind-pollinated relatives simply trust the wind to carry pollen from male to female flowers (Friedman & Barrett, 2009). And it is not only a plant thing: broadcast-spawning aquatic animals (Fig. 1, Opisthokonts), like many corals for instance, release millions of gametes simultaneously into the water, usually males and females alike (Parker *et al.,* 2018).

But sex is not about males and females either. First of all, males and females are not always separate individuals – think of well-known hermaphroditic plants (e.g. pine trees, first covering your world in yellow pollen, and then dropping cones on your head) or animals (e.g. snails) which have both male and female reproductive organs. In some species, for instance iconic fish like groupers or clownfish (Avise & Mank, 2009), individuals get to be both male and female in their life but sequentially – all told, if one excludes insects, one third of all animals are hermaphroditic (Jarne & Auld, 2006). In reality, "male" and "female" are less categories that apply to individuals than ways of describing two different *strategies*, that are organized in various ways within one species (Schaerer, 2017): one same



Fig. 1 "Protists constitute the majority of lineages across the eukaryotic tree of life. This schematic represents a synthesis of information on morphology, phylogenetic analyses (based on a few genes from a large diversity of organisms), as well as phylogenomic analyses (of many genes from representatives of major lineages). Seven "supergroups" are indicated by coloured wedges. Relationships of groups listed outside the supergroups remain contentious."

Picture and legend reproduced from Worden *et al.*, 2015. The root of the eukaryote evolutionary tree might lie **10**between Euglenozoa (Excavates) and all other eukaryotes (Cavalier-Smith, 2010).

individual can play one or the other its whole life, or both, either simultaneously or sequentially, opening the door to interesting allocation decisions (Wang *et al.*, 2016; Henshaw *et al.* 2015). Fundamentally, the male strategy is simply defined as producing small, motile gametes, while the female strategy is about producing big gametes well stocked with non-genetic resources.

But sex is not about big and small gametes either.

To start with, gametes need not be different sizes. The vast majority of eukaryotic taxonomic diversity is composed of unicellular organisms many of which are *isogamous*, which means that all the gametes they produce are the same size, and therefore contribute equally to the provisioning of the zygote (Lehtonen et al., 2016; note that some multicellular organisms can also be isogamous). Being the same size is not being equal, however, and indeed they cannot fuse indiscriminately - instead of belonging to different sexes, gametes now come in complementary mating types, with a zygote only viable when resulting from the fusion of two gametes of different mating types. And while the majority of isogamous species seem to have only two self-incompatible mating types, they do not all stop at that (Constable & Kokko, 2018) - the absolute record so far is held by the basidiomycete fungus Schizophyllum commune (Fig. 1: Opisthokonts) that boasts 23,000 potential mating types (Kothe, 1996). Mating types can for instance differ in their search behaviour (Lipinska et al., 2015), or the pheromones they are able to produce and receive (Hadjivasiliou & Pomiankowski, 2016), as well as the role they play in the inheritance of mitochondria (Gyawali & Lin, 2011; and see related discussions in Chapter II), but those differences might not be directly obvious to unsuspecting human scientists. This has led researchers to believe some organisms to be asexual, simply because they had failed for years to provide the unfortunate creatures with suitable mating partner (as happened to *Dictyostellium*, Fig. 1: Amoebozoa, Lahr et al., 2011).

But as if having potentially thousands of mating types was not enough to disorient a human observer (Fig. 1, Opisthokont), fungi (Opisthokont just as well) do not even always bother with gametes. When the hyphae (haploid filaments) of two compatible individuals meet, they can form a cytoplasmic bridge between two of their cells and let the nucleus of one migrate into the other. It can be some time before the two nuclei fuse and recombine, time during which the hypha keeps growing, while in this not-quite-diploid, but rather *dikaryotic* state. Finally, when the two nuclei finally fuse, they recombine, undergo meiosis to return to a haploid state, and spores are formed and dispersed (Anderson *et al.*, 2007; Lee *et al.*, 2010).

Sex, in the light of this asynchrony between genetic exchange and production of spores, suddenly ceases to seem to be about *reproduction* anymore. It even appears to be the opposite of reproduction when one considers the odd case of hypotrych Ciliates (Fig. 1, Alveolates), in which sex involves a net reduction in the number of individual cells. Instead of exchanging genetic information through a cytoplasmic bridge, two diploid adult hypotrych cells of a mind to mate simply undergo total fusion together and become one recombinant, still diploid cell, by some interesting nuclear arithmetic (made possible by juggling two different kinds of nuclei, but let us not indulge in further details; Heumann, 1975). To reproduce, on the other hand, Ciliates and the majority of unicellular eukaryotes simply divide asexually into two identical cells. Asexual reproduction, when one comes to think of it, really is a pleonasm, and sexual reproduction a contradiction - since an offspring born of two parents is a copy of neither, neither parent has been reproduced. One more proof, if need were, that textbooks on reproductive biology were not written by unicellular authors (Kokko, 2017).

Now that we have established everything that sex is not, are we left with anything that sex is? Yes, since fundamentally, sex is simply the process by which two individuals of a same species shuffle their genetic material. Eukaryotes, despite their usual insistence on never doing anything in any standardized way, do follow two (nearly) universal rules in the process of having sex: haploid and

diploid phases alternate thanks to the reductive operation of meiosis, and the two different genomes recombine, by randomly shuffling the chromosomes of each pair and/or by swapping DNA segments between homologous chromosomes (Loidl, 2016; Markov & Kaznacheev, 2016). This is what defines eukaryotic sex, and this was already present in LEKA, the Last Eukaryotic Common Ancestor, the most recent cell that all eukaryotes can be traced back to (Cavalier-Smith, 2010).

As we have seen through our meandering path towards its essence, though, sex does come in association with a great many extra features across the tree of eukaryotic life, and that makes it very difficult to find universal selection pressures responsible for its maintenance. This will become more and more apparent throughout this thesis and its discussion.

... THAT IS NOT SO UNLIKE WHAT NON-EUKARYOTES DO

Is sex, then, not to be found in non-eukaryotic organisms? Eukaryotes, which are the group of cellular organisms that possess a nucleus, share this planet with two other domains of cellular organisms, Archaea and Eubacteria (called prokaryotes, for want of a nucleus), as well as with Viruses - although the latter are often denied the right to be counted as "life", possibly as a result of textbook authors not being parasitic genetic entities themselves (Koonin & Starokadomskyy, 2016). If, as is often the case, one explicitly defines sex using uniquely eukaryotic characteristics (such as the reciprocity of the exchange of genetic material, for instance, or the existence of meiosis and haplo-diploid cycles), one can safely deduce that sex does not exist outside of the eukaryotic domain. But perhaps, instead of creating arbitrary boundaries, is it instructive to briefly look at what non-eukaryotes do that resembles the eukaryotic version of sex, in terms of mechanisms or outcomes.

Viruses are parasitic genetic elements made of DNA or RNA, that need to infect a host cell and use its cellular machinery in order to replicate themselves. If several viral strains infect the same host cell, reciprocal genetic exchange can occur, via breakages and reunion of the viral chromosomes (Chao, 1988; Pérez-Losada *et al.*, 2015). On top of that, genome segmentation is frequent in RNA viruses, and opens different possibilities. Segmented viruses possess several chromosomes, all of which must eventually find themselves in the same host-cell to complete the viral cycle (Sicard *et al.*, 2016). The viral chromosomes either all travel from host to host in the same compartment (mono-compartment viruses, like HIV), or all separately (multipartite viruses, Chao, 1988; in which chromosomes are actually much better team players than their puzzling transportation mode would suggest: Sicard *et al.*, 2019). Whenever several viruses infect the same host, chromosomes from different strains therefore find themselves reassorted in new combinations, with the new genome being born of as many potential parents as there are chromosome ("*Is this sex? If it is, it is genuine group sex*" – Szathmáry, 1992). Recombination and chromosome reassortment – that is indeed not so unlike what we have described in eukaryotes, and might well serve the same function (it seems at least to help with purging of deleterious mutations; Turner, 2003).

Eubacteria exchange genetic material a lot. So much so, that trying to define species and assign one genome per species has proved quite a discouraging task. This led to the emergence of the notion of pangenome, the sum of the "core genome" that all individuals of a taxonomic unit possess, and of the "accessory genome" that varies from cell to cell (Tettelin *et al.*, 2005; Bobay & Ochman, 2018). Three main processes of gene transfer are known in Eubacteria: plasmid-mediated conjugation, phage-mediated transduction, and natural bacterial transformation (Michod *et al.*, 2008). Conjugation is the only one that involves cell-to-cell interactions, and is under the control of plasmids: small circular genetic elements, spreading copies of themselves between bacteria (the typical vectors of antibiotic resistance). Transduction is not under bacterial control either, as it is the (likely accidental) transfer of DNA from one bacterial cell to the other by (sloppy) viruses. Transformation, lastly, is clearly a bacterial adaptation for DNA transfer. To carry out transformation, a bacterium must enter a state

called "competence", in which it can bind, take up, and recombine exogenous DNA into its chromosome, as well as actively exporting DNA itself into the environment. The DNA taken up for recombination is usually homologous and originates from a neighbouring cell of the same species, but it can, occasionally, be a heterologous fragment carrying totally novel genes. Bacteria have been found to enter a state of competence following DNA damage, for instance from UV radiations, suggesting that condition-dependent transformation might be used for DNA repair (Michod *et al.*, 2008). Alternatively, DNA is also good food to snack on (Finkel & Kolter, 2001).

Archaea, finally, are the prokaryotes to which eukaryotes are most related (indeed, the mitochondriacarrying eukaryotic cell can be seen as an Archaeon that has swallowed a Eubacterium and never felt quite normal again after that; Cavalier-Smith, 2010; Koonin, 2015). It is therefore no surprise that the version of prokaryotic sex the most disturbingly similar to what eukaryotes do has been found in one genus of Archaea, complete with cell-cell fusion, recombination of the two genomes (each composed of one circular chromosome) and separation (Rosenshine *et al.*, 1989). Aside from this particular case, Archaea use more or less the same means of genetic exchange as Eubacteria (Wagner *et al.*, 2017).

Whether all of the above should be called sex or not is solely a matter of semantics. From now on however, we will concern ourselves only with eukaryotic sex, the topic of this thesis – although non-eukaryotic sex will make a brief but important re-appearance in the general discussion.

Sex: can we agree on why it's there?

The vast majority of eukaryotes engage in sex at one point or another of their life-cycle (Lahr *et al.*, 2011). A great many exceptions certainly exist (if you live in central or northern Europe and it is still spring, take a peek through your window, you might see an asexual dandelion waving at you – wave back), but as we have seen, modern asexual eukaryotes all descend from sexual ancestors. To wonder why sex is so prevalent today is to ask two very distinct questions: why did the ancestors of all eukaryotes evolve sex, and why did most of their descendants keep it over such long evolutionary times?

THE ORIGINS OF SEX

The deep origins of eukaryotic sex are very difficult to reconstruct. This is no wonder, since sex appeared probably well over 2 billion years ago (Eme *et al.*, 2014; Dacks *et al.*, 2016), in organisms long disappeared, and at a time where the Earth was a very different place (Shaw, 2018). What is more, its origin is hopelessly entangled with that of every other innovation that makes the eukaryotic cell distinct from its (most-likely) archaeal-like ancestors: the presence of a nucleus, but also mitosis, a standardized number of specialized linear chromosomes, alternation between diploid and haploid phases through meiosis, a cytoskeleton, mitochondria, phagotrophy, etc. (Cavalier-Smith, 2010). This makes any hypothesis about the deep origins of eukaryotic sex speculative and eminently untestable – however we can still derive some level of intellectual comfort from the fact that an unlikely hypothesis can nonetheless be rejected on two aspects: the internal logic of its argument, best validated by modelling, and the robustness of its assumptions, informed notably by advances in phylogenetic reconstructions and comparative cell biology.

The selection pressures behind the evolution of eukaryotic sex must have heavily depended on the order in which novel eukaryotic features evolved. In the absence of any consensus at the moment, I shall not attempt here to review the literature, but rather provide a few example hypotheses in the way of illustrations. Let us take first a recent paper by Markov & Kaznacheev (2016). They chose as a starting point a population of archaea-like protoeukaryotes carrying a varying number of copies of their genome (no ploidy to speak of yet, as each chromosome is simply a copy of the entire genome). The main problems those protoeukaryotes are faced with are deleterious mutation accumulation, and

lack of control over the numbers and quality of genome copies passed down to each daughter cell during division. In their thorough computer modelling exercise, the authors show it is possible from this point to evolve frequent genetic exchanges between individuals, mitosis and meiosis, and chromosome specialization.

As a second example, let us turn to a model of a different nature, since it is verbal this time, but of an even more impressive thoroughness that I can hardly even try to it summarize here. Based on an unbelievably intimate knowledge of comparative cell biology, Cavalier-Smith (2010) proposes a stepby-step reconstruction of the evolution of meiosis and syngamy (cell-cell fusion), with this time as a starting point a protoeukaryotic cell that has no nucleus, but can conduct an error-prone mitosis, has a standard ploidy to maintain, and leads the "feast and famine existence" of the first phagocytotic predators. Meiosis, he argues, is a way to correct deleterious ploidy errors, and syngamy allows cells to form big diploid resting cysts in times of starvation, enabling DNA to be repaired from a different template in the process. Beyond DNA repair, improvement in the purging of deleterious mutations is a beneficial extra – and so was at the time the ability to bring together radically novel genetic innovations from different protoeukaryotic lineages, speeding up the stable transition to a new cell type qualitatively vastly different from its archaeal ancestor.

Finally, one branch of hypotheses about the origins of sex explores the idea that mitochondria had a role to play. Mitochondria, which are today considered a part of a cell's machinery (its "energy factory", in fact), used to be Eubacteria-like cells which one day took up residence inside the protoeukaryotic cell, and over time established the intimate symbiosis we observe today. Mitochondria can generate huge amounts of energy for the cell (Lane & Martin, 2010) but at the cost of generating very dangerous by-products: the dreaded ROS (reactive oxygen species). ROS are especially damaging to DNA, and their control still represents a challenge to modern eukaryotic cells: in the early days of the symbiosis, they could have imposed a consequent selection pressure to evolve efficient DNA-repair mechanisms, perhaps then under the form of sex (Hörandl & Speijer, 2018). In line with this hypothesis, some modern eukaryotes have been found to engage in condition-dependent sex under oxydative stress (Nedelcu *et al.*, 2004). Another type of hypotheses using mitochondria as their main suspect centres around the accumulation of deleterious mutation within mitochondrial genome, and the need to purge damaged mitochondria or co-evolve with them (e.g. Havird *et al.*, 2015). It is within this family of hypotheses that the work presented in Chapter II fits.

THE MAINTENANCE OF SEX

When thinking about the deep origins of eukaryotic sex, one frustrating realization was that none of the hypotheses we make can ever be really tested, since the actors of these events are long gone. Fortunately, this is not the case with hypotheses regarding the maintenance of sex, as sex is constantly being maintained all around us as we speak. Therefore, it might come as a surprise that it is still considered quite a mystery why we should observe sex anywhere at all. This all comes down to an analysis of costs and benefits that do not quite add up.

Sex has costs of different kinds, that have been extensively reviewed recently in Lehtonen *et al.* (2012) and Meirmans *et al.* (2012). Roughly, they can be seen as either demographic costs or genetic costs.

First of all, sex takes time. Meiosis itself takes much longer to complete than mitosis (up to ten times; Lewis, 1983), which might be irrelevant for a multicellular eukaryote with a long life-span, but is far from trivial for a unicellular organism: consider the number of mitotic divisions that competing cell lineages can complete while one is busy meiosing. Energy also needs to be spent conducting sex-related cellular processes, or expressing the right behaviours, e.g. related to mating - energy which could go instead towards mitotically produced offspring. Mating can increase predation or parasite transmission risks (including genetic parasites; Crespi & Schwander, 2012; Bast *et al.*, 2015). In species 14

with separate sexes, males represent a clear demographic cost, compared to an all-female asexual species. In the majority of cases, males do not contribute as much to offspring production as females do (this goes from contributing nothing more than genetic material, all the way to providing full paternal care). Therefore, devoting half of one's progeny to the male sex is the same as, in the worst case scenario, halving one's demographic output. This cost is not paid by individuals, but by lineages: an all-female, or at least female-biased population, has a faster growth rate than one with a balanced sex-ratio (Kobayashi & Hasegawa, 2016) and will eventually replace it. Finally, meiosis itself is a risky business and prone to errors that make its products nonviable (Levitis *et al.*, 2017), a cost also paid by humans in which a proportion of eggs cannot develop (Webster & Schuh, 2017).

On the genetic side, perhaps the most obvious objection to sex is: if the individual made it that far, surely it must mean that its genes and their combination are good? Why change anything, then? The costs of chromosome segregation and recombination lies in breaking up favourable allele combinations. These costs are exacerbated when there is a risk of inbreeding depression (if the parents are too related, deleterious recessive alleles become homozygous in the offspring, and are therefore expressed), outbreeding depression (if the parents are not related enough, alleles have not coevolved to function well together, or are adapted to different habitats) and hybridization (an extreme form of outbreeding depression; Edmands, 2007).

All the costs mentioned so far would be avoided as soon as the organism would resort to solely mitotic, uniparental means of reproduction – and are therefore the reason why obligately asexual lineages would be supposed to invade. In that, they have to be distinguished from entrenched costs, the ones that befell the species *because* of sex but would be paid just as much by a newly arisen asexual mutant (Stelzer, 2015). For instance, these costs can be of building showy ornaments, sperm-storage organs, or possessing sub-optimal traits due to intragenome sexual conflict between male and female optima. Given enough time, an asexual lineage can alleviate those costs through decay of the sexrelated traits (e.g. Kraaijeveld *et al.*, 2016; Schwander et *al.*, 2013; Parker *et al.*, 2019) – but they are not what gives asexual mutants their initial edge (Meirmans et al., 2012).

Seeing this rather forbidding catalogue of costs, surely sex must come with fantastic benefits, to be still found in most species of eukaryotes? In fact, within a decade or so after the paradox associated with the costs of sex was first identified, dozens of hypotheses had been proposed (Kondrashov, 1993; more recently reviewed in Meirmans & Strand, 2010; Neiman & Schwander, 2011; Hartfield & Keightley, 2012), some of which we describe below, but to this day there exists no general consensus as to whether there exists a universal mechanism behind the maintenance of sex. In models, a given hypothesis can rarely single-handedly explain the maintenance of sex within a reasonable parameter space, and in experiments, evidence remains inconclusive (reviews: Neiman *et al.*, 2018; Pesce *et al.*, 2016). This begs the question of whether one should really look for a single overarching evolutionary benefit of sex, or if the answer might lie in a pluralistic of benefits (Neiman et al., 2017). Idiosyncratic explanations or combinations thereof remain somewhat unsatisfying for a phenomenon so nearly universal as sex, and triggered considerations about how to ideally solve the trade-off between generalism, realism and precision of hypotheses (Meirmans & Strand, 2010).

In the long term, sexual lineages are expected to be longer lived than purely asexual ones, as seems to be confirmed by the "twiggy" phylogenetic distribution of purely asexual lineages in multicellular eukaryotes, where they occupy only the tip of phylogenetic branches (Schwander & Crespi, 2009; Neiman *et al.*, 2009). Sex allows faster differentiation, speciation, adaptation, purging of deleterious mutations, and prevention of genetic meltdown (Muller's ratchet), all factors that could explain why the world still contains a majority of sexual species, although asexual lineages might be able to replace sexual ones in the short-term (Hartfield & Keightley, 2012). While some authors argue that this form of lineage selection might be enough to explain the persistence of sex (de Vienne *et al.*, 2013), the fact that

most costs evoked above are paid immediately upon engaging in sex has led many to look for short-term benefits of sex too.

On an ecological time frame, hypotheses have focused on the idea that organisms engaging in sex are better able to keep up with their environment than their asexual counterparts. The "sex = variation" and "variation = good" argument is not as simple as it may seem (Otto & Lenormand, 2002), but sex is favoured over pure asexuality when selection pressures vary rapidly with time, with space, and when populations are small (therefore rapidly using up their standing genetic variation in the absence of regular recombination; Otto, 2009). The environmental fluctuations at play can be of different nature. For instance, the tangled-bank hypothesis (Bell, 1982) supposes spatial variation in selection pressures: in a spatially structured habitat, a set of offspring do better when they all occupy different microniches. The Red Queen hypothesis on the other hand (Bell, 1982), assumes that the biggest source of variation comes from the biotic environment of a species, with competitors, predators and especially parasites constantly coevolving with one another in a fast arms race. This is perhaps the ecological hypothesis that has received the most empirical support, but this support unfortunately stems mostly from a single system, making it hard to generalize (Neiman *et al.*, 2018).

Facultative sexuals seem to enjoy the best of both worlds in terms of genetic risk-taking: occasional sex allows them to try out different allele combinations, potentially hitting on a highly competitive one, while retaining the parental genotype in clonemates as a back-up. Rare sex is thought to provide the same genetic benefits as frequent sex (Green & Noakes, 1995). What is more, sex can then be made condition dependent, and only take place when its costs are the lowest, for instance when the population growth rate is low (Gerber *et al*, 2018), or when its benefits are the highest, for instance in the case of stress, DNA damage (Nedelcu *et al.*, 2004; Hörandl & Hadacek, 2013) or maladaptation (the "abandon-ship hypothesis" (Hadany & Otto, 2007; Gerber & Kokko, 2018).

Finally, we saw earlier how some costs can become associated with sex over the evolutionary history of a lineage, and therefore still be incurred by freshly evolved asexuals. Conversely, sometimes sex has become associated with beneficial features that cannot be done without anymore. For instance, the production of resting eggs is vital to many species of water flies (Fig. 1: Opisthokonta) in order to survive winter freezing or summer drought, and while most of the reproduction of those cyclical parthenogens is done through asexual reproduction, only sex can produce resting eggs (Stelzer & Lehtonen, 2016). A second example is the case of some unicellular diatoms (Fig. 1: Stramenopiles), which get smaller and smaller after each mitotic division, and need to restore their size by sexual fusion every few generations in order not to vanish (Vyverman, 2004).

Objectives

The maintenance of sex despite its short-term costs is still considered an unsolved problem, despite the wealth of hypotheses we have seen. Notably, it is difficult to find a proper balance between precise but idiosyncratic mechanisms, that might work in some species but not others, and universal but hardly testable explanations (Meirmans & Strand, 2010). One reason sex is so hard to study is that it is realized in different ways across time, space and taxonomic group, as testified by the first section of this introduction. To identify general principles, there is no way around it: one has to embrace sex in all its manifestations, and learn from exceptions as well as from common patterns.

In this thesis, I study sex under a variety of different angles, hoping to gain perspective into its many incarnations, and further our understanding (hopefully - at least it furthered mine) of why it is there, and why there it remained.

Thesis outline

To start from the beginning, **chapter II** opens up the thesis with modelling work about the origin of sex in Eukaryotes. To be more precise (and more humble), it explores one hypothesis regarding the evolution of cell-cell fusion, a necessary step towards eukaryotic sex. The model is staged in a world where (proto)mitochondria had already been acquired by the protoeukaryotic cells, where some level of coevolution between the two had taken place, and where accumulation of deleterious mutations in their mitochondria was beginning to prove a serious threat to protoeukaryotic cellular lineages. Assuming that complementation can happen between mitochondria within a cell, i.e. that a function is maintained at the cellular level if at least some of the mitochondria are able to carry it out, I find that the ability to undergo cell-cell fusion spreads through the population. If fusion is costly, the rate at which it occurs depends on the magnitude of that cost and the number of mitochondria per cell.

Chapter III - IV shift to the next question of the maintenance of sex, making use of the fantastic framework provided by geographic parthenogenesis: in a number of species, there exist populations that routinely engage in sex and populations that do not, providing a seemingly perfect set-up to investigate what selective pressures are responsible for maintaining sex. In **chapter III**, I review the literature on the topic of geographic parthenogenesis, the many patterns encountered, and the hypotheses formulated to explain them. Notably, broad scale geographic patterns have long been identified, where asexuality seems mostly present in high altitudes, latitudes, islands and disturbed areas. Those could indeed be environments in which asexuality is a superior strategy. But they are also the habitats that have been most recently (re)colonized, notably after the last deglaciation, and asexuality, when it allows to reproduce in the absence of a partner (which is not always the case, see chapter III), is famously known to be favoured in contexts of colonization. Therefore in **chapter IV**, I create a model to study what geographic patterns of sexuality emerge, when a mixed population of sexuals and asexuals is left to colonize a spatially homogeneous landscape, and discuss how they can be distinguished from adaptive processes in the field. The model is made to be applicable to a wide range of organisms, to embrace the diversity of sexual and asexual systems.

But there is more to sex than genetic shuffle and the necessity to find a mate. In **chapter V**, sadly still little more than a manuscript in early stages of preparation, but incorporated in this thesis for the sake of completeness, I turn to the consequences sex has on the evolution of other traits. Because shuffling one's genome should not be done with any partner, some level of mate choice is found across the tree of Eukaryotes. Sexual selection, the evolutionary force that results from this choice, is responsible for much wasted resources – but also for some of the most bizarre and baroque traits observed in biology. The example investigated is that of sperm competition in flies, and more precisely, the evolution of giant sperm.

Finally, **chapter VI**, provides a general discussion of the paradox of sex. Because the chapters of this thesis deal with very different aspects of sex, it is hard to extract what they collectively bring to the field. Instead, drawing on everything I have learnt during the preparation of this work, I present what I do not find that paradoxical anymore about the paradox of sex, what puzzles me instead, and some directions I would really like to see the field take, whether I am a part of it or not.

CHAPTER II Mitochondrial complementation: a possible neglected factor behind early eukaryotic sex

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Abstract

Sex is ancestral in eukaryotes. Meiotic sex differs from bacterial ways of exchanging genetic material by involving the fusion of two cells. We examine the hypothesis that fusion evolved in early eukaryotes because it was directly beneficial, rather than a passive side-effect of meiotic sex. We assume that the uptake of (proto)mitochondria into eukaryotes preceded the evolution of cell fusion, and that Muller's ratchet operating within symbiont lineages led to the accumulation of lineagespecific sets of mutations in asexual host cells. We examine if cell fusion, and the consequent biparental inheritance of symbionts, helps to mitigate the effects of this mutational meltdown of mitochondria. In our model, host cell fitness improves when two independently-evolved mitochondrial strains co-inhabit a single cytoplasm, mirroring mitochondrial complementation found in modern eukaryotes. If fusion incurs no cost, we find that an allele coding for fusion can invade a population of non-fusers. If fusion is costly, there are two thresholds. The first describes a maximal fusing rate (probability of fusion per round of cell division) that is able to fix. An allele that codes for a rate above this threshold can reach a polymorphic equilibrium with non-fusers, as long as the rate is below the second threshold, above which the fusion allele is counterselected. Whenever it evolves, fusion increases the population-wide level of heteroplasmy, which allows mitochondrial complementation and increases population fitness. The advantage of sex decreases with the number of mitochondria per cell, except at very low numbers. We conclude that beneficial interactions between mitochondria are a potential factor that selected for cell fusion in early eukaryotes.

Keywords - evolution of sex, eukaryogenesis, mitochondrial threshold effect, evolution of cell fusion

Introduction

The origin of sex in eukaryotes is a billion-year-old mystery. Phylogenetic studies and comparative genomics allow us to deduce that LECA, the Last Eukaryotic Common Ancestor that lived 1.0 to 1.6 billion years ago (Eme et al., 2014), already engaged in sex (Schurko & Logson, 2008; Goodenough & Heitman, 2014; Speijer et al., 2015). Sex for LECA, as for most of its descendants, can be defined as the fusion of two haploid cells, and the coming together of their nuclear chromosomes, to form a zygote (Lehtonen & Kokko, 2014). Meiosis then allows alternation between diploid and haploid phases and adds chromosomal recombination to the mix. Eukaryotic cells typically do not engage in sex every generation. Instead, multiple mitotic divisions take place between sexual bouts in both unicellular and multicellular organisms (Green & Noakes, 1995; Dacks & Roger, 1999).

It is difficult to reconstruct how LECA evolved its sexual cycle, as opposed to prokaryotic means of genetic exchange such as conjugation, transformation or transduction (Lehtonen & Kokko, 2014). The common ancestor of LECA and its closest extant prokaryotic lineage lived long before LECA itself arose (up to one billion years, Dacks et al., 2016), obscuring the order in which all defining features of eukaryotes were gained (including linear chromosomes, a nucleus, a cytoskeleton, mitochondria, and meiotic sex, Koonin & Yutin, 2010, Cavalier-Smith, 2010). Despite several false alarms, no intermediate forms are known to have survived to document the timeline of eukaryogenesis (Dacks et al., 2016; Zachar & Szathmáry, 2017). Additionally, the origin of sex was probably a response to different selective pressures than the ones responsible today for its maintenance (Hartfield & Keightley, 2012; Lehtonen et al., 2016), and those past selective pressures can only be inferred, not observed. Here we consider whether cytoplasmic fusion and mitochondria might be key features of eukaryotic evolution that prepared the ground for the evolution of sex. As such, we assume that mitochondrial symbionts were acquired before the evolution of sex (which is still debated: Koonin & Yutin, 2010; Pittis & Gabaldón, 2016a; Martin et al., 2017; Degli Esposti, 2016; Pittis & Gabaldón, 2016b).

Previous authors have proposed that the acquisition of mitochondria selected for the evolution of sex. Two verbal models focus on the genetic benefits of sex. Lane (2011) considers sex as a way for the host cell to maintain genome integrity against disruptions caused by mitochondria – either due to the reactive oxygen species generated by mitochondrial metabolism, or due to bombardment of the host's genome by mitochondrial DNA (Martin & Koonin, 2006). Havird et al. (2015) argue that sex aided mitonuclear coevolution, which was necessary to keep the symbiosis functional. Mathematical modelling has, so far, focused on the evolution of the seemingly simpler step of cell fusion (Radzvilavičius & Blackstone, 2015; Radzvilavičius, 2016a). Fusion is an intriguing phenomenon in its own right. It requires that the cells dissolve their cell walls and membranes, and it potentially enables transmission of cytoplasmic infections. It could therefore be seen as an inefficient way to exchange nuclei – especially since genetic material can be exchanged without requiring cellular fusion or much cytoplasmic mixing of the two partners, as exemplified in prokaryotes by use of "sex pili" during conjugation (Schröder & Lanka, 2005; Cabezón et al., 2014), and in eukaryotes by the formation of cytoplasmic bridges in ciliates (Adoutte & Beisson, 1972).

Nevertheless, fusion is a prerequisite for nuclear recombination in the vast majority of eukaryotes and is likely ancestral. This raises the question of whether fusion itself might have been selected for in early eukaryotes before becoming coopted as an integral part of the sexual cycle. A key issue is that mitochondrial inheritance cannot be assumed to have been uniparental from the start (Birky, 1995; Radzvilavičius & Blackstone, 2015; Radzvilavičius, 2016a), despite uniparentality being virtually universal in modern sexual eukaryotes, where elaborate machinery is required to enforce it (see Breton & Steward, 2015, for a discussion of the very few exceptions). In asexual cell lineages, uniparentality also occurs by default. However, any transitional state towards sex that involves cell

fusion should a priori lead to cytoplasmic mixing and biparental inheritance of mitochondria (Birky, 1995).

Since eukaryotic sex involves cell fusion, it appears necessary to consider two transitions in mitochondrial inheritance: from uniparental to biparental (when fusion first evolved), and back to uniparental. While the latter transition has been the object of significant theoretical effort in order to explain the prevalence of uniparentality (Hastings, 1992; Law & Hutson, 1992; Godelle & Reboud, 1995; Hadjivasiliou et al., 2013; Christie et al., 2015; Christie & Beekman, 2017a, b; Radzvilavičius et al., 2017a), the former has largely escaped attention. The transient evolution of biparentality can be thought of in three different ways: at this point of eukaryotic evolution when the transition occurred, biparental inheritance could have been (i) neutral, (ii) deleterious, but it evolved because other benefits of fusion overrode its costs, or (iii) beneficial and, being selected for in its own right, had the potential to drive the evolution of cell fusion. The last possibility (iii), which is the one this paper investigates, implies that selection pressures changed throughout eukaryote evolution, eventually making biparental inheritance counter-selected, as shall be addressed in the discussion.

Recently, the evolution of cell-cell fusion and cytoplasmic mixing has been modelled by Radzvilavičius & Blackstone (2015) and Radzvilavičius (2016b), who studied the spread of an allele triggering fusion in a population of otherwise clonal eukaryotes. In these models, mitochondria can be of one of two types: wild-type or mutated (with ongoing mutation from the former to the latter state). The fitness of a host cell depends on the number of mutated symbionts it possesses. Frequent fusion homogenizes the content of cells in the population, so that they all contain an intermediate proportion of mutated mitochondria (leading to high intra- and low inter-cellular variance). This can be beneficial for a cellular lineage, but only when maintaining a mediocre cytoplasm over generations is better than producing some offspring with high and some with low mitochondrial mutation load. A necessary, but not sufficient, condition for fusion to evolve was that the deleterious impact of an additional defective mitochondrion increases with the number of mutated symbionts already in the cell. Overall, the parameter space in which cell fusion evolves in these models is narrow, and the selective advantage is small, suggesting that controlling the number of mutated mitochondria within the cytoplasm might not have been the one major driving force behind the evolution of cell fusion.

In our model, we assume two different mitochondrial lineages. In contrast with models discussed above, we assume both lineages to be in a mutated state, but each with a different set of deleterious mutations. A cell homoplasmic for one type of mitochondria suffers the full phenotypic consequences of its mutated mitochondria and the associated fitness costs (Fig. S1A,D,E). A heteroplasmic cell, containing a mixture of both mitochondrial types, enjoys the benefits of complementation, and if there are sufficient numbers of both types present, the deleterious mutations are not expressed at the cell level (Fig. S1B,C,F). Complementation between mitochondrial strains carrying different mutations has been reported in extant eukaryotes (Takai et al., 1999; Gilkerson et al., 2008; Ma et al., 2014), even though one study also reported negative interactions happening between two otherwise healthy mitochondrial strains (Sharpley et al., 2012). Why complementation resulting from biparental inheritance could have played an important role at the onset of the symbiosis while being virtually irrelevant nowadays is addressed in our discussion. Note that we use the word complementation in a broad sense (following Sato et al., 2009) to include effects of masking (non-expression of a mutated allele thanks to the presence of its wild-type counterpart, reviewed in Rossignol et al., 2003) as well as complementation sensu stricto (restoration of a mitochondrial function when two strains carrying mutations on different genes related to this function are put together, e.g. Takai et al., 1999; Gilkerson et al., 2008; Nakada et al., 2002; Nakada et al., 2009; Ma et al., 2014). We show that cell-cell fusion can evolve under complementation, since fusion enables a cell to maintain heteroplasmy, offering a way to restore a fully functional cytoplasm.

Methods

GENERAL DESIGN

We assume an infinite population of haploid (proto)eukaryotic cells whose fitnesses depends on their (proto)mitochondria. The host cells have a life cycle that consists of viability selection, cell fusion (whether a cell fuses depends on genotype and population composition, see below), and asexual reproduction by mitotic division.

We consider a starting point where cells are asexual and fusion is absent. Cells are homoplasmic for one of two possible mitotypes, A or B. As both mitotypes A and B have their own set of deleterious mutations, a heteroplasmic cell, i.e. with a cytoplasm consisting of a mixture of A and B, is assumed to have higher survival than either type of homoplasmic cell, i.e. with A or B alone, due to mitochondrial complementation (Rossignol et al., 2003, see Fig. S1 for details). We then introduce a mutant allele that causes its carrier to fuse with another randomly selected cell in a proportion r of its reproductive cycles, and examine whether the benefits of heteroplasmy can overcome the costs of cell fusion. A mutant cell can initiate fusion with a non-mutant, consistent with unilateral fusion requirements possibly found in the gametes of early eukaryotes (Hernández & Podbilewicz, 2017). We run independent simulations that differ in the rate of fusion r expressed by the fusing genotype, which is consistent with facultative sex in modern unicellular eukaryotes. We vary the reproductive cost incurred by both partners of a fusion (which could for instance be a time cost), the shape of the complementation function, the number of mitochondria per eukaryotic cell, and the extent of mitochondrial turnover during a cell's lifetime (modelled by altering the variance in the cytoplasmic content of daughter compared to mother cells).

The simulation thereafter tracks the composition of the population in a deterministic manner, meaning that we omit drift. We monitor the evolution of frequencies of the cytoplasmic classes (i.e. classes of cells with a specific number of mitochondria of type A and B), as well as the frequency of the mutant allele coding for fusion in each class. We use f_t to denote the overall frequency of the mutant allele at generation t.

Each generation proceeds as follows.

LIFE CYCLE: 1. VIABILITY SELECTION

A cell's probability of surviving, φ , depends on the relative proportion of each of the two mitotypes present among its M mitochondria, following the description of the phenotypic threshold effect of mitochondrial mutations found in Rossignol et al. (2003; see also Fig. S1). For a cell with *i* mitochondria of type A among its *M*, we assume a complementation function

$$\varphi\left(\frac{i}{M}\right) = 1 - K\left(1 - 2\frac{i}{M}\right)^2 \tag{1}$$

where *K* is the survival probability difference between maximally heteroplasmic and homoplasmic cells (Fig. 1). Viability is highest when $i = \frac{1}{2}M$ (maximally heteroplasmic state), and lowest at the two possible homoplasmic states i = 0 or i = M. Note that symmetry implies that both mitotypes have a set of mutations impacting fitness with the same effect size. Some different complementation functions leading to qualitatively similar results are presented in the supplementary material (Fig. S4), including functions relaxing the assumptions that that the two mitotype have accumulated deleterious mutations with similar cumulated effect size (Fig. S10).

LIFE CYCLE: 2. FUSION

Prior to reproduction, a proportion r of mutant cells initiate fusion with a randomly selected partner in the population, mutant or not, with which they mix their cytoplasmic contents before separating again. Note that the proportion of cells that undergo fusion in the population differs from the frequency f of the mutant allele. This is because (i) a mutant cell only attempts fusion with probability $r \le 1$, and (ii) a cell that does not attempt to fuse (mutant or not) might still be chosen as a partner by a cell that does (Table 1).

When two cells fuse, mix their cytoplasm, and separate again, the probability that one of the resulting cells inherits *i* mitochondria of type A follows the hypergeometric distribution (sampling without replacement):

$$D(i|M,k) = \frac{\binom{k}{i}\binom{2M-k}{M-i}}{\binom{2M}{M}}$$
(2).

where *k* is the total number of mitochondria of type A present post-fusion in the double cell, and *M* denotes the number of mitochondria per single cell.



Fig. 1 Complementation function. Unless stated otherwise, it is the function used throughout the paper. It follows Eqn 1 with K = 0.3.

LIFE CYCLE: 3. REPRODUCTION

This stage is distinct from the fusion and fission above; all cells reproduce asexually regardless of whether they have participated in fusion before. However, cells that did engage in fusion (whether they initiated it or were simply chosen as partners) have decreased reproductive output compared to those that did not fuse: their relative contribution to the next generation drops from 1 to 1-c. In an infinite population with deterministic dynamics, each cell generates a distribution of daughters with all possible cytoplasmic contents, which is then scaled to sum up to a contribution to the next generation of 1 or 1-c. The probability for a daughter cell to inherit a certain cytoplasmic content is determined by binomial sampling (i.e. with replacement) from the mother cell's content. Sampling

with replacement is chosen to simulate mitochondrial turn-over and drift within the cytoplasm of the cell during its life. The following matrix gives the probability of obtaining a cytotype with *i* type A mitochondria among its *M*, from a parent cell with *k* mitochondria of that type among its *M*.

$$D_{ik} = d(i|M,k) = {\binom{M}{i}} \left(\frac{k}{M}\right)^{i} \left(1 - \frac{k}{M}\right)^{M-i}$$
(3).

Results obtained with sampling without replacement, i.e. a procedure leading to less variance in progeny content, can be found in the supplementary material (Figs S4–S9).

SIMULATIONS

A simulation starts with a population composed of 50% of cells homoplasmic for type A, and 50% of cells homoplasmic for type B mitochondria. These starting conditions yielded the same outcome as additional simulations where the starting point was a population of heteroplasmic cells at segregation-selection equilibrium (see more details below). A fusing allele is introduced at a low frequency (1%) among cells hosting one of the mitochondrial lineages. A simulation runs until the mutant allele has been lost (frequency $f < 10^{-8}$), has reached fixation (f > 0.99), or has reached a stable frequency ($\Delta f < 10^{-7}$ for 1000 generations). We run simulations for a range of fusion rates r to determine two thresholds, for each fusion cost *c* and number of mitochondria per cell *M*: the highest rate able to invade, as well as the highest rate able to reach a polymorphic equilibrium (beyond that rate, the fusing allele is counter-selected and goes extinct). As each simulation leads to a deterministic outcome, the threshold locations can be narrowed down efficiently with the bisection method.

To study the impact of fusion on population parameters such as fitness mean and variance, and heteroplasmy mean and variance, we compare the results of a run where all cells use the highest rate of fusion that we found to be able to fix, to the results of simulations with the same parameters but with no cell fusion. In those reference populations, heteroplasmy exists but is maintained at an equilibrium distribution solely by the balance between selection and segregation. While we create these reference populations mainly as a conceptual tool to be able to isolate the role of fusion in maintaining heteroplasmy and population fitness, we note that they are not mere hypothetical constructs but can also arise naturally. In some of our evolutionary simulations where the fusion allele eventually goes extinct, fusion persists nonetheless long enough to mix cytoplasms and generate heteroplasmy, which is thereafter maintained by selection-segregation (Fig. S2, S3). Here, to generate such populations for comparison's sake, we artificially start them with only asexual cells with maximum heteroplasmy, and let them reproduce until the population has reached segregation-selection equilibrium where the distribution of cytoplasmic types in the asexual population is stable (this is possible because the model is deterministic).

All simulations were implemented in R-3.4.3 (R Core Team, 2017).

Table 1 Composition of the population at generation t (f_t is the frequency of the mutant allele, $(1-f_t)$ the frequency of the resident allele, and r the rate of fusion of the mutants). When engaging infusion, mutants randomly select another cell in the population, irrespective of its genotype.

	Mutant allele	Resident allele	Total
Undergoes fusion Does not	$\begin{array}{l} f_t r + f_t (1-r)(f_t r) \\ f_t (1-r)(1-f_t r) \end{array}$	$(1 - f_{tj}(f_t r))$ $(1 - f_{tj}(1 - f_t r))$	$2f_t r - f_t^2 r^2 1 - (2f_t r - f_t^2 r^2)$
Total	f_t	$(1 - f_t)$	1

Results

We find that a mutant allele causing cell fusion and mitochondrial reshuffling can invade a population of non-fusing cells. For each combination of number of mitochondria and cost of fusion *c*, there exists a maximum fusing rate that can be fixed in a population (Fig. 2, 3; S1, S4), and a maximum fusing rate that reaches a stable intermediate frequency in the population (Fig. 3). Beyond the polymorphism threshold, the costs of fusion are incurred too frequently to outweigh its complementation-driven benefits, and the fusion-inducing allele goes extinct.



Fig. 2 Fixation success of a fusing allele. The logarithmic colour scale denotes the highest rate of fusion able to fix in a population, for a given combination of mitochondria number and relative reproductive success off users. The lowest rate tested in simulations was 0.005.



Fig. 3 Too frequent fusion is counter-selected. The three panels have identical parameter values for c and represent three horizontal "slices" of Fig. 2 according to the number of mitochondria: M = 4, 20 and 200 in (a), (b) and (c), respectively. For each combination of mitochondrial number and relative success of fusers, there exists a maximum fusing rate that can reach fixation (dark green area), and a maximum fusing rate that can reach a stable intermediate frequency in the population (light pink area). Above that rate, fusion is selected against and disappears. The higher the number of mitochondria, the smaller the polymorphic and fixation areas.

Unsurprisingly, higher fusion costs decrease the frequency of fusion that can fix. Without a cost, cells evolve to fuse every generation (1-c = 1, Fig. 2, S1, S4). The number of mitochondria *M* also impacts the profitability of fusion. For M = 4 and above, the higher the number of mitochondria, the lower the advantages of fusion. Since mitochondria are randomly segregated between daughter cells during cell division, heteroplasmic cells are more likely to generate homoplasmic daughters when they have few mitochondria than when they have many. This has consequences for the frequency of low-fitness, low heteroplasmy cells in the population (Fig. 4A), which are the cells benefitting the most from fusion (Fig. 4B). More cells with low heteroplasmy means more cells benefitting greatly from fusion, which translates to a higher expected benefit of fusion that is able to outweigh more severe costs.

This logic does not hold for very low numbers of mitochondria per cell, where random segregation is much more likely to produce homoplasmic cells. Here the benefits of fusion are easily outweighed by its costs. The rate of fusion required to maintain heteroplasmy is now so high that the associated costs become too severe. In other words, the problem of homoplasmy becomes too difficult to avoid, as random segregation operates too powerfully.

Whenever it evolves, fusion increases the average fitness of a population and the average population heteroplasmy (Fig. S2, S3). For low numbers of mitochondria, this fitness gain is associated with an increase in fitness variance and heteroplasmy variance in the population (Fig. 5, Fig. S4, S5). This is because an asexual population with few mitochondria is composed mainly of homoplasmic cells with low fitness (Fig. 4), and the evolution of cell fusion allows more of the fitter, heteroplasmic types to be maintained (Fig. 5B, S3). For high numbers of mitochondria, the fitness gain is associated with a decrease in fitness variance. Here, an asexual population can already maintain high levels of heteroplasmy and fitness (Fig. 4B), and fusion allows further narrowing of its distribution around that optimum.



Fig. 4 A population of asexual eukaryotes sets the stage for the evolution of fusion. (a-c) The stable distribution of cytotypes that is reached at equilibrium in an asexual population (no fusion). The equilibrium is attained when random segregation (which tends to erode heteroplasmy) and natural selection (which eliminates homoplasmic cells) reach a balance. In the case of M = 2 (a), random segregation is too strong for any heteroplasmic lineage to be maintained, despite the higher fitness of heteroplasmic cells. (d-f) The fitness benefit (expected viability) enjoyed by a mutant cell of a specific mitochondrial class, were it to fuse with a random partner. The red line indicates no relative advantage compared to a nonfuser. The more homoplasmic a cell, the more it would benefit from fusing, but to which extent depends on the population composition. By combining figures a-c and d-f, one can calculate the population average for the potential fitness advantage gained by fusing; it equals 1.054, 1.067, 1.029, for M = 2, 10 and 50, respectively, and matches well (i.e. is a good indication of) the fusing allele's invasion potential (Fig. S9).

Our results appear qualitatively robust regardless of the precise shape of the complementation function, but the exact parameter space in which fusion can evolve depends on our choice of this function and its parameters (see supplementary material Figs. S4 and S10 for instances where fusion is more, or less, likely to evolve than in the main example). Prospects for the invasion of fusion become weaker when sampling occurs without replacement (Fig. S5–S9), as this creates less variance between the cytoplasmic content of mother and daughter cells and improves an asexual lineage's ability to remain heteroplasmic. An asymmetric fitness function also reduces the parameter space in which fusion evolves (Fig. S10), because in many cases the mitotype with less severe mitochondrial mutations will fix in the population before fusion can spread. Finally, we find that the benefits of cell fusion, measured as the cost that fusion can carry and still evolve (as seen on Fig. 2), are predicted well by the initial fitness advantage a fusing mutant gets in a population of non-fusers at segregation-selection equilibrium (Fig. S9).

Discussion

MITOCHONDRIAL COMPLEMENTATION CAN SELECT FOR CELL FUSION

We explored the possibility that cell fusion – nowadays closely intertwined with meiotic sex – could have initially evolved to enable complementation between different mitochondrial strains in the same cytoplasm. Our model explores the conditions under which a mutation triggering occasional fusion spreads in a population of protoeukaryotes. Cell fusion is beneficial because it counteracts the effects of random segregation and therefore enhances heteroplasmy in daughter cells (Radzvilavičius, 2016b). In line with the general statement that rare sex may often yield a better cost-benefit balance than obligate sex (Burke & Bonduriansky, 2017), we find that fusing every generation is only selected for if



Fig 5 Fusion impacts variance both in fitness (a) and heteroplasmy (b) within the population. To produce (a), the heteroplasmy of a cell was calculated as 1 - | 1 - 2i/M |, with *i* the number of mitochondria of type A among the *M* in the cytoplasm of that cell. Therefore, it ranges from 0 (homoplasmic, i.e. the cell contains either 0 or 100% of type A mitochondria) to 1 (maximally heteroplasmic, i.e. the cellcontains 50% of type A and 50% of type B mitochondria). "+" signs indicate when variance increased compared to what it was in the asexual population; variance decreased where there is no sign. Mean fitness and heteroplasmy increased everywhere due to fusion (Fig. S5).

fusion is cost-free; costly fusion leads to it being employed cyclically, with several rounds of clonal reproduction taking place between bouts of fusion.

Occasional sex together with long periods of asexual reproduction is common among extant unicellular eukaryotes (Dacks & Roger, 1999; Nieuwenhuis & James, 2016), and is also expected from theoretical models on the maintenance of sex and recombination (Green & Noakes, 1995; Burke & Bonduriansky, 2017), though for reasons different from the ones modelled in this paper, since mitochondrial inheritance is nowadays uniparental. For instance, the order of magnitude of the frequency of sex has been estimated as once every 10^2 to 10^5 generations in the marine unicellular Pseudoperkinsus tapeti (Marshall & Berbee, 2010), every 10³ in the wild yeast Saccharomyces paradoxus (Tsai et al., 2008), and every 10 to 10⁴ generations in the budding yeast S. cerevisiae (Ruderfer et al., 2006; here the estimate is of the outcrossing rate). In our model, the descendants of a heteroplasmic cell become progressively more homoplasmic over multiple clonal generations, reaching a switching point after which the benefits of fusion exceed the (fixed) costs. While we do not assume cells to be able to monitor their own heteroplasmy, frequencies of fusion that can evolve reflect the speed at which a mitotically-dividing cellular lineage loses heteroplasmy. This speed is increased by the variance between the cytoplasmic content of a mother and its daughter. This explains our finding that the lower the number of mitochondria per cell, the higher the fusion frequency that can be selected for: a clonal lineage becomes homoplasmic faster when there are only few mitochondria. Additionally, a procedure reducing the variance between mother and daughters during division decreases the optimal fusion frequency (Fig. S4).

Our model uses a range for the numbers of mitochondria consistent with modern unicellular eukaryotes: Okie et al. (2016) gathered data for 23 species, where they found that the number of mitochondria scales with cell size, and that 90% of the species had less than 260 mitochondria per cell, with a median number of 43 (range of 2-17'700, Jordan Okie, personal communication).

HOW DO OUR RESULTS RELATE TO OTHERS'?

One of our results is that the benefits of fusion tend to decrease with the number of mitochondria per cell. This is congruent with earlier results obtained by Radzvilavičius & Blackstone (2015) and Radzvilavičius (2016b), albeit from a different standpoint. There, mitochondria are modelled as being either cooperative, or selfish with a replication advantage, and the authors investigate whether cell fusion can spread. Fusion is a double-edged sword in this case: it can allow a cell to mitigate its number of selfish mitochondria, but also favours the transmission of faster replicating selfish mitochondria. Fusion in this setting can evolve if the replication advantage enjoyed by selfish mitochondria is low, and fusion frequency is high. Like in our model, lower numbers of mitochondria per cell (50 vs 200 or 20 vs 50 in their case) increases the likelihood that cell-cell fusion evolves. The reason is that for small numbers of mitochondria, segregation generates higher variance between daughter cells, and allows a stronger purifying selection to operate, constraining the spread of selfish symbionts, and making fusion a safer process.

Our main assumptions contrast in two ways with other models. First, discussions of the evolution of cell fusion (e.g. Lane, 2012) typically do not include the possibility of complementation between mitochondrial lineages. Second, we assume that mitochondrial lineages trapped in different clonal lineages of asexual protoeukaryotes diverge, which contrasts with a coevolutionary scenario (between the nucleus and mitochondria) presented by Havird et al. (2015). Their verbal model is placed in a setting where tight interactions between nuclear and mitochondrial proteins have already evolved, and assumes that a high rate of mitochondrial mutation selects for nuclear genomes to increase their rate of adaptation, which they achieve by recombining (an argument akin to the Red Queen hypothesis). Without a mathematical model, it is difficult to evaluate if nuclear adaptation to a given mitochondrial background is facilitated or impaired by shuffling alleles between cells, if each lineage has accumulated different mutations and potentially adapted to them.

LIST OF ASSUMPTIONS AND LIMITATIONS

Our simple proof of principle that mitochondrial complementation could have played a role in the evolution of cell fusion relies on a number of assumptions, which, if proven unlikely in the future, can be used to reject complementation as a potential contributor to the origin of sex. It is also worth noting that our model focuses on the origins of cell fusion, not on its maintenance; hence its assumptions are tailored to fit the onset of eukaryogenesis rather than any selection pressures acting in its later stages. Indeed, biparental inheritance and maintenance of heteroplasmy, the cornerstones of our model, are clearly not selected for in extant eukaryotes.

The three main assumptions we detail below relate to the timing of endosymbiosis, the mechanistic potential for complementation, and the genetic potential for complementation, assumed to have changed through eukaryogenesis.

First, our model assumes that the acquisition of the bacterium relative to alpha-proteobacteria that later became the mitochondrion happened early in eukaryogenesis, preceding the evolution of cell fusion and sex. An early onset of the symbiosis clearly has the potential to dramatically affect the subsequent evolution of the host, and it has been argued by some to be the driving force behind eukaryogenesis (Lane, 2011; Martin et al., 2016; but see Cavalier-Smith, 2010). Still, the "mito-early vs mito-late" debate has yet to be resolved unambiguously (Keeling, 2014; Pittis & Gabaldón, 2016a; Martin et al., 2017; Degli Esposti, 2016; Pittis & Gabaldón, 2016b). More specifically, our model requires that fusion evolved at a time when coadaptation was sufficiently advanced for mutations in the symbiont to reduce the fitness of the host.

A second major assumption of the model is the coexistence of mitochondrial lineages with different deleterious mutations. Muller's ratchet, the irreversible accumulation of deleterious mutations in asexual genomes, is typically studied by tracking the dynamics of the loss of the least-mutated class that is to say, by focusing on the number of mutations, not their identity (e.g. Bergstrom & Pritchard, 1998, Metzger & Eule, 2013, Christie & Beekman, 2017a, Radzvilavičius et al., 2017a, in mitochondria; though see Gordo et al., 2002, for a model of neutral genetic diversity in a ratchet setting). However, a mutational class (i.e. all individuals harbouring a given number of mutations) can comprise different lineages carrying different mutations. If the ratchet has led to the establishment of separate mitochondrial lineages, with different sets of mutations, but leading to comparable declines in host fitness, our process of host fitness restoration through complementation becomes conceivable; if the ratchet operates differently, our mechanism may work less well (Fig. S10). Note that while we have used accumulation of deleterious mutations as our conceptual framework, the complementation function we used can also be reformulated in terms of beneficial mutations: in the absence of recombination between organelles, the only way for a host to enjoy the combined effects of two beneficial mutations that arose on different mitochondrial lineages is to harbour both lineages simultaneously (Park & Krug, 2007, but see Christie & Beekman, 2017a, on the benefits of uniparental inheritance to circumvent clonal interference, by increasing the fixation rate of beneficial mitochondrial mutations as they arise).

Third, our hypothesis relies on complementation (*sensu lato*, i.e. including masking) being possible and of sufficient efficiency between early mitochondria. It is still poorly understood how mitochondrial complementation occurs in modern organisms, but the fusion/fission cycles of mitochondria appear to play a role (Gilkerson et al., 2008). Such mitochondrial dynamics seem to be a common feature of extant eukaryotes: it is found in organisms as varied as yeast (Rafelski, 2013), animals (Chan, 2006), amoebozoa (Schimmel et al., 2012) and plants (Arimura et al., 2004; Seguí-Simarro et al., 2008). Still, fusion and fission are not behaviours displayed by eubacteria (Wagner et al., 2017), which suggests a derived origin. However, metabolic complementation has also been found between different bacterial endosymbionts sharing an insect host (Rao et al., 2015), indicating that complementation is possible in nascent endosymbioses.

Proposing mitochondrial complementation as a positive outcome of fusion is perhaps contentious, since the near-ubiquity of uniparental inheritance suggests that cytoplasmic mixing is somehow detrimental. Direct deleterious interactions between mitolines would indeed lead to the evolution of uniparental inheritance (Christie et al., 2015), but are not required for that, as shown by a variety of models based on other processes (Hastings, 1992; Law & Hutson, 1992; Godelle & Reboud, 1995; Hadjivasiliou et al., 2013; Christie & Beekman, 2017b; Radzvilavičius et al., 2017a). Experimentally, direct detrimental interactions have only been clearly reported in one study to our knowledge (Sharpley et al., 2012 in mice), although some confusion might result from the use of the phrase "deleterious heteroplasmy" in biomedecine. It refers to situations where a deleterious mutant only starts to negatively impact the phenotype of a cell after its frequency exceeds the threshold beyond which it is no longer masked by healthy mitochondria (Rossignol et al., 2003). Importantly, the phrase does not refer to any negative interaction between mitochondrial strains.

Positive interactions, on the other hand, have been reported somewhat more widely, both between closely related strains within a patient's cells, and between diverged strains artificially put together in the lab. Complementation sensu stricto has been found in Drosophila, humans and mice (Takai et al., 1992; Nakada et al., 2002; Gilkerson et al., 2008; Nakada et al., 2009; Sato et al., 2009; Ma et al., 2014; but see Enriquez et al., 2000 for an argument on the rarity of the phenomenon), and masking is a well-known phenomenon in the medical literature, where a de novo deleterious mutation often starts impacting the phenotype only after exceeding a certain prevalence threshold within the cells

(Rossignol et al., 2003). Finally, the fitness benefits of heteroplasmy do not need to be very high for fusion to be selected for (Fig. S4B, K=0.1).

BIPARENTAL INHERITANCE: FROM BENEFICIAL TO DETRIMENTAL?

When fusion evolved in eukaryotic cells, mitochondrial inheritance switched from uniparental to biparental, only to subsequently revert back to uniparental. Theory has so far mainly focused on explaining the second transition. In this paper, we focused on the first one. We explored the possibility that biparental inheritance was originally selected for, and drove the evolution of fusion, as opposed to fusion being directly selected for and biparental inheritance arising only as a by-product. The mechanism we tested was complementation between mitochondrial strains, and we showed that it could indeed have led to the evolution of fusion.

Why, though, would complementation have been particularly relevant for early eukaryotes and not modern ones? What changed to eventually make biparental inheritance selected against? We propose three arguments, related to the decline of the mutation rate, to the reduction in the number of mitochondrial genes and their relocation into the nucleus, and to the invention of recombination that made the maintenance of heteroplasmy obsolete.

The genome of the protomitochondrial symbiont soon after the beginning of the symbiosis was large compared to that of modern mitochondria (Gray et al., 2001). It was also subject to a high mutation pressure due to poorly controlled oxidative phosphorylation and its mutagenous by-products (Hörandl & Hadacek, 2013; Speijer, 2014), and was probably evolving within a small population of protoeukaryotes. Such factors can lead to the rapid accumulation of different mutations trapped in different cellular lineages, setting the stage for our model: the only way to recover functional copies of the mutated genes was by bringing them together in one cytoplasm, resulting in a large fitness advantage to fusing cells.

Maintaining heteroplasmy by fusion is a short-term solution that cannot last indefinitely. At this stage, a functional mitochondrial genome could have been reconstituted if mitochondrial recombination was taking place at that time – a possibility that is difficult to verify at present. Homologous recombination between different molecules of mitochondrial DNA within a cell can occur in extant eukaryotes, as has been shown in some plants, fungi and animals (reviewed in White et al., 2008), although the taxonomic span and evolutionary history of that ability are not well assessed yet. Recombination has also been found among other bacterial symbionts of eukaryotes, e.g. Wolbachia (Baldo et al., 2005). Theoretical modelling shows that combining mitochondrial recombination with paternal leakage (i.e. moderate biparental inheritance) more efficiently counters Muller's ratchet in mitochondria than paternal leakage alone (Radzvilavičius et al., 2017b). Nevertheless, strict uniparental inheritance is yet more efficient at clearing deleterious mutations than paternal leakage with recombination, resulting in lower mutation loads within cells (Radzvilavičius et al., 2017).

Regardless of whether mitochondria do – or did – recombine, nuclear genes clearly do. Maintaining heteroplasmy for the purpose of complementation can become obsolete through the migration of most mitochondrial genes (or gene functions) into the nucleus, together with the evolution of nuclear recombination. In modern eukaryotes, fewer than 70 core proteins and RNAs are still encoded within the mitochondrion (Gray et al., 2004), while all other proteins (from ten to a hundred times as many) involved in mitochondrial function are encoded in the nucleus (Bousette et al., 2009; Boengler et al., 2011; Gray, 2015). The few genes left in mitochondria are highly conserved and appear to be under strong purifying selection (Mamirova et al., 2007; Popadin et al., 2012; Allen, 2015), probably due to the dangers associated with dysfunctional mitochondria.

The evolution of biparental and uniparental inheritance solve two different problems. The first transition, according to the hypothesis explored in our model, concerns organisms with a history of being clonal, currently in the process of domesticating a symbiont, and comprising of different cellular lineages losing different symbiotic functions due to high mutation rates and low population sizes. Under those conditions, biparental inheritance allows quick recovery of those functions via complementation. The second transition, on the other hand, presumably took place in sexual organisms in which most of the mitochondrial functions had been taken over by the nucleus, leaving mitochondrial genomes small, streamlined, and homogeneous. Such organisms experience a set of novel problems: how to best protect their established mitochondrial genomes against mutation (Radzvilavičius et al., 2017), to increase mito-nuclear co-adaptation (Hadjivasiliou et al., 2013), and to avoid the spread of selfish organelles (Hastings, 1992, Law & Hudson, 1992, Hadjivasiliou et al., 2013).

Conclusion

Our model is a proof-of-principle for a potential evolutionary pathway taking protoeukaryotes, hosting protomitochondria, from a clonal life cycle to a life cycle involving cell-cell fusion. This endpoint is still far removed from the putative state of our Last Eukaryotic Common Ancestor. Nuclear sex, uniparental inheritance, and a small and streamlined mitochondrial genome still had to evolve — which has been the focus of most models of eukaryogenesis. Cell fusion itself, however, remains a puzzle. Why mix cytoplasm, allowing biparental inheritance of mitochondria, if biparental inheritance is counterselected in extant organisms, and mechanisms allowing transfer of genetic material without cytoplasmic mixing were possible? Our model suggests mitochondrial complementation could have played a role.

Code availability

The full R code running the model and producing the figures is available freely at: https://github.com/Redsiana/Escape-from-mitochondria

As well as on Dryad: <u>https://doi.org/10.5061/dryad.j331mj8</u>

Authors' contributions

A.T. designed the project and implemented the model, A.T., J.R.C. and H.K. analysed the model and wrote the paper.

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APPENDIX CHAPTER II


Fig. S1 Fixation success of a fusing allele, according to different complementation functions and rates of mitochondrial turn-over. The logarithmic colour scale denotes the highest rate of fusion able to fix in a population, for a given combination of mitochondria number and relative reproductive success of fusers. The lowest rate tested in simulations was 0.005. Daughter cells inherit mitochondria from their mother cell either via a sampling procedure with replacement (simulating a high mitochondrial turn-over, and increasing mother-daughter variance in cytoplasmic content) or without replacement (low mitochondrial turn-over, low variance). The different complementation functions used are

A) $\phi(x) = 1 - K (1 - 2\frac{i}{M})^2$ with K = 0.3,

B)
$$\varphi'(x) = \left(\frac{K}{1+e^{-B\left(\frac{i}{M}-h\right)}} + b\right) \times \left(\frac{K}{1+e^{B\left(\frac{i}{M}-h\right)}} + b\right)$$
 with $K = 0.3$, $B = 100$, $h = 0.2$, $b = 1-K = 0.7$,

C) $\varphi'(x)$ with K = 0.3, B = 100, h = 0.4, b = 1-K = 0.7,

D) $\varphi'(x)$ with K = 0.4, B = 100, h = 0.4, b = 1-K = 0.6



Relative reproductive rate of fusers (1-c)

Relative reproductive rate of fusers (1-c)

36

Fig. S2 **Fusion increases the mean fitness within the population.** Note the only exception found, on panel D, without replacement, where a minor decrease was recorded – this area is also associated with an increase in variance (Fig. S4). The Before / After panels correspond to before and after the evolution of cell fusion. "+" signs indicate when variance increased compared to what it was in the asexual population; variance decreased where there is no sign. Daughter cells inherit mitochondria from their mother cell either via a sampling procedure with replacement (simulating a high mitochondrial turn-over, and increasing mother-daughter variance in cytoplasmic content) or without replacement (low mitochondrial turn-over, low variance). The different complementation functions used are the same as in Fig. S1 (see legend)



Relative reproductive rate of fusers (1-c)

Relative reproductive rate of fusers (1-c)

Fig. S3 **Fusion increases the mean heteroplasmy within the population.** Note the only exception found, on panel D, without replacement, where a minor decrease was recorded – this area is also associated with an increase in variance (Fig. S5). The Before / After panels correspond to before and after the evolution of cell fusion. The heteroplasmy of a cell was calculated as 1-|1-2i/M|, with i the number of mitochondria of type A among the M in the cytoplasm of that cell. Therefore it ranges from 0 (homoplasmic, i.e. the cell contains either 0 or 100% of type A mitochondria) to 1 (maximally heteroplasmic, i.e.the cell contains 50% of type A and 50% of type B mitochondria). "+"signs indicate when the mean increased compared to what it was in the asexual population; the mean decreased where there is no sign. Daughter cells inherit mitochondria from their mother cell either via a sampling procedure with replacement (simulating a high mitochondrial turn-over, and increasing mother-daughter variance in cytoplasmic content) or without replacement (low mitochondrial turn-over, low variance). The different complementation functions used are the same as in Fig. S1 (see legend)









Relative reproductive rate of fusers (1-c)

Relative reproductive rate of fusers (1-c)

Fig. S4 **Fusion impacts the fitness variance within the population.** The Before / After panels correspond to before and after the evolution of cell fusion. "+" signs indicate when variance increased compared to what it was in the asexual population; variance decreased where there is no sign. Daughter cells inherit mitochondria from their mother cell either via a sampling procedure with replacement (simulating a high mitochondrial turn-over, and increasing mother-daughter variance in cytoplasmic content) or without replacement (low mitochondrial turn-over, low variance). The different complementation functions used are the same as in Fig. S1 (see legend). Note that on panel D without replacement, the area for which a minor decrease in mean fitness was recorded (Fig. S2) is also associated with a decrease in variance.



Relative reproductive rate of fusers (1-c)

Relative reproductive rate of fusers (1-c)

Fig. S4 **Fusion impacts the heteroplasmy variance within the population.** The Before / After panels correspond to before and after the evolution of cell fusion. The heteroplasmy of a cell was calculated as 1-|1-2i/M|, with i the number of mitochondria of type A among the M in the cytoplasm of that cell. Therefore it ranges from 0 (homoplasmic, i.e. the cell contains either 0 or 100% of type A mitochondria) to 1 (maximally heteroplasmic, i.e.the cell contains 50% of type A and 50% of type B mitochondria). "+" signs indicate when variance increased compared to what it was in the asexual population; variance decreased where there is no sign. Daughter cells inherit mitochondria from their mother cell either via a sampling procedure with replacement (simulating a high mitochondrial turn-over, and increasing mother-daughter variance in cytoplasmic content) or without replacement (low mitochondrial turn-over, low variance). The different complementation functions used are the same as in Fig. S1 (see legend). Note that on panel D without replacement, the area for which a minor decrease in mean heteroplasmy was recorded (Fig. S3) is also associated with a decrease in variance.





Sampling without replacement

Number of mitochondria per cell

44

Fig. S6 **The initial fitness advantage a fusing mutant gets in a population of non-fusers is a good predictor of the rate of fusion that will be able to fix (Fig.S1).** The fusing advantage (y-axis) is a function of the number of mitochondria per cell (x-axis); insets are a zoom into the region spanning 2-20 mitochondria. Daughter cells inherit mitochondria from their mother cell either via a sampling procedure with replacement (simulating a high mitochondrial turn-over, and increasing mother-daughter variance in cytoplasmic content) or without replacement (low mitochondrial turn-over, low variance). The different complementation functions used are the same as in Fig. S1 (see legend). The initial fitness advantage is calculated as the relative viability of a cell which would mix with a randomly selected partner, compared to one that wouldn't, in a population of asexuals in segregation-selection equilibrium. The graphs are good predictors of the results obtained in Fig. S1.



S7.

Fig. S7 Using an asymmetric fitness function decreases the parameter space where fusion can evolve. Even when fusion would be a beneficial trait, the mitotype associated with the least deleterious fitness effects often fixes in the population before fusion has time to spread. We use a symmetric function throughout the paper based on the assumption that between two clicks of Muller's ratchet, the predominant class of symbionts is the least-mutated, fittest one, but that this class may comprise different mitochondrial lineages that accumulated different deleterious mutations, rendering complementation possible. Mitochondrial sampling procedure: with replacement

CHAPTER III What does the geography of parthenogenesis teach us about sex ?

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Abstract

Theory predicts that sexual reproduction is difficult to maintain if asexuality is an option, yet sex is very common. To understand why, it is important to pay attention to repeatably occurring conditions that favour transitions to, or persistence of, asexuality. Geographic parthenogenesis is a term that has been applied to describe a large variety of patterns where sexual and related asexual forms differ in their geographic distribution. Often asexuality is stated to occur in a habitat that is in some sense marginal, but the interpretation differs across studies: parthenogens might predominate near the margin of the sexuals' distribution but might also extend far beyond the sexual range, they may be disproportionately found in newly colonizable areas (e.g., areas previously glaciated), or in habitats where abiotic selection pressures are relatively stronger than biotic ones (e.g. cold, dry). Here we review the various patterns proposed in the literature, the hypotheses put forward to explain them, and the assumptions they rely on. Surprisingly few mathematical models consider geographic parthenogenesis as their focal question, but all models for the evolution of sex could be evaluated in this framework if the (often ecological) causal factors vary predictably with geography. We also recommend broadening the taxa studied beyond the traditional favourites.

Keywords: evolution of sex, local adaptation, colonization, clonality, species range

Introduction

The term "parthénogenèse géographique" was coined in 1928 by Albert Vandel (Vandel, 1928), a French zoologist and early biospeleologist (i.e. a researcher of cave-dwelling organisms). His work replaced the earlier concept of "geographic spanandry" — a term used to describe that males of some species became rarer with higher latitudes. In the arthropods he studied, Vandel noticed that spanandry was due to the increasing prevalence of obligate parthenogenetic forms of the same morphospecies, making the phrase "geographic parthenogenesis" a more precise explanation for the rarity of males. He recognized that the latitudinal pattern, which he found in some species but not others, was but an instance of geographic parthenogenesis in general, that he defined as a phenomenon where a sexual and a parthenogenetic form of the same species occupy distinct geographic areas, though with potential overlap (Vandel, 1928). Modern definitions concern more broadly asexual organisms that have different distributions from their closest living sexual relatives (a pragmatic solution to the problem that species concepts enter a zone of ambiguity once some lineages are asexual).

Since then, other trends have been proposed to the distribution of asexuals compared to their sexual counterparts, and all appeared for the first time in 1978 in a paper by Glesener & Tilman (1978). Modern introductions to the phenomenon of geographic parthenogenesis often focus on a co-occurrence of three patterns (e.g. Hörandl, 2009; Verhoeven & Biere, 2013): first, parthenogens tend to have a wider distribution than their sexual counterparts Hörandl, 2009; second, they tend to occupy higher latitudes (mostly studied in the northern hemisphere ; Bierzychudek, 1985, but see Kearney, 2003 and Buckley *et al.*, 2009), and third, they tend to occur in higher altitudes (Kearney, 2005). In addition to the above broad patterns, authors proposed a disproportionate occurrence of parthenogens in arid habitats compared to their sexual counterparts (e.g. Kearney, 2003, and Uzzell & Darevsky, 1975 in Australian lizards), on islands or island-like habitats (e.g. Cuellar, 1977), or 'disturbed' habitats (Vrijenhoek & Parker, 2009), which may or may not be associated with humans (see Hoffmann *et al.*, 2009).

Intriguingly, one of the early patterns proposed by Glesener & Tilman (1978), namely that parthenogens disproportionately occupy continental rather than maritime regions, has disappeared from the literature. Their statement concerning a trend to drier habitats appears to be based on one single example, *Trichoniscus elisabethae*, cited in Vandel (1928) and Suomalainen (1950), where a parthenogen was found both in the coldest and driest edges of the sexual distribution. To this day it is difficult to judge to what extent aridity is a general trend among parthenogens (see section 3.1).

Some sexual and asexual pairs do not follow any of the trends listed above (e.g. Toko *et al.*, 2006; Grismer & Grismer, 2010), or even show an opposite pattern (e.g. Tanaka *et al.*, 2014). It is difficult to estimate what fraction of geographic parthenogenesis cases they represent, as exceptions may receive less attention than cases that confirm the expectations, ending up being ignored in synthetic works and reviews on the topic (e.g. Kearney, 2005; Lundmark & Saura, 2006). While some authors implicitly use "geographic parthenogenesis" to specifically refer to the occurrence of patterns in the expected (directional) direction, we will here stick to the broad definition given by Vandel which includes any kind of geographic difference in reproductive mode.

A MARGINAL HABITAT?

Overall, the geographic margins of a species distribution, potentially occupied by parthenogens, are often equated with ecological marginality, and various authors expand differently on the term. Descriptions include low-stability, transient or disclimax habitats (Cuellar, 1977; Bell, 1982; Lynch, 1984; Levin, 1975), metapopulation structure with colonization-extinction cycles (Vrijenhoek, 1985; Haag & Ebert, 2004), low amount or diversity of resources (Glesener & Tilman, 1978; Gaggiotti, 1994), 50

low population productivity (Peck *et al.*, 1998), low density (Baker, 1965; Hörandl, 2006), high openness and habitat vacancy (Levin, 1975, Kearney *et al.*, 2009), and higher abiotic than biotic selection-pressure (Glesener & Tilman, 1978).

The diversity of characterizations above highlights a problem: it is difficult to pinpoint the precise meaning of 'marginal' habitats, given that most reports of geographic parthenogenesis are purely descriptions of sexuals and asexuals' range. It should be obvious that numerous biotic and abiotic factors will vary along the way from the 'core' to the 'margin', which gives a researcher perhaps too much freedom to focus on any one dimension of variation. Explaining species distribution in general has been a long-standing question in ecology, but field evidence of the factors limiting any species boundaries are still surprisingly scarce, lagging well behind theory (Sexton *et al.*, 2009). In the case of geographic parthenogens, it is difficult to judge which definition of 'marginality' is likely to suit most systems. Only in recent years have studies begun to characterize the actual niche differences between sexuals and asexuals in some model species. For instance, Schmit *et al.* (2013) suggested that sexual *E. virens* persisted only in ponds with a sufficiently unpredictable hydroperiod, whereas asexuals dominated more stable ponds; and Verduijn et al. (2004) identified microecological preferences of sexual and asexual dandelions which might explain their large-scale distribution, considered a typical instance of geographic parthenogenesis.

Finally, the notion of marginality, either geographic or ecological, is difficult to defend in species where asexuals occupy a much wider geographic area than sexuals (e.g. buttercup *Ranunculus auricomus*, Hörandl, 2009; weevil *Otiorhynchus scaber*, Stenberg *et al.*, 2003). Larger ranges seem instead more consistent with the second feature of many areas occupied by parthenogens: they have been more recently opened to colonization. This argument extends over variable timescales, from habitats recently created by humans (e.g. ponds, Schmit *et al.*, 2013; or forest tracks, Foucaud *et al.*, 2009), to islands (Cuellar, 1977), and, most notably, to zones that became progressively available after the last ice age (Kearney, 2005; Suomalainen, 1950). Note that longitudinal differences in distribution are not considered a typical pattern of geographic parthenogenesis, but as emphasized by Bell (1982), they can nonetheless reflect the direction the climate envelope moved, opening new habitats in its wake. Distance from the closest glacial refugia might in this respect be the relevant metric, with differences in latitude being only one of its most obvious correlates.

This debate points at interesting differences between explanations that all at first, appear superficially to be simply different manifestations of the term 'marginal'. There are aspects of the pattern that encourage researchers to seek ecological explanations inherent to current habitat, while others emphasize the importance of temporal aspects of the evolutionary history.

TO WHAT EXTENT DO THE PARTHENOGENS SHARE COMMON FEATURES?

Leaving aside the definitional minefield of prokaryotic sex or asex (see Cohan & Aracena, 2012; Redfield, 2001; Lehtonen & Kokko, 2014), examples of parthenogenesis can be found in all major groups of eukaryotes. Given that parthenogenesis is a derived trait (sex in eukaryotes being ancestral), it is unsurprising that it does not manifest itself in the same way in all instances. Broadly, parthenogenesis is defined as a form of asexual reproduction where the zygote derives from an unfertilized female gamete (Rice, 2009). It thus contrasts with selfing, where fertilization occurs between gametes produced by the same individual (e.g. in plants Grossenbacher *et al.*, 2015; animals, Casu *et al.*, 2012; fungi, Yun et al., 1999), and with vegetative reproduction, where the new individual stems from a collection of somatic cells which usually results in relatively lower dispersal compared with the production of zygotes (e.g. plants, Richards, 1997; animals, D'Souza & Michiels, 2009; fungi, Saleh *et al.*, 2012; algae, Tatarenkov *et al.*, 2005).

The founder of the term geographic parthenogenesis himself remarked that a northerly distribution of asexuality was probably not restrained to parthenogenetic species: Vandel cites instances of aquatic angiosperms that reproduce solely via bulbils in the North of Europe (Vandel, 1928). However, vegetative reproduction is often ignored in discussions concerning the evolution of sex, thus the parallel drawn by Vandel has been left largely unexplored. Yet, selfing, vegetative reproduction and parthenogenesis share some common traits; and conversely, there is much diversity among parthenogens themselves. One instance is that the well-known demographic cost of male production (the twofold cost of sex) does not apply to hermaphrodites, for which the cost of sex results from a different process (Lehtonen *et al.*, 2012).

The origins of parthenogenesis are not always known. Some cases have been linked to the action of bacterial endosymbionts (many arthropods, Huigens & Stouthamer, 2003); parthenogenesis-inducing mutations have been identified in a few cases (e.g. in *Daphnia*, Lynch *et al.*, 2008; aphids, Jaquiéry *et al.*, 2014; some angiosperms, Bicknell & Koltunow, 2004); and large-scale genomic events are often suspected to have either directly or indirectly caused the evolution of parthenogenesis (Cuellar, 1977). In many lineages, parthenogenesis is indeed associated with past hybridization, often along an increase in ploidy (Vandel, 1928; Cuella, 1977). A few autopolyploid lineages (i.e. polyploids resulting from genome duplication) are also found to be parthenogenetic. Note that polyploidy is a frequent feature of selfing plants too (Barringer, 2007).

Those correlates can have different implications for the fitness of parthenogens (see section 2). Moreover, the developmental routes that have evolved to circumvent standard meiosis are also diverse, and this can lead to differences in key genetic and ecological properties of parthenogens (Stenberg & Saura, 2009), as discussed further.

Reproduction with no need for a partner is a feature of vegetative reproduction, self-fertilization and many forms of parthenogenesis, but not all: cases exist where zygote development requires sperm or pollen as a trigger of embryogenesis, but the offspring still develops parthenogenetically as the sperm or pollen makes no genetic contribution. This quirky system is known as gynogenesis in animals (Schlupp, 2005) and pseudogamy in plants (most parthenogenetic angiosperms, Hörandl, 2010).

Clearly, geographic distributions of the parasitic asexual form cannot expand past their sexual hosts, when parthenogens strictly rely on a male function as a developmental trigger. Still, some pseudogamous species are very widespread, thanks to one of two tricks. First, the absence of karyogamy can make the spectrum of suitable host species wider, allowing the parthenogens to expand outside of, and exceed, their original sexual parents' range (e.g. earthworm Lumbricillus lineatus, fish Poecilia formosa (Lynch, 1984); see also Schwander & Oldroyd (2016) for an invasive hermaphroditic clam that uses and rogenesis: sperm 'hijacks' eggs produced by other hermaphrodites which then develop as clones of their father, as the maternal genome is eliminated). Second, hermaphrodites can combine the production of parthenogenetic eggs and sperm, which then can be used to trigger parthenogenetic reproduction in either other conspecifics (e.g. sperm-trading planarians, D'Souza & Michiels, 2009) or themselves (self-compatibility commonly evolves in pseudogamous plants, Hörandl, 2010). Interestingly, the former category frees the parthenogens from the constraint of having to coexist with the parental sexual species, which can be ecologically a very difficult form of coexistence (Lehtonen et al., 2013). Instead, reproduction is now dependent on the presence of conspecific parthenogens: it is therefore a rare case of parthenogenesis without the capacity for fully uniparental reproduction. The latter category is demographically extremely similar to self-fertilization (selfing), though with potential genetic differences.

Selfing is an extreme form of inbreeding, which eventually leads to complete homozygosity. Some forms of parthenogenesis similarly involve the ploidy-restoring fusion of two products of meiosis, and

if the fusion occurs late in the process of oogenesis (terminal fusion automixis, Nougué *et al.*, 2015), the genetic consequences of parthenogenesis and selfing are identical. Total homozygosity, on the other hand, can instantly occur in lineages using gamete duplication to restore ploidy (Stenberg & Saura, 2009). Some other types of asexuality will typically 'freeze' the levels of heterozygosity in a clonal lineage. This happens in vegetative reproduction, as the propagule contains somatic cells, and also in some forms of parthenogenesis. Two types of parthenogenesis can achieve this: in central fusion automixis the two products of the reductional division of meiosis fuse to restore ploidy, while in apomictic parthenogenesis meiosis is totally suppressed, which makes parthenogenesis functionally mitotic (note that this statement uses a definition different from the broad botanical use of the term 'apomixis' where it refers to all forms of asexual production of seeds, van Dijk, 2009). Apomictic parthenogenesis that, instead, use fusion of meiotic products, recombination can still occur. As this results in erosion of heterozygosity, some lineages are thought to have evolved to suppress recombination for this reason (e.g. Altiero *et al.*, 2015), yet this might also provide ways of purging deleterious mutations (Neiman & Schwander, 2011).

On top of intra-individual genetic diversity, population-level diversity also varies widely in parthenogens: it can go all the way from a unique clonal genome (e.g. Caron *et al.*, 2014) to a diversity higher than sexuals (e.g. in Collembola: Niklasson *et al.*, 2000). Intriguingly, some species comprise single clones over very large areas (e.g. millipede *Nemasoma varicorne* in Denmark, Hoy Jensen *et al.*, 2002), while in others, each clonal genotype occupies a very narrow range (e.g. *Ranunculus auricomus*, Hörandl, 1998). This diversity can be ancient and stem from several independent origins. Alternatively, the generation of new asexual genotypes can still be ongoing, for instance via mutation (Birky & Barraclough, 2009), continuing hybridization of, or with, the sexual parents (Lutes, 2011), contagious asexuality via endosymbiont transmission (Huigens *et al.*, 2000) or rare crossings with sexuals (e.g. in hermaphrodite flatworms, D'Souza & Michiels, 2009; in *Daphnia*, Paland *et al.*, 2005; and in *Artemia* due to rare parthenogenetic sons, Maccari *et al.*, 2014), or forms of "parasex" (Schwander, 2016) such as horizontal gene transfer between individuals (bdelloid rotifers, see Debortoli *et al.*, 2016) or introgression of environmental DNA (anhydrobiotic rotifers or tardigrades, Gladyshev & Arkhipova, 2010).

If, as seems to be the case, asexuality comes in different 'flavours' – species can be found that are various combinations of polyploid, hybrid, host of manipulative symbiont, autonomous or sperm-dependent, relatively homo- or heterozygous, with low or high genetic diversity – then it may be hard to predict clear rules for geographic parthenogenesis that could be repeatably observed across taxa. It is therefore no wonder that it has been difficult to find a unifying explanation for the phenomenon.

Explanations for the main patterns of geographic parthenogenesis

We now turn our attention to published arguments (both verbal and mathematical) to explain the geographic distribution of parthenogens in higher altitudes, latitudes, islands, disturbed environments, or over a larger area than their sexual relatives. Table 1 lists mathematical models that either directly address the phenomenon or that include a conclusions section where the authors discuss the model's implications for geographic parthenogenesis. Given the diversity we have discussed in the preceding section, it is clear that none of the formal nor verbal models apply to all types of parthenogens, nor to all potential patterns that have been discussed in the literature. One can nevertheless identify what the ultimate goals should be, aside from scenarios where sexuals might not reach certain areas in the first place, while asexuals, once they have emerged, can. Any model should

explain : (i) why sexuals are not outcompeted to extinction due to the various costs associated with sex; and (ii) conversely, once it predicts that sexuals can persist, why they do not do so throughout the entire range. In other words, it should address the two sides of the coin: why sex (here), and why asex (there)? Intriguingly, the default state, and hence the question asked, subtly differs between models. Some assume a baseline demographic cost to sex (Table 1, 'Democost' \geq 2), and by proposing a counteracting advantage, they look for conditions where this advantage is realized sufficiently to favour sex. On the other hand, some models take the overall superiority of sex for granted ('Democost' <1), and seek special properties of asexuality that confer it an advantage in some contexts, making the question of geographic parthenogenesis more about "why asex?" than "why sex?"

NEUTRAL MODELS REGARDING ASEXUALITY

In order to assess the explanatory power of adaptive hypotheses concerning the distribution of parthenogenesis, it is important to first consider expectations under a neutral model that does not resort to benefits or costs of asexuality *per se*, but to spatially varying probabilities of appearance (Glesener & Tilman, 1978; Suomalainen, 1950) or persistence (Kearney, 2005; Lynch, 1984) of new asexual lineages.

The environments where asexual lineages originate could offer elements of explanation for their current distribution, if the probability of transition from sexuality to asexuality varies spatially. Hörandl (2009) describes a scenario of 'opportunistic geographic parthenogenesis': climate change can make species ranges split, contract or expand, which then can create opportunities for hybridization, which in turn are known to have played a role in the origins of many parthenogenetic species (e.g. direct experimental evidence: Schultz, 1973; genetic inference: Kearney *et al.*, 2009). Moreover, cold spells, variable temperatures and water stress can sometimes trigger the production of unreduced gametes (Ramsey & Schemske, 1998). If climate impacts the transition rate to asexuality, 'hotspots' for parthenogens can be predicted to be in areas with fast-paced climate change in the recent evolutionary past. Elevated occurrence of asexual forms in previously glaciated areas, in particular, has been argued to support this idea.

Table 1 (opposite). Mathematical models of geographic parthenogenesis differ in their assumptions about parthenogens and in their ecological outcomes. Notation: YES: explicit focus of the model, Y: model implicitly applies to it, ~: model would need modifications to include this assumption, N: model structure conflicts with this assumption. First 3 columns concern the assumptions made regarding parthenogens' degree of uniparentality (autonomous/pseudogamous/pseudogamous hermaphrodite), the next two columns indicate assumptions regarding homozygosity (apomictic/automictic). The 'sexes' column indicates whether the model assumes a gonochorous species (Q+d) or an outcrossing hermaphrodite (q^* (out)). The 'democost' column indicates the reproductive output of asexuals compared to sexuals in the model; 2 means that total fecundity is the same but: in a gonochoristic context the number of female offspring is doubled; in a hermaphroditic context male function resources are reallocated to female function. Models also differ in their assumptions about spatial structure, mating among sexuals (or possibility for crosses between asexual male function and sexual females), dispersal, and clonal diversity, as indicated. The final columns include whether clonal diversity is a requirement of the mechanism leading to a geographic parthenogenesis pattern (column 'diversity required?'), what this mechanism is, where the asexuals are predicted to prevail, whether coexistence of sexuals and asexuals is possible, and the temporal pattern predicted.

Paper	Autonom.	Pseudog.	Pseudog + hermaphro.	Apomictic	Automictic	Sexes	Democost	Space	Mating	Dispersal	Clonal diversity	diversity required ?	Mechanism leading to geographic parthenogenesis	Habitat of asexuals?	coexistence?	Stable ?
Variability and competition for resources																
Gaggiotti, 1994	YES	Y	Y	Y	Y	♀ (out)	[1;2]	Patches with heterogeneouscompeti tion	random (space, genotype)	random	Implicit, lower than sexuals	N	Competitive asymmetries	lower competition / more structured resource / lower diversity of sexuals	partial asexual refugium	stable
Weeks, 1993	YES	Y	Y	Y	Y	⊄ (out)	2	1 patch, stable or fluctuating resource	random (no space)	NA	Explicit (ongoing lineage generation)	Y	Fluctuating environment favours phenotypic diversity	more stable resource	dynamic equili	brium
Scheu et al., 2007	YES	Y	Y	Y	Y	₽+ď	2	1 patch, regrowing resources	random (no space)	NA	Explicit (ongoing lineage generation)	Y	Competitive asymmetries	lower competition = higher mortality / more stable resource / regrows faster / larger pop	Ν	stable
Song et al., 2011	YES	Y	Y	Y	Y	₽+ď	2	continuous, latitudinal gradient in resource / death rate	local, random	short-distance, random (migration rate affects coexistence)	Explicit (ongoing lineage generation)	Ν	Competitive asymmetries	lower competition = higher mortality / more stable resource / regrows faster / larger pop	Y (for intermediate migration rate)	stable
Barrier against outbreeding																
Peck et al., 1998	YES	Y	Y	Y	~ (਼ਰੀ (out)	[0.7 ; 1[discrete, South-North gradient of productivity	local, random	random	Low (ongoing lineage generation and selection)	Y/N	Outbreeding depression of sexuals	sink habitat	Y (gradient)	stable
Mutation accumulation in space																
Salathé et al., 2006	YES	N	Y	Y	~ (਼ਰੀ (out)	2	Homogeneous grid	local, random	short-distance, random	Deleterious mutation accumulation	Ν	Mutational meltdown prevents invasion of large pop	small pop	Ν	stable
Contagious asexuality																
Britton & Mogie, 2001	YES	N	~	Y	N	਼ਰਾ (out)	<1	Continuous, homogeneous	local, random; asexual ♂ X sexual ♀	local (diffusion)	NA	N	Contagious asexuality	newly opened (verbal extrapolation)	yes (long-term)	slow asexual spread

Emergence must be followed by persistence for geographic parthenogenesis to be observable. A newly arisen parthenogenetic lineage is surrounded by sexuals, and as a rare cytotype, is endangered by destabilizing hybridization with the parental sexuals (Lynch, 1984; Levin, 1975). Persistence through this critical phase should be easier if an asexual lineage can exist in an area free of sexuals. Climate change provides newly opened environments, by driving the retreat of glaciers or deserts, or simply by shifting a species' suitable climatic envelope over new territories. Only those asexuals that happened to colonize them (by chance, as under the neutral model, they are not better colonizers *per se*) remain observable today. Boundaries could then get stabilized by evolution of reproductive barriers, or a lethal hybrid zone equally detrimental to sexuals as asexuals (Kearney, 2003; Lynch, 1984; Levin, 1975).

Geographic differences arise in this 'neutral' model because asexuals are simply assumed to persist better when they are not coexisting with sexuals, and the two types do not reach all areas. This hypothesis does not require differences in dispersal capacity between reproductive modes, but a limited capacity of both: in the absence of differences in competitive ability, the establishment patterns are expected to maintain significant geographic structure only if areas are not continually bombarded by sustained invasion efforts of each type. This hypothesis also relies on the fact that transitions from sexuality to asexuality are much more frequent than the reverse.

MODELS IN WHICH CORRELATES OF PARTHENOGENESIS RATHER THAN

PARTHENOGENESIS ITSELF MATTER

There is also a class of models that, unlike the null models, accept that asexuals and sexuals can differ, but retain the view of the null models that asexuality per se might not be the driver behind patterns of geographic parthenogenesis. Instead, these models focus on traits that covary with asexual reproduction: polyploidy and hybridity (Cuellar, 1974). The former was already present in the writings of Vandel (1928) and Suomalainen (1950), while the latter remained little discussed until Kearney et al. (2005). While hybridization between two species or genetically distant populations is usually deleterious (Johnson, 2008), hybrids sometimes enjoy hybrid vigour and are fitter than either parent (Chen, 2013; Pekkala et al., 2012). If associated with parthenogenesis (itself possibly a direct outcome of hybridization, Schultz, 1973), the fitness advantage will be conserved. Similarly, polyploidy is argued to confer a set of advantages such as larger cells, more gene expression, less sensibility to deleterious mutations and more evolutionary potential thanks to gene redundancy (Comai, 2005), which might provide an advantage in colonizing harsher environments (te Beest et al., 2011), although this argument should not be taken at face value. For instance, in a species of Japanese ferns, sexuals are found at higher latitudes, altitudes, and colder places than parthenogens, as the latter's larger, polyploid cells are more sensitive to freezing due to their high water content (Tanaka et al., 2014).

Because most asexual species featured in the geographic parthenogenesis literature are both hybrid and polyploid, disentangling these effects is tricky. This triggered a debate that appears to have ended without a resolution, in the absence of any clearer way to cut than a few available counter-examples in both camps (Kearney, 2005; Lundmark & Saura, 2006; Kearney, 2006). Of course, there is no reason why the two hypotheses should be mutually exclusive (while it remains a valid question which one might be the stronger driving force).

It is clearly instructive to look at parallel questions asked in purely sexual lineages, as hybridity and ploidy changes can both occur without a transition to asexuality. Here, studies focusing on range size have given mixed results. The effect of diploid hybridity seems unpredictable (review in Rieseberg & Wendel, 1993). Autopolyploidy (i.e. genome duplication) has been stated to lead to larger ranges than diploid parents in plants (van Dijk & Bakx-Schotman, 1997; but see Hörandl, 2006, for an opposite

view), but we know of no formal meta-analysis of the question. Allopolyploidy, i.e. hybrid polyploidy, does not seem to correlate to larger ranges either (Brochmann *et al.*, 2003), but intriguingly, two extensive studies in plants show it is more frequent in high latitudes (Brochmann *et al.*, 2004) and in invasive plant species (Pandit *et al.*, 2014). The abundance of polyploid morphs in northern biota, termed 'geographic ploidy', was already an object of speculation by Vandel (1928).

Of course, parthenogenetic reproduction may interact with the above advantages. If asexuality helps stabilize a hybrid or polyploid genome against meiotic disturbances or deleterious introgression, it could allow a species to make the most of the advantages of either (Hörandl, 2006). As a whole, one could imagine fruitful ways to bring some new light into the debate by focusing on taxonomical groups where parthenogenesis occurs without hybridity or polyploidy, for instance in certain haplodiploid insects and mites made parthenogenetic by an endosymbiont (Stouthamer, 1997; Weeks & Breeuwer, 2001), or diploid scale insects (Ross *et al.*, 2010). These are largely absent from the geographic parthenogenesis literature so far.

UNIPARENTALITY AND ITS EFFECT ON COLONIZING ABILITIES

We now turn our attention to ideas involving demographic effects of parthenogenesis. Uniparentality is an obvious potential explanation for the increase in frequency of parthenogens the further away from the glacial refugia, in disturbed areas, islands, and marginal ecological niches. All else being equal, the ability to establish a population from a single individual predicts improved colonizing abilities, and there are two components to asexuality that can help: reproductive assurance, which is the ability to reproduce without finding a mate, and demographic advantage, incurred if parthenogens avoid paying the twofold cost of sex. This is an extreme form of the general demographic principle that female-biased populations can invade faster (Miller & Inouye, 2013; Freedberg & Taylor, 2007). Note that dispersal ability *per se*, i.e. the ability to cover (and survive) a certain distance, need not differ. To our knowledge no difference in dispersal abilities between asexual and sexual propagules has been reported, apart from the obvious differences between short-distance vegetative (asexual) spread and the longer-distance spreading of (sexual or asexual) seeds. Potential differences should be investigated more closely, as dispersal in time and space can provide an alternative to sex in dealing with parasites and environmental uncertainty (Wilson, 2011).

Reproductive assurance is only advantageous in cases where sexuals suffer from a low density of potential mates (mating Allee effect, Gascoigne *et al.*, 2009): for instance in newly open, resource-poor or high mortality habitats, areas with a low availability of pollinators, or a short growing season (e.g. Kramer & Templeton, 2001, in mixed populations of sexual and asexual *Drosophila*). The disproportionate presence of asexual plants in newly deglaciated areas compared to their sexual parents (Brochmann *et al.*, 2003), suggests that the wave of recolonization from glacial refugia was largely dominated by asexuals (Hörandl, 2009). The temporal dynamics are obviously of interest here. This is a challenging research question as it is far easier to gain access to 'snapshot' data than long time series. Even so, cases have been documented where sexuals are catching up on asexuals (Cuellar, 1977, in cricket *Saga pedo*; Soreng & Van Devender, 1989, in grass *Poa fendleriana*), lending support to the idea that asexuals were simply faster in colonizing. Theoretically, the opposite can happen with contagious asexuality, which can make the distribution of sexual population shrink over time (e.g. Paland *et al.*, 2005, in *Daphnia pulex*; Britton & Mogie, 2001).

As in section 2.2, it is useful to draw parallels with fully sexual systems. In selfing plants, "Baker's law" (Baker, 1955; Stebbins, 1957) was formulated to refer to the enrichment of self-compatible species in island ecosystems. A review of the geographic distribution of self-pollinating plants (Grossenbacher *et al.*, 2015) shows that selfing species consistently have larger ranges than their strictly outcrossing relatives. In a review of the true implications of this law, Pannell *et al.* (2015) show that uniparentality

is expected to be advantageous in four contexts, similar to those discussed in geographic parthenogenesis: colonization of remote places by long-distance dispersal (island-like habitats), range expansion, colonization by invasive species, and repeated colonization in metapopulations with frequent extinctions.

EARLY SELECTION ON CLONAL LINEAGES: FROZEN NICHES AND GENERAL PURPOSE GENOTYPES

In order to observe successful parthenogens, not every transition to asexuality has to succeed; it is sufficient that some do. As pointed out by Lynch (1984), as selection acts on some newly emerged asexual lineages, the survivors will be the ones fortuitously blessed with favourable combinations of traits (clonal selection). Compared to those highly fit asexuals, sexuals incur a genetic cost of sex, as it breaks beneficial allelic combinations. This has led to two hypotheses to explain ecological success of parthenogenesis provided many independent transitions within a lineage: the frozen niche variation, and the general purpose genotype. Empirical support for both hypotheses is synthesized in Vrijenhoek & Parker (2009).

First, the frozen niche variation hypothesis (Vrijenhoek, 1979) states that a diverse array of clones, all representing a different microspecies, can together occupy more niches than sexuals, as sex pulls the phenotypic distribution of the latter always back towards the mean (Weeks, 1993). Note that the name should not be taken to imply that the population dynamics are in any way 'frozen': e.g. in snails *Potamopyrgus antipodarum*, a diverse array of clones has been shown to resist to co-evolving parasites via successive frequency-dependent selection of different genotypes (Jokela *et al.*, 2003).

Second, the general purpose genotype hypothesis (Baker, 1965; Parker *et al.*, 1977) states that clonal selection in a fluctuating environment would favour a clone that freezes a polyvalent genotype, which allows it to survive many conditions (a possibility enhanced by hybridization, Lynch, 1984; Baker, 1965). If the asexual population consists of such generalists only, they are expected to be outcompeted by specialized sexuals (or specialized clones) wherever local conditions are stable enough (and dispersal is limited enough) for local adaptation to be important. However, in highly variable habitats at the margins of their competitors, general purpose genotypes would thrive while specialists go extinct (bet-hedging, see Starrfelt & Kokko, 2012). This echoes the vision of parthenogens as fugitive or 'weed' species (Baker, 1965; Wright & Lowe, 1968).

ECOLOGICAL CONDITIONS IMPACT THE RELATIVE BENEFITS OF SEX

In this section we review hypotheses that focus on how the benefits of sex might vary with ecology. Each hypothesis takes for granted a demographic advantage of asexuality (no cost of male production), and highlights how counteracting benefits of sex can vary spatially, tipping the balance in favour one or the other reproductive mode.

One of the strongest candidate theories so far to explain how sex persists despite its demographic costs is that of the Red Queen (Hartfield & Keightley, 2012). The permanent need to escape biotic (and hence evolving) challenges, such as parasitism or interspecific competition, drives an evolutionary arm race, in which non-recombining lineages cannot keep up. This led Glesener and Tilman (1978) to formulate their verbal biotic interaction model, where the distribution of parthenogens in marginal habitats is explained by the lesser parasitism, competition or predation they experience there (note that while they did not use the word 'marginal', their list of characteristics of habitat matches those discussed above in section 1.1). When the need to constantly adapt is removed, asexuals outcompete sexual lineages. An intriguing aspect of this hypothesis is the complex identity of 'marginality': it first assumes that marginal habitats are generally difficult to persist in, as this is clearly required to explain the absence of parasites, competitors or predators; thereafter, for those few species that do make it

there, life can become 'easier', i.e. requiring less continual adaptation, than it was in the core habitats. The gradient assumed here is that from core to marginal habitats, the selection pressures shift from biotic to abiotic.

While much of the Red Queen literature, being vast, is beyond the scope of this review, it is noteworthy that in some cases, there is geographic variation in the prevalence of parasites, and rates of sex have been shown to be indeed higher in parasitized zones (e.g. freshwater snails: King *et al.*, 2009; plants: Verhoeven & Biere, 2013; see also Busch *et al.*, 2004, for analogous results with outcrossing vs. selfing). But as noted in section 2.4, asexuality can present itself as a genetically diverse array of asexuals, and such a coexisting set can be as well-equipped to deal with parasitism as a sexual population, given alternating frequency-dependent selection that acts on the different clones (Jokela *et al.*, 2003).

The second prerequisite for a Red Queen interpretation of geographic parthenogenesis is that biotic interactions decrease with latitude. This was firmly thought to be the case for a long time, until recent meta-analyses over large geographical scales yielded conflicting results (Schemske *et al.*, 2009, versus Moles *et al.*, 2001, Moles *et al.*, 2011). However, some authors argue that such broad scale studies will miss the relevant patterns occurring within species (Johnson & Rasmann, 2011), as for instance the latitudinal gradient in plant-enemy interactions documented in the dandelion, a textbook example of geographic parthenogenesis (Verhoeven & Biere, 2013).

Resource competition is another context where sex is expected to be beneficial. The tangled bank hypothesis and its variations (Bell, 1982; Ghiselin, 1974) propose that the phenotypic diversity of sexuals enables them to share the resource space more efficiently than a homogeneous array of clones interfering with each other's success. This idea has led to the expectation that sexuals should prevail in habitats with diverse and structured resource, whereas the benefits of diversity vanish in simple habitats where all compete for the same resource, allowing asexuals to express their demographic advantage. The argument extends to habitats where disturbances are so frequent that they cannot develop much structure (Bell, 1982). This is, again, in line with a vision of parthenogens as fugitive species (Baker, 1965). Note that the above line of thought requires sexuals to be the more diverse population (see 1.2).

Resource diversity appears to have been the only idea to receive a substantial amount of modelling attention in the specific context of geographic parthenogenesis (Table 1). Gaggiotti's model (1994) assumes a coarse-grained environment (each individual experiences one type of resource) with dispersal between patches, and predicts persistence of either sexuals or asexuals depending on intraand interspecific competition coefficients. His model does not explicitly include genetic diversity, but outlines the rules of competitive interactions that allow for the persistence of each type. In biological terms, asexuals win when the diversity of resources is low (as everyone competes for the same thing), or when sexuals have a diversity low enough to lose their competitive advantage. Another series of papers (Scheu & Drossel, 2007; Song et al., 2011) explore the idea that sexuals benefit by being able to exploit underused resources. Resource use is determined by the match between genotype and environment, and the models explore how sexuals may be better at finding underused resources because offspring are not clones of their parents (shown for a single habitat: Scheu & Drossel, 2007; multiple habitats along a one-dimensional gradient: Song *et al.*, 2011). To simulate a change from the centre to the boundary of the species range, Song et al. (2011) impose a gradient in terms of death rate, variety of resource types, and/or resource re-growth rate. They find that asexuals (with the demographic advantage of no male production) are favoured by small population sizes, high death rates, and high resource growth rate, all of which decrease competition, as well as by the availability of fewer resource types. These factors all reflect their core assumption, that sexuals' diversity allows them to utilize resources that would otherwise remain unused.

The above models tend to assume, implicitly or explicitly, that asexuals exhibit lower phenotypic diversity than sexuals. It should also be kept in mind that the diversity within a reproductive mode can also depend on location: in a broad meta-analysis of 115 species of animals and plants (regardless of their reproductive mode), Eckert et al. (2008) found a decline in the diversity of neutral genetic markers towards the range margin in 64% of the studies they reviewed. Caution has to be exerted when extrapolating from neutral to non-neutral diversity (Kirk & Freeland, 2011), and the adaptive micro-niche variation on which relies the tangled bank hypothesis might be more resistant to drift than neutral markers; however a parallel decrease in polymorphism can still be expected, especially with a population history of founder effects. Finally, stability of the environment has received attention as an explanatory factor (see also section 2.4), but it is currently difficult to judge to what extent this explains geographic patterns. Not only is it challenging to provide generalizations about whether marginal habitats are less stable than core habitats: some habitats can vary much but do so in a relatively predictable manner (seasonality). In addition, asexuals have been predicted to fare better in either stable or in unstable habitats, depending on the definition. Stability has been argued to favour them because of their inability to adapt otherwise (theory: Weeks, 1993; empirical evidence: Becks & Agrawal, 2013). But unstable environments have likewise been argued to favour asexuals, either because they are a general-purpose genotype (see section 2.4) or because of an idea rooted in the r-K selection literature: environmental variation selects for fast growth rates (potentially achieved by avoiding the costs of sex) over competitive abilities (Cuellar, 1977; Bell, 1982; empirical evidence: Becks & Agrawal, 2013). The relationship between environmental stability, predictability and the prevalence of sex appears to be an area of much potential for future work, especially because species with facultative sex provide additional hints. Asexual life cycles of such species typically go on as long as conditions remain relatively unchanged, while deteriorating conditions lead to sex (e.g. cyclical parthenogens have sexually produced resting eggs that can withstand winter and/or drying out Decaestecker et al., 2009). Theoretical work in this area appears lacking, while empirically, Schmit et al. showed in *Eucypris* clams that pond hydroperiod mattered less than predictability in explaining the presence of asexuals (Schmit et al., 2013).

THE IMPORTANCE OF GENE FLOW

Sexual species can experience variations in gene flow and heterozygosity in a manner that has no direct analogy in parthenogenetic lineages (genes of course do 'flow' if parthenogens migrate from one area to another, but they do not enter new genetic backgrounds in the process). This has several potential consequences for geographic parthenogenesis.

Insufficient gene flow can lead to inbreeding depression, brought about by small population sizes or genetic bottlenecks. These are characteristic of invasion of new environments or metapopulations dynamics, with cycles of extinction and recolonization. In those contexts, apomictic parthenogens can be particularly good colonizers, as they travel with 'frozen' levels of heterozygosity, and can also benefit from reproductive assurance and high growth rate in the absence of strong competition (Vrijenhoek, 1985; Haag & Ebert, 2004).

Asexuality also provides protection against outbreeding depression. For instance, mating with immigrants can result in the loss of locally adaptive alleles (Lenormand, 2002); see Rossi & Menozzi (2012) for the only evaluation so far of outbreeding depression in a context of geographic parthenogenesis, but with inconclusive results. This is problematic in the context of asymmetric migration from a source to a sink environment. The matter is complex, however, because gene flow from the core to the margin can either facilitate adaptation of sexuals, by increasing the genetic variance for selection to operate on, or impair it, because of core-adapted genes swamping any local adaptation (Kawecki, 2008; Lagator *et al.*, 2014). To what extent asexuals can achieve local adaptedness depends on the frequency of transitions: if sexuality regularly gave rise to new asexual lineages, some

of them might be based on the locally best genotypes which they can then retain better than the sexuals; but if transitions to asexuality are rare (and if they first have to overcome problems with perfecting an asexual life cycle, Bengtsson, 2009), and asexuals have to reach new areas by dispersal instead of being newly created at the local site, achieving local adaptation via asexual means can be much harder.

Peck et al. (1998) built a model of outbreeding depression based on a landscape with an environmental gradient, where individual fitness depends on the degree of match between phenotype and environment, in the presence of short-distance dispersal as well as mutation (a mutation is required to create the first asexual individual; further mutations potentially enable its descendants to adapt locally). Population productivity is set to decline from an area called 'south' to the 'north', which yields asymmetric gene flow and prevents northern individuals from fully adapting to their environment (gene swamping). The model is able to produce a pattern where the north eventually becomes populated by locally adapted asexuals, which maintain higher fitness than sexuals by escaping the swamping. Peck et al.'s assumptions of a very large (three-fold) difference in productivity between the core and the margin likely contribute to the neatness of the pattern, but outbreeding depression preventing local adaptation is a reality in the wild (Kawecki, 2008). In an alga, Lagator *et al.* (2014) showed experimentally that both sex and migration on their own were beneficial in helping a sink population to adapt, but that asexuality did better than sexuality in the case of high immigration rates, by preventing outbreeding depression.

Lack of recombination might be a short-term benefit, but it becomes a problem for asexuals in the long run, which adds a temporal dimension to the above results. Muller (1964) pointed out that deleterious mutations fixing at a higher rate in the absence of recombination are a particularly severe problem for asexual lineages. This mutational meltdown will decrease their fitness compared to sexuals (Muller's ratchet). Space interacts with time to play a role in determining whether an asexual lineage with a demographic advantage will succeed in displacing a sexual parent before its fitness is decreased too much (Hartfield *et al.*, 20120, and references therein).

In this race against the clock, any factor that slows down asexual spread makes their fixation less likely. Salathé *et al.* (2006) modelled a situation where asexuals in principle have a superior growth rate, but because of short-range dispersal, they compete essentially among themselves. This prevents them from realizing fully their advantage and slows down their invasion (Table 1). Invading a large sexual population may therefore prove too much of a challenge for asexuals: they will eventually accumulate too many mutations before the invasion is complete, and the process ends with asexuals being outcompeted by sexuals. Salathé *et al.* (2006) state that their model fits patterns of geographic parthenogenesis if one takes small population. Note, however, that despite an explicit spatial model being included in their paper, it does not produce a spatial gradient from sex to asex; instead the pattern is the fixation of either, depending on parameter values. We suspect that showing simultaneous persistence of reproductive modes, in different regions of the simulated world, might require extending the spatial scale such that the dynamic processes can occur relatively independently in different parts of the world.

In another model featuring metapopulations, this time with clear between-deme structure, Hartfield *et al.* (2012) repeat many findings of earlier work (high levels of subdivision, with low migration and genetic flow between the demes, make asexual spread slow), and additionally connect the findings to explicit predictions of F_{ST} levels. They show that sex maintains at the metapopulation level only for high levels of F_{ST} .

While the models discussed here have not explicitly sought conditions of stable geographic patterns of parthenogenesis (the emphasis being on predicting which reproductive mode will eventually reach total fixation in the landscape), the outcomes often suggest that fixation over a large scale needs a long time. Given how little is known about the long-term stability of geographic parthenogenesis (see section 2.3), such outcomes are not irrelevant, and some published models give explicit temporal dynamics (see e.g. Fig. 4 in Salathé *et al.*, 2006).

Interestingly, invading a large population of sexuals can prove difficult, but once established, a large population of asexuals should resist mutational meltdown (and extinction) longer than a small one. Ross *et al.* (2013) found that in scale insects, asexuality is more common in polyphagous, pest, or widespread species, and turn the traditional causal argument around: instead of explaining their distribution by their reproductive mode, they propose that species that produce very large populations in the first place resist Muller's ratchet for longer, thus remaining observable today. In support of this direction of causation, they also report that sexual species of genera containing parthenogens have relatively large geographic ranges. These two predictions, that asexuals should be able to invade better small populations, but to persist better in large ones, highlight the importance of the time scale of invasion and persistence of asexuals (see also 2.3). They also provide additional reasons why the most favourable scenario for asexuals is to colonize a new environment: there, no sexual competitors will slow them down, nor outcompete them once mutational meltdown has reached critical levels (see also 2.1).

How much is known empirically about the above models' assumptions regarding inbreeding and outbreeding depression, metapopulation dynamics and mutational meltdown? In a review of sexual species' range limits over a broad taxonomic scale, Sexton *et al.* (2009) found mixed support for marginal populations being smaller or exhibiting lower fitness or density. Gaston (2009) in his review of the literature finds stronger support for systematic core-to-edge variation in levels of occupancy than in local population density or size. Once more, it would be invaluable to monitor the contemporary spread of asexual species, and possibly the recovery of sexuals, and gather data on population dynamics, structure, and on chronology.

Synthesis of the hypotheses

Geographic parthenogenesis does not occupy the centre of the stage when it comes to theoretical questions surrounding sex. Perhaps it should, given that it is discussed as one of the few settings to provide repeatable clues as to which of sex or asex wins (though, as section 2.2 shows, the identity of the causal factor behind it can be difficult to disentangle from the various covariates). The enigma of why sex persists at all has received much more attention. Theoretical work has identified ecological parameters that are thought to favour sexual reproduction, ranging from the presence of coevolving parasites (Red Queen, Glesener & Tilman, 1978) to the complexity of the environment (Tangled Bank, Gaggiotti, 1994; Scheu & Drossel, 2007; Song *et al.*, 2011) or its temporal unpredictability (Weeks, 1993; see Hartfield & Keightley, 2012, for a recent review. Any model where one parameter impacts the likelihood that sex is maintained can predict repeatable patterns of geographic parthenogenesis, if this key parameter correlates, in some systematic way, with geography. However this step remains largely untested. There seems to be no way to avoid the difficult task of going beyond simple geographic mapping of sexuals and asexuals, and of characterizing in details the ecological niche and population processes at play in their respective ranges (including what happens at any range overlap).

Future directions

Geographic parthenogenesis is an 'old' research topic that could be revived using a multitude of approaches. We present a non-exhaustive list of ideas in Table 2, and discuss below the rationale and potential pitfalls in developing such a research program.

BETTER DOCUMENTATION OF THE PATTERNS

The concept of geographic parthenogenesis primarily refers to species where both a sexual and an asexual form exist and are geographically more or less distinct (Vandel, 1928). However, the term has been taken by some authors to specifically refer to species, or pairs of sister species, where parthenogens are found in wider areas, higher latitudes, altitudes, disturbed areas, or island-like habitats, considered typical patterns. What appears to be largely missing is a quantitative characterization of sexual and asexual ranges that is done in a systematic way, avoiding pre-selecting species based on an interesting pattern already being noted. Whether a case study matches an expected pattern is open to subjectivity, and a statistical validation of the soundness of the suspected trends is therefore desirable.

Plants appear better studied than animals in this respect. Bierzychudek was the first to test whether parthenogens span larger and colder ranges than their sexual counterparts (Bierzychudek, 1985). She examined 43 cases (10 genera) of plants where the distribution of both forms were known: in 76% of the cases, the asexuals' range was larger and more northerly. She also compiled data of 130 sexual and asexual species regarding whether they occupied a previously glaciated area or not. Significantly more sexual species were found only in non-previously glaciated areas, and more asexuals were found only in previously-glaciated areas.

While Bierzychudek's study was based on precise distribution data and statistical testing, caveats need be mentioned. The species constituting the dataset did not satisfy assumptions of random sampling across families as they were imposed by the availability of data, leading to a strong taxonomic bias: more than half of included cases are from the well-studied Asteraceae which contains a high proportion of apomicts (Richards, 1997), and happens to harbour almost all the angiosperms with autonomous, rather than pseudogamous, parthenogenesis (Hörandl *et al.*, 2008). Thus we can conclude that geographic parthenogenesis appears a consistent pattern in autonomous Asteraceae, but patterns in pseudogamous angiosperms have not been investigated in detail (Hörandl *et al.*, 2008).

Since the work of Bierzychudek (1985), no broad scale quantitative test has been published to validate the assumed typical differences in the range size, localization and properties of sexuals and asexuals (though note the valuable study of Grossenbacher *et al.*, 2015, of the equivalent question in selfing plants, see section 2.3). The geographic parthenogenesis literature has clear 'favourite taxa': temperate angiosperms with distributions in Europe and Northern America, lizards (Kearney *et al.*, 2009), and Coleoptera (Suomalainen *et al.*, 1987). Reviews can be found on plants and animals by Kearney (2005), on angiosperms by Hörandl *et al.* (2008), on arthropods by Lundmark & Saura (2006). Expanding the discussion to other groups is crucial to determine if asexuality affects distribution patterns universally and in a consistent fashion (a best case scenario, because then geographic parthenogenesis could give the crucial clue to the mystery of the persistence of sex as a whole), or if recurring geographic patterns are merely a quirky phenomenon restrained to a few groups. In the latter case it could heavily interact with ecological specificities of these groups to explain the distribution of their asexual forms (as already pointed out in Cuellar, 1977; Lynch, 1984; Ross *et al.*, 2013). For instance, Asteraceae are widespread exploiters of pioneer habitats (Funk *et al.*, 2005).

The enormous group of internal and external parasites is remarkably absent from the debate (though their sexuality is not always straightforward to assess, Weedall, 2015). Sexual reproduction is commonly viewed as a way to keep up with parasites, but it is less often discussed how parasites might keep up with their hosts. Successful and widespread species of asexual pathogens exist (Tibayrenc & Ayala, 2012), but to our knowledge they have not been compared with sexual sister taxa in any geographic context. Being an agricultural pest has also been shown to often be associated with parthenogenesis (Hoffmann *et al.*, 2008; Ross *et al.*, 2013). Some globally spreading pest species of

Table 2. A list of suggestions for future research. (*GeoP = Geographic Parthenogenesis, GPG = General Purpose Genotype, FNV = Frozen Niche Variation*)

What needs to be done	Why?	State of the field
Broad scale patterns: quantify and statistically compare geographic distributions of sexuals vs asexuals over a wide phylogeny (range, latitude, habitat type)	Patterns still need to be validated. Do they give us universal information about contextual benefits of sexual vs asexual reproduction, or are results taxonomically restricted and/or idiosyncratic?	 Some meta-analyses already exist: selfing plants (Grossenbacher <i>et al.</i>, 2015), scale insects (Ross <i>et al.</i>, 2013), pests (Hoffmann <i>et al.</i>, 2008). New developments in functional biogeography (Violle <i>et al.</i>, 2014) could include intra- and interspecific variation in reproductive mode.
Broaden the taxonomical scope and form a database of parthenogens' characteristics (ecology, autonomous reproduction, heterozygosity protection, polyploidy, hybridity); include selfing and vegetative reproduction and traits of sexual sister species (or taxa)	Find out if geographic patterns associate mostly with certain characteristics of asexuals, over a wide range of organisms. Does the absence of sex itself matter, or rather its correlates?	 Tree of Sex (2014) is a starting point. Neiman & Schwander (2011) outline how research could benefit from comparing different genetic consequences of various parthenogenesis systems across sister species and taxa.
Document the genetic diversity of asexuals and their sexual counterparts	A diverse assemblage of clones, or the possibility of cryptic sex, can perform as well as sex in ecological times. Models sometimes assume asexuals are more diverse, sometimes less. Only parthenogens surviving with little diversity can tell us about contexts where sex is not necessary (Neiman & Schwander, 2011)	 New molecular methods make genotyping increasingly easy, as well as detection of rare sex signatures e.g. early reviews: genetic diversity in asexual reptiles (Kearney <i>et al.</i>, 2009); comparison of sexual vs asexual mitochondrial genetic diversity available in 10 systems (Fontcuberta <i>et al.</i>, 2016)
Identify evidence for different stages of asexual or sexual spread; document the temporal dynamics of contact zones and boundaries	If the range of the sexual species shrinks, asexuals may be showing their demographic advantage, or asexuality may be contagious. If asexuals spread beyond the sexual range, this is indicative of better colonizing abilities or broader niche (via FNV, GPG, hybridity, polyploidy) If the asexual range shrinks, they may have been faster colonizers but sexuals are catching up; or they may display decreased fitness from mutational meltdown.	Temporal dynamics of sexual/asexual systems are still little known, apart from indirect inferences based on some habitats not having been available for very long.

Small scale patterns: niche characterization of overlapping sexuals vs asexuals; displacement and competition experiments; experimental evolution	Coexistence or competitive exclusion experiments will inform us whether the experimentally created ecological conditions favour sex or asex; if multiple conditions are tested and their geographic distribution is known, studies will link ecological patterns behind geographic parthenogenesis to the general theories of sex.	Examples: ostracods and predictability (Schmit <i>et al.</i> , 2013), woodlouse and abiotic preferences (Fussey, 1984), dandelions and plant-enemy interactions (Verhoeven & Biere, 2013). Many experimental evolution studies on sex exist, but conditions of experiments have typically not been interpreted in ways that make potential links to the geography of natural populations clear.
Existence of geographic clines between sexual core and contact zone with asexuals with respect to important characteristics of the population or its environment? (population size, genetic diversity, biotic interactions, resource structure)	Many general theories for the maintenance of sex can lead to GeoP if there is a geographic trend in its parameters, but this needs testing.	Recent meta-analyses about latitudinal reduction in biotic interactions (Schemske <i>et al.</i> , 2009, versus Moles <i>et al.</i> , 2011a, Moles <i>et al.</i> , 2011b) or core to margin genetic diversity decline (Eckert <i>et al.</i> , 2008) Applied to GeoP systems: Verhoeven & Biere (2013)
Document mating interactions between sexual males and parthenogenetic females; find evidence for sperm or pollen limitation.	Reproductive assurance might boost the spread of asexual organisms if mate availability is limiting. On the flipside, sexual conflict predicts that it is in the male (but not always in the female) interest to have females reproducing sexually. If population density is high, sexual harassment by males is a more severe problem for females, and if this leads to more frequent sex this can turn facultative sex into a pattern of geographic parthenogenesis via effects of local population density (Gerber & Kokko, submitted manuscript). Finally, neutral hypothesis for geographic parthenogenesis would get support from deleterious hybridization.	Examples of spatial reproductive assurance studies in plants: Cosendai <i>et al.</i> (2013), Randle <i>et al.</i> (2009), Petanidou <i>et al.</i> (2012). Gerber & Kokko, submitted manuscript, provides a theoretical basis of the conflict idea.
Develop theory more systematically; stop treating geographic parthenogenesis as a 'separate' topic from the rest of the theoretical literature on sex	Many hypotheses presented in this paper exist in the literature as verbal ideas only. General models on the evolution of sex miss out on opportunities to test them if they do not phrase their predictions in contexts where geography might covary with parameter settings.	Table 1 contains a list of what already exists.

fungi are known to use only vegetative reproduction outside their native range, where they are normally only facultative asexuals, despite several independent introductions being documented (Saleh *et al.*, 2012).

Finally, sections 1 and 2 emphasize that current explanations for geographic parthenogenesis patterns tend to be based on ideas about the emergence, invasion and demographic nature of asexual lineages that cannot all be shared by existing asexual lineages. To identify which of these candidate explanations are most relevant, it would be extremely valuable to document how well model assumptions match the properties of each category of parthenogens, and whether some categories are more strongly associated with a given geographic pattern. For instance, a database such as the Tree Of Sex (2014) provides a good starting point for cataloguing species where both sexual and asexual populations are found, their respective distribution, and candidate characteristics to explain geographic patterns (ecology, ploidy, hybridity, type of asexuality...). Given the tremendous amount of biogeographic data now available, fine scale geographic analyses could also prove useful, as would filling in the gap between the time since the last glacial maximum and much more ancient processes (see Horne & Martens, 1998, for arguments why this could be important).

While addressing these goals is clearly beyond the scope of this review, it is instructive to lay out some of the pitfalls that would have to be avoided. One tends to assume that reproduction is sexual until proven otherwise (indeed, it was the curious scarcity of males that led Vandel on the right track), and detection of asexuality might happen far more easily when the asexual range is large than when it is small – particularly if asexual and sexual forms overlap in range. Occurrence of asexuals in 'human-associated habitat' is prone to another detection bias. The only species of snake known to be obligately parthenogenetic is the fossorial species *Ramphotyphlops braminus*, also called 'flowerpot snake' after its worldwide introduction along the global potted plant trade (Gibbons & Dorcas, 2005). Here it seems reasonable to assume that a closer look at its reproductive system was encouraged by it is cosmopolitanism and association with humans. This kind of detection bias has to be considered when arguing for a causal relationship between parthenogenesis and association with humans (see Kearney *et al.*, 2009).

Another factor to consider is potential nonlinearities in data: in a recent field survey, the relationship between altitude and parthenogenesis in plants proved not as straightforward as previously thought, as above the altitude where asexuals increase in frequency, sexuals may take over again (Hörandl *et al.*, 2011). Finally, there is the problem already mentioned in section 1.1: how to deal with the proliferation of environmental patterns claimed to exist. For instance, the claim that asexual populations tend to inhabit more arid environments than sexuals is documented mainly in species of the Australian desert (Kearney, 2003; Kearney, 2005; Kearney *et al.*, 2006). As with the 'high latitude' pattern, drier habitats of the parthenogens might just reflect the direction the climatic envelope moved, opening new areas to colonization. There are also subtle differences in how the question has been asked, with consequences for the characterization of the pattern. Rather than showing that parthenogenetic sisters occupy consistently drier habitats than their sexual sisters, the data suggest that the desert harbours more asexual taxa (along with their sexual counterparts) than other Australian environments (Kearney, 2003, Kearney *et al.*, 2006).

AT WHAT SCALE ARE WE EXPECTED TO FIND THE PATTERN?

Should the distribution of asexual and sexual forms be different enough that a satellite could in principle map these? If we require this to be the case, then interesting causalities might remain hidden. For instance, Fussey found that sexual woodlice associate with more calcareous microhabitats than their asexual counterparts, but the scale of such environmental variation being very small and patchy, it led to no identified pattern of geographic parthenogenesis (Fussey, 1984).

Bell used 'ecological parthenogenesis' to refer to environments or lifestyles for which the incidence of parthenogenesis is higher or lower than average for a given clade (Bell, 1982, p311) (e.g. in agricultural environments, Hoffmann et al., 2008). Reality may offer us a continuum from small-scale habitat preferences and/or outcomes of competition, to large-scale patterns. For instance, all known examples of parthenogenesis in tardigrades involve freshwater, and not marine, species (Bertolani et al., 1990). Similarly, parthenogenesis is common in non-marine ostracods (Bell, 1982), but only one marine species has so far been confirmed to be parthenogenetic (Hull & Rollinson, 2000). In the Baltic Sea, which offers a wide gradient of salinity, several normally strictly sexual algae reproduce solely by vegetative propagation in freshwater areas of the sea (Tatarenkov et al., 2005; Gabrielsen et al., 2002). An explanation is that gametes, having evolved in seawater, face osmotic problems in freshwater, which could favour a switch to asexual reproduction (Serrão et al., 1996). Another hypothesis considers the marine environment more stable with more biotic interactions, while freshwater systems pose fluctuating selection pressures - but even if such a generalization was justified, as we highlighted above (section 2.5) there are difficulties in predicting which way this should impact sex. For instance, parthenogenetic mites and springtails tend to inhabit the stable soil horizon, while sexuals dwell above, in the more variable litter (Chahartaghi et al., 2006; Chahartaghi et al., 2009); somewhat frustratingly, earthworms show the opposite pattern (Jaenike et al., 1980).

If sex and asex are each favoured in different ecological conditions, then large scale patterns of geographic parthenogenesis are analogous to small scale patterns of 'ecological parthenogenesis', only organized over a wide geographic gradient. Alternatively, large scale patterns can stem from large scale events such as glacier retreat. Integrating data from organisms of all sizes and on various spatial scales might be a key step in disentangling the causes of geographic variation in reproductive mode. We also emphasize that 'marginal' and 'geographical' are human constructs based on scales that are relevant for us, and those concepts have to be carefully adapted to the reality of the studied organisms.

Conclusions: Mind the diversity!

The dazzling differences among cases of parthenogenesis, their putative habitats and reasons why they are there, are poorly reflected in current modelling efforts. Despite the theoretical attachment to a twofold cost of sex, empirical estimates and more detailed theory point towards frequently lower costs in the wild (Lehtonen *et al.*, 2012; Stelzer, 2015), and mathematical modelling of geographic parthenogenesis should incorporate some flexibility and realism in this parameter (Table 1). The twofoldness of any cost is based on certain assumptions about the role of males (Lehtonen *et al.*, 2012), yet it is noteworthy that apart from one reference in Table 1 (Britton & Mogie, 2001), males (or male function) are not considered. Their behaviour towards parthenogenetic females, or intralocus sexual conflict, could have important consequences (Connallon *et al.*, 2010).

Another noteworthy point is that all models of Table 1 explicitly or implicitly concern autonomous parthenogens, though some results could probably be extended to pseudogamous cases whenever coexistence is possible, or self-fertilization. Apomictic parthenogenesis appears to be a necessary requirement for only one of the models of Table 1 (Peck *et al.*, 1998), but it is currently unknown whether automixis would shift each model outcome towards an asexual disadvantage (it is an extreme form of inbreeding), or whether the benefit of freezing favourable adaptations would be of overriding importance. Because the way the zygote is produced determines how the actual genetic benefits of sex arise, assessing the ecological and evolutionary success of parthenogens with different reproduction modality is especially relevant (see Neiman & Schwander, 2011, for suggested research avenues). Verbal models also display a tendency to build scenarios tailored to one particular category of parthenogen and pattern. There appears to be much to be gained if we remember to celebrate the diversity of how and where asexuals appear and persist, both theoretically and empirically.

It also appears timely to connect the geographic parthenogenesis literature much better with related questions. We have already highlighted how, if patterns prove consistent with one explanation but not others, we could gain much in terms of understanding sex in general. Other 'neighbouring' fields include the ecology of selfing organisms, which offer similar geographic trends (sections 1.2 & 2.3) while sharing another trait with parthenogens: the twiggy nature of their phylogenetic distribution, and an unfortunate reputation of being evolutionary dead-ends (but see Schwander & Crespi, 2009; Igic & Busch, 2013). Studies of vegetatively reproducing organisms are also largely disconnected from other forms of asexuality, and papers reporting a latitudinal decrease in the use of sex and increase in vegetative reproduction often propose idiosyncratic explanations, without linking it to the general literature on geographic parthenogenesis (e.g. Dorken & Eckert, 2001). Vegetative reproduction is also interesting because it poses strong constraints on dispersal distances — though this did not prevent the Mediterranean sea from being colonized by a single, vegetatively reproducing genotype of sea-star, which remarkably also happens to be a male (Karako *et al.*, 2002)!

The study of invasion dynamics and range expansion are particularly relevant for geographic parthenogenesis. First because asexuality is frequently found in the non-native range of invasive species, and second because some of the conditions we discussed here as potentially favourable to asexuality can be found at the edge of an expanding population: enemy release, low-density of mates, inbreeding, and low diversity of sexuals (Chuang & Peterson, 2016). To date, no model of geographic parthenogenesis has focused on how well asexuals would do on the front of an expansion (but see Peischl *et al.*, 2015, for a first step in that direction). Finally, there is a large body of work on local adaptation and its coevolution with dispersal (e.g. Blanquart & Gandon, 2014; Berdahl *et al.*, 2015), but intricacies of sex are rarely discussed in this context (but see Lagator *et al.*, 2014); taxa with sexual and asexual forms could offer much insight into this question as well.

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AT drafted the article, AT and HK gave critical revisions, both approved of the final version to be published.

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CHAPTER IV Geographic parthenogenesis in expanding populations: what are the expected patterns?

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Abstract

When a species reproduces sexually in only part of its range, asexual reproduction at high latitudes or altitudes is the typical pattern. Does geographic parthenogenesis inform us about the selection pressures responsible for maintaining sex, or reflect a transient advantage of uniparental reproduction during recent recolonization? We study the latter option with an individual-based model of a sexual population expanding along a spatially homogeneous corridor, with mutations towards asexuality. We cover a diversity of sexual and asexual reproductive strategies under differing selective regimes (cost of homozygosity, genetic basis of resource competition). We find that typical geographic parthenogenesis patterns arise during range expansion whenever asexuals enjoy reproductive assurance that help them spread ahead of sexuals, or when asexuals are sperm-dependent hermaphrodites who interfere with the sexuals' reproduction. Once asexuals have established populations at the population margin, they then either expand back towards the core or become gradually displaced by sexuals catching up, depending on genetic and ecological parameters. Our results show that geographic parthenogenesis patterns can emerge from expansion dynamics without any ecological gradient. Adaptive explanations for the presence of asexuality at higher latitudes should therefore be carefully tested in order to reject processes unrelated to the effects of recombination as the sole drivers.

Keywords – Population expansion, Invasion, Apomixis, Evolution of selfing, Vegetative reproduction, Baker's law

Introduction

Obligate sex is surprisingly common among multicellular eukaryotes despite numerous short-term costs relative to asexual reproduction (Lehtonen *et al.*, 2012). A key endeavour has therefore been to look for counteracting short-term benefits (Neiman *et al.*, 2018). Geographic parthenogenesis, the phenomenon where a species reproduces asexually in only a part of its range (Vandel, 1928; Glesener & Tilman, 1978), offers an arguably underutilized set-up to identify the ecological conditions and selection pressures that favour sexual or asexual reproduction (Tilquin & Kokko, 2016). While a diversity of patterns exist, a general, large-scale trend seems to be that asexuals occur in higher altitudes and latitudes than their sexual counterparts (Glesener & Tilman, 1978; Bierzychudek, 1985; Kearney, 2005; Johnson *et al.*, 2010; Randle *et al.*, 2009; van der Kooi *et al.*, 2017), also true for selfers: Grossenbacher *et al.* (2015), as well as frequently occupying much larger ranges (van der Kooi *et al.*, 2017; Ross *et al.*, 2013; Grossenbacher *et al.*, 2015).

Hypotheses as to why asexuality should be adaptive at high latitudes must make and test two assumptions: first, what is the selection pressure favouring either sex or asex, and second, whether it covaries with latitude (reviewed in Tilquin & Kokko, 2016). Examples of mechanisms that could underly selection are that marginal environments at high latitudes are physiologically demanding, which favours polyploidy (a frequent characteristic of parthenogens (Lundmark, 2006), that population densities are low so uniparentality protects from mating Allee effects (Pannell & Barrett, 1998), that gene flow from the more productive core impairs adaptation to marginal habitats unless recombination is stopped (Peck *et al.*, 1998), or that abiotic challenges replace biotic ones at high latitudes, decreasing the power of Red Queen dynamics (Glesener & Tilman, 1978; Verhoeven & Biere, 2013) or tangled-bank competition (Bell, 1982; Gaggiotti, 1994; Song *et al.*, 2011) to maintain sex.

Assessing ecological explanations is made more challenging by the fact that populations at higher altitudes and latitudes are younger, often resulting from recolonization following the Holocene glacial retreat (19,000-10,000 years ago, (Clark *et al.*, 2012; Bell, 1982). Hence the conundrum: is a latitudinal shift in ecological conditions responsible for the transition in reproductive mode, or might a similar pattern result solely from different colonization abilities of sexuals and asexuals (Hörandl, 2009)? Tests of ecological hypotheses remain unsatisfying as this contrast is rarely made (Tilquin & Kokko, 2016), but see Kirchheimer (2018). Importantly, colonization ability as a driver of geographic patterns might be influenced by the mating system and specific genetic consequences of each reproductive mode: geographic pathenogenesis has been reported in very diverse organisms (Tilquin & Kokko, 2016; Tatarenkov *et al.*, 2005; Grossenbacher *et al.*, 2015), but whether it is more associated with some forms of asexual reproduction than others is not clear.

Here, we present a model of the evolution of geographic parthenogenesis, focusing on colonization dynamics in the absence of an environmental gradient, while varying the characteristics of organisms (Table 1). We consider sexual reproduction either in the form of gonochorism (i.e. with separate sexes) or hermaphroditism, and hermaphrodites can be either obligate outcrossers or facultative selfers. Asexual mutants can be reproductively autonomous, or need mating to trigger reproduction ("sperm-dependent parthenogenesis" in animals, "pseudogamous apomixis" in plants (Hörandl *et al.*, 2008; Van Dijk, 2009). Further, we assume asexuals produce diploid offspring via a mechanism that is either comparable to apomixis (in the zoological acceptation, where the mother passes her entire genome down to her progeny, freezing heterozygosity into the asexual lineage; genetically similar to vegetative reproduction), or gamete duplication (which results in a fully homozygous genome in one generation). Those two mechanisms are extreme points of a spectrum that also contains selfing and various types of automixis (Neiman & Schwander, 2011).
The above alternatives differ in the degree to which reproductive assurance or inheritance mechanisms favour (or disfavour) the spatial spread of asexuals ahead of sexuals. Our model additionally investigates two consequences that genetic composition can have on fitness, acting either alone or jointly. First, survival probability can depend on heterozygosity (the 'homozygosity penalty' scenario). This approximates effects such as the unmasking of deleterious recessive mutations in homozygous genomes (Chapman *et al.*, 2009) and/or poorer viability due to lower immune system diversity (Ellison *et al.*, 2012). Such effects are particularly detrimental in asexuals with gamete duplication, and in inbred sexuals. Second, the intensity of local competition for resources can depend on local genetic diversity (the 'tangled bank' scenario (Bell, 1982; Wuest & Niklaus, 2018); this is particularly detrimental for sexual populations genetically impoverished through founder effects or for monoclonal assemblages (Tagg *et al.*, 2005). Finally, our model also comments on the stability of geographic patterns over time — a factor rarely reported in empirical studies due to obvious challenges, but clearly of interest when interpreting distributions measured at a particular point in time.

Table 1 *Parameters used in the model. A single set of 200 random parameter combinations was created by drawing each parameter from its corresponding uniform distribution. That same set of parameters was then used in each scenario defined by the categorical variables (a), as per Table 2.*

Parameter	Symbol	Value (min-max, or discrete set)	
GEOGRAPHY			
Dimensions of the corridor	$X_{\max}Y_{\max}$	200×10	
Dimensions of the starting population	X_0Y_0	10×10	
DEMOGRAPHY			
Patch carrying capacity	Κ	5-40	
Fecundity of sexuals	f_s	4-10	
Fecundity of asexuals (= handicap × fecundity of sexuals)	f_a (= ρf_s)	Hermaphroditic species: $\rho = 1$	
		Gonochorous species: $\rho = 0.5$	
GENETICS			
Number of resource-related genes	G	2-20	
Survival probability of a fully homozygous individual ^a	b	0.8 (homozygosity penalty), 1 (no penalty)	
Mutation rate to sexuality	μ	5×10 ⁻³ , 5×10 ⁻⁴ , 5×10 ⁻⁵	
RESOURCES			
Type of competition ^a		Tangled-bank or Scramble competition	
DISPERSAL			
Mean distance dispersed (in geographic units)	d	0.5-3	
Shape parameter of the kernel	С	0.5, 0.8, 1, 1.2, 5	
REPRODUCTION			
Number of sexes in the species ^a		2 (Gonochorous), 1 (hermaphroditic)	
Types of sexuals ^a		Obligate outcrosser, facultative selfer (if hermaphroditic)	
Types of asexuals ^a		Autonomous apomictic, autonomous with gamete-duplication, sperm-dependent apomictic, sperm-dependent with gamete- duplication	

Methods

MODEL OVERVIEW

We initiate our model with a purely sexual population at the beginning of an invasion corridor, ready to spread. Thereafter, occasional mutations turn females into asexuals. We record the range of asexuals along the invasion corridor once it has been fully colonized, and again at a later time to document the temporal stability of geographic parthenenogenesis.

GEOGRAPHY AND LIFE-CYCLE OVERVIEW

The corridor consists of a lattice of 200×10 discrete patches (each a square with side of 1 unit). For convenience, we refer to the *X*-dimension as the 'latitude', and to comply with this interpretation, the boundaries at X = 0 and X = 200 are reflecting, while the *Y* axis uses wrapped boundaries to avoid edge effects.

Generations are discrete. Newborns become juveniles with a probability that may depend on their genotype. Juveniles then disperse out of their natal patch, reach adulthood after surviving competition (each patch contains resources allowing maximally K individuals to survive), and reproduce (asexually or sexually). Each individual carries a genotype of L+1 biallelic unlinked loci. L loci code for resource use, and one locus for the reproductive mode.

The details of the life cycle are as follows (all parameters listed in Table 1).

1. Offspring survival

Newborns become juvenile with a probability that depends on their genotype in the 'homogyzosity penalty' scenario. Heterozygosity at the *L* loci serves as a proxy for the whole genome, with survival probability *p* decreasing with homozygosity 1-h:

$$p = 1 - (1 - b)(1 - h)^2$$

Here *h* is the proportion of heterozygous loci, and *b* the survival of entirely homozygous individuals. *b* can either be 1 (no effect of homozygosity) or lower, according to the scenario modelled.

2. Dispersal

Juveniles disperse with a distance distribution that follows an exponential power kernel of mean distance *d* (Supp. Mat.), using a shape parameter *c* allowing for thin-tailed (c > 1), exponential (c = 1), as well as fat-tailed (c < 1) distributions, modulating the frequency of very long-distance dispersal events (Klein *et al.*, 2006). Direction of dispersal is chosen randomly, and each individual is assigned to the patch that encompasses its landing location.

3. Local competition

Each patch can fully meet the needs of at most *K* individuals. We model two ecological scenarios: either all individuals are equivalent competitors ('scramble competition'), or competition depends on genotype ('tangled bank').

In the 'scramble competition' scenario, each of the *n* local individuals obtains K/n resource units. Each then survives with probability K/n, yielding *K* as the expected number of survivors. If $K/n \ge 1$, all local individuals survive.

In the 'tangled bank' scenario, competition is more intense if allelic diversity is low. We achieve this by assuming that, for each of the L loci, an individual's survival is improved if it has the rarer of the two alleles (a or b) at that locus. A focal individual's survival probability is

$$\frac{1}{L}\sum_{i=1}^{L} \left(\frac{K}{N_{\mathrm{a}i}}n_{\mathrm{a}i} + \frac{K}{N_{\mathrm{b}i}}n_{\mathrm{b}i}\right)$$

where n_{ai} is the individual's number of a alleles at the *i*th locus (this value can be 0, 1 or 2), n_{bi} is its number of b alleles at this locus, and N_{ai} and N_{bi} give the total number of each in the local population (including the focal individual). Again, if the survival expectation exceeds 1, the individual is guaranteed to survive. Note that *K* individuals are expected to survive if the two alleles are at equal frequency at each locus; otherwise, fewer survive and the carrying capacity *K* is not reached.

4. Reproduction

Reproduction follows species-specific rules (Supp. Mat.), where 'species' refers to the combination of traits described in Table 1 (Reproduction). Asexuals have the same fecundity as sexuals in hermaphroditic species, and half of it in gonochoric species. This handicap is to make their demographic output comparable, in order to focus on other effects than the cost of males, but is also not an unrealistic assumption (Levitis *et al.*, 2017).

Mutation can turn a sexually-produced female into an asexual, but back mutations are not allowed. Resource acquisition loci do not mutate. Inheritance is Mendelian for sexuals, with all loci considered independent; in asexuals, offspring either inherit the full parental genome ("apomixis") or only one half, that is duplicated ("gamete duplication").

After reproduction, the parental generation dies, and the life cycle begins again at step 1.

Population initialization

We initiate each simulation with the leftmost $X_0 \times Y_0$ patches (Table 1) at carrying capacity for juvenile sexuals ready to disperse, therefore starting the cycle at the 2nd step described above. The *L* resource-use loci are initiated at an equilibrium distribution for independent, neutral alleles (genotypes aa, ab and bb present in proportion $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{1}{4}$ respectively). In dioecious species, sex (male, female) is assigned randomly to each individual.

End of a run and information gathered

Once the corridor has been entirely colonized (time T_1 : when each of the highest latitude patches is occuped by at least one individual), we record the position of asexuals individuals along the invasion corridor. We then keep the simulation running for the same number of generations again, and repeat measurements at time $T_2 = 2T_1$. We stop simulations early if asexuals clearly outcompete sexuals, that is, when the proportion of asexuals exceeds 90% of the global population. At T_1 and T_2 , population state is summarized with two key measures: the global proportion of asexuals in the landscape, and their median latitude.

Experimental design and parameter values

To determine i) the outcome (geographic pattern) for each type of asexuality in an invasion context, and ii) the mechanisms behind the outcomes, we make use of a full factorial experimental design, testing each combination of sexual ancestor/asexual mutant (10 combinations, referred to as 'species', plus 3 control cases, Table 2), under 4 different selective scenarios: with or without a homozygosity penalty, and scramble or tangled bank competition. This results in $13 \times 4 = 52$ study cases. In control 74

runs, mutations do not have any phenotypic effect and act as a neutral marker in order to examine gene surfing, i.e. the stochastic invasion of the front of expanding populations by neutral alleles, which can then spread over large areas (Klopfstein et al., 2005). Results obtained with non-neutral mutations must be interpreted against this background. In control simulations involving gonochoric species, the mutation (or 'marker') is only passed down the female line, while it is transmitted to all offspring in hermaphroditic species; this is to make the demographic fate of the marker comparable to that of the asexual mutation in the test simulations.

All other parameters of the species' biology, which are not of primary interest to our study, are drawn randomly from sensible ranges (Table 1) to create a unique collection of 200 different parameter sets. For maximum comparability, each of the 52 cases of interest is replicated 200 times using that unique collection.

The code was written in R (R Core Team, 2017) making use of RStudio (RStudio Team, 2016) and the packages *ggplot2* (Wickham, 2009), *RColorBrewer* (Neuwirth, 2014) and *beepr* (Bååth, 2015).

	Separate sexes?	Partner needed?	Inheritance mechanism?	
Ancestral:	Gonochorous	Obligate sexual	Mendelian	
Mutant:	Gonochorous	Obligate sexual	Mendelian	(control)
	Gonochorous	Autonomous	Apomictic	
	Gonochorous	Autonomous	Gamete-duplication	
	Gonochorous	Sperm-dependent	Apomictic	
	Gonochorous	Sperm-dependent	Gamete-duplication	
Ancestral:	Hermaphrodite	Obligate sexual	Mendelian	
Mutant:	Hermaphrodite	Obligate sexual	Mendelian	(control)
	Hermaphrodite	Autonomous	Apomictic	
	Hermaphrodite	Autonomous	Gamete-duplication	
	Hermaphrodite	Sperm-dependent	Apomictic	
	Hermaphrodite	Sperm-dependent	Gamete-duplication	
Ancestral:	Hermaphrodite	Facultative selfer	Mendelian	
Mutant:	Hermaphrodite	Facultative selfer	Mendelian	(control)
	Hermaphrodite	Autonomous	Apomictic	
	Hermaphrodite	Autonomous	Gamete-duplication	

Table 2 *We test* 13 *combinations of ancestral sexual species and asexual mutants, varying in the number of sexes, reproductive autonomy, and inheritance mechanism.*

Results

Typical GPG patterns arise from reproductive assurance

The simulations reveal a variety of potential geographic patterns, varying in how the median latitude of asexuals relates to their abundance (example: Fig. 1, Supp. 1). The archetypal geographic parthenogenesis pattern has all asexuals concentrated at the far end of the corridor (upper edge of the grey triangle in Fig. 1). We therefore conservatively define the 'GPG-score' for each scenario as the proportion of simulations that lie on that line (Fig. 2).

The highest scores, with more than 50% of runs yielding a clear geographic parthenogenesis signal, arise when the asexuals are reproductively autonomous (Fig. 2). This score rises to above 95% if we focus on cases where asexuals form more than 10% of the population (Fig. Supp. 2), a coexistence more likely to be discovered on the field. Reproductive assurance gives autonomous asexuals better colonizing abilities than sexuals, which depend on the presence of a partner on a patch to reproduce. This effect is somewhat stronger in gonochorous species than hermaphrodites (Fig. 2): for the former, colonizing an empty patch requires the simultaneous immigration of both a male and a female, while for the latter, any two individuals will do.

For three of our modelled species-types, asexuals do not enjoy any reproductive assurance advantage over sexuals.

First, sperm-dependent asexuals of a gonochorous species cannot colonize patches without the presence of their sexual hosts, and hence must follow, and not precede, the spread of sexuals. Although they can reach high densities (Fig. Supp. 4), they do not do so on the margin, resulting in a null GPG score (Fig. 2a).

Second, for a species where sexuals are facultative selfers, autonomous asexuals only differ in the way they restore offspring ploidy. Despite an *a priori* expectation that this might favour asexuals near the margin (see Discussion), results are indistinguishable from control runs in terms of GPG-scores (Fig. 2c), although differences remain in the prevalence reached in the landscape (Fig. Supp. 4).

Third and last, sperm-dependent asexuals arising from a hermaphroditic outcrossing species yield a high GPG-score (Fig. 2b) despite no better reproductive assurance than sexuals, leading us to the next finding.

ASYMMETRIC MATING INTERACTIONS CAN RESULT IN GEOGRAPHIC PARTHENOGENESIS

In the hermaphroditic species we model, sperm-dependent asexuals can use sperm from both sexual and asexual donors. Sexuals, on the other hand, cannot be fertilized by sperm from an asexual. This choice allows to avoid the complications of contagious asexuality, a matter outside our scope, but is not altogether unrealistic (Dobeš *et al.*, 2017). One consequence of this choice is that reproductive interactions between the two subspecies are asymmetric, and favour asexuals at the margin where sperm availability may be limiting. The GPG-scores of sperm-dependent hermaphroditic parthenogens are 31% and 26% for apomixis and gamete duplication respectively, but breaking down this average reveals a strong effect of the competition scenario (Fig. 2b). When competition depends on genotype (tangled-bank), GPG-scores are very low, but when it is independent of genotype (scramble), GPG-scores are higher and comparable to those reached by their autonomous asexual counterparts. The explanation is as follows. In tangled-bank competition, asexuals are at an ecological disadvantage. Their low genetic diversity limits their resource acquisition, while immigrating sexuals possessing locally rare alleles acquire underused resources.



Fig. 1 Clear geographic parthenogenesis patterns emerge in gonochoric species with autonomous apomictic asexuals (for other species types see Fig. Supp. 1). Population states observed at T₁ (a, b) and T₂ (c,d,) for a purely sexual gonochoric species in which neutral mutations are tracked (control, a, c), or a gonochoric species where arising autonomous, apomictic asexuality is tracked (b, d). Each simulation run is characterized by the prevalence of asexuals (or marked individuals in the controls) in the landscape as a whole, and their median latitude. The prevalence measure, $\frac{N_A}{K \times Y_{max}}$, divides the total number of asexuals by the carrying capacity of one latitudinal slice of the landscape, and thus indicates how many latitudinal slices they would occupy if they all clustered together geographically. Median latitude is the latitude above which half of the asexuals are found. All results are necessarily contained within a triangle: dots along the upper diagonal indicate that asexuals all cluster on the far side of the corridor (the archetypal pattern of geographic parthenogenesis); dots on the lower diagonal imply that they have replaced sexuals at the original core. Each panel shows 800 runs, i.e. the replication of 200 parameter combinations in 4 scenarios (Table 1, 2). Colours code for three mutation rates.

Under scramble competition, however, the asexuals indirectly interfere with the sexuals' reproduction by competing for the same resources (while not acting as mating partners). For sexuals to establish in a patch held by asexuals, their propagule pressure must be high enough that at least two immigrants survive the competition with the asexuals and reach adulthood – while only a single surviving asexual can reproduce in a patch dominated by sexuals.

GEOGRAPHIC PARTHENOGENESIS CORRELATES WITH FASTER INVASIONS

Populations took between 17 and 1267 generations (median: 111) to invade a 200-patch long corridor; the pronounced differences result from the range of dispersal parameters sampled (Table 1). When asexuals take over the margin (species with high GPG-scores), invasion speed increases compared to control runs. Species types rank similarly in terms of GPG-score and reduction of colonization time (between 73 generations saved in gonochoric species with autonomous, apomictic asexuals, and 6 generations in hermaphroditic species with sperm-dependent, gamete-duplicating asexuals, Table Supp. 1).

\mbox{GPG} patterns can be transient, or reinforce over time

From T_1 to T_2 , all GPG-scores decrease mildly in a similar fashion across scenarios (Fig. Supp. 3), indicating that the initial spatial separation between sexuals and asexuals is eroding. Spatial overlap develops when asexuals spread back and establish populations near the original core, or when sexuals catch up and displace the asexuals from the edge, as revealed by the changes in frequency of either type in the landscape (Fig. 3, Supp. 5).

Broadly, asexuals spread back and increase in number over time, regardless of species type, in two cases: (i) when asexuality is associated with neither deleterious genetic nor ecological effects (i.e. scramble competition & no homozygosity penalty, Fig. 3, light blue), and (ii) in all scenarios where asexuals are apomictic, especially so when homozygosity is costly (dark red and blue) as then sexuals may suffer from inbreeding. The alternative pattern of sexuals catching up is found in scenarios where asexuals use gamete-duplication and suffer from homozygosity penalty.

Overall, apomictic asexuals gain more ground over sexuals when homozygosity is costly than when it is not, whereas gamete-duplicating asexuals gain more ground (or lose less) when homozygosity is not costly. Greatest stability in population composition is found in scenarios with tangled-bank competition and no homozygosity penalty (light red, see also Fig. Supp. 4).

As expected, asexuality is selectively neutral and behaves identically to control populations in hermaphroditic species with facultatively selfing sexuals, when there is no ecological nor genetic consequence to asexuality (light blue), as confirmed by GPG-score (Fig. 1), prevalence (Fig. Supp. 4) and evolution over time (Fig. 3). Additionally, asexuality behaves much like a selectively neutral mutation in hermaphroditic species with apomictic, sperm-dependent asexuals, competing in a tangled bank with no homozygosity penalty (light red). In this case, the similarity probably reflects counteracting selection pressures reaching an equilibrium. Neither sexuals nor asexuals suffer from inbreeding depression nor benefit from reproductive assurance, but asexuals do have a reproductive edge due to asymmetric mating interactions, apparently enough to offset their competitive inferiority due to low inter-individual variability in resource use (mitigated by intra-individual variability frozen by apomixis).

$C {\rm LONAL} \ {\rm DIVERSITY} \ {\rm Helps} \ {\rm Asexual} \ {\rm Maintenance} \ {\rm in \ tangled-bank} \ {\rm Scenarios}$

In our model, as exuals are continuously generated from sexuals by mutation. While this choice was made to increase the likelihood that as exuals establish during the time of the simulation (reporting GPG patterns is impossible if they do not), it is unrealistic in magnitude (spontaneously arising as exuality is rare in nature (Neiman *et al.*, 2014). Across the mutation rates we used (5 10⁻³, 5 10⁻⁴, 78 $5 \cdot 10^{-5}$), higher rates obviously increase the establishment rate of asexual populations as well as their final density. Less obviously, mutation rate also has non-linear effects on clonal diversity, and hence competition. While clonal diversity does not matter in scramble scenarios, a monoclonal population cannot reach high densities in tangled-bank scenarios and is vulnerable to sexual invasion.

At the lowest two mutation rates considered, asexuals tend to be less prevalent and decline more between T_1 and T_2 in tangled-bank scenarios than in scramble-competition scenarios, especially when homozygosity is not costly (Fig. Supp. 4b, Supp. 6). In scramble scenarios, the aexual population on the edge typically remains monoclonal (although new clones can spread by drift), whereas in tangledbank scenarios, the original clone is usually invaded successively by different clones originating from the edge of the sexual population, resulting in stable polymorphic clonal populations with increased density (see Fig. 4 for typical runs). At the highest mutation rate however, competition regime does not substantially impact asexual prevalence and persistence, presumably because mutation generates enough diversity to make asexuals and sexuals compete on equal terms even in tangled-bank scenarios.

Finally, although the ecological rules described above also govern the likelihood of a sexual genotype invading a patch held by asexuals, sexuals face an additional difficulty: lack of mates. While mating failure is an issue in both empty patches and patches occupied by asexuals, the problem is exacerbated in the latter due to resource competition.



Fig. 2 The strength of geographic parthenogenesis depends on species characteristics and ecological scenario. The GPG-score is the proportion of runs where all asexuals cluster at the population edge (upper diagonal of Fig. 1). Grey translucent bars are average GPG-scores for each species type. Coloured bars are that average broken down for the four ecological and genetic scenarios (TgB = tangled-bank competition, SC = scrambled competition, HP = homozygosity penalty). Each scenario was replicated using the same set of 200 parameter combinations, except for Selfers where the parameter set was doubled to decrease the sampling error due to the low probability of GPG



Fig. 3 Difference between the proportion of asexuals in the landscape T_2 and T_1 , for all species and ecological and genetic scenarios modelled (TgB = tangled-bank competition, SC = scrambled competition, HP = homozygosity penalty). Positive values indicate an increase in asexual population size. The five cases where boxplots are mere lines indistinguishable from 0 correspond to cases where asexual do not maintain significant populations at neither T_1 nor T_2 (<2%, Fig. Supp. 4)

Discussion

Typical geographic parthenogenesis patterns – asexuals occupying higher latitudes and potentially much bigger areas than sexuals – are readily formed in our range expansion model as soon as asexuals enjoy reproductive assurance and sexuals do not. This is in line with traditional expectations (Bierzychudek, 1985; Law & Crespi, 2002; Kearney, 2005; Pannell *et al.*, 2015) as well as recent findings from models of the spread of vegetatively reproducing *Fucus* algae in the Baltic (Rafajlović *et al.*, 2017), or buttercup recolonization of the Alps after the last glaciation (Kirchheimer *et al.*, 2018).

We did not find an effect of the genetic inheritance mechanism on its own on the probability of geographic parthenogenesis: GPG-scores of runs where both sexuals and asexuals could reproduce uniparentally were identical to control runs (Fig. 2), despite theoretical grounds to expect the relative fitness of different inheritance mechanisms to vary along a species' expanding range. Indeed, sexual populations tend to lose heterozygosity and genetic variability at the expansion front due to founder effects and inbreeding (see model by Peischl *et al.*, 2015). This at first sight suggests better prospects for asexuals on the front than in the core. Firstly, apomicts could outcompete sexuals near the margin as a result of 'freezing' heterozygous genotypes (Vrijenhoek & Parker, 2009), although this would require that asexual genotypes be "captured" in the core to then reap benefits on the front. Secondly, gamete-duplicating asexuals, which are disadvantaged in the core by their extreme homozygosity, would compete on more equal terms with inbred sexuals near the margin. The decline in the genetic diversity of sexuals near the margin underlying those hypotheses, however, does not appear in our model (data not shown), possibly due to the short time-scale investigated compared to the model by Peischl *et al.* (2015).



Fig. 4: Example runs resulting in a GPG-pattern and showing clonal diversity dynamics typical of each competition regime. The y-axis shows the number of individuals of different types occupying each latitudinal slice: one slice is 10 patches, each with a carrying capacity of K=11, leading to an expected number of individuals of 110 per slice (dashed line) if resources are optimally shared. Black: sexual individuals. Colours: the 5 most prevalent asexual clones, labelled according to the generation the lineage emerged. Grey: other minor clonal lineages. a,c) Scramble competition allows a single clone to take over the margin, and reach high densities. b,d) Tangled-bank competition prevents a single clone from reaching high densities. The first clone to take over the margin is being invaded successively by different clonal lineages originating from the sexual gene pool. They form polymorphic populations and achieve higher densities than monomorphic ones. Species modelled: hermaphrodites with autonomous, gamete-duplicating asexuals; other parameters are b = 0.8, μ = 0.005, K = 11, f_s = f_a = 8, G = 17, d = 0.9, c = 0.5.

Our study highlights what data are required for showing that a given geographic pattern reflects the respective merits of sex and asex in different environments, and not simply the sorting that occurred during expansion. In some cases, it might be known that an asexual population established among sexuals at a time when no range expansion was going on (e.g. Paland *et al.*, 2005). Alternatively, expansion and establishment of asexuality might have been concomitant, in which case it is possible that asexuals did not only benefit from reproductive assurance, but were also better adapted than sexuals to the higher latitudes *per se*. In that case, one would expect their respective ranges to eventually stabilize around some identifiable ecological boundary.

An observable asexual range shift per se (for instance see Hoy Jensen et al. (2002) where sexuals catch up on asexuals) cannot be taken as evidence for either ecological selection or spatial sorting. In our spatially homogeneous model, most simulations show shifting ranges, with either sexuals catching up or asexuals pushing back. Those shifts are due to differences in resource use (clonal assemblages may be better or worse than sexuals at exploiting a structured resource (Weeks, 1993), for an empirical study showing the former see Lavanchy et al., (2016) and/or viability, which depends here on heterozygosity and is therefore impacted by reproductive mode. Real-life fertility differences between sexuals and asexuals can favour either mode (Levitis et al., 2017; Hörandl et al., 2008; Lehtonen et al., 2012). Under adaptive hypotheses, ranges are expected to change until sexuals and asexuals fully occupy the respective habitats that favour them, and any subsequent shifts should then reflect environmental change. Range stability, a rare outcome in our model under any scenario, could thus be a hint that ecological selection is at play. However, ranges can also be stabilized by a variety of processes from deleterious hybridization (Barton & Hewitt, 1985) to Allee effects (range pinning, Keitt et al., 2001), highlighting the need to test adaptive hypotheses experimentally (e.g. reciprocal transplant of sexuals and asexuals or common garden experiments (Weeks & Hoffmann, 2008; Verhoeven & Biere, 2013; Lavanchy et al., 2016).

If the goal is to use geographic parthenogenesis to identify what maintains sex among multicellular eukaryotes, a difficult task remains: to establish what environmental factors (if any) select for or against out-crossing *per se*. First, even if experiments confirm the superiority of each reproductive mode in its natural environment, alternative interpretations exist: the trait aiding success may be a correlate of asexuality (e.g. hybridity, polyploidy (Lundmark, 2006; Kearney, 2006), or be a local adaptation that took place after the original invasion and spatial sorting (Lombardo & Elkinton, 2017; Kirchheimer *et al.*, 2018). Second, comparing the success of each reproductive strategy requires accounting for the genetic diversity of both populations. An advantage of sex precisely lies in generating and maintaining diversity, yet it would be unfair to compare a newly derived and heavily bottlenecked asexual population to an ancient and diverse ancestral sexual population.

In our model, resource structure and transition rate to asexuality impact clonal diversity. When resources are completely unstructured (arguably an unlikely scenario in the wild), a single clone tends to dominate most of the asexual range, while stable clonal assemblages readily form in tangled-bank scenarios ("frozen niche variation" (Vrijenhoek & Parker, 2009), for experimental evidence see Weeks & Hoffmann (2008) ; Tagg *et al.* (2005), increasing resistance of asexuals to sexual invasion (as in a model by Weeks, 1993). In nature, a single clone rarely occupies a large area: either single-clone patches are small and attributable to founder effects, or widespread clones live in sympatry with very local ones (Hörandl *et al.*, 2008; Van Dijk, 2003). This is compatible with our results once taking into account that we model a corridor. Despite being two-dimensional, a corridor yields more homogeneous populations than unlimited two dimensional landscapes, in which 'genetic sectoring' occurs (Hallatschek & Nelson, 2010; Peischl *et al.*, 2015).

While our model applies to many types of asexuality, we have not exhausted all the possibilities offered by real life. We briefly mention five caveats here. First, vegetative reproduction is a type of asexuality associated with reduced dispersal (although this has exceptions (Ronsheim, 1994), a possibility we do not implement. Second, we assume that asexuals arise by mutation (a rare occurrence (Neiman *et al.*, 2014), ignoring the ecological and genetic consequences of hybridity or polyploidy (Lundmark, 2006; Kearney, 2006), or of contagious asexuality (Britton & Mogie, 2001). Third, we assume a fixed cost of homozygosity, which is an oversimplification of the dynamics of inbreeding depression at range margins, where deleterious mutations may fix or become purged (Hargreaves & Eckert, 2014). Fourth, we model annual (semelparous) species, where reproductive assurance is more beneficial than in perennial (iteroparous) ones (Pannell *et al.*, 2015), but

parthenogenetic plants are typically perennials (Hörandl, 2010). Finally, our model of pure sexuals or asexuals does not consider mixed-mating strategies, where the rate of asexuality/selfing can evolve in a graded manner, potentially maintaining some gene flow between core and front (Pannell *et al.*, 2015; Hargreaves & Eckert, 2014).

Although the above suggests that more tailored modelling would improve precision for some groups, our results likely generalize well to the various types of asexuals that enjoy reproductive assurance. This suggests that repeatable patterns of geographic parthenogenesis (Johnson *et al.*, 2010; Grossenbacher *et al.*, 2015; van der Kooi *et al.*, 2017; Randle *et al.*, 2009) could, in large part, be attributed to recolonization history and reproductive assurance. Any adaptive hypothesis (e.g. Weeks, 1993; Gaggiotti, 1994; Peck *et al.*, 1998; Britton & Mogie, 2001; Haag & Ebert, 2004; Salathé *et al.*, 2006; Song *et al.*, 2011) therefore needs to reject this spatial process as a sole cause. Interestingly, despite receiving most of the attention, larger ranges and higher latitudes and altitudes are not the only geographic patterns of parthenogenesis. In many species the distribution of sexual and asexual populations does not follow such broad-scale patterns, and could instead be called "ecological parthenogenesis" following Bell (Bell, 1982; Tilquin & Kokko, 2016; Fussey, 1984; Gregor, 2013). These cases might provide the clearest assessment potential for the ecological costs and benefits of sex.

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APPENDIX CHAPTER IV



Fig. Supp. 1 Geographic parthenogenesis patterns for all species modelled, at T1 and T2..



Fig. Supp. 2 GPG scores calculated on runs where asexuals form more than 10% of the population.



Fig. Supp. 3 GPG scores calculated on all runs at T_1 and T_2 .



Fig. Supp. 4 Difference between the average proportion of asexuals in the landscape between T₁ (black dot) and T₂ (coloured dot) for all species and scenarios.



Fig. Supp. 5 Evolution of the geographic patterns over time (T₂ - T₁), in terms of the proportion of asexuals in the landscape (x-axis), and their median position (y-axis). For instance, asexuals which have taken over the margin, and subsequently spread back towards the core, will be on the diagonal of the bottom (i.e. decrease in median) right (i.e. increase in number) quarter.



Fig. Supp. 6 Difference for all runs in GPG-score between T₁ and T₂ (calculated as T₂- T₁), broken down by species, scenario and mutation rate.

CHAPTER V Sperm Olympics: the effect of male condition on optimal sperm size-number allocation

To be co-first-authored with Benjamin Hopkins, University of Oxford

Supervised by Hanna Kokko

Abstract

Post-copulatory selection in small animals can involve mechanical interactions among sperm cells, and between sperm and female reproductive tract. Sperm size is therefore an important trait for competition, and males need to find the optimal allocation of their reproductive budget between sperm size and number. We ask how this optimal strategy varies according to a male's own phenotypic condition and its competitive environment. We model a condition-structured population competing for fertilizations and vary the number of eggs available per female clutch, the competition risk, and the variance in condition in the male population. In a preliminary version of the model, we find that males of intermediate condition are the first ones to sacrifice sperm numbers when competition increases, in order to produce more competitive sperm. Males of poor condition tend to invest more in numbers and make the most of non-competitive situations. Optimal sperm size is a better indicator of male condition than sperm number.

Keywords: sperm competition, post-copulatory selection, cryptic choice, giant sperm

Introduction (preliminary, thesis version)

Eukaryotic sex is costly in many ways (Lehtonen *et al.*, 2012). One particular family of costs are those associated with sexual selection (Kuijper *et al.*, 2012), especially in lineages where gametes have evolved asymmetric zygote provisioning (i.e. anisogamy , Lehtonen *et al.*, 2016). Through making different investment choices between gamete size and number, the female and male strategies follow divergent reproductive interests and selection pressures, setting the stage for the "primordial sexual conflict" (Parker, 1979). Ancestral sex roles in species with separate sexes are that females, whose large eggs are a limiting resource, invest time and energy in maximizing the quality of the males who will sire their offspring, while males instead attempt to maximize the number of eggs they fertilize (Lehtonen *et al.*, 2016). To do so, males must interact successfully both with females and other males, using sexually selected traits broadly falling into the categories of "ornaments" and "armaments", respectively (Berglund *et al.*, 1996). The interactions in question can happen both before and after mating.

Post-copulatory selection takes place in a female's reproductive tract, and acts on the ability of sperm cells to meet female-imposed requirements (Lüpold et al., 2013) and to out-compete sperm from competitors. For a long time, sperm competition was thought to be a simple lottery: the more cells, the better (Lüpold & Pitnick, 2018). The history of models of sperm competition is comprehensively reviewed by Parker (Parker & Pizzari, 2010). Foundational work in this field originally sought to explain why sperm are usually so tiny and many (Parker, 1982), with the focus on 'raffle'-like ideas: the more sperm cells one male offers for a lottery, the higher the chances this male will win and become the sire. The field then gradually developed towards a finer understanding of the multiple trade-offs faced by a male, in terms of total investment, remating rate, sperm number, sperm size, or seminal fluid volume (Parker & Pizzari, 2010). Conditional investment models have been developed to predict how males should plastically adjust their investment according to the social situation, in terms of competition risk, female mating status, or role (i.e. whether the first or second male to mate with the female has a competitive advantage). Models generally use game theory, two classes of males and various mating asymmetries, and identify conditions under which the favoured, or least favoured male, should invest more in ejaculate volume or sperm number (Parker, 1982; Engqvist & Reinhold, 2006; Parker et al., 2010)

It is therefore now recognized that sperm-sperm and sperm-tract interactions can make the outcome of competition more complex than a random 'raffle', with sperm size being a key trait influencing fertilization success (Lüpold & Pitnick, 2018). A male is therefore expected to manage its reproductive budget facing several trade-offs: should it invest in the number of matings, the quantity of sperm cells per ejaculate, sperm size ...? Depending on the situation, this leads to different "ejaculate economics", where several different possibilities are covered by previous theory (Parker & Pizzari, 2010), but with an assumption that we aim to relax here: that there are no total budget differences between males. Here, we ask whether males who differ in condition should evolve the same sperm size (such that high-budget males will produce more numerous sperm), the same sperm number (such that high-budget males will produce no more, but larger, sperm than low-budget males), or some other combination along the size-number trade-off. We are interested in an analogy from sports (which occasionally offers useful insight for mating-related economics, see e.g. Fromhage & Kokko, 2014, but also Getty, 2006) : if countries vary in the absolute budget they can spend on sport, should they invest in a few strong competitors who are sent to an international competition, or instead spread the training funds to many citizens, in the hope that through a numbers game one will emerge as a winner on international arenas as well?

Across species of Metazoa, comparative analyses have shown the size of the species to influence the relative merits of investing in sperm number *versus* size (Immler *et al.*, 2011; Lüpold & Fitzpatrick, 2015). In large animals, competition occurs via diluting other males' ejaculates, so sperm cells tend to be small and many. In animals with small reproductive tracts, sperm quality may be favoured over quantity due to the importance of direct physical interactions: bigger sperm might, for instance, be better at displacing others, at competing for limited sperm-storage space, or at resisting being flushed away by the female (Bjork *et al.*, 2007; Parker *et al.*, 2010; Lüpold et al., 2012).

Within groups of organisms of comparable size, the very broad-scale negative relationship between organism size and sperm length, as described above, breaks down: within drosophilid fruit flies, larger-bodied species also have longer sperm (Lüpold *et al*, 2016). Drosophilids have established themselves as a model group in the study of post-copulatory selection and show broad interspecific variation in sperm size, including a species with the longest know sperm cells in the animal kingdom (*Drosophila bifurca*, reaching a length of 58mm, Pitnick *et al.*, 1995}, Fig. 1). In species with giant sperm, it is interesting to note that the male gamete may now be much bigger than the female gamete. The anisogamy-based definitions of 'male' and 'female' are nevertheless not reversed in the sense of which sex provisions the zygote, since the resources expended on sperm cells are mostly "wasted" on competition rather than used to form a larger zygote. However, because of their high production costs, giant sperm cells may also become a limiting resource, with consequences on ejaculate economics as we will see below (Lüpold et al., 2016).

Within a species, several factors can influence sperm size-number allocation. The intensity of sperm competition, when increased experimentally (by manipulating the number of matings per female), has been shown to lead to the evolution of larger sperm cells (in a nematode: LaMunyon & Ward, 2002; in a flour beetle: Godwin *et al.* (2017). The condition of a given male is also expected to affect its reproductive budget and potentially its allocation strategy. Some sexually selected traits have been argued to function as honest indicators of male quality, which is expected to happen when the marginal cost of exaggerating the trait decreases with male condition (handicap principle, Zahavi, 1975; Getty, 2006). If sperm size belongs to that category of traits, it has to covary with condition. In a recent study, Lüpold *et al.* (2016) manipulated male condition in one species of *Drosophila* by rearing larvae in benign *versus* severe conditions. Males reared in severe conditions had a markedly smaller body size, but this did not translate into any difference in sperm size. Sperm number, on the other hand, did correlate with body size in 7 species of *Drosophila* tested (sperm number was indirectly measured as the number of offspring sired given unlimited access to virgin females, Lüpold *et al.*, 2016).

These findings prompted us to ask the question: what is the optimal investment in sperm number *versus* size for a male given its condition, in different competitive contexts? When should number be sacrificed for size? We present here a preliminary model of a size-structured population, where we vary the weight given to sperm size in competitiveness, the competition intensity, the number of eggs per female, and the distribution of male condition in the population.



Fig. 1 Scanning electron micrograph of *Drosophila bifurca* sperm (picture: R. Dallai, U. of Siena; borrowed from Lüpold & Pitcnick, 2018, only for this thesis), displaying its high noodling potential.

Methods (preliminary)

SPERM-COMPETITION

We model a condition-structured population of males with reproductive budget *B* normally distributed around a mean of \overline{B} with a standard deviation of σ_B , representative of the naturally occurring condition variation in the population. The population is divided according into *c* discrete condition classes of equal density, each represented by its average budget B_i . The reproductive strategy of class *i* is characterized by n_i , the number of sperm cells produced with budget B_i . The size of the produced sperms relates to their number following the relationship

$$s_i = \left(\frac{B_i}{n_i}\right)^{\alpha}$$

Due to constraints, for instance set by the female reproductive tracts, the minimal sperm size required to be able fertilize an egg is s_{min} .

Females are not explicitly modelled, but we assume that for each mating, a male has a probability p of being immediately preceded or followed by another male, creating sperm competition for the E eggs of the female. In this first version of the model we assume no advantage to being first or second to mate.

In the absence of competition, the fitness of a male of class *i* is $min(E, n_i)$.

If competition occurs between two males \mathcal{J}_i and \mathcal{J}_j , each ejaculate monopolizes a fraction of the eggs proportional to its relative competitiveness. If this leads to an ejaculate monopolizing more eggs than can be fertilized, the leftover eggs become available again for the other male's leftover sperm. This happens as follows.

The relative competitiveness $r_{i,j}$ of the ejaculate of \mathcal{J}_i facing \mathcal{J}_j is given by:

$$r_{i,j} = \frac{n_i s_i^{\gamma}}{n_i s_i^{\gamma} + n_j s_j^{\gamma}}$$

where sperm size disproportionately affects competition compared to sperm number, as controlled by the exponent γ , henceforth called the *noodling coefficient* (for self-explanatory reasons, see Fig. 1). In the context of fruit flies, competitiveness can be roughly viewed as the ability to displace, or resist displacement by, other sperms, and to jam the female's seminal receptacle.

When $E > n_i + n_j$, there are more eggs than sperm; every sperm cell fertilizes one egg, and the fitness of \mathcal{J}_i is n_i .

When $E < n_i + n_j$, the ejaculate of \mathcal{J}_i monopolizes a fraction $r_{i,j}$ of the eggs, therefore fertilizing $\min(n_i, r_{i,j}E)$ eggs and leading to a number of leftover sperm cells of $L_i = E - \min(n_i, r_{i,j}E)$.

Once both males have fertilized as many eggs as is possible among those they monopolized, the number of leftover eggs is given by

$$G_{i,j} = E - \min(n_i, r_{i,j}E) - \min(n_j, r_{j,i}E)$$

Those eggs then become available to the leftover sperm cells, if any exist.

As a result, the expected fitness w_i of a male belonging to class *i* and playing strategy n_i is the average of its returns without competition and with competition, integrated over its competitive environment, i.e. all potential competitors identity and their strategies. The population is composed of *c* condition classes each playing a strategy forming together the competitive environment $\pi = \{n_1, n_2, ..., n_c\}$. Therefore, w_i is calculated as:

$$w_{i} = p \min(E, n_{i}) + (1 - p) \frac{1}{c} \sum_{j=1}^{c} \left(\min(r_{i,j}E, n_{i}) + \min(G_{i,j}, L_{i}) \right)$$

The model as written above considers a single mating event per male, but a different interpretation is consistent with its structure: males can also be considered to acquire multiple independent matings, as long as each class of males gets to mate the same number of times. The number of matings is therefore not a variable males can adjust according to their budget.

STRATEGY OPTIMIZATION

We use an iterative algorithm to try and approach a stable vector of optimal strategies across condition classes, π^* . During each iteration, the algorithm randomly selects one focal class, and computes the fitness derived for each possible strategy it could play. Possible strategies for class *i* are all the integers comprised between $n_{imin} = 1$ and $n_{imax} = \frac{B_i}{s_{min}^{\frac{1}{4}}}$, the latter being the size-

minimizing strategy where sperm cells are of size s_{min} . In a first version of the algorithm, we update the focal class strategy to the one yielding the highest fitness (or if more than one exist, pick randomly one strategy among the equally best), before moving on to the next iteration. We find that the fitness of each class eventually stabilizes, but the population vector of strategies π does not. Some classes appear to oscillate between several equivalent strategies, which in their turn influence the optimal strategies of other classes.

To visualize the results, we therefore plot for each class the distribution of strategies successively adopted over a thousand iterations after fitness has stabilized. This reveals a "ragged" pattern, with neighbouring classes potentially having distributions centred around very different strategies

(high-n *vs* high-s). Estimating one's own condition precisely is a difficult task in nature, so it seems hardly realistic that strategies should be so discontinuous. We therefore add errors in a second tentative version of the algorithm, loosely following McNamara *et al.* (1997). The results presented in the next section were obtained with this method. After selecting a focal class *i* the algorithm now calculates the fitness returns of each possible strategy if played by class *i* itself, as well as if played by class i - 1 and class i + 1. The chosen strategy is the one yielding the highest average score (using weights of 0.5, 0.25 and 0.25 for i, i - 1 and i + 1 respectively; or of 0.66, 0.33 if dealing with the lowest or highest condition classes). This does successfully smoothen the curve of the strategy as a function of condition, but variability remains in the strategy played by each class.

Results (preliminary)

To begin this first analysis, we restrain the set of parameters analysed to three, and set the others constant. All results reported below are obtained for a fixed reproductive budget in the male population ($\bar{B} = 100$), allometry coefficient of sperm cost ($\alpha = 1$, i.e.the cost of a sperm varies linearly with its size), minimum required sperm size ($s_{min} = 1$) and noodling coefficient ($\gamma = 2$). We explore the impact of three factors on the optimal sperm size-number allocation strategy: the risk of sperm competition *p* (varied between 0 and 1 with 0.1 increments), the number of eggs per female ($E = \{20, 50, 80, 100, 120, 150, 180\}$), and, to a lesser extent, the standard deviation in condition (σ_B), that is set to 30 for all runs analysed except one series, for comparison's sake ($\sigma_B = 10$, E = 100 with varying *p*, Fig. 2, right panel).

The risk of sperm competition proves to strongly shape male strategies. Below a certain threshold of risk, denoted θ , males maximize the number of fertilizations in the absence of competition, and invest in sperm size only when they have extra resources (i.e. condition), which is beneficial when competition occurs. This is summarized by the simple rule: when $p < \theta$, males with a budget too small to fertilize all of a female's eggs ($B_i < E$, since $\alpha = 1$ and $s_{min} = 1$) produce as many sperm cells as possible, and males with a large enough budget ($B_i > E$) produce exactly *E* sperm cells (Fig. 2, 3).

When the risk of sperm competition exceeds θ , some classes of males begin sacrificing the number of sperm cells in order to increase their size (and hence competitiveness). This comes at the cost of missed paternity when competition does not take place. The first classes to begin investing more in sperm size are intermediate, followed by higher condition classes, but never the lowest condition classes (e.g. Fig. 2, 3).

The value of θ is not a monotonous function of the number of eggs: it takes a value of $0.7 < \theta < 0.8$ for $E = \{80, 100, 120, 150\}$ and of $0.8 < \theta < 0.9$ for $E = \{20, 50, 180\}$.

Let us now turn to the relationship between male condition and investment strategy. Sperm size and sperm number both increase monotonously with male condition in only one case: when females produce more eggs than can be fertilized by any male alone (E = 180). Outside of that special case, sperm number is rarely a monotonous indicator of male condition, but sperm size usually increases with male condition (especially when p = 1, e.g. Fig. 2, 3). This rule, howev



Fig 2 Optimal condition-dependent allocation in sperm number *versus* size across competition risk p, contrasted between populations with different variance in condition. Due to instability in the optimization procedure, best strategies are shown as distributions of the values taken over 1000 iterations. The graphs are organized in three columns showing different aspects of the optimal strategies: i) the number of sperm cells produced n (red dots show the maximum number of sperm cells affordable for each condition class; the dotted line shows the number of eggs per female E); ii) the resulting size of sperm cells s (the dotted line is the minimum required size s_{\min}); iii) the number of fertilizations achieved, w (dotted line: E). Left-hand panels: $\sigma_B = 30$; right-hand panels: $\sigma_B = 10$. Lower values of p are omitted as they yield a pattern similar to that obtained for p = 0.7. Other parameters are E = 100, $\overline{B} = 100$, $s_{\min} = 1$, $\alpha = 1$, $\gamma = 2$.



Fig. 3 Optimal condition-dependent allocation in sperm number *versus* size across competition risk p, for contrasting number of eggs per females *E*. For both panels $\sigma_B = 30$. Legend as for Fig. 2. 98

comes with exceptions: in the vicinity of $p = \theta$, the relationship between sperm size and male condition can show very complex, non-monotonous patterns (Fig. 2, 3), and lead to males of intermediate condition classes having much bigger sperm than the highest quality males. Fig. 4 shows a particularly dramatic example.

When competition is universal (p = 1), the pattern observed in all cases is that the worst condition classes invest more in sperm number than the next classes, leading to a dip in sperm number before it rises again when moving to higher condition males (e.g. Fig. 2, 3).

Finally, and perhaps counter-intuitively, we find that increasing sperm competition risk decreases reproductive inequalities. The fitness difference between the best and worst classes decreases as p increases, mainly through a reduction in the fitness of the best classes (e.g. Fig. 2, 3).

The results obtained for a population with smaller variance in reproductive budget follow the same general patterns as described above despite there being more noise in the optimal strategies (Fig. 2, left *vs* right panel), but a larger parameter space needs to be analysed to establish whether this truly generalizes.



Fig. 4 Illustration of a case where the optimal strategy of males belonging to the highest condition classes is to produce exactly *E* sperms, while males of intermediate condition sacrifice number for size and produce much bigger sperm. Despite variation, the lowest condition classes, like the highest, show a predominant strategy of investing in number and not size (parameters *E* = 100, $\sigma_B = 10, p = 0.78$)

Discussion (preliminary)

The optimal investment in sperm number *versus* size is somewhat analogous to the much better studied life history question of quality-quantity trade-offs in offspring production (reviewed in Marshall *et al.* (2018). The analogy appears strong especially in cases where large offspring (e.g. large seeds (Kavanagh & Burns, 2014) are better at displacing competitors who might attempt to establish themselves at the same site, because ultimate success then depends on the relative size of one's own competitors (seeds, or sperm cells) with respect to those of the others (Mesterton-Gibbons & Hardy, 2004). In our context, increasing the investment per offspring in the hope that it recruits to the breeding population is analogous to a specific sperm cell being sufficiently large to

outcompete all of its competitors for a given egg; in both cases, the success depends both on the size and the number of competitors, and in both cases, there may be among-parent differences in total budget.

We found that a male may profit from increasing either the size or the number (or both) of its sperm when enjoying a larger total budget, depending its condition relative to other males, and on the competitive context. The preliminary condition-structured model presented here, despite the narrow parameter space so far analysed, gives insight to how these rules may matter. First, above a certain risk of sperm competition, some males, typically those of intermediate condition, begin sacrificing sperm number in order to increase their competitiveness. This comes at the cost of missed fertilization opportunities in the absence of competition. Second, optimal sperm size does not always vary monotonously with male condition. Third, increasing competition decreases fitness variance in the population. We first discuss the broader evolutionary context in which those results should fit, then urn towards a more methodological critique of the model itself and how to expand on it.

The model presented in this manuscript is an optimization procedure: it does not track evolutionary changes over generations. Thus, while the strategy played by each class of males influences the optimum for others, we have assumed the condition structure of the population to remain unchanged over generations, since there is no genetic basis underlying condition for selection to act upon. The environment provided by the female's reproductive investment and tract also stay fixed. In reality, coevolution has occurred between the length of female reproductive tract and sperm size in drosophilids, the size of both being correlated (Pitnick *et al.*, 1999). When correlating competition risk and male total reproductive investment across species, Immler *et al.* (2011) find that both sperm number and size increase with competition. Competition increases size more than it increases sperm numbers, though, which Immler *et al.* hypothesize to occur due to the fact that ejaculate volumes underlie sperm competition.

One could expect that the female seminal receptacle evolves to become longer if this allows screening for high-quality sires, assuming a correlation between male sperm size and genetic quality. But within several species, Lüpold *et al.* (2016) show that sperm number, not sperm size, correlate with condition. These authors argue that by imposing a demanding lower limit on sperm size, females allow male condition to become apparent in the number of sperm they can afford to produce. Insofar as females benefit from having a high (rather than low) condition male fertilize their eggs, this may in principle be achieved by any combination of size and number that can be best optimized by those with a high budget. In this context, it is interesting that males in our model achieved more equal success (reduced fitness variance) when we assumed multiple mating to be common. This suggests that it might be difficult for females to have a definitive 'last word' on sire identity based on postcopulatory processes alone (but see the discussion below for whether alternative model formulations could restore high variance in male success at high competition risk).

The complexity of evolutionary interactions between female choice and male traits is beyond the model presented in the present manuscript, where we simply focused on the male side. However, the results of Lüpold *et al.* (2016) do not appear consistent with size-driven competition. Instead, they appear consistent with hard selection imposed by females on sperm size (s_{min} in the current model), followed by sperm competition based on sperm number. Size-driven sperm competition as we focused on in the model only rarely resulted in males of low and high quality producing similarly sized sperm, and even then, this equality was only found between particular classes rather than as an overarching solution across all classes. Instead, we found a general correlation

between sperm size and male condition, which suggests that size could be used as signal of male quality in this context. Signalling in this context means that 'poorer' males tend to reveal their low budgets by producing less competitive sperm, but note that honesty does not need to be absolute for female choice to be stable. A proportion of low-condition males may exaggerate their trait without affecting too much the average returns of choosing males based on that trait (Kokko, 1997).

The next logical step in the project is to explore further the parameter space afforded by the current version of the model, and then to replicate the analysis for versions that differ structurally. Empirical knowledge about the mechanisms underlying sperm competition within the female reproductive tract is still largely lacking, and we likewise have only limited information on how males divide their mating budget between pre- and postcopulatory investments including sperm size and number (Lüpold & Pitnick, 2018; Godwin et al. 2017; Firman, 2018). It is therefore difficult to justify particular structural modelling choices, and examining variations remains an important task given their high potential to change the outcome. For instance, the particular shape of the solution we found at high competition risk, where the lowest condition classes invest more in sperm numbers that the next classes, might result from our assumption of redistribution of so far non-fertilized eggs. In the current model formulation, if males that invest in few but competitive sperm are not able to fertilize all the eggs they have successfully monopolized, they leave eggs unfertilized, providing opportunities for a competitor. Low quality males that do not stand a chance in competition exploit this rule in the current version of the model, by giving up on competitiveness and simply trying to fertilize as many of the left-over eggs as possible. Alternative formulations might not give them this opportunity to exploit.

Another structural assumption which needs to be studied is the shape of the size-number trade-off. In the current model, this shape is determined by the allometric coefficient α , which can be justifiably criticized for having no independent effect that is not captured in a mathematically identical manner by changes in the noodling coefficient γ . It would therefore be of interest to make the marginal costs of increasing sperm size higher for males in low condition (a common feature in models of 'honesty', Grafen, 1990; Biernaskie et al., 2018; which in the current context could be biologically interpreted as the maintenance of big sperm being more 'draining' for small males). This might yield a clearer split between low-quality n-strategists and high quality s-strategists. Furthermore, we expect this to restore the variance in reproductive success in the male population at high competition risk, by exacerbating the impact of differences in condition. In the current formulation, more competition results in less disparity in male reproductive output.

The model presented in this manuscript differs in several respects from many traditional game theory models (reviewed in Parker & Pizzari, 2010) that often feature two males differing in nothing else than whether they mated first or second with a given female (or, more generally, if they act in a 'favoured' or 'disfavoured' role). In the current model, males differ in condition too, and instead of condition being partitioned in only two classes, we model it as a semi-continuous, normally distributed trait. It is especially important since the optimal strategy for any given male depends on the competitive environment formed by all others. Population-level variance in condition can clearly affect the results by altering the investment levels that can lead to appreciable success against competitors. We also make explicit assumptions about the number of gametes for both males and females, allowing depletion to be part of the equation, which form further differences to classical weighted raffle models (e.g. Fromhage *et al.*, 2008; Parker& Ball, 2005).

In our model, the optimal strategy is clearly impacted by whether there is a shortage or an excess of eggs compared to sperm cells; although the latter case is probably rare in nature, it must be taken into account in a model where sperm cells can evolve to be large, and hence potentially limiting. Indeed, in species with giant sperm, the number ratio of the two gametes can be surprisingly close to unity: Bjork & Pitnick (2006) estimated that only 5.8 sperms are produced for every egg in *D. bifurcata*, and while still clearly different from 1:1, this number does not yet take into account the further reduction in numbers between sperm offered, sperm stored, and finally sperm used (Bressac *et al.*, 1994). Moreover, females of species with giant sperm remate more often than related species, presumably because they rapidly use up all the sperm transferred by males (Bjork & Pitnick, 2006). Finally, on top of the trade-off between sperm size and number, there is a third dimension that we do not take into account in the model but is likely to matter in terms of optimal strategy: the number of matings a male engages in over its life.

To conclude in a way that puts this chapter in the general perspective of this thesis, the model presented here is an interesting illustration of the intense costs that sex, and sexual selection, can impose on individuals and populations. Aside from the obvious drain on male resources (illustrated by the fact that males with giant sperm modulate their sperm production according to perceived competition risk, Bjork *et al.*, (2007a), the model we analyse suggests that the demographic output of the population might even be reduced due to sexual selection, if males can no longer fertilize all eggs produced by the females. While this has been shown to occur in various species as a result of male-male competition (Warner *et al.*, 1995; Wedell *et al.*, 2002; Smith *et al.*, 2009), it is typically a result of males running out of sperm after multiple matings. In contrast, in the theoretical case modelled here where giant sperm can evolve, sperm shortage can result from the total number of sperm cells produced by all males of the population not matching the total number of eggs available.

CHAPTER VI General Discussion

What have we learnt, between the introduction and now?

In Chapter II, we took a peek at the very early evolutionary history of eukaryotes, as I explored a new hypothesis to explain why our proto-eukaryotic ancestors started fusing with one another on a regular basis. It turns out that if they already possessed and depended on mitochondria, and if the genomes of those mitochondria had started degenerating, then cellular fusion could in principle have evolved as an emergency response to restore vital mitochondrial functions. How often cells should have engaged in fusion back then depends notably on the number of mitochondria they possessed. Trying to assess the likelihood of my most major assumption, I also stumbled across an interesting social phenomenon. There happens to be a heated argument ongoing, about whether mitochondria were domesticated early or late in eukaryogenesis. Following the publication of an analysis favouring the mito-late hypothesis, a second set of authors published a dismissively written response claiming to invalidate the argument, which was in turn addressed in a blogpost by the first party. The story is complete with rather insulting comments on the said blogpost, kindly contributed by a friend of the mito-early party (and a certainly better established than behaved professor). None of this left me the wiser, since both sides were making technical points I am utterly unable to judge. And while this decidedly has more entertainment value than your usual literature search, it is nonetheless a very disagreeable situation, for how can one trust scientists with no apparent capacity for self-doubt and collaboration? I confess I wish my model relied on the hypothesis championed by the polite team (if their attitude is more rational, surely their methods must also be?), sadly it is not so. What this digression says about how scientific ideas progress, I will not venture to speculate on.

Chapter III is set about 2 billion years later than Chapter I, in the present. Based on a broad literature survey, I reviewed the main patterns of distribution of sexual and asexual forms observed nowadays, mostly in plants and animals. Three points are, I find, particularly interesting to underline. First, while the concept of geographic parthenogenesis was originally meant to embrace any difference in distribution between sexuals and asexuals of any form (Vandel, 1928), its use has been impoverished over time in the literature. It is now not uncommon to see it equated with "the phenomenon that asexuals have larger distributions" or "more northerly" than sexuals, at the author's will - which, although those trends have indeed been quantitatively validated in various taxa by recent meta-analyses (Johnson et al., 2010, Ross et al., 2013, Grossenbacher et al., 2015, van der Kooi et al., 2017), vastly undersells the richness of the concept. It also promotes some level of reporting bias, by which only patterns that can be more or less made to fit that particular definition are reported as geographic parthenogenesis, reinforcing even further the disproportionate attention paid to those two particular trends. A second interesting observation to me was that, although many adaptive hypotheses have been put forward to explain those and other geographic patterns, very few have been experimentally tested, and none validated. This does not prevent some hypotheses from being generally accepted as explanations in what seems to be little more than storytelling. Finally, I came to realize (and in great part, after the review was published), that many fascinating instances of geographic parthenogenesis go unreported as such, because they are not found in parthenogens, but in other types of asexuals (like those using vegetative reproduction or selfing - the latter being, arguably, not so different from automictic parthenogenesis). It is unfortunate that the words chosen to coin a concept a hundred years ago can cause similar phenomena in different organisms to be studied independently – perhaps, following the principles of inclusive writing, should one now prefer the term of "geographic asexuality"?

Motivated in part by the three concerns mentioned above, I developed the model presented in Chapter III to address the two most reported patterns of geographic parthenogenesis (larger ranges and higher latitudes), taking into account as large a variety of sexual and asexual modes of reproduction as I could manage, specifically trying to provide a theoretical base against which to assess adaptive hypotheses. The difficulty, indeed, in identifying a selection pressures which varies with latitude and could influence the distribution of sex, is that latitude also correlates with colonization history. The model shows that reproductively autonomous asexuals readily take over the front of an invasion wave, occupying as a result large areas of the most recently colonized habitat – precisely the patterns found in nature. As a conclusion, it seems that validating adaptive hypotheses regarding the distribution of sex cannot be done without transplant experiments.

Finally, Chapter IV provided a more modest ending for the body of this thesis, as it turned away from deep history, wide spaces and profuse diversity, to focus instead on the particular consequences sex had on a specific group of species. Sexual selection and male-male competition, in small bodied species like drosophilids, can drive the evolution of rather large sperm cells, which engage in fierce competition for fertilizations inside the female reproductive tract. In what is unfortunately still little more than a preliminary model, I showed that size-mediated sperm competition can lead males of different phenotypic quality to invest differently in gamete size versus numbers. In particular, when competition risk increases, males of intermediate quality are the first to reduce the number of their gametes in order to increase their competitiveness, thereby sacrificing fertilization opportunities when competition does not occur. This imposes costs on individuals, and reduces further the demographic output of the species, adding to the already present "twofold of males".

Now if we briefly summarize the main characteristics of sex as practiced by the protagonists of each chapter, we reach the following catalogue. Unicellular protoeukaryotes, just like the vast majority of unicellular modern eukaryotes, engage in sex infrequently, with many rounds of asexual divisions in between. The species appearing in the geographic parthenogenesis literature are, on the other hand, multicellular organisms, and mostly belonging to (or discussed as though they belong to) obligately sexual taxa. In truth, there is no good reason for that. It probably comes from the fact that it is more conspicuous when a normally obligately sexual species abandons sexual reproduction entirely, than when a facultatively sexual species simply reduces its rate of sex. Different types of parthenogenesis were discussed, which differ in the amount of recombination that takes place. Vegetative reproduction, overall, was rather swept under the carpet, by me as well as many cited authors, both in cases where it becomes the only mode of reproduction, and in cases where it coexists with sex within one organism. Finally, drosophilids are among the most studied model organisms biological sciences have in store, have a score of fascinating and costly traits related to sexual selection, and are obligate sexuals despite one parthenogenetic species known and a propensity to produce the odd asexual egg (Markow, 2013).

Facultative sex, vegetative reproduction, obligate sexual reproduction. Shall we now go through that diversity again but more slowly, and see if the paradox of sex retains its paradoxical nature after all? Is the prevalence of sex, given its known costs, surprising?



Dunn CW, et al. 2014. Annu. Rev. Ecol. Evol. Syst. 45:371–95

Fig. 1 Phylogenetic tree of Metazoa, borrowed from Dunn et al., 2014

The vast majority of eukaryotes have sex – yes, but how much?

Central to the paradox of sex is the notion that eukaryotes must somehow be having too much sex. Too much for theoreticians' taste, at least, based on how costly it is supposed to be. But how much is too much? Let us first have a look at how the "paradox of sex" is introduced in a selection of recent papers on the topic, to get a general feel: "the question why most organisms shuffle their genetic information instead of just producing exact copies of themselves" (Stelzer, 2015); "The ubiquity of sexual reproduction across eukaryotes" (Stelzer & Lehtonen, 2016) "why sexual reproduction is so widespread" (Otto & Lenormand, 2002); "why so many eukaryotic species produce offspring via sexual reproduction" (Neiman et al., 2018); "Why sexual reproduction predominates in nature remains a mystery" (Neiman et al., 2017). So, is the problem that all eukaryotes have sex all the time? As we have seen throughout this thesis, this is hardly the case, when one considers the whole diversity of eukaryotes, most of which is unicellular and not so keen on entering meiosis at the drop of a hat.

This selection of quotes was non-random, and many other papers do not make the same overstatement (e.g. "That most higher organisms reproduce sexually at least during part of their life cycle is traditionally considered paradoxical", Meirman et al., 2012, or "Sexual reproduction entails a number of costs, and yet the majority of eukaryotes engage in sex, at least occasionally", Otto, 2009). What all the papers quoted have in common, though, is that they do aim to address the paradox of sex directly, and not simply mention it as an introduction to frame a specific sub-question. To the naïve eye that was mine three years ago, the impression that all eukaryotes have sex all the time, and that the cost of sex is somehow twofold (an assumption coming from species with separate sexes and no paternal care) was maintained mostly by being repeated time and again in the introduction of papers that focus on one particular species for which this is true – a case of taxonomic chauvinism (Bonnet et al., 2002). But where is this framing of the paradox of sex coming from?

In the time of Fisher, sex was not thought to be much of a problem (Dagg, 2016). Its long term benefits in terms of lineage persistence seemed obvious and the notion of a trait being there "for the good of the species" was still uncritically accepted (Wilson & Wilson, 2008). It is only some 30 years later, in the 60's, that Williams and Maynard-Smith looked at the problem differently, and wondered what was in for the individual. Specifically, it was the twofold cost of males that made them ask what immediate benefits of sex prevented sexual individuals from being rapidly outcompeted by asexual counterparts (Dagg, 2016). The paradox of sex was born, and it was born of a species with males, females, and obligate sex.

Following that, sexual theory has mainly been tailored for multicellular organisms (to be honest: animals and plants), because unicellular eukaryotes were considered primitive and often assumed to be asexual (Lahr et al., 2011), and because fungi were, well, it's complicated (my interpretation). Sex was therefore viewed as something necessary to reproduction, or, at the very least, part of the scheduled life-history of every individual, vegetative reproduction being arguably a form of growth (we will come back to that). But this view does not fit well with protists, and most eukaryotes are protists. This statement is not necessarily to be taken in terms of numbers of species: there are only around 200,000 species of protists described (Cordliss, 2002, although this is anyway bound to be a dramatic underestimation), but in terms of evolutionary diversity. The group of insects, for instance, might well be extremely speciose - they are phylogenetically so related as to count as a single data point, when considering a deep phylogeny of eukaryotes. The multiple supergroups of protists, on the other hand, are separated by very long evolutionary histories (Intro, Fig. 1), and their evolution can be considered independent. Moreover their unicellular, facultatively sexual lifestyle is likely to be relatively similar to that of our Last Eukaryotic Common Ancestor (Speijer et al., 2015) - and all multicellular taxa are nested within groups of protists. So, do those archetypal eukaryotes have sex too often?
Measuring the frequency at which unicellular eukaryotes have sex is not easy, but it has been estimated as once every 10^2 to 10^5 generations in the marine unicellular *Pseudoperkinsus tapeti* (Marshall & Berbee, 2010), every 10^3 in the wild yeast *Saccharomyces paradoxus* (Tsai et al., 2008), and every 10 to 10^4 generations in the budding yeast *S. cerevisiae* (Ruderfer et al., 2006; all three are Opisthokonts, so relatively close to animals). There is only one case of obligate sexuality reported to my knowledge, in the genus *Corythion* (Rhizaria; Lahr et al., 2011). As for obligate asexuality, after close inspection it does not seem to be a characteristic of any ancient group, although absence is much harder to prove than presence (Lahr et al., 2011).

Costs of sex are high for a unicell, especially in the form of the lengthiness and riskiness of meiosis (Lewis, 1983, Levitis et al., 2017). But sex can be timed to coincide with a period of low costs (e.g. low population growth rate) and maximum benefit (low general fitness, stress, or DNA damage; Nedelcu et al., 2004; Hörandl & Hadacek, 2013). Under this angle, although there is still certainly much to learn about the costs and benefits of sex and their dynamics in protists, it does not seem like eukaryotes are frankly having too much sex compared to expectations. But what happens to the ease of choosing between mitotic or meiotic reproduction, when one is a multicellular?

The vast majority of eukaryotes reproduces asexually – animals included!

If you are a protist, your cell is your body, your soma, your germline. Multicellular eukaryotes (i.e. some red and some green algae, some fungi, some plants, and all animals; Parfrey & Lahr, 2013) grow their body via mitosis, and produce gametes via meiosis. The direct equivalent of the protist's asexual division is therefore what is called vegetative reproduction: the creation of a new individual from a propagule made of somatic cells. Coming from a multicellular organism, propagules can be made of one or several cells. Multicellular propagules have a survival advantage from already being big, although modelling shows that unicellular ones might allow growth rate maximization (Pichugin et al., 2017). Gametic cells, however, need to be single in order to fuse with another (after a thought experiment, Grosberg & Strathmann, 1998, daring to ask the real questions, conclude that multicellular gamete aggregates would be inefficient, run the risk of ending up a partly unfertilized chimera, and foster conflict due to a non-homogeneous genetic composition). From there it follows that, once organisms become multicellular, sexual and asexual reproduction take very different evolutionary roads, as the former requires a unicellular bottleneck, and the latter likely involves multicellular propagules.

Plants are sessile organisms in which vegetative reproduction is a common ability (possessed by 80% of Angiosperms, Klimeš et al., 1997). The original asymmetry in propagule size might be one reason why dispersal is now carried out by pollen and seeds, while vegetative propagules are not much good at dispersion, so much so that vegetative reproduction is sometimes not considered reproduction but simply growth (although there exists some variation, between the very local tubers or tillers, and the more travel-happy runners, bulbils or floating propagules, Vallejo-Marín et al., 2010). On top of this speculative ancestry argument, there are adaptive reasons why sex should be associated with dispersal, and clonality with philopatry (Gerber & Kokko, 2018). Conversely, asexual dispersal structures have evolved, for instance, in fungi in the form of mitospores: unicellular dispersive asexual propagules (Möykkynen, 1997). Nonetheless, be it only by historical convenience, sexual reproduction in plants is usually more associated with dispersal therefore becomes an "entrenched" benefit of sex. Stopping sexual reproduction to instead focus on the ancestral mode of asexual reproduction, i.e. vegetative reproduction, comes at the immediate cost of reduced dispersal abilities. One way to by-pass this

cost is to evolve apomixis, which is the secondary co-option of structures that have evolved for sexual reproduction, to reproduce asexually instead. Plants possess an unparalleled diversity of options to reproduce, between species but also within a single individual: some can do vegetative propagation, sexual reproduction (both selfing and outcrossing), and apomictic reproduction all at the same time (Barrett, 2015). In plants, an analysis of the costs and benefits of sex cannot be done without taking into account the characteristics of, and allocation to, the different ways of reproducing both sexually and asexually, and the confounding factor of dispersal. But aside from the obvious complexity of the system, does the rate of sex in plants represent an overall paradox?

Animals, the very taxon that has inspired the paradox of sex, are also widely capable of vegetative reproduction (Meirmans et al., 2012), much more widely than I had originally realized. Although it seems to be absent in Chordata, Mollusks, Rotifera, and the big group of Ecdysozoa (and probably other smaller groups), the ability to reproduce vegetatively is found broadly in all the rest of the tree (see Fig. 1), e.g. in Porifera, by gemmules and budding (Ereskovsky & Tokina, 2007), in Cnidaria, by budding and strobilation (Technau & Steele, 2011), in Echinodermata, by fission (Karako et al., 2002), in Bryozoan, by budding and fission (Thorp et al., 2010), in Annelida, by fission (Bely & Wray, 2001), in Platyhelminthes, by fission (Malinowski et al., 2017) etc. Interestingly, the ability to reproduce vegetatively and the ability to regenerate missing parts do not appear coupled in the phylogeny of Metazoans (Alvarado, 2000), although they are within planarians (Egger et al., 2007).

So, how often do those animals make use of their asexual powers to reproduce? I wish I had more time to investigate the incidence of sexual versus vegetative reproduction in Metazoa for this discussion, but the first two examples that came my way will have to do in the matter of a first impression: when Barker & Scheibling last checked (2008), up to a third of the sea stars of a population had recently undergone fission (see Dolmatov, 2014, for factors affecting fission rates in echinoderms); while Malinowski et al. (2017) tell us that the planarian *Dugesia japonica* undergoes fission once per month per worm (but it needs to be dark).

From what we have just seen, eukaryotes are ancestrally capable of both meiotic, sexual reproduction, and mitotic, asexual reproduction (also called vegetative reproduction). Although they might come with different, lineage specific correlated traits, having the choice seems like a luxury worth keeping. Why, then, did some taxa mostly remarkable by the fact that they write the textbooks, end up losing the ability to reproduce asexually?

The real weirdoes: obligate sexuals and obligate asexuals

The suggestion is getting more pressing that what constitutes perhaps the greatest paradox of sex is the existence of obligately sexual taxa (Kleiman & Hadany, 2015; Burke & Bonduriansky, 2017, Neiman et al., 2018). The problem is often framed in those terms: "to explain the paradox of obligate sex, theory must account for the capacity of obligately sexual populations to resist invasions by facultatively asexual mutants" (from Burke & Bonduriansky, 2018; see also Kleiman & Hadany, 2015; Burke & Bonduriansky, 2017; Neiman et al., 2018). That would certainly help, but given that obligate sexuals descend from organisms that could do both sex and vegetative reproduction, should not the first question to ask be why some lineages abandoned the ability to reproduce asexually in the first place? (Hadany & Otto, 2007)

When placing phylogeny and ancestral states at the core of the matter, one ends up with different questions. It is no longer solely about the theoretical costs and benefits of sex, outcrossing, or recombination, taken in isolation from the rest of the body. The question of the loss of asexual reproduction becomes about pluripotent cells, ontogeny and body plan. Things of which I know

nothing. I do suspect, however, that losing the capacity to reproduce vegetatively must have been the price to pay for some lineages (e.g. among animals: Chordata, Mollusks, Rotifera, Ecdysozoa) to explore new advantageous body plans, and that it seemed like a good idea at the time it evolved. As a result, those lineages got stuck with sex as the only possible way of reproducing themselves. Paradoxically, this can also be viewed as the benefits of sex reaching an all-time high: they become no less than reproduction itself. But by the same token, the costs need now be paid every single generation, which is especially heavy in terms of needing to find a partner, and producing males in species with separate sexes (males who, incidentally, became an even worse demographic burden the moment they lost the ability to reproduce themselves vegetatively). This situation is not very stable, as illustrated by the numbers of secondary transitions to asexuality (van der Kooi et al., 2017).

At that stage, the only way to restore the capacity of asexual reproduction is to hijack the structures already used for sexual reproduction, but removing the sex - that is called parthenogenesis. Parthenogenesis is therefore the derived type of asexual reproduction, as opposed to vegetative reproduction, which is ancestral. What is really meant by "removing the sex" is left to the discretion of the species (Meirmans et al., 2012): for some it means fully replacing meiosis by mitosis (apomixis), but for some others meiosis is still part of the process, which can be seen as some form of self-fertilization (automixis, with benefits of meiosis, in terms of DNA repair, potentially still reaped, Hörandl & Hadacek, 2013), while some still require interactions with a male partner in order to complete their own asexual reproduction (pseudogamy). Having to repurpose structures and processes that have evolved for sexual reproduction does not make the transition easy (Rice, 2002); for instance, restoring offspring ploidy from unfertilized gametes can be hazardous (Neiman & Schwander, 2011; Levitis et al., 2017). Moreover, when a transition to parthenogenesis occurs, the asexuals are cut-off from the rest of the species gene pool and go through a severe bottleneck. All those constraints make it difficult to compare pairs of sexual and asexual lineages simply based on the presence or absence of sex - all other things are definitely not equal. The fitness of parthenogens is often worse than that of their sexual counterparts (Levitis et al., 2017), and tellingly, successful transitions are found more in species with large population sizes (Ross et al., 2013).

The relatively young age of obligately asexual taxa leads to suppose that they eventually get extinct in the absence of the long term genetic benefits of sex (but see Schwander & Crespi, 2009), although the genomic consequences of asexuality have not been found to be as dramatic as expected in recent analyses (Jaron et al., 2018). When an asexual lineage lasts, it might be thanks to the evolution of non-canonical mechanisms that fulfil the functions of sex (Lahr et al., 2011), as in the case of the famously ancient "asexual scandal", bdelloid rotifers, which display high frequency of horizontal gene transfer, and strong DNA repair mechanisms (Schwander, 2016; Hecox-Lea & Welch, 2018).

Responding to the problems posed by obligate sexuality by going fully asexual might justifiably seem a little drastic. Facultative sexuality combines the best of both worlds, and despite the evolutionary challenge of being able to do both from the same ancestral machinery, it has evolved in several groups of ancestrally obligately sexual animals (Jalvingh et al., 2016, Schneider & Elgar, 2010). Why it is not more common is still an open question, but it seems that the stability of such systems is reinforced by a sex determination system that allows male production from parthenogenetic eggs, and the association of sex with a critical feature of the species life-cycle such as diapause (Burke & Bonduriansky, 2018; Stelzer & Lehtonen, 2016).

Conclusions

After ambling through the diversity of eukaryotic sex and asex to better understand what is referred to as the paradox of sex, I am left feeling rather more confused than when I started. I still find that a lot of my thinking is shaped by pluricellular oddities, especially animals, and I expect it will take a lot of time to finally embrace the relative complexity found in the sexual lives of plants, fungi, protists, and see them as the new normal.

Perhaps the root of my confusion also lies in the very definition of sex, after all. For defining sex by its features (e.g. meiosis, alternation of haploid and diploid phases) does little to our understanding of its adaptive nature – but defining sex by its function is almost a case of circular reasoning, when one looks precisely for the reasons explaining its ubiquity. Is a selfing plant having sex? It is definitely undergoing meiosis and recombination, although it is not exchanging genes with another individual than itself. Is a rotifer picking up environmental DNA and recombining with it having sex? There is no meiosis, there is no reciprocal exchange, and yet this mechanism has proved to allow the long term striving of asexual rotifers, in a way that is seen as successfully replacing "canonical" sex. How about viruses and prokaryotes..?

What is more, sex has become over evolutionary time associated with a host of different features, in a very taxon-specific manner, like dormancy, dispersal, cellular bottleneck, germline, reproduction itself... Therefore even in a species that does both sex and asexual reproduction, identifying the conditions that trigger sex is not necessarily enough to tell apart whether it is sex itself, or one of its associated features, that is being called upon.

Because of that, I have come to think that the most promising systems to understand the most fundamental costs and benefits of sex are unicellular eukaryotes, particularly species where sex does not lead to subsequently entering a particular physiologic state (no association with dormancy, for instance). Asking one cell, capable of both meiosis and mitosis, which one it prefers to undergo given a specific set of environmental cues. Does it get any more straightforward? To go even further, it might even be beneficial to forget the distinction between eukaryotic sex and non-eukaryotic sex, since the very same questions are being asked in bacteria (e.g. is sex rather a conservative or innovative process, Ambur et al., 2016? in what contexts do species lose or gain competence, Mell & Redfield, 2014?).

Finally, as a theoretician, I now realize that we perhaps too often neglect to consider phylogeny, and the order in which evolutionary innovations really took place in the history of eukaryotes. For instance, what do we learn about the world, from a model showing that obligate sex could facilitate the evolution of anisogamy, when obligate sex is only known to have appeared in anisogamous lineages, a billion year after anisogamy itself (Lehtonen et al., 2016)? How to best balance theoretical abstraction and generality, with relevance to transitions that actually happened, and organisms that actually exist or have existed? Projects like the Tree of Sex provide new exciting resources to better address such questions (Ashman et al., 2014).

Perhaps, after all, the best remedy to my growing confusion in the midst of eukaryotic diversity is simply to amble some more, and get to know that great big tree better until it starts making more intimate sense.

"I don't understand!" says Mammal. "Everyone is weird but me!"

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SCIENTIFIC CV

Personal information

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Education & training

- 2015 2019 **PhD student in Evolutionary Biology**, supervised by Hanna Kokko, University of Zürich, Switzerland
- 2012 2015 : **Master's degree obtained with honours, top student**: Ecology, Evolution, Biometry, Université Claude Bernard, Lyon 1 (UCBL), France
- 2009 2012: **Bachelor's degree with high honours, top student**: Biology of Organisms and Populations, UCBL
- 2009: Baccalauréat of Science with highest honours, Lycée Lumière, La Ciotat, France

Papers published

All manuscripts have been submitted to society journals or non-for-profit publishers.

- Tilquin, A., Christie, J. R., & Kokko, H. (2018). Mitochondrial complementation: a possible neglected factor behind early eukaryotic sex. Journal of evolutionary biology.
- Tilquin, A., & Kokko, H. (2016). What does the geography of parthenogenesis teach us about sex? Phil. Trans. R. Soc. B, 371(1706), 20150538.

Side-activity report

- Public Outreach and Event Organization
 - Scientific speed-dating, side event at Evolution 2018, August 20th 2018, Montpellier

- **Scientific busking**, with the "Biology means life" outreach project, 4th April 2018, on Irchel campus (Zürich) *Biodiversity of sounds in the sea*
- Scientific speed-dating, side event at Biology18, February 14th 2018, Neuchâtel
- **Organization of a workshop**, Special Topic Network in association with ESEB 2017, Lund *Bridging the gap between local adaptation and sex-differences*
- **Ignite Zürich**, Zürich, Switzerland, March 3rd 2017 5 minute TED-like talk: *The songs of fish the noise of humans*
- Massive Online Open Course in Biodiversity, UZH, 2016 Creation of a 9 minute video, shot together with Hanna Kokko, on the evolution of sex and mating diversity
- o Scientific speed-dating, side event at Biology16, February 10th 2016, Lausanne
- Oral Presentations
 - **Evolution 2018**, Montpellier, France, August 19-22th 2018 *Asexuals take over the front of an invasion wave*
 - **Biology18**, Neuchâtel, Switzerland, February 15-16th 2018 *Endosymbiosis, the original sin? How cell fusion could have evolved to mitigate mitochondrial meltdown*
 - **IEU PhD Retreat**, Switzerland, June 6-8th 2017 Original sin: mutation accumulation *in mitochondria at the origins of sex?*
 - **Behaviour, Ecology, Environment and Evolution Seminars**, Zürich, Switzerland, May 30th 2017 *Sex in Eukaryotes: mitochondria as the original sin*
 - **Biology17**, Bern, Switzerland, February 2-3rd 2017 Flash-talk: *Asexuality takes over the front of invasion waves: a new explanation for geographic parthenogenesis*
 - **Zürich Interaction Seminar**, Zürich, Switzerland, October 17th 2016 –*Why isn't everybody asexual already?*
 - **Biology16**, Lausanne, Switzerland, February 10-13th 2016 *The geography of parthenogenesis: what we really know*
 - **Retreat with the Schwander lab (University of Lausanne)**, Switzerland, February 7-10th 2016 *Weird sex and the evolutionary origins of sexual reproduction*
 - **Center of Excellence in Biological Interactions Annual Meeting**, Lammi, Finland, October 2015 *Geographic parthenogenesis: first steps to a review*
 - SFA (Société Française d'Acoustique) meeting, Journées d'étude Acoustique et Applications Navales, Paris, France, October 27th 2015 Le Monde du Vacarme : impact des activités humaines sur l'univers acoustique d'un poisson
 - **EMPSEB 2015**, Sterling, Scotland, September 2015 *Why mating*? *Modelling the spread of a sex-strike allele in haplodiploids*
- Posters
 - **Biology19**, Zürich, Switzerland, February 7-8th 2019 *Why would anyone have sex every single generation?*
 - **European Marine Biology Symposium (EMBS53),** Ostend, Belgium, September 17-21st 2018 Your research on our boat? A citizen science project around the world aboard a 10m yacht
 - ESEB 2017, Groningen, The Netherlands, August 20-25th 2017 Endosymbiosis at the origin of sex? How cell fusion could have evolved to counter mitochondrial meltdown – Tilquin, Christie, Kokko
 - **Biology17**, Bern, Switzerland, February 2-3rd 2017 *Asexuality takes over the front of invasion waves: a new explanation for geographic parthenogenesis* Tilquin, Kokko

- Sfécologie2016, International Conference on Ecological Sciences, Marseille, France, October 24-28th 2016 – Why mating? Modelling the spread of a sex-strike allele in haplodiploids – Tilquin, Fauvergue
- Center of Excellence in Biological Interactions Annual Meeting, Jyväskylä, Finland, August 6-9th 2016 – Asexual reproduction: where, when and why? – Gerber, Tilquin, Booksmythe, Kokko
- Awards
 - Runner-up for the team spirit and research productivity prize, Center of Excellence in Biological Interactions Annual Meeting, Jyväskylä, Finland, August 6-9th 2016
 - **Runner-up for the best talk**, Center of Excellence in Biological Interactions Annual Meeting, Lammi, Finland, October 2015
- Reviewing work, peer-reviewed articles
 - Ethology (February 2018)
 - Proceedings of the Royal Society B: Biological Sciences (February 2017, October 2017)
 - Journal of Evolutionary Biology (January 2017)
 - Scientific Reports (December 2016)
 - Journal of Behavioural Ecology And Sociobiology (March 2016)
- Reviewing work, grant proposals
 - Grant proposal for the Agence Nationale pour la Recherche (French National Research Agency), 439k€ (May 2018)
- Courses given
 - **Calculus:** teaching practicals and grading copies in calculus (MAT182, 1st year biomedicine) Fall 2016 (120 hours)
 - Modelling: one practical given on linear algebra applied to sustainable harvesting
 Spring 2016 (3 hours)
 - **Statistics:** practicals in statistics using R Fall 2016 (30 hours); tutoring of a group of master students Fall 2015 (30 hours)
- Courses taken
 - Science Videos for the Web (Transferable skills) Spring 2018 1 ECTS
 - Scientific Busking (Transferable skills) Spring 2018 1 ECTS
 - Introduction to UNIX/Linux and Bash Scripting (BIO609) Spring 2017 1 ECTS
 - Logic and Reasoning for Scientists (Transferable skills) Spring 2017 1 ECTS
 - Scientific Writing for Evolutionary Biologists (BIO555, Transferable skills) Fall 2016 1 ECTS
 - o The Evolution of Human Nature (BIO215) Fall 2016 2 ECTS
 - **Post-Beginner German** (Transferable skills) Fall 2016 1 ECTS
 - Zürich Interaction Seminars (ECO401) Fall 2015, Spring 2016, Fall 2016 1 ECTS
 - **Science Photography** (BIO367) Fall 2016 2 ECTS
 - **Topics in Evolutionary Biology** (BIO554) Fall 2015 1 ECTS

(A) SEXUAL REPRODUCTION

WHERE, WHEN & WHY?

by Nina Gerber, Anaïs Tilquin, Isobel Booksmythe & Hanna Kokko

WHY TO HAVE SEX

• Why did early Eukaryotes evolve sex, the organized exchange of genetic material between two individuals as a result of meiosis? Could this innovation be linked to their recent incorporation of mitochondria, and the pressure of coevolution? We plan modelling work on the topic with new team-members.

• Why is sexuality still so prevalent today compared to asexuality? To find out, we study when and where organisms resort to sex and not asex.



University of



Sex is often coupled with dispersal in space or time (Daphnia, aphids, plants...). We look for conditions that lead to such a coevolution, in an environment that changes in space and over time. Ongoing modelling project.



THE ROLE OF MALES

Sexual harassment affects the maintenance of sex in a facultatively sexual species. Our model shows that resisting mating pays off at low population densities, leading to full asexuality and extinction of males. In a narrow range of higher densities, facultative sex persists. At high densities, resisting males is too costly and fully sexual reproduction wins. This could explain the rarity of facultative sexuality, and some patterns of geographic parthenogenesis.





Endosymbiosis at the origin of sex? How cell fusion could have evolved to counter mitochondrial meltdown

University of Zurich¹²⁴

Anaïs Tilquin¹, Arunas Radzvilavičius² & Hanna Kokko¹ ¹University of Zurich, Switzerland; ²University of Pennsylvania, USA



Birth of the eukaryotes: the Different mitochondrial strains can evolution of sex and mitochondria complement each other Host 1 During the early days of viability Early Hypothetical Extant eukaryogenesis, mutations eukaryotes eukaryotes step accumulate fast in mitochondria 0.5 **Complementation function** Cell In a heteroplasmic cell, different fusion 0 mitochondria can mask each Mitochondrial conten No sex other's mutations (<u>;</u>) **Biparental** inheritance Yes! Uniparental Uniparental Cell fusion increases heteroplasmy inheritance inheritance of of Countering random segregation of mitochondria during cell mitochondria mitochondria division, which tends to restore homoplasmy: ...Can biparental inheritance be beneficial ? In simulations, fusion evolves to maintain high levels of heteroplasmy



If there is no cost to fusing (x=1), cells evolve to fuse and mix every generation (z=1), maximizing heteroplasmy

If there is a cost to fusing (x<1), cells can evolve to fuse at a rate lower than once per generation (z<1)

The fewer mitochondria (y), the faster random segregation restores homoplasmy, and the more fusion is needed (z)

Also: the shape of the complementation function influences the optimal rate of fusion that evolves (results not shown, but just ask me)

Conclusions and outlook

 The assumptions we make about eukaryogenesis: mitochondria predated sex; different deleterious mutations accumulated in different mitochondrial lineages; heteroplasmy and complementation can restore a "wild-type" mitochondrial phenotype
 The results we obtain: cell fusion can evolve to maintain a heterogeneous cytoplasm ➤ Further speculations: cell fusion could have been the first step allowing the evolution of the nuclear interactions called 'sex'. Then, as more mitochondrial genes were transferred to the nucleus, sex and recombination made biparental inheritance and heteroplasmy obsolete. Uniparental inheritance evolved... But that is another story!

