Theoretical Studies on the Role and Evolution of Mating Types and Two Sexes

Zena Hadjivasiliou

May 2014

A Thesis submitted for the degree of Doctor of Philosophy

CoMPLEX
UCL
Physics Building
Gower Street
London, WC1E 6BT

I, Zena Hadjivasiliou, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Στη γιαγιὰ τη Νίνα, την δόκτορα του σμιλιοὺ, με αγὰπη,

Acknowledgements

I am deeply grateful to my supervisors Andrew Pomiankowski and Nick Lane for their guidance and patience, for inspiring and challenging me, and for encouraging me to pursue my own curiosity and ideas. I am also thankful to Rob Seymour who is no longer with us but who was a great supervisor, without whom the work carried out in the first part of this thesis would not have been possible. I would like to thank Yoh Iwasa for hosting me at his lab at Kyushu University and for his valuable contribution to the development of the model presented in Chapter 5. I am also grateful to all the people in the Iwasa lab for their hospitality and stimulating discussions, particularly Marko Josup and Joung Hun Lee. I am indebted to many people for insightful and challenging discussions that have undoubtedly contributed to the development of this work, in particular, Joe Bailey, Marko Josup, Lorette Noiret, Elena Savvidou and Buzz Baum. I would also like to express my gratitude to Bram Kuijper, Joe Bailey and Lorette Noiret for their invaluable comments on the work presented in Part II of this thesis, and Zoe Hadjivasiliou for help with editing. I also thank all the people that made CoMPLEX a stimulating and wonderful environment to work in: Lorette, Sam, Mae, Ed, Dorothy, Lisa, Liz, Sami, Victor, Sophie, Zhuoiy and especially Abi and Chris that made administration so easy. Finally, a warm thank you to my parents for giving me the chance and for their support throughout, and to Joe for his endless patience.

Abstract

Why there are two distinct sexes has received little attention compared with that lavished on the value of sexual reproduction. While sex requires two parents, there is no obvious need for these to be of different sexes. Furthermore, self-incompatible gametes seemingly reduce the likelihood of finding a partner. What causes mating types and sexes to predominate in nature remains a conundrum.

The uniparental inheritance (UPI) of mitochondria (in which only one sex, usually the female, passes on its mitochondria) is widespread among sexual organisms. Theoretical work suggests that the evolution of two sexes can be understood in the light of mitochondrial inheritance. However, the exact role of UPI is not clearly understood. Part I of this thesis considers the evolution of self-incompatible mating types in relation to this perspective, using probability theory and population genetics. Chapter 2 studies the impact of UPI on interactions between genes in the mitochondria and the nucleus, in an effort to elucidate the role of UPI itself. In Chapter 3, I develop a new, explicit theoretical model that challenges the prominent view that selection for UPI leads to the establishment of self-incompatible mating types and sexes.

An alternative hypothesis proposes that mating types evolved as a consequence of selection for asymmetry in gamete attraction and recognition. This idea is based on the assumption that an asymmetry in gamete communication leads to more effective attraction and recognition. In Part II of this thesis, I examine this idea further. In Chapter 4, I perform an extensive literature review of mating type interactions and provide empirical support for the prediction that an asymmetry in signalling is indeed common in nature. The underlying assumptions of this hypothesis are linked to the physical constraints that gametes experience during sex, and the role of polarity in cell-cell interactions. To assess the impact of these constraints rigorously, in Chapter 5 I develop a biophysical model for signaller-detector dynamics based on chemical diffusion, chemotaxis and individual cell movement that can be tested *in silico* and *in vitro*.

This thesis examines the role and origins of self-incompatible mating types and sexes. The novel theoretical methods and perspective on the empirical literature presented here place this evolutionary question in a fresh context and encourage further theoretical and empirical work.

Contents

1 Introduction **12**

1.1 Sex, sexes and mating types 12

1.1.1 Sex 12

1.1.2 Mating cells 13

1.2 Why do we need sexes? 16

1.2.1 The paradox 16

1.2.2 Models for mating type evolution: a review 17

1.2.3 The number of mating types 23

1.3 Thesis aims and modelling approach 24

1.4 Thesis layout 26

I Dynamics and evolution of mitochondrial inheritance **28**

2 Selection for mitonuclear coadaptation and the evolution of two sexes **30**

2.1 Introduction 30

2.2 Model of mitonuclear coadaptation 33

2.3 Results 36

2.3.1 Simple model with no bottleneck 38

2.3.2 Effect of a bottleneck 40

2.3.3 Varying μ and ν 40

2.3.4 Rates of evolution 42

2.4 Discussion 42

2.5 Appendix 46

2.5.1 Mathematical Derivations 46

2.5.2 Simulations . . . 50
2.5.3 Extensions to the model . . . 50

3 Dynamics of mitochondrial inheritance in the evolution of mating types and two sexes 56

3.1 Introduction . . . 57
3.2 Model outline . . . 59
3.3 Results . . . 60
3.3.1 Mitochondrial mutation pressure . . . 60
3.3.2 Mutants with a replicative advantage . . . 63
3.3.3 Mitonuclear coadaptation . . . 64
3.3.4 Mating types . . . 65
3.4 Discussion . . . 67
3.5 Appendix . . . 70
3.5.1 Mathematical Derivations . . . 70
3.5.2 Extensions to the model . . . 73

II Cell-cell signalling and the evolution of mating types 83

4 Literature review of mating-type-specific functions 85

4.1 Introduction . . . 85
4.2 Method . . . 87
4.3 Literature review . . . 88
4.3.1 Plantae (Green Algae) . . . 88
4.3.2 Chromalveolates . . . 90
4.3.3 Unikonta . . . 93
4.3.4 Homothallic mating . . . 98
4.4 Discussion . . . 101
4.5 Appendix . . . 104

5 Cell-cell signaling in sexual chemotaxis and the evolution of mating types 113

5.1 Introduction . . . 114
5.2 How mating types can avoid self-stimulation: a model . . . 117
5.2.1 Chemotaxis in eukaryotes . . . 117
5.2.2 Cell Sensing and Movement . . . 118

5.2.3 Chemical Diffusion . . . 121
5.3 Results . . . 124
5.3.1 Two cells . . . 124
5.3.2 Multiple cells . . . 131
5.4 Discussion . . . 134
5.5 Limitations and further work . . . 136
5.6 Appendix . . . 138
5.6.1 Numerical solution . . . 138
5.6.2 Stability Conditions . . . 140
5.6.3 Grid size . . . 141
5.6.4 Tables . . . 141

6 Summary, further work and conclusions **145**
6.1 Summary and further work . . . 146
6.1.1 Mitochondria in the evolution of mating types and sexes . . . 146
6.1.2 Signalling in the evolution of mating types and sexes . . . 150
6.2 Some further issues . . . 153
6.2.1 The number of mating types . . . 153
6.2.2 From mating types to sexes . . . 154
6.3 Conclusion . . . 156

References **158**

List of figures

1.1 Sexes and mating types 15

2.1 Fitness as function of mitonuclear coadaptation 33
2.2 Life cycle diagram 34
2.3 Equilibria states 36
2.4 Population fitness density under coadaptation 38
2.5 Fitness advantage of uniparental inheritance over biparental inheritance and contour plot for varying B and M 39
2.6 Fitness under uniparental and biparental inheritance against mutation rates 41
2.7 Mean time until mean fitness stabilizes for uniparental and biparental inheritance 42
2.8 Fitness function with added heterozygote advantage 55
2.9 Fitness function for M = 50 and n_{th}=40. 55

3.1 Life cycle 58
3.2 Fitness function and the three equilibria 60
3.3 Normalised mean fitness of genotypes and genes 61
3.4 Genotype frequencies and mean genotype fitness through time 62
3.5 Leakage diagram 63
3.6 Fitness distribution for different modes of mitochondrial inheritance 64
3.7 Equilibria with mating types 66
3.8 Fitness density plot 75
3.9 Mean fitness of genes and genotypes for a concave fitness function and A x A matings uniparental 76
3.10 Mean fitness of genes and genotypes for a convex fitness function 77
3.11 Probability of sampling a mutant mitochondria under the assumption of selfish conflict. . . 78

3.12 Equilibirum gene frequencies against replicative advantage 79
3.13 Genotype and gene frequency over time assuming fluctuating environments 80
3.14 Change in genotype frequency with self incompatible mutants starting with $p_{aa} = 1$ 81
3.15 Equilibrium frequency of A_1 for different M and μ 82
3.16 Change in genotype frequency with mating types and recombination 82

5.1 The consequences of self-secretion 115
5.2 Receptor occupancy and difference in receptor occupancy against concentration 117
5.3 Numerical approach on a two dimensional grid 119
5.4 Concentration around a moving cell 123
5.5 Mean time until fusion for random walkers 124
5.6 Cell trajectories 125
5.7 Diffusive behaviour 126
5.8 Mean time until fusion for two cells for fixed S/K_d 127
5.9 Parameter analysis for M=2. 128
5.10 Impact of cell density and cell speed for random movement 131
5.11 Parameter Analysis for M>2 133
5.12 Grid size effects 142

6.1 Possible evolutionary transitions towards mating types and sexes 155

List of Tables

2.1 Summary statistics capturing fitness of population 37

2.2 Probability of mutating from nuclear state k to nuclear state i; k, i = 1,2,3 for states (0 0), (0 1) and (1 1), respectively. 47

2.3 Summary statistics for different parameter sets (M, B, μ, ν) for heterozygote advantage c. . 53

2.4 Summary statistics assuming $n_{th} < M$ 54

4.1 Mating-type-specific functions 98

4.2 Mating-type-specific functions in Green Algae 105

4.3 Mating-type-specific functions in Chromalveolates 108

4.4 Mating-type-specific functions in Fungi and Amoebazoa 111

5.1 Parameters, variables and symbols 120

5.2 Parameter interpretation 130

5.3 Mean time until fusion for $M = 2$ 143

5.4 The time until 75% of cells pair up ($M > 2$) 144

Chapter 1

Introduction

Sexual reproduction requires two parents, but the need for the two partners to be different remains a conundrum in evolutionary biology. Indeed, the gametes of nearly all sexual organisms are divided into different groups – sexes, or mating types – and gametes of the same sex or mating type cannot unite. Self-incompatible gamete classes, however, seemingly reduce the likelihood of finding a partner. Why are they then so widely spread in nature? The evolution of sexes, i.e. male and female gametes, has received ample attention and is largely ascribed to disruptive selection. The adaptive benefits of mating types, however, where morphologically identical gametes are divided into self-incompatible mating groups, remain a mystery. The evolution of mating types in a previously pan-sexual population, where any gamete could mate with any other, received attention during the last decades of the 20th century, but it has thereafter laid largely dormant. This work addresses the evolution of mating types by focusing on explicating the functional significance of mating cells. In this thesis I reevaluate past theoretical work using new mathematical approaches and place previous workers' and my own findings within our current understanding of cell biology. Furthermore, I explore novel ideas that elucidate the functional role of self-incompatible mating types, and the adaptive benefits of asymmetric gamete fusions. This work aims to provide a fresh perspective on the evolution of sexes while encouraging further theoretical and experimental work.

1.1 Sex, sexes and mating types

1.1.1 Sex

The ability to generate progeny goes hand in hand with life itself. Unicellular organisms such as bacteria and some protists reproduce asexually through simple cellular divisions, producing offspring that are identical to

their parents. Genetic variation in asexual species emerges from mutation and lateral gene transfer between organisms (Zhaxybayeva and Doolittle, 2011). In contrast to clonal division, sexual reproduction requires two partners. Typically, the genomes of the two parents undergo meiotic division producing haploid gametes which then combine in the zygote, a process known as fertilization. This gives rise to a new and unique organism.

The majority of eukaryotic organisms reproduce sexually at least at some point during their life cycle. In fact, sex is considered to be a primordial and central characteristic of all eukaryotes (Ramesh et al., 2005; Lane, 2011a). Furthermore, the persistence of sex across complex life forms has led to an extensive body of work that elucidates its evolutionary advantages (Maynard Smith, 1978; Bell, 1982; Otto, 2009). The origin of sex however, remains the greatest mystery in evolutionary biology.

The means employed by organisms to achieve sexual reproduction vary greatly. In animals, morphologically distinct males and females with specialized sexual organs produce sexual cells (the sperm and egg) that fuse together to form a zygote. In plants, the female and male organs and sexual cells are produced within the same individual. In that sense individual organisms are neither male nor female being classified as hermaphrodite. Unicellular eukaryotes such as some fungi and algae, typically reproduce vegetatively via mitotic division and they engage in sexual reproduction when exposed to starvation or stress. Considerable variation in the sexual process exists within different eukaryotic groups. Notwithstanding this diversity in modes of sexual reproduction across species, there is a common underlying necessity for two specialized sexual cells to come together and fuse (or conjugate and exchange nuclei; (Miyake, 1974)). The role and evolution of these specialized sexual cells – the gametes – is the focus of this thesis.

1.1.2 Mating cells

Gametes are sexual cells. They contain a haploid set of chromosomes, they are derived through meiosis and are fusion competent; two gametes are combined in sexual reproduction to form a diploid zygote. Sexual reproduction that involves the fusion of two morphologically or behaviourally dissimilar gametes is referred to as anisogamy. Oogamy is an extreme form of anisogamy that predominates in animals and plants but is also seen in other groups such as red and brown algae (Brawley and Johnson, 1992; Kim, 1997). The gametes of oogamous species are either large and non-motile (the egg or female gamete) or small and highly motile (the sperm or male gamete). Furthermore, the egg and sperm are highly specialized cells with the egg being the ‘receiver’ and the sperm being the ‘donor’ cell. Isogamy on the other hand, is the fusion of two morphologically identical gametes and is common amongst unicellular protists.

Sexes and mating types

Sexes are easy to define in oogamous species: the female is the egg-producing sex whereas the male is the sperm-producing sex. In anisogamous but not oogamous species, the female is defined as the sex producing larger or less motile gametes, whereas the male is the sex producing the smaller or more motile gametes. Anisogamy does not necessarily imply oogamy, for example the size difference between the two types of gametes may only be marginal or both gametes may be equally motile (but differ in size).

Defining sexes becomes challenging in isogamous species where all gametes are morphologically the same. Notwithstanding this apparent similarity, isogametes are also divided into self-incompatible groups so that only gametes from different groups can fuse together. In that sense, sexual fusions remain asymmetric even in isogamous species. Sexual differentiation in morphologically identical gametes was first discovered in the fungal group *Mucorales* by Blakeslee as early as 1904 (Blakeslee, 1904). The intercompatible mating groups in isogamous organisms are referred to as 'mating types', and are usually genetically determined. An additional complexity is that many isogamous species have more than two mating types, the number of which can range from two to up to several thousands – the basidiomycete *Schizophyllum commune* for example, has more than 20 000 mating types (Raper, 1966). In such occurrences, only gametes and/or nuclei that do not belong to the same mating type can freely mate.

It is important to distinguish between mating types and the self-incompatibility (SI) types seen in many hermaphrodite species, best studied in angiosperms (Takayama and Isogai, 2005). SI systems are present in multicellular, oogamous and herpmaphordite organisms and they impose an additional level of complexity on the function of sexes, by acting as a barrier to fusions between the egg and sperm produced by the same individual (i.e. they avoid inbreeding). Mating types, on the other hand, are mostly a feature of unicellular organisms with isogametes, and as illustrated in Chapter 4, their function goes well beyond that of a mere SI system. In that sense, mating types can be thought of as morphologically identical sexes although establishing a clear distinction between sexes and mating types can also be challenging.

Sexes or mating types?

Although mating types determine sexual identity, they differ from sexes. Still, specifying the distinction between mating types and sexes is perplexing. Evidence across lineages suggests that anisogamy evolved from isogamy several times independently (Grell, 1973; Sonneborn, 1978; Miyake, 1996; Nozaki et al., 2006). In addition, theoretical work on the evolution of anisogamy has often begun from the reasonable assumption of isogamous ancestors. In that sense, isogamy precedes anisogamy and the selective forces behind the evolution of sexes and anisogamy lie in the roots of mating type type evolution. Nonetheless,

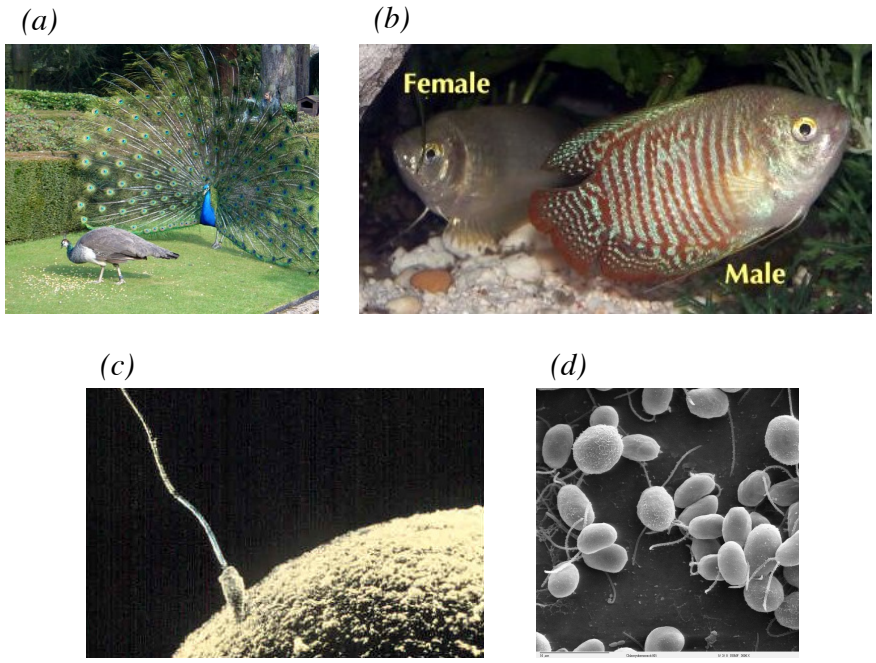

Figure 1.1: Sexes and mating types. (*a*): Peacock and peahen. The male is larger than the female and displays its colourful tail as a means to attract the female (Image: ToastyKen (Own work) [CC-BY-3.0 (http://creativecommons.org/licenses/by/3.0)], via Wikimedia Commons), (*b*): Male fish are larger and more colourful than female fish. This is an exemplification of sexual selection; males evolved characteristics that are attractive to the females, (*c*): A sperm attempting to enter the egg. In oogamous species the female gamete (egg) is many times larger than the male (sperm) and is immobile in contrast to the sperm, (*d*): *Chlamydomonas* cells. Cells of opposite mating type (+ and -) are morphologically identical and for many species of *Chlamydomonas*, so are their gametes (picture taken from (Smith and Lefebvre, 1996))

many species incorporate both mating types and sexes, imposing a distinction between them. For example, the filamentous ascomycete *Neurospora crassa* has two mating types *and* two sexes. Individuals produce both female and male gametes but male gametes can only fertilize the female gametes of the opposite mating type (Coppin et al., 1997). This is true for many other fungi and some ciliates where migratory and stationary nuclei are produced by the same individual but only nuclei from different mating types can be exchanged (Phadke and Zufall, 2009; Coppin et al., 1997). This distinction between isogamous mating types and anisogamous sexes leads to the question why the two are not always linked. I discuss this further in the final chapter of this thesis.

1.2 Why do we need sexes?

1.2.1 The paradox

The persistence of binary mating systems in sexual organisms constitutes a well-known conundrum in evolutionary biology. In the majority of sexual organisms two parents of different sex or mating type are necessary. Moreover, two is the most common number of mating types (sexes are always two). Why this is the case is not obvious. At face value, two sexes seem to be the worst of all possible worlds: individuals are restricted to mating with half the population, which must have a selective cost if there is any difficulty in finding a mate. Either a single sex or multiple sexes should be better, as both would enable individuals to mate with a larger proportion of the population. The two sexes in complex multicellular organisms typically assume highly specialised roles throughout mating, at both the organism and gametic level. For example, female and male animals are highly specialized to attract partners (e.g. bright colours in fish and birds) and the egg and sperm evolved complex mechanisms that lead to attraction and fusion (Fig.1.1(*a*)-(*c*)). In addition, fertilization is internalized in many multicellular organisms where the two sexes evolved specialised organs facilitating copulation. The asymmetry between the sexes is seemingly indispensable in most multicellular organisms with an obvious distinction between male and female function, and an alternative mating system without any sexes is admittedly a challenge to fathom.

This is not true when it comes to unicellular organisms with isogametes. Unicellular protists of different mating type are morphologically the same, and so are their haploid gametes (e.g. Fig.1.1(*d*)). Despite this apparent similarity, only gametes of different mating type can fuse, with unions between gametes of the same mating type being surprisingly rare. This is also true of organisms with more than two mating types. Hence at the most rudimentary level, the question of the evolution of sexes becomes a question of mating incompatibility as exemplified by mating types.

The evolution of anisogamy has been the focus of many studies, indicating that it evolved through disruptive selection (Parker et al., 1972; Bell, 1978; Cox and Sethian, 1985; Dusenbery, 2000; Bulmer and Parker, 2002; Dusenbery, 2006). Opposing selective pressures for optimal gamete encounter rates (through asymmetric use of chemotaxis and varying gamete size), the number of gametes produced, and zygote viability, endow an advantage to either very small (and so numerous and highly motile) gametes or much larger (and so stationary and robust) gametes. Still, why isogamous mating types persist remains a subject of debate amongst evolutionary biologists (Billiard et al., 2011; Hoekstra, 2011; Billiard et al., 2012; Perrin, 2012). Various hypotheses have been constructed to elucidate the selective forces behind the evolution and maintenance of mating types, which I briefly review in what follows.

1.2.2 Models for mating type evolution: a review

Inbreeding and avoidance of mating between same clones

This model proposes that mating types safeguard the benefits of sex and that therein lie their evolutionary advantages (Charlesworth and Charlesworth, 1979; Uyenoyama, 1988b,a; Czaran and Hoekstra, 2004). The benefits of sex are most frequently associated with its recombinatorial advantages. The breaking down and reconstruction of chromosomes may produce new allele combinations providing grounds for selection to proceed further (Otto, 2009). These recombined genomes can also act to repair damaged DNA following the accumulation of deleterious mutations. Syngamy between two identical clones (same clone mating) or two related gametes (inbreeding) restricts the recombinatory benefits of sex.

The first class of models are those proposed by Charlesworth and Charlesworth (1979) and Uyenoyama (1988a,b). These models posit that mating types evolved because they avoid inbreeding and so prevent the generation of homozygous deleterious alleles while promoting the elimination of recessive alleles, something that same-clone or related-gamete fusions fail to achieve. The general assumptions are that individuals can only reproduce sexually and that same-clone fusions and fusions between related gametes are less viable than random fusions between unrelated cells. These assumptions allow modifiers that impose self-incompatibility, and so restrict inbreeding, to spread. Under certain assumptions these modifiers can spread to fixation leaving a population consisting only of self-incompatible individuals. These models were primarily focused on angiosperms, where their conclusions find ample support (see for example, Kubo et al. (2010)). However, most of their assumptions cannot be readily applied to unicellular lineages that are predominantly haploid. For example, Charlesworth and Charlesworth (1979), Uyenoyama (1988a) and Uyenoyama (1988b) assume that reproduction is predominantly sexual which is not true in most unicellular protists.

There is extensive evidence that both selfing and inbreeding are detrimental in complex organisms (Crnokrak and Roff, 1999; Hedrick and Kalinowski, 2000). Nonetheless, this model finds less support across isogamous protists. This is for two main reasons. Firstly, inbreeding depression in multicellular organisms is due to the accumulation of recessive alleles in the genomes of the mature organisms throughout its life. Gametes of the same diploid parent carry the same mutational load and so fusions between them cannot mask these mutations. However, this does not readily apply to unicellular protists. Many isogamous protists such as some yeasts and algae are predominantly haploid and unicellular. During their haploid phase these organisms grow mitotically and are fully exposed to natural selection at the single cell level which purges deleterious mutations while promoting beneficial ones. The second challenge to this theory lies in the observation that many diploid lineages have evolved sophisticated genetic and epigenetic mechanisms

to allow selfing. A well known example is that of mating type switching in some yeasts where sister cells change their mating type allowing unions between them (Dalgaard and Klar, 1999; Klar et al., 1982). This is also well documented in ciliates where mating type can be mediated through either genetic, epigenetic, environmental or stochastic factors. Therefore, different mating types can be expressed in the identical progeny of a single conjugating pair (Phadke and Zufall, 2009). Another relevant case is the budding yeast which proliferates mainly at its diploid state. When the diploid cells, which are heterozygote for the mating type genes, are starved, they undergo sporulation producing four gametes, two from each mating type. In nature, these mate immediately to restore the diploid phase and so inbreeding is the rule in this species even though mating types are present (O Morgan, 2007). Similar occurrences are known in other fungi where mating types are present but often do not fully restrict sibling matings (for example (Fowler and Vaillancourt, 2007)). These considerations indicate that inbreeding depression or same-clone mating are not necessarily relevant in unicellular organisms.

An alternative model was proposed by Czaran and Hoekstra (2004). This model also evokes the disadvantages of same-clone mating but not on the premise of mutation accumulation. Instead, Czaran and Hoekstra (2004) propose that mating types provide an adaptive advantage by allowing fusions between dissimilar gametes. Same-clone or highly inbred matings provide no grounds for the generation of new types and do not increase variation in the population, limiting the advantages of sex. Assuming an advantage to outbreeding and that gametes have limited dispersal abilities, so that fusions are highly probable between sister cells, the authors show that two mating types could spread to fixation. This model can only be applied to unicellular organisms where mating type is determined at the haploid level and with limited gamete dispersal. So the model is readily applicable to some fungi and non motile algae but less appropriate for more motile algae or some ciliates that are both highly motile and chiefly diploid, or cases such as that of the budding yeast outlined above. It follows that although the model proposed by Czaran and Hoekstra (2004) is indeed compelling and is considered by many evolutionary biologists as the ultimate explanation for the evolution and persistence of mating types, it is not without challenges both on theoretical and empirical grounds. This theory deserves further examination to assess its merits and difficulties in light of the issues I have discussed briefly.

Developmental switch

The developmental-switch model posits that the adaptive benefits of mating types lie in their capacity to allow fertilised cells to regulate developmental pathways and so recognise and respond to their ploidy status. This was first suggested by van der Meer and Todd (1977) and later by Herskowitz (1985) and Haag

(2007). Perrin (2012) has recently endorsed this hypothesis and provided empirical support to support it. The underlying idea is that mating types, encoding mating-type-specific genes, facilitate the formation of heterodimers in the diploid zygote that are necessary and unique indicators of the cell's diploid state. In predominately unicellular organisms, this prevents further syngamy and can trigger meiosis. Therefore, the selective advantages of mating types lie in their capacity to signal developmental switches during the cell's life cycle.

This hypothesis has been expressed verbally but it has not been examined through the construction of theoretical models thus far. An evolutionary model could provide grounds supporting or challenging this idea. For example, a model can provide a quantitative account comparing putative benefits and drawbacks and so encourage expansion of the developmental switch theory.

In his 2012 report, Perrin provides ample evidence in algae, ciliates and fungi to support this hypothesis (Perrin, 2012). Interestingly, heterodimers in the zygote appear to be indispensable for post fertilization events and proper development in many species. For example in *Chlamydomonas*, mating-type-specific transcription factors form a heterodimer that was shown to be necessary and sufficient to stop further syngamy, to trigger the zygotic program and to coordinate meiosis (Zhao et al., 2001). Importantly, if heterodimerization fails, the diploid zygote cannot enter meiosis at the appropriate point, which can be lethal for the cell (Ebersold, 1967; Galloway and Goodenough, 1985). One problem with this assertion is that mating types are highly evolved, and enforcing same mating type unions in species where fusions only occur between different mating types may be problematic for reasons not directly related to the lack of heterodimerization. Furthermore, empirical evidence supporting heterodimer formation as an important mechanism for sexual development in modern eukaryotes does not imply that the adaptive merits of mating types lie in their capacity to regulate developmental switches. The evolutionary benefits of heterodimer formation in otherwise identical partners are difficult to assess in modern mating types. The study of rare species where unions between same mating types are possible, and the presence or absence of heterodimerization therein could clarify matters.

Mating between gametes of the same mating type are rare, albeit possible. This poses another challenge to this theory, as it suggests that cells may be able to recognise their ploidy level and developmental stage through alternative mechanisms. How do organisms manage to monitor their ploidy and developmental changes in the absence of any hetetozygocity? Perrin (2012), argues that this does not necessarily oppose the developmental switch theory on the premise that same mating type unions often involve some degree of heterozygosity. However, further experiments are required to support this reasoning. Such investigations should focus on determining whether heterodimers are present in same mating type fusions, and the way

developmental switches are regulated in species where mating types are not necessary for mating.

Asymmetric fusions all along

Another model, put forward independently by Hoekstra (1990a) and Bell (1993), proposes that the evolution of asymmetric cell fusions precedes the evolution of sex. According to this model, an asymmetry with respect to cell fusion was driven by a genetic element or a cytoplasmic replicator (for example, a virus). This replicator can be inherited vertically through mitotic cell division or horizontally by promoting fusion of its host cell with another cell thereby spreading in the population. The next step requires a mutation that causes the symbiont to avoid fusion with another, already infected cell. This mutant replaces the original symbiont and the population ends up with uninfected cells and infected cells that promote fusions with uninfected cells. For this, an assumption that fusions between cells are costly is required. The model also assumes that the original cytoplasmic element can be lost with some non-zero probability to prevent it going to fixation early on following its appearance.

One prediction of this model is that cells will subsequently evolve asymmetric molecular mechanisms to improve cellular fusion. Although such mechanisms are indeed present in many lineages, their evolutionary origin and function do not necessarily relate to the predictions of this model nor is there any obvious way of assessing whether they do. In addition, this hypothesis predicts that only the gamete types that are the descendants of the infected cells should actively utilize signals that promote fusions, which is contradicted by bipolar systems of mutual attraction and recognition in many fungi and ciliates (Billiard et al., 2011). Finally, Bell's hypothesis (Bell, 1993) predicts that mating-type genes are derived from transposons, something that has thus far found no consistent evidence (Hoekstra, 2011).

Control over cytoplasmic inheritance

A widely shared view posits that mating types present an adaptive advantage by controlling mitochondrial (and chloroplast) inheritance (Hoekstra, 1990b; Hastings, 1992; Hurst and Hamilton, 1992; Hutson and Law, 1993; Randerson and Hurst, 1999). Mitochondria are descended from free-living bacteria that were engulfed by another cell early in eukaryotic evolution. In the early evolution of mitochondrial symbiosis, mitochondria lost a large fraction of their genes through reductive evolution, but they maintained their own tiny genome (Gray et al., 1999; Esser et al., 2004). The ability of eukaryotic cells to produce energy (ATP) depends on these organelles, and mitochondrial mutations can result in cell death and serious disorders at the level of the organism (Wang, 2001; Zeviani and Di Donato, 2004). These models are based on the assumption that uniparental inheritance is beneficial because it limits the spread of deleterious cytoplasmic

elements, safeguarding these precious organelles.

The idea that the evolution of mating types and anisogamy follow from selection for uniparental inheritance, and so fit mitochondria, was first proposed by Cosmides and Tooby (1981). Heteroplasmy, which is the presence of different cytoplasmic genotypes in the same host, provides grounds for competition between organelles. Mutant organelles with a replicative advantage but an impaired contribution to the cell's performance increase in frequency, at the expense of the host's fitness. Nuclear mutations that enforce uniparental inheritance can then provide an adaptive advantage by minimising conflict between different mitochondrial (or chloroplast) genomes.

Assuming that uniparental cytoplasmic inheritance provides a selective advantage, how is it to be regulated? The models of Hoekstra (1990b), Hastings (1992), Hutson and Law (1993), Hurst and Hamilton (1992) and Randerson and Hurst (1999), differ in their assumptions about the nature of the modifiers imposing uniparental inheritance and fitness effects. However, all assume a nuclear mutation imposing uniparental cytoplasmic inheritance, which spreads because it limits cytoplasmic mutations that have a replicative advantage but are deleterious to the host. After the uniparental mutant spreads to some intermediate frequency, a further requirement is the appearance and spread of nuclear mutations imposing mating incompatibility, so that all gamete fusions have uniparental inheritance with two sexes that regulate it. As noted by Hoekstra (2011), one problem associated with these models is the requirement that the nuclear mutations imposing uniparental inheritance appear while the population is polymorphic for the harmful (or selfish) and wild type cytoplasms. In addition, these models assume a fixed fitness reduction for hosts carrying selfish mutants and a fixed fitness increase for uniparental zygotes independently of the residual mutational load in the cytoplasm. In support of these models, evidence for conflict in the cytoplasm has been reported. Experimental and theoretical work on the yeast *Saccharomyces cerevisiae* by Taylor et al. (2002) found that within cell selection favoured parasitic mitochondria. Note that *S. cerevisiae* do not have uniparental inheritance and they attain homoplasmy through multiple mitotic divisions following sex (Jensen and Hobbs, 2000). Conflict between different cytoplasms was also inferred in the mushroom *Agaricus Bitorquis*, where homoplasmic zygotes were shown to grow much faster than hetreroplasmic ones (Hintz et al., 1988).

Another, more recent idea, proposes that two sexes facilitate better coadaptation of mitochondrial and nuclear genes (Lane, 2006, 2011a). There is ample evidence across eukaryotes that the nuclear and mitochondrial genomes have adapted to each other, facilitating the key mitochondrial function: oxidative phosphorylation (Blier et al., 2001; Dowling et al., 2008; Burton and Barreto, 2012). This hypothesis is based on the premise that uniparental inheritance, and so two sexes that regulate it, lead to better coadaptation between the nucleus and the mitochondria by means of avoiding the breaking of mitonuclear states

favoured by selection. The relevance of this theory to the simple mutation model is not obvious and may depend on the nucleus in a variety of ways (e.g. homozygote versus heterozygote nucleus, nuclear imprinting). Chapter 2 presents a mathematical model testing this hypothesis and discussing its similarities and differences to the simple mutation model, something that was previously lacking.

These hypotheses propose that uniparental inheritance is the driver of the evolution of sexes. This finds ample support across species. In most anisogamous groups mitochondrial inheritance is strongly correlated with sexes, with only the female usually passing on its mitochondria. This is not always the case but sex-specific roles in mitochondrial inheritance are ubiquitous in complex multicellular organisms. For example, in the doubly uniparental inheritance system of bivalve mussels, males receive mitochondria from both parents, but these then segregate, with male mitochondria entering the gonads and female mitochondria committed to the soma (Zouros, 2013). A range of unicellular protists with isogamous mating types also abide to this rule: one mating type transmits its mitochondria while the other does not. However, there is a series of important observations that contradict this model. For example, many yeasts have biparental inheritance and two mating types. Also, lineages that do not undergo cytoplasmic fusion during sex , such as ciliates, maintain mating types. Finally, some species have both mating types and uniparental inheritance but the two are not linked (Birky, 1995, 2001; Xu, 2005).

This class of models, the evolution of uniparental inheritance and its role in the evolution of mating types and sexes are the focus of Chapters 2 and 3.

Gamete communication

Hoekstra (1982) also proposed that mating types evolved as a consequence of selection for asymmetry in gamete attraction and recognition. This theory is based on the assumption that gametes secrete specific molecules that allow recognition and/or attraction between partners prior to fusion. The underlying assumption of this model is that self secretion leads to receptor saturation, thereby compromising the ability of cell's to respond to external signals.

The mathematical model constructed by (Hoekstra, 1982) assumes an initial population where all gametes are the same and any gamete is free to fuse with any other. Mating is mediated via signalling: all gametes produce and are able to respond to the same signal. The signal functions either as a recognition mechanism or as a means for chemoattraction. In addition, this model assumes that mating is not possible without the generation or detection of signals. This is a reasonable assumption as some form of a species-specific signal must be in place to indicate the presence of a possible partner and so initiate fertilization. A further assumption is reduced fitness for individuals that generate and respond to the same signal on the

premise that receptor saturation impairs a cell's ability to respond in a single chemical-receptor system. Mutant individuals with a non-functional receptor or signal are therefore more efficient in mating and have higher fitness. Under certain conditions, selection favours linkage between the receptor and signal loci and the initial self-fertile genotype disappears at the advantage of gametes with a functional copy of only the receptor or signal gene. One drawback associated with this model is that the impact of receptor saturation or the interference due to self secretion are not explicitly modelled. Instead, the model assumes that generating and detecting the same signal is costly, without directly assessing the difficulties associated with such a system of gamete interaction. This lead to the assumption of a fixed, but hard to quantify, cost for cells that generate and sense the same signal.

Although assuming that a species-specific signal must be in place to achieve mating, and that receptors may saturate due to self-signalling are both reasonable assumptions, no substantial empirical evidence supporting these assumptions was presented. In that sense, experimental reports are necessary to validate Hoekstra's assumptions. In addition, the lack of empirical evidence means that a quantification of the costs of self-secretion, which is central to the model, is missing. This will be the focus the second Part of this thesis. Chapter 4 addresses the shortage in experimental evidence supporting this model. Chapter 5 investigates the impact of self-secretion in a single chemical-receptor system in an effort to specify appropriate costs for self-secretion in an evolutionary model.

1.2.3 The number of mating types

An obvious question that follows from the evolution of distinct mating classes concerns the optimal number of mating types. If organisms have any mating types at all, then the mating restrictions imposed by mating incompatibility decrease as the number of mating types increases. Why then, do most species have only two mating types? Furthermore, what determines the number of mating types in lineages such as ciliates and slime molds that have several mating types, or in some basidiomycetes where the number of mating types ranges from two to several thousand?

One explanation is that mating kinetics and the cost of finding a mate in a limited time period determine the number of mating types (Iwasa and Sasaki, 1987). Other answers could lie in the molecular or genetic mechanisms that determine mating types or the evolutionary origin of mating types in specific lineages (Billiard et al., 2011). This thesis does not explicitly consider the evolution of the number of mating types. I do, however, discuss this issue in the light of the findings and conclusions of this work in Chapter 6.

1.3 Thesis aims and modelling approach

Evolutionary theories intend to unravel the processes via which living forms change, by studying why and how continuous biological modification and adaptation take place. In this thesis I aim to uncover the adaptive benefits of asymmetric partner fusions that are nearly ubiquitous in sexual reproduction, thereby explicating the evolutionary roots of mating types and sexes. Ascribing causation is admittedly not an easy task. Here, I focus on the functional significance of mating types and sexes by emphasizing the constraints that organisms encounter during sex, and the capacity of mating types to overcome these difficulties. In this context, it is important to determine firstly what favourable adaptations the presence of different mating partners may confer and secondly, to indicate why couldn't these improvements be achieved in the absence of mating types, through alternative mechanisms. The questions of *why* and *how* go hand in hand in evolution – claiming to understand one but not the other would be to fall far short. For instance, important changes often occur during the spread of specific modifiers, changing their relative adaptive benefits. Thus, given the adaptive benefits of mating types, one needs to ask how it is that these evolve, i.e. how do genes pertinent to specific mating type functions spread, and how could they become linked to mating incompatibility.

The first Part of this thesis concerns the evolution of genes that impose uniparental inheritance of mitochondria and mating incompatibilities. Mathematical models of evolution assume that the fate of a new gene in a population will depend on its relative fitness with regards to other alleles on the same locus. Fitness can be thought of as a viability or reproduction-success coefficient for specific genotypes. Consider a single biallelic locus where A and a are the two alleles and p and q are their corresponding frequencies. Then, three genotypes are possible: AA, Aa and aa. Assuming that genetic inheritance is Mendelian, that the genotype fitnesses are the same and that mating is random, the expected frequencies of the three genotypes at equilibrium are as follows:

$$p_{AA} = p^2 \tag{1.1}$$

$$p_{Aa} = pq + qp = 2pq \tag{1.2}$$

$$p_{aa} = q^2 \tag{1.3}$$

This also known as the Hardy-Weinberg equilibrium (Edwards, 2008). Natural selection is a concept admittedly hard to define and embody in a mathematical model. One way of representing the works of natural selection mathematically is by assuming that different genotypes are associated with different repro-

ductive or viability rates. In a situation where different genotypes have different corresponding fitnesses, the frequency of each genotype can then be altered by a factor proportional to its fitness. When modelling natural selection throughout this thesis, I define fitness as the viability of individuals that are free to randomly mate with one another. It follows that letting w_{AA}, w_{Aa} and w_{aa} be the fitness of genotypes AA, Aa and aa respectively, and p'_{AA}, p'_{Aa}, p'_{aa} the expected frequencies of the genotypes AA, Aa and aa following a single round of selection we obtain,

$$p'_{AA} = p^2 \frac{w_{AA}}{\bar{w}} \tag{1.4}$$

$$p'_{Aa} = 2pq \frac{w_{Aa}}{\bar{w}} \tag{1.5}$$

$$p'_{aa} = q^2 \frac{w_{aa}}{\bar{w}} \tag{1.6}$$

where p and q are the frequencies of alleles A and a respectively prior to selection, and $\bar{w}$ is the mean fitness so that $\bar{w} = p^2 w_{AA} + 2pqw_{Aa} + q^2 w_{aa}$. These equations can be used to predict the expected frequency of AA, Aa and aa at equilibrium (i.e. when selection no longer changes the genotype frequency).

This simple formulation can lead to surprisingly complex dynamics once more than two alleles or a single locus are examined (Kirkpatrick et al., 2002), or when dealing with small populations where stochasticity has significant effects (Hartl and Clark, 1997). These considerations have led to a vast body of developments in evolutionary biology, aiding our understanding of genetic evolution. The assumption of fixed fitness coefficients in these formulations allows for clear predictions based on analytically derived formulas that relate the frequency of an allele at equilibrium to its fitness relative to other alleles in the population. Despite the compelling advantages of assuming a fixed fitness, this is not always a reasonable assumption. For example, the fitness of an allele may change as its frequency increases in the population (Cockerham et al., 1972), or depend upon intrinsic complexities specific to a particular problem, that may themselves change in the course of evolution.

Previous workers studying the spread of mutants imposing uniparental inheritance of mitochondria and mating incompatibility assumed a fixed cost for cells that carry mutant mitochondria and a fixed benefit for zygotes with uniparentally inherited mitochondrial (Hoekstra, 1990b; Hastings, 1992; Hurst and Hamilton, 1992; Hutson and Law, 1993; Randerson and Hurst, 1999). This generated some interesting dynamics leading many to the conclusion that uniparental mutants and mating types that regulate mitochondrial inheritance, can readily spread.

In Part I of this thesis (Chapters 2 and 3) , I re-examine the fate of mutants imposing uniparental

inheritance by defining fitness as an explicit function of the mitochondrial mutational load. This is done in the context of a model comprising a detailed life cycle model with mitochondrial segregation in cell division being explicitly considered. The resulting model is a more accurate representation of protists' life cycles but the residual complexity does not allow for an analytical solution. Despite the apparent limitation, this more thorough model provides a counter-intuitive interpretation that deepens our understanding of the evolution of uniparental cytoplasmic inheritance and challenges the now orthodox view that the main adaptive benefits of sexes and mating types lie in their capacity to regulate UPI, so that one sex passes its mitochondria while the other does not. The assumption of fixed fitness coefficients is particularly problematic in the context of mitochondrial inheritance evolution because mutation and adaptation occur at two levels: that of the nucleus and that of the mitochondria. Treating the latter as static falls short of representing the actual population dynamics and interaction between different levels of selection within and between organisms.

In Part II of this thesis (specifically, Chapters 5) , I present a model of gamete chemotaxis to study the impact of asymmetric gamete roles during mating, with a focus on partner attraction. My model was motivated by the anticipation that a moving cell secreting a chemoattractant will not itself be able to effectively detect external signals (based on the same chemoattractant) and so move towards potential partners. Individual cell movement, and the way in which this can interfere with the ability of cells to respond to external cues is the centre of this study and holds a key role in defining the chemotactic system. This can only be analysed through an agent based approach resulting in a model more complex than many of the classic chemotaxis formulations that are founded on collective cell movement (Hillen and Painter, 2009). As in the models of Part I, an analytic solution to this problem is not possible. However, the agent-based formulation, serves to explicitly quantify evolutionary weaknesses and benefits that were undetermined in previous models, and provides grounds for a detailed evolutionary model.

Throughout this thesis, I try to maintain a clear and simple approach towards problem formulation and model construction, while appreciating that certain complexities are indispensable for one to reach meaningful conclusions through mathematical modelling.

1.4 Thesis layout

This work is split into two parts. Part I (Chapters 2 and 3), concerns the class of models proposing that the uniparental inheritance of mitochondria can explain the evolution of mating types and sexes. Part II (Chapters 4 and 5), investigates the evolution of mating types and sexes in the light of the adaptive benefits of a polarised recognition and attraction system.

Chapter 2 studies the impact of the uniparental inheritance (UPI) of mitochondria on interactions be-

tween genes in the mitochondria and the nucleus, in an effort to elucidate the role of UPI. In particular, it examines whether selection for adaptation between the nuclear and mitochondrial genomes (mitonuclear coadaptation) could in principle have promoted uniparental inheritance of mitochondria. This work shows that selection for mitonuclear coadaptation favours the evolution of uniparental inheritance.The relevance of these findings to the evolution of two distinct mating types and sexes is discussed.The chapter is adapted from the published paper by Hadjivasiliou et al. (2012).

In Chapter 3, I develop a new, explicit theoretical model that challenges the prominent view that selection for UPI leads to the establishment of self-incompatible mating types and sexes. This model suggests that as UPI increases in the population its relative fitness advantage diminishes in a frequency-dependent manner. It follows that while some degree of UPI is favoured, linked mating types cannot evolve to fixation. The complexities of this model are presented and discussed. We conclude that uniparental inheritance of mitochondria is unlikely to have driven the evolution of self-incompatible mating types and relate our findings to patterns of mitochondrial inheritance seen in nature. The chapter is adapted from the published paper by Hadjivasiliou et al. (2013).

Chapter 4 is an extensive literature survey of mating type interactions, undertaken to provide empirical support for the prediction that an asymmetry in signalling between mating partners is widespread. The findings largely support the hypothesis that asymmetric mating type specific functions improve mating, while providing grounds for further theoretical and experimental work.

Chapter 5 is an investigation into the role of signalling during sexual chemotaxis and the impact of self-signalling on partner-finding efficiency. In this Chapter I argue that mating types pose a solution to the apparent inevitability of self-excitation throughout gamete interactions that are crucial for sex. I develop a physically realistic model for signaller-detector dynamics based on chemical diffusion, chemotaxis and individual cell movement. As anticipated, the model indicates that self-secretion impairs the ability of gametes to detect external signals and move towards potential partners, under a wide range of physiological conditions. The findings of this chapter can form the basis for an evolutionary model. This work is at a preliminary stage and further implementations that will be considered in the future are discussed at the end of Chapter 5.

Finally, in Chapter 6 I summarize this work, discuss the implications of my findings, draw conclusions about the evolution of mating types and sexes and outline possible directions for future work on this topic.

Part I

Dynamics and evolution of mitochondrial inheritance

A large body of theoretical work along with the prevalence of uniparental inheritance (UPI) of mitochondria endowed ample support to the proposition that the adaptive benefits of mating types and two sexes lie in their capacity to regulate UPI. However, *why* and *how* exactly UPI improves mitochondrial fitness is not clearly understood. In Part I of this thesis I use theoretical techniques to further examine these issues. In Chapter 2, I develop a mathematical model that can be employed to contrast UPI to biparental inheritance of mitochondria (BPI). I specifically examine a new hypothesis asserting that the adaptive benefits of UPI lie in its capacity to regulate mitochondrial-nuclear interactions that are crucial for oxidative phosphorylation. In Chapter 3, I study the spread of nuclear modifiers that can impose UPI and/or mating incompatibility while explicitly considering mitochondrial mutation and selection. This is a new modelling approach as it dynamically considers the mutational load in the mitochondria that directly determines cell fitness, rather than assuming a fixed selective advantage for UPI. This method, greatly changes the evolutionary dynamics revealed in the past, and challenges the conclusions reached by other workers.

Chapter 2

Selection for mitonuclear coadaptation and the evolution of two sexes [1]

Mitochondria are descended from free-living bacteria that were engulfed by another cell some two billion years ago. A redistribution of DNA led to most genetic information being lost or transferred to a large central genome in the nucleus, leaving multiple copies of a residual genome in the mitochondria. Oxidative phosphorylation, the most critical function of mitochondria, depends on the functional compatibility of proteins encoded by both the nucleus and mitochondria. We investigate whether selection for adaptation between the nuclear and mitochondrial genomes (mitonuclear coadaptation) could in principle have promoted uniparental inheritance of mitochondria. Using a mathematical model, we explore the importance of the radical differences in ploidy levels, sexual and asexual modes of inheritance, and mutation rates of the nucleus and mitochondria. We show that the major features of mitochondrial inheritance, notably uniparental inheritance and bottlenecking, enhance the coadaptation of mitochondrial and nuclear genes and therefore improve fitness. We conclude that, under a wide range of conditions, selection for mitonuclear coadaptation favours the evolution of uniparental inheritance and discuss the relevance of our findings to the evolution of two distinct mating types and sexes.

2.1 Introduction

The advantages and disadvantages of sexual reproduction are well known, if disputed (Keightley and Otto, 2006). The reason for the existence of two sexes in the vast majority of sexual organisms is less celebrated

[1] This study was conducted in collaboration with Nick Lane, Andrew Pomiankowski and Rob Seymour and has been published in the *Proceedings of the Royal Society B* (Hadjivasiliou et al., 2012)

and understood. While sex requires two parents, there is no obvious need for these parents to be of different sexes. At face value, two sexes seem to be the worst of all possible worlds: individuals are restricted to mating with half the population, which must have a selective cost if there is any difficulty in finding a mate. Either a single sex or multiple sexes should be better, as both would enable individuals to mate with a larger proportion of the population.

Sexual dimorphism is grounded in anisogamy, in which one sex, by definition the female, produces a few large, immobile eggs, while the male produces greater quantities of small, motile sperm. Parker et al. (1972) proposed that anisogamy evolved from an isogamous population via disruptive selection. The hypothesis assumes that zygote fitness increases with size, and that gamete production has a number-size trade-off. While this may be true, such trade-offs cannot explain the existence of two sexes (or strictly, mating types) in isogamous species, where gametes are morphologically identical. Thus, the basis of two sexes precedes the evolution of anisogamy and sexual dimorphism, and cannot be ascribed solely to disruptive selection.

The distinction between two sexes is frequently associated with the inheritance of cytoplasmic genes (Birky, 2001). One sex, usually the female, passes on mitochondrial genes, the other does not. In isogamous species with mating types, uniparental inheritance of mitochondria (UPI) is also widespread. Many of the exceptions typically conform to the spirit of this generality. For example, in the doubly uniparental inheritance system of bivalve mussels, males receive mitochondria from both parents, but these then segregate, with male mitochondria entering the gonads and female mitochondria committed to the soma (Zouros et al., 1994). Likewise, the multiple mating types of some slime moulds such as *Physarum polycephalum*, and the thousands of mating types in fungi such as *Schizophyllum commune*, do not contravene the principle of uniparental inheritance of mitochondria (Kawano et al., 1987; Raper, 1966). From this point of view, an explanation for the asymmetry of the sexes could lie in the selective forces that led to uniparental cytoplasmic inheritance.

Most theoretical work on the evolution of uniparental cytoplasmic inheritance has concentrated on its role as a mechanism to minimize selfish conflict between cytoplasmic elements (Cosmides and Tooby, 1981; Hoekstra, 1990a; Hurst and Hamilton, 1992; Hutson and Law, 1993). It is argued that mixing cytoplasmic elements from different parents may result in conflict amongst them (Hurst and Hamilton, 1992) or selection for good competitors (Hutson and Law, 1993), in both cases at the cost of cell fitness. Various authors have modelled these frameworks and concluded that nuclear mutations that enforce uniparental transmission of the cytoplasm are favoured by selection, thereby eliminating the opportunity for conflict in the zygote or the spread of selfish mutants (Cosmides and Tooby, 1981; Hoekstra, 1990a; Hurst and Hamilton, 1992; Hutson and Law, 1993). This seems reasonable even though there are some constraints on the models. For

instance, as noted by Birky (1995) and Hoekstra (2011), mutations that induce uniparental inheritance are only selected during the brief time window when a selfish mutant is present, and before it spreads to fixation. If such selection only operates occasionally, selfish conflict might fall short of a general explanation for the near-universality of uniparental inheritance.

In this Chapter we explore a novel hypothesis for the evolution of uniparental inheritance. In the early evolution of mitochondrial symbiosis, a large fraction of the mitochondrial genome migrated to the nucleus (Gray et al., 1999; Esser et al., 2004). This means that adaptive evolution of the key mitochondrial function, oxidative phosphorylation, depends on proteins encoded by two different genomes. There is strong evidence across many eukaryotic orders, from fungi and plants to invertebrates and mammals (including humans) that the mitochondrial and nuclear genomes have adapted to each other over evolutionary time (Blier et al., 2001; Dowling et al., 2008). This evidence includes a concordance between the evolutionary rates of mitochondrial and nuclear genes encoding respiratory-chain subunits, a decline in respiratory function in nuclear-cytoplasmic hybrids (cybrids) and hybrid breakdown in introgressed populations caused by mitonuclear incompatibilities (Lane, 2011b,c).

Does uniparental inheritance of mitochondria facilitate better coadaptation of mitochondrial and nuclear genes? And if so how does this relate to the evolution of two sexes (Lane, 2006, 2011c) ? Here we explore this possibility using a mathematical model of evolution in a unicellular organism with the ancestral state of biparental inheritance of mitochondria. That uniparental inheritance of mitochondria may preserve mitonuclear coadaptation is rooted in the idea that uniparental inheritance ensures that at least one nuclear gene with a full set of coadapted mitochondria will always enter the zygote. Biparental inheritance on the other hand, could disturb coevolved nuclear and mitochondrial gene combinations by mixing two independent mitonuclear states in the new cell. Our model explores the different modes and tempi of inheritance and evolution of nuclear and mitochondrial genes: different copy number (1 or 2 in the nucleus vs. many in mitochondria), different mutation rates (typically lower in the nucleus) and different patterns of inheritance (Mendelian in the nucleus; uni/biparental and bottlenecks in mitochondria).

The model allows us to consider the consequences of selection for mitonuclear coadaptation. We do not consider direct competition between uni- and biparental inheritance of mitochondria here. The dynamics of the two modes of inheritance are known to be complex (e.g. see Hutson and Law (1993)) and we leave their investigation for the next Chapter. Here we demonstrate that mitonuclear coadaptation is indeed improved with uniparental inheritance and mitochondrial bottlenecks under a wide range of conditions. This indicates the requirement for coadaptation as a potential force in the evolution of uniparental inheritance of mitochondria.

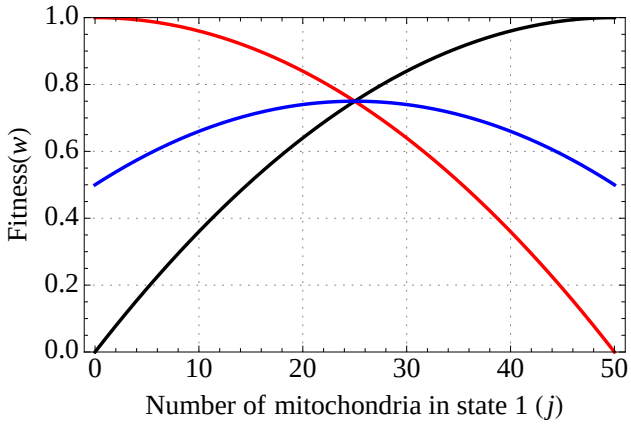

Figure 2.1: Fitness function for $M = 50$. Red, black and blue curves for nuclear states (00), (11) and (01) respectively.

2.2 Model of mitonuclear coadaptation

To model coadaptation between the nucleus and mitochondria, we consider a single gene in the nucleus that interacts with a single gene in the mitochondria. Both genes have two allelic states, 0 and 1. We assume that each cell contains a fixed number M of haploid mitochondria. Let the diploid nuclear state $i = 1, 2, 3$ represent the three possible genotypes (00), (01), (11) respectively. Let the mitochondrial state j, where $j \in \{0, 1, ..., M\}$, represent a cell with j mitochondria in state 1 and $M - j$ mitochondria in state 0. Under this model, there exist 3 possible nuclear states and $M + 1$ mitochondrial states. It follows that any cell in the population can be in $3(M + 1)$ possible mitonuclear states. Fitness is a function of the degree of matching between genes in the nucleus and the mitochondria defined by,

$$w(i, j) = \begin{cases} 1 - \left(\frac{j}{M}\right)^2 & \text{if } i = 1 \\ 1 - \frac{1}{2}\left(\frac{j}{M}\right)^2 - \frac{1}{2}\left(\frac{M-j}{M}\right)^2 & \text{if } i = 2 \\ 1 - \left(\frac{M-j}{M}\right)^2 & \text{if } i = 3 \end{cases} \tag{2.1}$$

where i is the nuclear state and j the mitochondrial state. Since a cell contains many mitochondria, mitonuclear mismatches that are present in only a few of a cell's mitochondria are likely to have a very minor fitness effect, as is borne out by the relatively high threshold of mitochondrial mutations within a cell required to cause a significant decline in oxidative phosphorylation in mitochondrial diseases (Adkins et al., 1996). The decline in fitness should become increasingly steep with greater mismatch, which justifies the choice of the quadratic functions in Eq.(2.1) to describe fitness (Fig. 2.1). Optimal fitness is achieved when the mitochondrial and nuclear genes are fully matched.

To model the evolution of the system, we suppose a life cycle composed of five steps (see Fig. 2.2).

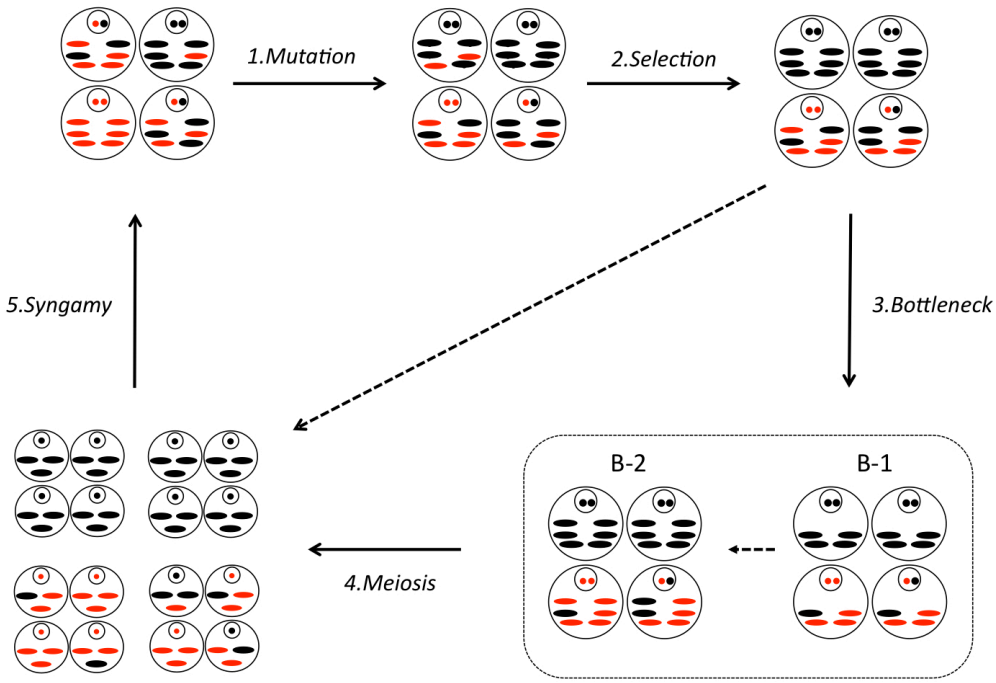

Figure 2.2: Schematic representation of the life cycle. Unicellular organisms (large circles) containing a number of haploid mitochondria (ovals) and a nucleus (smaller circle) undergo steps 1-5 described in the main text. The mitochondria are shaded or left blank to represent the two states the mitochondrial genes may assume. The smallest circles in the nucleus represent nuclear genes that are shaded or left blank to represent the two states genes may assume. B-1 and B-2 are the two bottleneck stages as described in the main text. The dashed arrow represents the case where no bottleneck is assumed.

The population of unicellular organisms undergoes clonal expansion during which it is subject to mutation and selection (the model's logic also applies to multicellular organisms). We do not explicitly model this, but for simplicity impose mutation (step one) followed by selection (step two). The pair of nuclear genes mutate independently of each other and of the mitochondrial genes with probability ν. Mitochondrial genes mutate independently of each other and of the nuclear genes with probability μ. After mutation, selection is imposed, with the change in the relative frequency of each mitonuclear genotype being proportional to its fitness as defined in Eq. (2.1).

Surviving cells then enter the sexual phase in which they undergo meiosis and syngamy to produce the next generation. We assume that there is a mitochondrial bottleneck before meiosis (Fig.2.2, step three). This imposes two rounds of sampling: the first without replacement from a mitochondrial population of size M down to the bottleneck size B, and the second with replacement from B up to M. The bottleneck is simply a process of sampling and amplification in the mitochondrial population of a cell, and the precise mechanism by which this is achieved (e.g. physical bottleneck or non-random segregation) is not relevant.

Each cell then undergoes meiosis (step four). The cell's population of mitochondria is doubled to $2M$ and then reduced through two cell divisions to produce four haploid gametes each with $M/2$ mitochondria. At each meiotic cell division, the mitochondrial genotypes of the parent cell are randomly segregated between the two daughter cells (i.e. sampling without replacement). Gametes then randomly fuse with each other to form the next generation of cells that re-enter the life cycle (step five). Depending on the mode of mitochondrial inheritance assumed, only one (uniparental inheritance) or both (biparental inheritance) parents transmit their mitochondrial genomes to the offspring (new cell). With uniparental inheritance, the $M/2$ mitochondria inherited from the transmitting sex are sampled with replacement to restore the original number M. With biparental inheritance, the mitochondrial genomes of the two parents are conjoined to form a set of M mitochondria.

We assume an infinite population of cells thus neglecting drift in nuclear genes. Note however that the population of mitochondrial genes is of finite size, M, and drift in the mitochondria is explicitly considered. This life cycle can be described mathematically in an exact manner (see Appendix). However, the complexity encompassed by the biological process prevents us from solving analytically for the equilibrium states. In order to investigate the asymptotic behaviour of the system we used numerical simulation. The initial frequency of each mitonuclear genotype was assigned from a uniform distribution Uni(0,1) and then normalized so that the frequencies sum to 1. We let the population evolve according to steps 1-5. We assume that equilibrium has been reached when the maximum of all changes in relative genotype frequency across a generation is smaller than an appropriately small value (see Appendix).

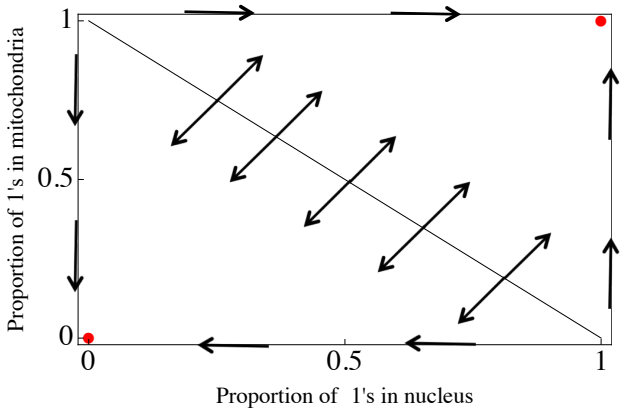

Figure 2.3: Schematic representation of the two equilibria found here (with a homozygote nucleus 00 or 11). The mitochondria are homoplasmic matching the nucleus at equilibrium (minus some mutational noise). The two red dots at the bottom left and top right indicate the equilibria.

2.3 Results

We ran simulations for a variety of parameter values (see Appendix) and compared the genotype distributions at equilibrium under uniparental and biparental mitochondrial inheritance. Depending on p_0 , the initial frequency of allele 1 in the nucleus, and q_0, the initial frequency of allele 1 in the mitochondria, the population converged on either nuclear state (11) or (00) (Fig. 2.3). This was the case with both uniparental and biparental inheritance of mitochondria. The heterozygous case (01) was never found to be attractive within the parameter sets employed in our study. This outcome follows from the assumption of additive effects, as the mitochondria can match a homozygous nucleus better than a heterozygote (see Eq(2.1)). With a heterozygous nucleus, mitochondrial and nuclear genes can never be in full agreement. As a result, the nucleus always converges to one of the homozygous states along with matching mitochondria, dependent on initial conditions (Fig. 2.3).

In order to compare the fitness under uniparental or biparental inheritance of mitochondria, we plotted the population fitness distributions at equilibrium. These were generally skewed to the right (the fittest states, Fig. 2.4). A number of statistical measures were calculated in order to capture the distribution of genotypes at equilibrium, in particular the population mean fitness and variance ($\bar{w}$ and σ^2 respectively), as well as P_w the percentage of the population having ftness greater than a value w (e.g. $w = 0.9$ or 0.95). The latter measures act as good indicators of the population concentration around the fittest state. We also measured the average mitochondrial variation within individuals in the population, h. This is a measure of mitochondrial heteroplasmy in the population. The values of these statistics are given for a wide range of parameter values in Table 2.1.

Table 2.1: Summary statistics for different parameter sets (M, B, μ, ν). The statistics $\bar{w}$ and σ^2 are the mean and variance of the population fitness and $P_{0.95}$ and $P_{0.9}$ are the proportion of the population with fitness greater than 0.95 and 0.9 respectively. The parameter h is the within cell variance in the mitochondria indicating the degree of heteroplasmy.

	Uniparental					**Biparental**				
(M, B, μ, ν)	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$	h	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$	h
A. Simple model										
1. $(200, -, 0.01, 0.001)$	0.945	0.0034	61.6	83.8	0.151	0.826	0.0013	$1.06 * 10^{-4}$	1.04	0.242
2. $(150, -, 0.01, 0.001)$	0.951	0.0036	66.2	85.6	0.138	0.841	0.0016	0.113	5.33	0.238
3. $(100, -, 0.01, 0.001)$	0.965	0.0042	79.7	89.8	0.119	0.865	0.0020	0.728	21.2	0.230
4. $(50, -, 0.01, 0.001)$	0.972	0.0048	85.9	91.5	0.0866	0.904	0.0026	19.0	56.6	0.208
B. Effect of a bottleneck										
5.$(200, 100, 0.01, 0.001)$	0.960	0.0039	75.7	88.5	0.113	0.871	0.0021	1.42	29.2	0.225
6. $(200, 50, 0.01, 0.001)$	0.966	0.0042	80.5	90.0	0.0930	0.896	0.0025	11.0	53.7	0.210
7.$(200, 10, 0.01, 0.001)$	0.978	0.0059	89.9	93.6	0.0396	0.950	0.0035	64.8	85.7	0.139
8. $(100, 50, 0.01, 0.001)$	0.969	0.0045	83.3	90.4	0.0821	0.909	0.0027	22.2	63.4	0.2008
9. $(100, 25, 0.01, 0.001)$	0.973	0.0050	86.4	91.7	0.0639	0.928	0.0030	42.9	76.2	0.179
10.$(100, 10, 0.01, 0.001)$	0.978	0.0060	90.5	93.9	0.0388	0.952	0.0035	67.2	85.9	0.136
11.$(50, 25, 0.01, 0.001)$	0.977	0.0056	89.2	92.9	0.0466	0.938	0.0032	55.0	79.9	0.167
12.$(50, 10, 0.01, 0.001)$	0.979	0.0063	91.5	94.1	0.0320	0.955	0.0034	71.2	86.2	0.129
C. Varying μ and ν										
13.$(50, 10, 0.001, 0.001)$	0.997	0.0011	98.9	99.2	0.00352	0.993	0.00074	97.5	99.2	0.0358
14.$(50, 10, 0.001, 0.01)$	0.988	0.0055	97.1	97.4	0.00359	0.983	0.0051	95.1	97.0	0.0391
15.$(50, 10, 0.1, 0.01)$	0.821	0.040	37.6	48.3	0.163	0.778	0.015	7.17	17.3	0.228
16.$(50, 10, 0.5, 0.01)$	0.711	0.022	4.97	9.48	0.214	0.734	0.0095	1.11	4.22	0.237

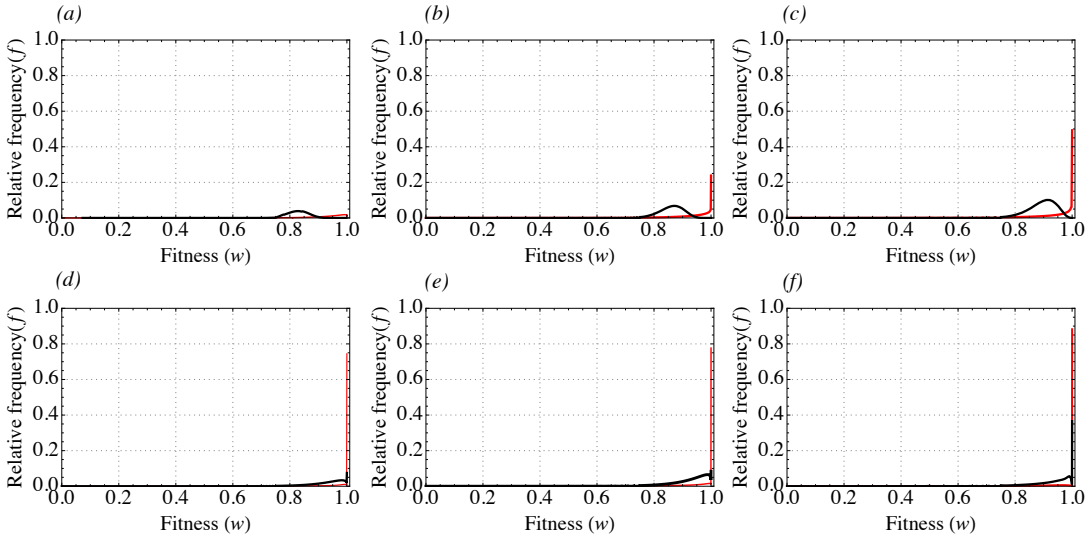

Figure 2.4: Population fitness density under uniparental (red) and biparental (black) mitochondria inheritance for μ = 0.01; ν=0.001 and different values for the pair (M, B). (a)-(c) Parameter values with no bottleneck (M, B) equal to (200,-), (100,-) and (50, -) respectively. (d)-(f) Parameter values with bottlenecks (100,10), (50, 10) and (50, 5) respectively.

2.3.1 Simple model with no bottleneck

In the absence of a bottleneck, uniparental inheritance always gave a higher mean and variance of the population fitness (Table 2.1A). The values of P_w were also higher with uniparental inheritance and this can be seen from the heavy skewness of the distribution under uniparental inheritance (Fig. 2.4 (a)-(c)). So the higher variance under uniparental inheritance was due to a highly skewed distribution with a high concentration of genotypes in the fittest states plus a long tail. In contrast, biparental inheritance generated a more normally distributed range of fitness around the mean. Finally, mitochondrial heteroplasmy was notably lower under uniparental inheritance (Table 2.1A) as has been shown previously (Bergstrom and Pritchard, 1998).

Mean fitness decreased with larger numbers of mitochondria per cell (M) under both modes of inheritance (Table 2.1A). Likewise P_w values dropped and heteroplasmy increased (Table 2.1A). Uniparental inheritance, unlike biparental inheritance, maintained high levels of mitonuclear matching for larger values of M (Fig. 2.5 (a), (b)). The fitness advantage of uniparental inheritance, both in mean fitness and $P_{0.9}/P_{0.95}$ increased with M (Fig. 2.5 (a), (b)). Likewise the heteroplasmy measure h, increased with M, under both modes of inheritance, albeit was substantially higher with biparental than with uniparental inheritance (Fig. 2.5 (c)).

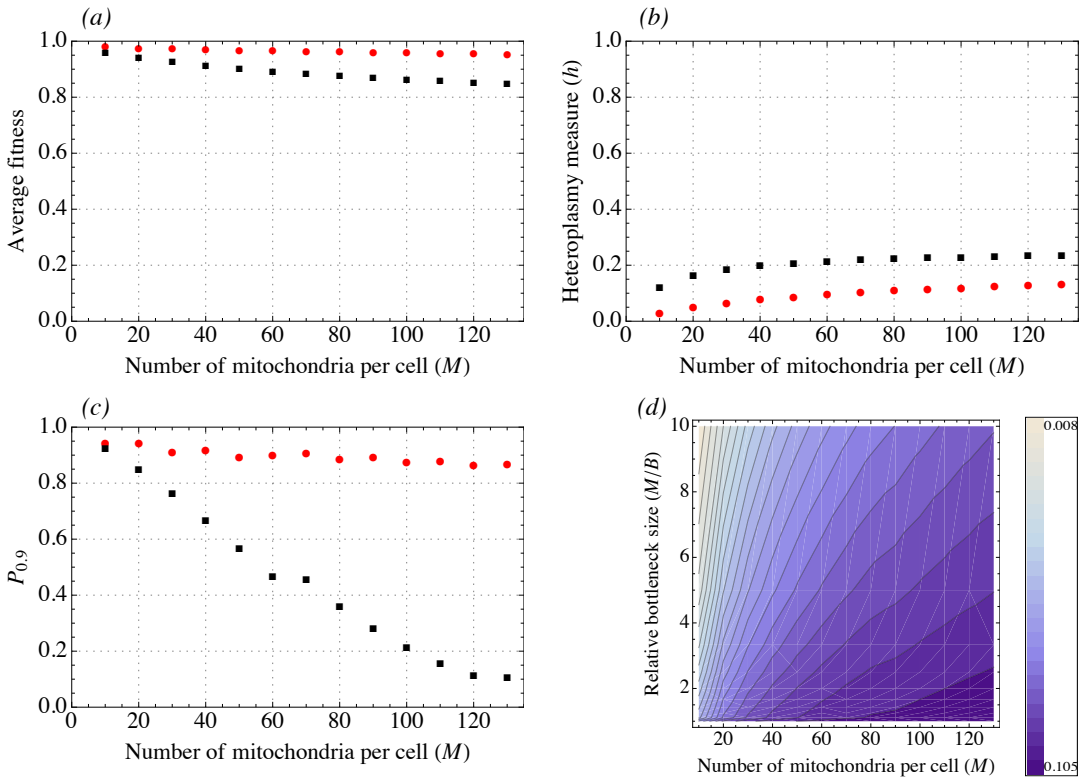

Figure 2.5: (*a*), (*b*), (*c*) Fitness advantage of uniparental inheritance over biparental inheritance. Contrasting values of $\bar{w}$, $P_{0.9}$ and h for variable numbers of mitochondria per cell (M) with no bottleneck (red points for uniparental inheritance and black for biparental inheritance). Baseline parameter values for (*a*) - (*c*), M=50, μ = 0.01; ν = 0.001, no bottleneck. (*d*): Contour plot for the difference in $\bar{w}$ for different number of mitochondria per cell (M) and the relative bottleneck size (M/B). Other parameter values, μ = 0.01; ν = 0.001.

2.3.2 Effect of a bottleneck

When a bottleneck was included in the model, the population fitness distribution improved under both modes of inheritance. The tighter the bottleneck, the better the resulting fitness distribution (both and $P_{0.9}/P_{0.95}$) and the lower the level of mitochondrial heteroplasmy (Table 2.1) under both modes of inheritance. Bottlenecks had the general effect of decreasing the distinction between uniparental and biparental inheritance (Fig. 2.4 (d)-(f) and Table 2.1B). Interestingly, when a very tight bottleneck was assumed, the number of mitochondria per cell seemed to have less of an effect on the fitness distribution. This can be seen with a bottleneck $B = 10$, contrasting the number of mitochondria $M = 200$, 100 and 50 (Table 2.1B, rows 7, 10 and 12 respectively). There was little difference in mean fitness under uniparental and biparental inheritance with this very tight bottleneck. The opposite was the case without a bottleneck (Table 2.1A, rows 1, 3 and 4). To illustrate the dynamic effect of the coupling (M, B) on the distinction between the two modes of inheritance, we generated a contour plot for the difference in mean fitness ($\bar{w}$) under the two modes of inheritance (uniparental minus biparental), for different values of M and relative bottleneck size M/B (Fig. 2.5(d)). This shows that the advantage of uniparental inheritance is greater for high values of M and less tight bottlenecks.

2.3.3 Varying μ and ν

In the analysis above we assumed that the mitochondrial mutation rate (μ) exceeded the nuclear mutation rate (ν). When this pattern of mutation was reversed (Table 2.1 C, row 13 $\nu = \mu$, row 14 $\nu > \mu$), the advantage of uniparental over biparental inheritance was smaller (Fig. 2.6). On the other hand, increasing μ while keeping ν fixed resulted in a greater advantage of uniparental inheritance (Table 2.1 C, row 14-15). However, when μ was increased beyond a threshold value the distinction between uniparental and biparental inheritance decreased and biparental inheritance gave higher average fitness, although the value of $P_{0.9}$ and $P_{0.95}$ were always higher for uniparental inheritance (Table 2.1 C, row 16; Fig. 2.6).

This initially puzzling result can be explained as follows. When the mitochondrial mutation rate becomes very high, all cells are kept in a state of considerable mitochondrial heteroplasmy (Table 2.1 C). This selects for heterozygosity in the nucleus. Even though segregation of the nuclear genes results in a high frequency of homozygotes, net selection can favour a more even representation of the 0 and 1 alleles in the mitochondria of progeny. So above a high threshold of mitochondrial mutation rate, biparental inheritance is favoured over uniparental inheritance. This is unlikely to be of relevance under natural circumstances.

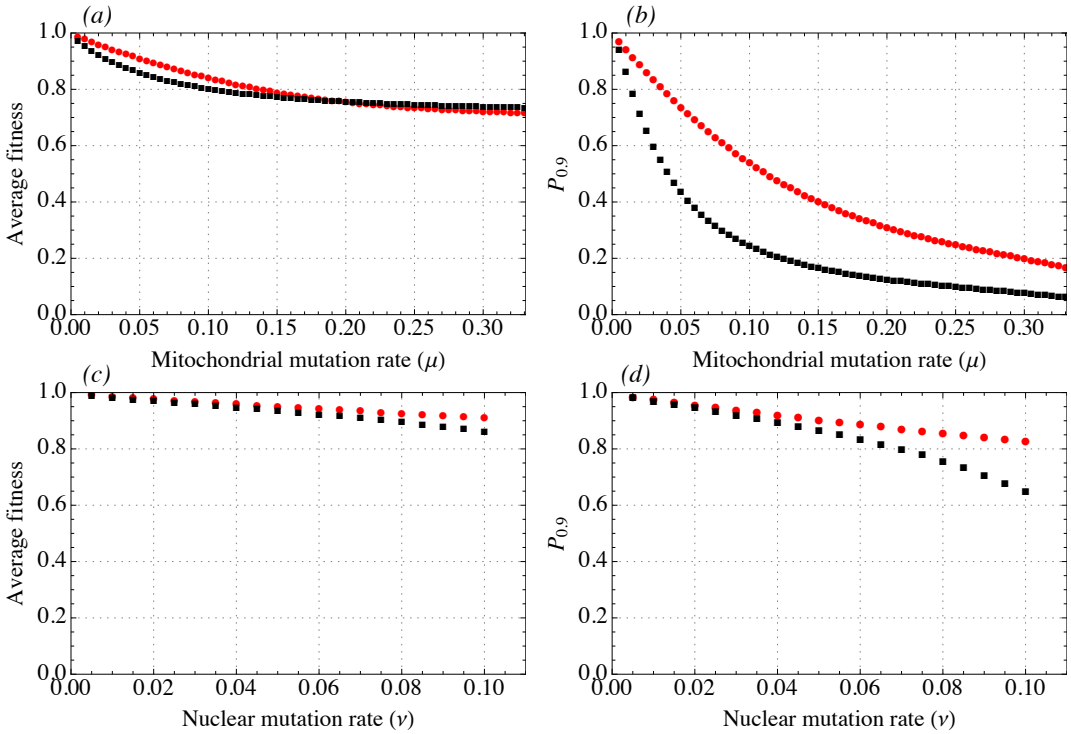

Figure 2.6: Fitness under uniparental (red points) and biparental (black points) inheritance given variation in the mutation rates. (*a*), (*b*): Variation in mitochondria mutation rate μ given a fixed nucleus mutation rate $\nu = 0.001$. (*c*), (*d*): Variation in nuclear mutation rate given a fixed mitochondria mutation rate $\mu = 0.001$. Other parameter values, $M = 50$; $B = 10$.

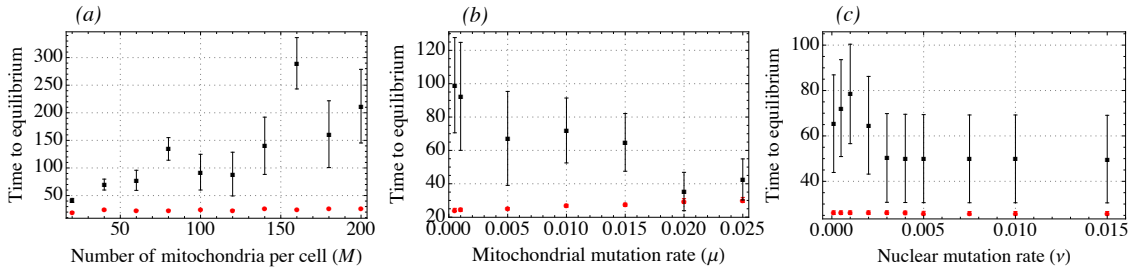

Figure 2.7: Mean time from the onset of the simulation until mean fitness stabilizes within 10^{-5} under uniparental (red points) and biparental (black points) inheritance, (*a*): against the number of mitochondria per cell (μ=0.01, ν=0.001), (*b*): against the mitochondrial mutation rate (M=100, ν=0.001) and (*c*): against the nuclear mutation rate (M=100, μ=0.01). The results are averaged over 10 simulations.

2.3.4 Rates of evolution

The rate at which uniparental and biparental populations evolve from a randomly coadapted state to their corresponding equilibria are different. In particular, we found that the rate at which uniparental populations move towards the optimal coadapted state is many times faster than of biparental populations. The plots in Fig. 2.7 shows the mean time until stability is reached starting from a randomly coadapted population against the number of mitochondria per cell M, the mitochondrial mutation rate μ and the nuclear mutation rate ν.

The distinction in the rates of evolution is a consequence of the difference in variation under the two modes of inheritance: higher variation under uniparental inheritance means that selection operates more swiftly. When the number of mitochondria per cell increases, there is a slight increase in the time until equilibrium for both modes of inheritance (Fig. 2.7(*a*)); more mitochondria imply more mitonuclear states and therefore more time until the population is pushed to its optimal state. Higher mitochondrial mutation rates have the opposite effect under uniparental and biparental inheritance (Fig. 2.7(*b*)). As μ increases, the increase in mutational noise and heteroplasmy benefit the heterozygote state and biparental inheritance (see Section 2.3.3) which explains why biparental inheritance results in faster rates of evolution for larger μ. Finally varying ν had a less severe impact on the rates of evolution for both uni- and biparental inheritance Fig. 2.7(*c*).

2.4 Discussion

Our results suggest that mitonuclear coadaptation is improved under UPI. Uniparental inheritance increased the mean population fitness ($\bar{w}$) and the proportion of the population with high fitness genotypes ($P_{0.9}$ and $P_{0.95}$) (Table 2.1, Fig. 2.4 (*a*)-(*c*)). These outcomes can be explained in two ways. Biparental inheritance

reduces the variance in mitochondrial states between cells and increases heteroplasmy (i.e. within cell variance) in zygotes. This is disadvantageous as it interferes with coadaptation, which requires matching of the mitochondria population to the nuclear background. In our model, the optimal state towards which the population evolves contains a homozygous nucleus (either (00) or (11)) with corresponding homoplasy in the mitochondria (state 0 or 1 respectively). This is equivalent to full coadaptation between the nucleus and mitochondria. Once a population is near to this state, fitness is improved if heteroplasmy is minimized. This is better achieved by uniparental inheritance, which precludes the mixing of mitochondrial populations in the zygote. A second way of formulating this advantage is to note that the higher variance between individuals generated under uniparental inheritance improves the efficiency of selection. This permits selection to amplify the frequency of optimal genotypes, allowing the population to evolve closer to the optimal state more quickly. We can see this in the skewed distribution of fitness under uniparental inheritance with a high frequency of individuals attaining maximum fitness (Fig. 2.4 (*a*-*c*)). This outcome follows from our assumption that selection is concave down and so reaches a plateau as the number of matching mitochondria increases (Fig. 2.1 and Eq. (2.1)).

We also examined the effect of a mitochondrial bottleneck before meiosis. This had a beneficial effect on mitonuclear coadaptation under both modes of inheritance (Fig. 2.4). Both the mean population fitness ($\bar{w}$) and the proportion of the population with high fitness genotypes ($P_{0.9}$ and $P_{0.95}$) were improved when a bottleneck was assumed (Table 2.1). Decreasing the bottleneck size had a positive effect on the fitness distribution (Table 2.1, Fig. 2.4 (*d*-*f*)). The effects of a bottleneck were similar to those generated by uniparental inheritance. Bottlenecks are a sampling process that reduces heteroplasmy and increases the variation between zygotes, and so increases the efficiency of selection (Table 2.1), a conclusion drawn before in other modelling contexts (Bergstrom and Pritchard, 1998; Roze et al., 2005). In general, bottlenecks had larger effects on biparental inheritance, reducing the advantage of uniparental over biparental inheritance. This was especially true when the bottleneck was very tight (Fig. 2.5 (*d*), high M/B).

Our model explicitly considers an idealised unicellular lifecycle (Fig. 2.2). In this context, we can interpret the bottleneck step as equivalent to mitochondrial segregation during cell division in the clonal expansion phase of the lifecycle. The results therefore suggest that if unicellular species originally had few mitochondria and biparental inheritance, there would only have been weak selection in favour of the evolution of uniparental inheritance. Mitochondrial segregation during cell division, might have been sufficient to restrict heteroplasmy and so maintain adaptation. However, in lineages where the number of mitochondria per cell increased, there would have been a much greater advantage generated by the switch to uniparental inheritance. Thus uniparental inheritance may have been a prerequisite for mitonuclear coadaptation in

multicellular organisms with higher energy requirements and larger populations of mitochondria per cell. It is notable that organisms such as yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) have a small number of mitochondria (<100) and do indeed lack uniparental inheritance (Thrailkill and Birky, 1980; Birky, 1995; Hermann and Shaw, 1998). Their mode of inheritance involves biparental inheritance of mitochondria followed by mitochondrial segregation. Plainly this is sufficient to maintain mitonuclear function, as predicted by our model.

Our model also has implications for multicellular organisms, where true germline bottlenecking occurs (Bergstrom and Pritchard, 1998; Roze et al., 2005). In line with our results, true bottlenecks are only observed in large organisms with large numbers of mitochondria (Hauswirth and Laipis, 1985; Jansen and de Boer, 1998; Rand, 2001). The tightness of the bottleneck varies across species and correlates with litter size (species with small litters having tighter bottlenecks) (Krakauer and Mira, 1999). This fits our expectations, because the smaller the litter, the greater the need for offspring fitness to be assured, hence likewise for mitonuclear coadaptation. From our results, we also predict that species with higher aerobic capacity, such as birds with powered flight, should also exhibit very tight bottlenecks (Lane, 2008, 2011b,c).

In our model, optimal fitness can only be achieved by the homozygote states. This relates to our assumption that the nuclear genes are additive in their effect on mitochondria and both alleles are equally active. The additive assumption seems a natural one, as the population of mitochondria are likely to interact with the gene products of both nuclear alleles. To ensure that the preference for homozygous states was not an artefact of the additive assumption in our model, we considered a situation in which there was an advantage for heterozygotes (see Appendix). Even in these cases, the population converged to the homozygous state. This is related to the pattern of Mendelian inheritance. A population can never be fully heterozygous because heterozygous parents will always give rise to 50% of homozygous offspring. Therefore, even if the heterozygotes reach optimum fitness their homozygous offspring will be significantly less fit eventually pushing the population to one of the homozygous states. This was true for low and high heterozygote advantage and uniparental inheritance, and for low heterozygote advantage and biparental inheritance. When the advantage attached to heterozygotes crossed a threshold and mitochondrial inheritance was biparental, however, a new equilibrium was obtained with a heterozygote nucleus (see Appendix).

The preference for homozygote states in our model also means that at this coadapted equilibrium, mitochondria can be thought of as wild type (matching the nucleus) or mutant (in disagreement with the nucleus). Although this is conceptually different to a simple mutation framework, once the equilibrium is achieved the two models become very similar. This could perhaps be viewed as a limitation of our model in capturing the full effect of mitonuclear interactions. Further work should examine other possibilities such as

the presence of several interacting alleles in the nucleus and the mitochondria, or mechanisms other than a heterozygote advantage that would force a nuclear polymorphism to persist (e.g. negative frequency dependent selection on nuclear alleles). Another scenario not considered here is possible imprinting or epistatic effects, although there is no evidence to support this possibility. Further work elucidating the way in which nuclear and mitochondrial genes interact is important. One distinction that remains between our model and that of simple mitochondrial mutations is the rate of evolution to a new coadapted state which will be faster under uniparental inheritance. Therefore, if changes such as environmental shifts favour a new coadapted configuration, uniparental inheritance would be more efficient at reaching the new optimal state.

We focused on mutation rates in which the mitochondrial rate (μ) was 10 times faster than the nuclear rate (ν). This difference seems appropriate for animals and fungi, where mitochondrial evolution rates are typically an order of magnitude greater than nuclear rates (Lynch et al., 2006; Nabholz et al., 2008). Note that a mutation in our model signifies a shift from one state to another, and is therefore commensurate with long-term evolutionary rates rather than mutations in nucleotide sequence, which can range over several orders of magnitude. In general, we found that uniparental inheritance was favoured whenever the mitochondrial mutation rate was greater than the nuclear mutation rate (Table 2.1, Fig. 2.6 (a, c)). We also considered the reverse case (Table 2.1, Fig. 2.6 (b, d)) which is perhaps more representative of plants, where nucleotide substitution rates are lower in the mitochondria than in the nucleus (Palmer and Herbon, 1988; Wolfe, 1982). In this case, the benefit of uniparental inheritance was lower. This might help to explain why heteroplasmy is more common in angiosperms having biparental inheritance (Zhang, 2003).

In conclusion, our model suggests that selection for mitonuclear coadaptation may favour the evolution of uniparental inheritance in unicellular organisms, particularly when the number of mitochondria is large (i.e. in highly energetic cells). Likewise, our model predicts the combination of uniparental inheritance with germline bottlenecking in larger multicellular organisms. Our work illustrates a fundamental principle of uniparental inheritance, namely the capacity to generate greater between cell variation and lower within cell variation and so facilitate faster selection for mitonuclear coadaptation. Conversely, biparental inheritance, by mixing different populations of mitochondria, restricts the evolution of optimal mitonuclear combinations. To relate these findings to the evolution of uniparental inheritance of mitochondria and two sexes, further work exploring the evolutionary invasion of uniparental mutants in biparental populations is needed. We expect that, given the difference in fitness between the two modes of inheritance illustrated here, the requirement for mitonuclear coadaptation will be an important force favouring the establishment of uniparental inheritance.

2.5 Appendix

2.5.1 Mathematical Derivations

We present a derivation of the equations modelling the life cycle outlined in the main text. We define $f(i, j)$ to be the relative frequency of cells with mitonuclear genotype (i, j) (i^{th} nuclear and j^{th} mitochondrial state). The nuclear states $i = 1, 2, 3$ represent the states (00), (01) and (11) respectively. A mitochondrial state j represents a cell with j mitochondria in state 1; $j = 0, 1, ..., M$. It follows that there are $3(M + 1)$ possible mitonuclear states and their frequencies sum up to one,

$$\sum_{i=1}^{3} \sum_{j=0}^{M} f(i, j) = 1.$$

We derive the change in the relative frequency of each genotype following each step of the life cycle 1-5 as outlined in the main text (Fig. 2.2). We assume an infinite population size and so ignore drift. We let $f(i, j)$ and $f'(i, j)$ be the frequencies at the onset and after each step respectively.

Relative frequency recalculation

1. Following mutation

To compute the relative frequency of each genotype $f'(i, j)$ we need to consider all possible genotypes that can mutate to (i, j). We can write,

$$f'(i, j) = \sum_{k,l} P((k, l) \rightarrow (i, j)) f(k, l),$$

$$P((k, l) \rightarrow (i, j)) = P_{nuclear}(k \rightarrow i) P_{mitochondrial}(l \rightarrow j),$$

since nuclear and mitochondrial genes mutate independently.

For the mitochondria, we note that each mitochondrial gene can mutate *only once* in each round. That is, there can be a mutation from 0 to 1 or a mutation from 1 to 0, but there can be no back mutations in the same round. This means that, if the initial mitochondrial genome state is l (number of 1s), then the number of new mutations from 0 to 1 follows Binomial$(\mu, M - l)$ and the number of new mutations from 1 to 0 follows Binomial(μ, l). We therefore obtain,

$$P_{mitochondrial}(l \rightarrow j) = \sum_{x=max(0,j-l)}^{min(M-l,j)} P(x \text{ new mutations from 0 to 1}) \, \mathrm{P}(l - j + x \text{ new mutations from 1 to 0})$$

Table 2.2 below summarizes the probabilities for the nuclear transitions, $P_{nuclear}(k \rightarrow i)$.

Table 2.2: Probability of mutating from nuclear state k to nuclear state i; k, i = 1,2,3 for states (0 0), (0 1) and (1 1), respectively.

$i\backslash k$	1	2	3
1	$(1-\nu)^2$	$(1-\nu)\nu$	ν^2
2	$2(1-\nu)\nu$	$(1-\nu)^2+\nu^2$	$2(1-\nu)\nu$
3	ν^2	$(1-\nu)\nu$	$(1-\nu)^2$

2. Following selection

Using a standard population genetic model of generational frequency change in a large population,

$$f'(i,j) = \frac{f(i,j)w(i,j)}{\bar{w}}$$

where $\bar{w} = \sum_{i,j} f(i,j)w(i,j)$ and $w(i,j)$ is the fitness of a cell with mitonuclear genotype (i,j) as defined by Eq. (2.1) in the main text.

3. Following the bottleneck

There are two rounds of sampling in a bottleneck, B_1 and B_2. First from M, the original number of mitochondria are reduced by sampling without replacement down to B, the bottleneck size. Second from B, the population is restored by sampling with replacement back to M. Following the bottleneck we have,

$$f'(i,j) = \sum_{X=0}^{M}\sum_{Z=0}^{B} f(i,X)P_{B_1}(X \to Z)P_{B_2}(Z \to j),$$

where $P_{B_1}(X \to Z)$ is the probability that a cell with X mitochondria in state 1 before the bottleneck has Z mitochondria in state 1 after the first bottleneck step and $P_{B_2}(Z \to j)$ is the probability that a cell with Z mitochondria in state 1 before the second bottleneck step has j mitochondria in state 1 after the bottleneck. These probabilities are given by,

$$P_{B_1}(X \to Z) = \frac{\binom{X}{Z}\binom{M-X}{B-Z}}{\binom{M}{B}}$$

and

$$P_{B_2}(Z \to j) = 1, \text{ when } j = 0 \text{ and } Z = 0$$

$$P_{B_2}(Z \to j) = \binom{M}{j}\left(\frac{Z}{B}\right)^j\left(\frac{B-Z}{B}\right)^{(M-j)}, \text{ otherwise.}$$

4. Following meiosis

Finding the relative frequencies of each genotype following the two meiotic divisions is more complex computationally. The derivation is performed in two steps.

STEP 1: first meiotic subdivision

In this step, each nuclear gene and each mitochondrion is first duplicated, and then two diploid daughter cells are formed by random segregation, each with M mitochondria. We use $fm1(i, j)$ to denote the relative frequency of cells in nuclear state i and mitochondrial state j after the first meiotic division, where $1 \leq i \leq 3$ and $0 \leq j \leq M$. Let,

$$P_{(i,k)}(j) = P\left[\text{an } (i,k) \text{ cell has a daughter with } j \text{ mitochondria in state 1}\right],$$

then,

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

We therefore obtain,

$$\begin{aligned}
fm1(1,j) &= \sum_{k=j/2}^{M} P_{(1,k)}(j) f(1,k) + \sum_{k=j/2}^{M} \frac{1}{6} P_{(2,k)}(j) f(2,k), \\
fm1(2,j) &= \sum_{k=j/2}^{M} \frac{2}{3} P_{(2,k)}(j) f(2,k), \\
fm1(3,j) &= \sum_{k=j/2}^{M} P_{(3,k)}(j) f(3,k) + \sum_{k=j/2}^{M} \frac{1}{6} P_{(2,k)}(j) f(2,k).
\end{aligned}$$

STEP 2: second meiotic subdivision

In this step, each diploid cell resulting from the first meiotic division randomly segregates to produce two haploid gametes, each containing $M/2$ mitochondria. We denote by $m1(i, j)$ a daughter with nuclear state i and mitochondrial state j following the previous (first) meiotic division. Like before, we denote by $fm2(i, j)$ the relative frequency of gametes with nuclear state i and mitochondrial state j , where now $i = 1, 2$ represents a (haploid) nuclear gene in state 0 or 1, respectively, and j takes values in the range $0, ..., M/2$, where we assume that M is even. Let,

$$P_{m1(i,k)}(j) = P[\text{An } m1(i,k) \text{ cell has a daughter with } j \text{ mitochondria in state 1}],$$

then,

$$P_{m1(i,k)}(j) = \frac{\binom{k}{j}\binom{M-k}{M/2-j}}{\binom{M}{M/2}}.$$

We therefore obtain,

$$\begin{aligned} fm2(1,j) &= \sum_{k=j}^{M} P_{m1(1,k)}(j) fm1(1,k) + \sum_{k=j}^{M} \frac{1}{2} P_{m1(2,k)}(j) fm1(2,k), \\ fm2(2,j) &= \sum_{k=j}^{M} P_{m1(3,k)}(j) fm1(3,k) + \sum_{k=j}^{M} \frac{1}{2} P_{m1(2,k)}(j) fm1(2,k). \end{aligned}$$

5. Following syngamy

A. Assuming biparental inheritance

Taking $fm2(i,j) = 0$ for $j > M/2$, we have,

$$\begin{aligned} f'(1,k) &= \sum_{j=0}^{k} fm2(1,j) fm2(1,k-j), \\ f'(2,k) &= 2\sum_{j=0}^{k} fm2(1,j) fm2(2,k-j), \\ f'(3,k) &= \sum_{j=0}^{k} fm2(2,j) fm2(2,k-j). \end{aligned}$$

B. Assuming uniparental inheritance

We assume that the mitochondrial genome of one parent is discarded, and that of the other is doubled through sampling with replacement,

$$\begin{aligned} f'(1,k) &= \left\{\sum_{l=0}^{M/2} fm2(1,l)\right\} \sum_{m=0}^{M/2} fm2(1,m) \binom{M}{k} \left(\frac{2m}{M}\right)^k \left(\frac{M-2m}{M}\right)^{(M-k)}, \\ f'(2,k) &= \left\{\sum_{l=0}^{M/2} fm2(1,l)\right\} \sum_{m=0}^{M/2} fm2(2,m) \binom{M}{k} \left(\frac{2m}{M}\right)^k \left(\frac{M-2m}{M}\right)^{(M-k)}, \\ &\quad + \left\{\sum_{l=0}^{M/2} fm2(2,l)\right\} \sum_{m=0}^{M/2} fm2(1,m) \binom{M}{k} \left(\frac{2m}{M}\right)^k \left(\frac{M-2m}{M}\right)^{(M-k)}, \\ f'(3,k) &= \left\{\sum_{l=0}^{M/2} fm2(2,l)\right\} \sum_{m=0}^{M/2} fm2(2,m) \binom{M}{k} \left(\frac{2m}{M}\right)^k \left(\frac{M-2m}{M}\right)^{(M-k)}. \end{aligned}$$

2.5.2 Simulations

At each run in the simulations the genotype frequencies went through the recursions described above. The simulations were stopped when the maximum of the difference in the relative frequency between every genotype from one generation to the next was below ϵ for $\epsilon = 10^{-9}$. We detected two stable nuclear equilibria (00) and (11), as discussed in the main text.

The simulations were run for a wide range of parameter sets. Below is a summary of the parameter sets for which we produced simulation outcomes. The equilibria of all simulations were in agreement with the results we present in the main text.

1. **Varying** M. We initially fixed the mutation rates to $(\mu, \nu) = (0.01, 0.001)$ and varied M in the range $\{10, 20, 30, ..., 200\}$. No bottleneck was included in these simulations.

2. **Varying** B **and** M. For each M in the range $\{10, 20, 30, ..., 200\}$ we varied B in the range $\{ M/10, 2M/10 , ..., 9M/10, -\}$ where $-$ represents the case where no bottleneck was assumed. The mutation rates were set to $(\mu, \nu) = (0.01, 0.001)$ for these runs.

3. **Varying the mitochondrial mutation rate** μ. Keeping M fixed at 50, assuming a relatively moderate bottleneck at B =10 and a low nuclear mutation rate ν=0.001 we varied μ in the range $\{0.005, 0.01, 0.015, ..., 0.3\}$.

4. **Varying the nuclear mutation rate** ν. Keeping M fixed at 50, assuming a relatively moderate bottleneck at B =10 and a low mitochondrial mutation rate μ=0.001 we varied ν in the range $\{ 0.005, 0.01, 0.015, ..., 0.1\}$. We also considered a single more extreme case where μ=0.5. The nuclear equilibrium in that extreme case was not purely homozygote. This is because extreme nuclear mutation rates do not allow the population to settle down a homozygote state even at the mutation-selection balance.

The results of all simulations were in agreement with the conclusions presented in the main text.

2.5.3 Extensions to the model

Role of heterozygote state

It may appear at first sight that convergence to the homozygote states is related to the homozygote advantage built into the fitness function. To investigate this, we modified the fitness function to incorporate heterozygote advantage. The fitness function in this case is defined by Eq. 2.2 (Fig. 2.8),

$$w(i,j) = \begin{cases} 1 - \left(\frac{j}{M}\right)^2 & \text{if } i=1 \\ 1 - \frac{1}{2}\left(\frac{j}{M}\right)^2 - \frac{1}{2}\left(\frac{M-j}{M}\right)^2 + c & \text{if } i=2 \\ 1 - \left(\frac{M-j}{M}\right)^2 & \text{if } i=3 \end{cases} \tag{2.2}$$

where c is a constant, taken to be large enough to confer heterozygote advantage.

The main effect of adding heterozygote advantage was to weaken selection against the heterozygote state. Under uniparental inheritance the population always converged to a homozygote state, with and without a bottleneck. Convergence to this state was significantly slower than it was when no heterozygote advantage was assumed. Under biparental inheritance the population got stuck on the heterozygote state (50 % of the population being heterozygotes and 25 % in each heterozygote state - Table 2.3). This was not the case when the heterozygote advantage, c, was small ($c = \frac{1}{4}$) and a bottleneck was included (Table 2.3; rows 2 and 4). For larger values of c ($c = \frac{3}{8}$) the biparental population could not escape from the heterozygote state even with a relatively tight bottleneck ($B = 10$). In fact, after running the simulations for a very large number of generations (of the order of 10000) the biparental population showed a tendency to diverge slightly from the 50%, 25%, 25% equilibrium but never actually converged to a homozygote state.

These observations suggest that uniparental inheritance would push the population to the homozygote state even when a significant heterozygote advantage is assumed. This convergence is slow due to weak selection against the heterozygote state. Nevertheless, as explained in the main text, a heterozygote state is problematic because Mendelian inheritance will always result in the production of many homozygote offspring. Therefore well co-adapted heterozygotes (with the additional fitness advantage implemented here) will give rise to less fit homozygote offspring. Thus, despite heterozygote advantage, the population performs better overall when it is nearly homogeneous homozygote. Uniparental inheritance and a bottleneck are sufficient for convergence to the homozygote state. Biparental inheritance however, increases heteroplasmy (see main text). It follows that the heterozygote state becomes stable in biparental populations when a significant heterozygote advantage is assumed. When the advantage for the heterozygotes is moderate, biparental inheritance with a bottleneck also converges to the homozygote state.

Adding a threshold to the number of mismatches a cell can tolerate

It is likely that there is a threshold in the number of mitochondrial mutations beyond which fitness declines to zero (Smith et al., 2002). The threshold may vary across species or organs, being higher in organisms or organs with greater energy demands (e.g. muscle and brain tissue, or pollen growth). To reflect this we

included a parameter n_{th} in the fitness function,

$$w(i,j) = \begin{cases} 1 - \left(\frac{j}{n_{th}}\right)^2 & \text{if } i = 1 \text{ and } j > n_{th} \\ 1 - \frac{1}{2}\left(\frac{j}{n_{th}}\right)^2 - \frac{1}{2}\left(\frac{M-j}{n_{th}}\right)^2 + c & \text{if } i = 2 \text{ and } M - n_{th} < j < n_{th} \\ 1 - \left(\frac{M-j}{n_{th}}\right)^2 & \text{if } i = 3 \text{ and } j > M - n_{th} \\ 0 & \text{otherwise.} \end{cases} \tag{2.3}$$

We ran simulations for different values of n_{th} (Table 2.4). Changes in n_{th} did not have a major effect on the equilibrium fitness distribution, except to decrease the frequency of individuals with mismatches with increasing n_{th}. Selection becomes stronger as n_{th} becomes smaller (i.e. selection is more intense the lower the tolerance of mismatches). Decreasing the value of n_{th} resulted in a higher concentration in the fittest states under both modes of inheritance (see $P_{0.9}$ and $P_{0.95}$ in rows 2 and 3 in Table 2.4).

Note that the decrease in mean fitness in these cases may be misleading. A reason for the drop in mean fitness is related to the role of n_{th} in the definition of the fitness function (Equation (2.3)). An increase in the denominator n_{th} means that even if the mitonuclear composition of a cell remains fixed, the fitness of that cell for that new value of n_{th} will decrease. Therefore equal, or even improved, levels of coadaptation may result in reduced fitness. The general observation that uniparental inheritance resulted in better results than biparental inheritance also holds true when n_{th} is implemented in the model.

Table 2.3: Summary statistics for different parameter sets (M, B, μ, ν) for heterozygote advantage c. The statistics $\bar{w}$ and σ^2 are the mean and variance of the population fitness and $P_0.95$ and $P_0.9$ are the proportion of the population with fitness greater than 0.95 and 0.9 respectively.

Uniparental						**Biparental**				
A. $c = \frac{1}{4}$										
(M, B, μ, ν)	(p_{00}, p_{01}, p_{11})	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$	(p_{00}, p_{01}, p_{11})	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$
1. $(50, -, 0.01, 0.001)$	(98.9,0.0113, 3.216*10^{-5})	0.972	0.0048	85.2	90.8	(0.25, 0.5, 0.25)	0.871	0.0184	49.7	50.1
2. $(50, 10, 0.01, 0.001)$	(1.76*10^{-5}, 0.00836, 0.992)	0.979	0.0062	91.0	93.6	(0.975, 0.0251, 1.6*10^{-4})	0.953	0.0035	69.2	84.8
3. $(100, -, 0.01, 0.001)$	(1.12*10^{-4}, 0.0210, 0.979)	0.957	0.0037	73.6	86.3	(0.25, 0.5, 0.25)	0.873	0.017	50	50
4. $(100, 10, 0.01, 0.001)$	(1.99*10^{-05}, 0.00888, 0.991)	0.977	0.0058	89.6	93.0	(2.24*10^{-04}, 0.0295, 0.970)	0.949	0.0035	64.8	84.3
B. $c = \frac{3}{8}$										
$(M, B, \mu, \nu,)$	(p_{00}, p_{01}, p_{11})	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$	(p_{00}, p_{01}, p_{11})	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$
5. $(50, -, 0.01, 0.001)$	(0.944, 0.0553, 8.13*10^{-4})	0.969	0.0052	82.5	88.9	(0.25, 0.5, 0.25)	0.933	0.038	50.0	50.7
6. $(50, 10, 0.01, 0.001)$	(0.974, 0.0258, 0.000171)	0.978	0.0064	89.7	92.4	(0.25,0.5,0.25)	0.922	0.045	52.9	57.9
7. $(100, -, 0.01, 0.001)$	(0.732, 0.248, 0.0209)	0.946	0.016	64.4	79.2	(0.25, 0.5, 0.25)	0.935	0.036	50.0	50.1
8. $(100, 10, 0.01, 0.001)$	(0.970, 0.0295, 2.24*10^{-4})	0.976	0.0061	88.4	91.6	(0.25, 0.5, 0.25)	0.923	0.044	52.3	57.5

Table 2.4: Summary statistics for different parameter sets $(M, B, \mu, \nu, M/n_{th})$. The statistics $\bar{w}$ and σ^2 are the mean and variance of the population fitness and $P_0.95$ and $P_0.9$ are the proportion of the population with fitness greater than 0.95 and 0.9 respectively.

	Uniparental				**Biparental**			
$(M, B, \mu, \nu, M/n_{th})$	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$	$\bar{w}$	σ^2	$P_{0.95}$	$P_{0.9}$
1. $(50, 10, 0.01, 0.001, 1)$	0.979	0.0063	91.5	94.1	0.955	0.0034	71.2	86.2
2. $(50, 10, 0.01, 0.001, 0.8)$	0.977	0.0082	93.1	95.6	0.952	0.0051	81.1	92.3
3. $(50, 10, 0.01, 0.001, 0.6)$	0.974	0.011	94.9	97.0	0.948	0.0074	89.9	96.7

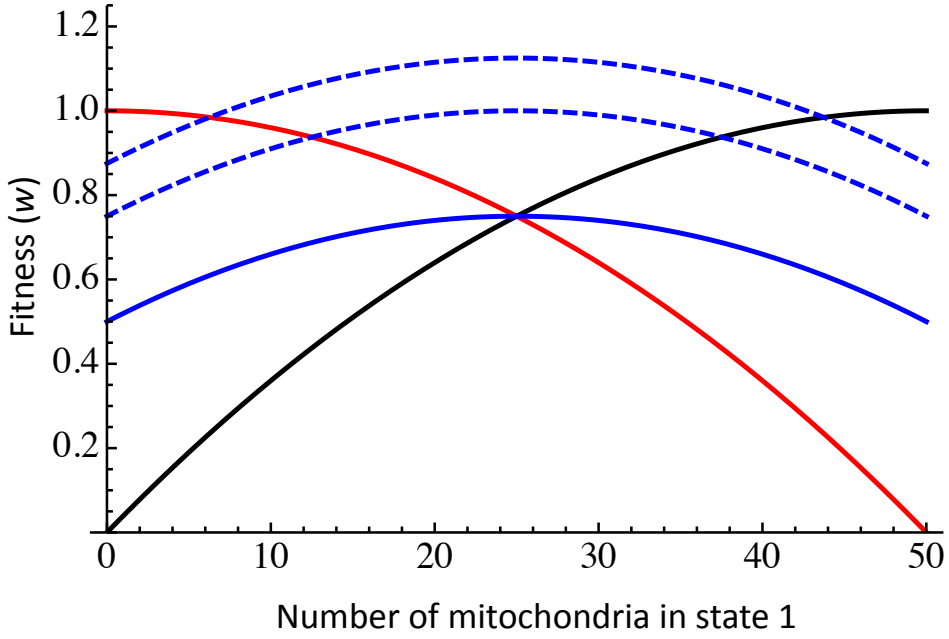

Figure 2.8: Fitness function for M = 50. Red, black and blue curves for nuclear states (00), (11) and (01) respectively. The dotted lines represent the heterozygote fitness when the heterozygote advantage, c, is equal to $\frac{1}{4}$ and $\frac{3}{8}$

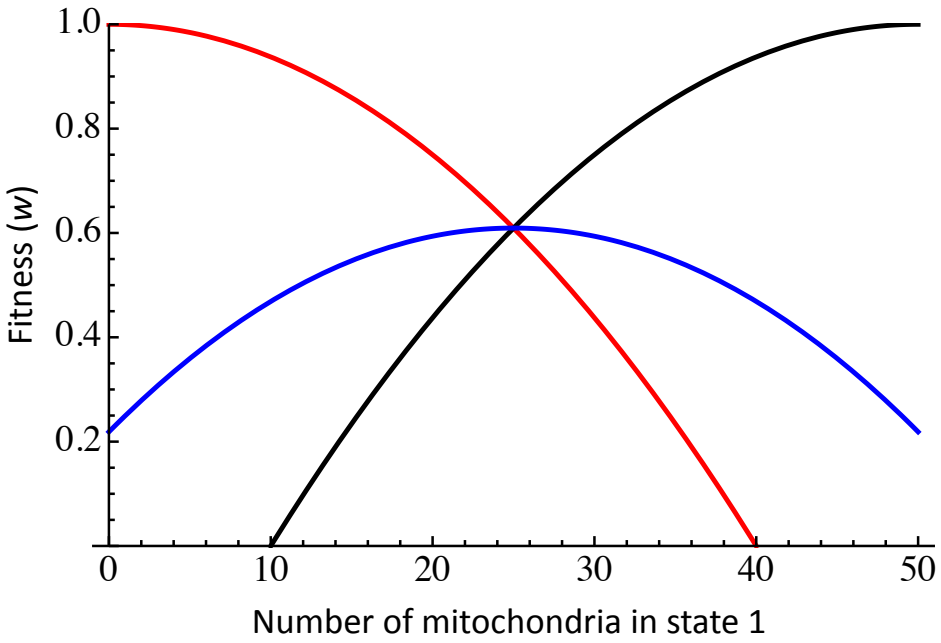

Figure 2.9: Fitness function for M = 50 and n_{th}=40. Red, black and blue curves for nuclear states (00), (11) and (01) respectively.

Chapter 3

Dynamics of mitochondrial inheritance in the evolution of mating types and two sexes[1]

The uniparental inheritance (UPI) of mitochondria is thought to explain the evolution of two mating types, or even two sexes with anisogametes. However, the exact role of UPI is not clearly understood. Here we develop a new model, which considers the spread of UPI mutants within a biparental inheritance (BPI) population. Our model explicitly considers mitochondrial mutation and selection in parallel with the spread of UPI mutants and self-incompatible mating types. In line with earlier work, we find that UPI improves fitness under mitochondrial mutation accumulation, selfish conflict and mitonuclear coadaptation. However, we find that as UPI increases in the population its relative fitness advantage diminishes in a frequency dependent manner. The fitness benefits of UPI 'leak' into the biparentally reproducing part of the population through successive matings, limiting the spread of UPI. Critically, while this process favours some degree of UPI, it does not lead to the establishment of linked mating types; nor the collapse of multiple mating types to two. Only when two mating types exist beforehand can associated UPI mutants spread to fixation under the pressure of high mitochondrial mutation rate, large mitochondrial population size and selfish mutants. Variation in these parameters could account for the range of UPI actually observed in nature, from strict UPI in some *Chlamydomonas* species to BPI in yeast. We conclude that uniparental inheritance of mitochondria alone is unlikely to have driven the evolution of self-incompatible mating types.

[1]This study was conducted in collaboration with Andrew Pomiankowski, Nick Lane and Rob Seymour and has been published in the *Proceedings of the Royal Society B* (Hadjivasiliou et al., 2013)

3.1 Introduction

Notwithstanding the prominence of theoretical work linking uniparental inheritance of mitochondria (UPI) to the evolution of the sexes, the reasons UPI is so widespread are uncertain. UPI is nearly universal among multicellular animals and plants, where one sex (usually the female) passes on its mitochondria while the other does not (Hoekstra, 2000). UPI is also widespread amongst isogamous unicellular organisms with morphologically identical gametes, which nonetheless often correspond to two mating types (Birky, 1995; Billiard et al., 2011). A well-founded hypothesis that associates the evolution of mating types and sexes to that of UPI must primarily be grounded in a solid understanding of the value and evolution of UPI. A rigorous analysis of the transition from biparental inheritance (BPI) to UPI, and the evolution of associated self-incompatible mating types or sexes should then follow.

There are three main hypothesis elucidating the significance of UPI. First, UPI may purge deleterious mitochondrial mutations. Specifically, it has been shown that UPI decreases the variance in mtDNA within cells, and increases the variance in mtDNA between cells, facilitating selection against deleterious mitochondria (Bergstrom and Pritchard, 1998; Roze et al., 2005). In contrast, biparental inheritance (BPI) averages the number of mutant mitochondria of parents, hindering selection for the lower mutation load. However, there is no explicit evolutionary model of the transition from BPI to UPI based on the ability of UPI to restrain mtDNA mutations.

A second hypothesis proposes that UPI minimizes selfish conflict between mitochondria and the host cell. Mixing mitochondria (or other cytoplasmic elements) from different parents may drive selection for mutations that are beneficial for the mitochondria (providing a replicative advantage) but harmful to the host cell (Eberhard, 1980; Cosmides and Tooby, 1981; Partridge and Hurst, 1998). This hypothesis has received the greatest attention. Evolutionary models using a fixed cost for cells carrying selfish mitochondrial mutants have shown that uniparental mutants in the nucleus are favoured in a biparental population (Randerson and Hurst, 1999) and under specific conditions can lead to the evolution of two mating types (Hurst and Hamilton, 1992; Hutson and Law, 1993). The assumption of a fixed cost for BPI in these models is dubious, however, and needs to be further investigated as fitness reduction is expected to depend on the number of selfish mutations carried. Such consideration was made in an earlier analysis by Hastings (1992), who concluded that the spread of selfish mutants led to the evolution of UPI and anisogamy. However, this model actually showed that only moderate levels of UPI evolve; and it did not consider the possible invasion of associated mating types.

The third hypothesis relates to the work of the previous Chapter, demonstrating that mitonuclear coadaptation improves with UPI under a wide range of conditions. This hypothesis is based on the fact that

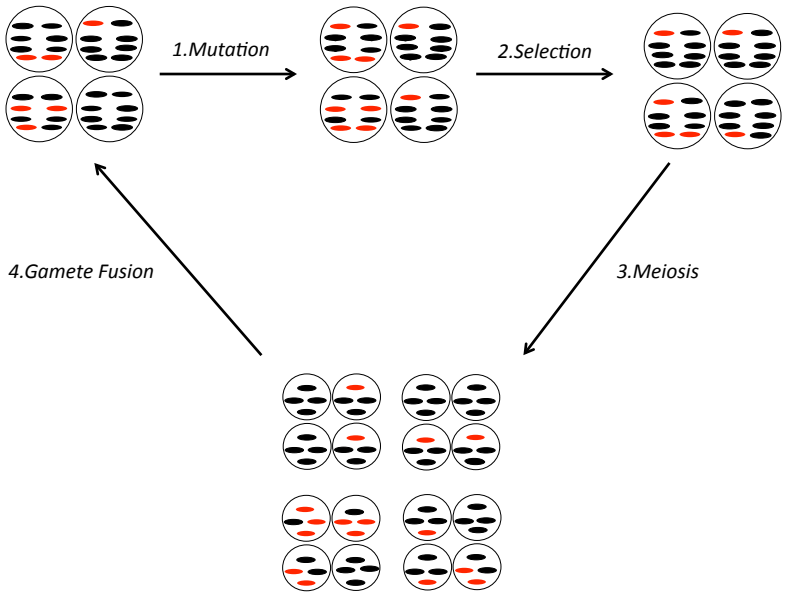

Figure 3.1: Model life cycle. Big circles are cells while ovals represent mitochondria (black for wild-type and red for mutant).

oxidative phosphorylation requires multiple interactions between proteins, RNA and DNA encoded in the nucleus and mitochondria (Burton and Barreto, 2012). Strong evidence across many eukaryotic organisms demonstrates that the two genomes have indeed adapted to each other over evolutionary time (Blier et al., 2001; Mishmar et al., 2006; Lane, 2011c). The theoretical work in the previous chapter again supports an advantage to UPI, but also lacks formal consideration of the evolution transition from BPI to UPI.

These limitations and different models make it difficult to determine the conditions under which the fitness benefits of UPI are sufficient to drive the evolution of self-incompatible mating types in unicellular organisms. Here, we assess this evolutionary question for all three hypotheses by developing a novel, more extensive model. We explicitly define a nuclear mechanism of mitochondrial inheritance and ask whether UPI could evolve in an ancestral population where mitochondrial inheritance is biparental. By explicitly incorporating mitochondrial mutation and selection in the model, while introducing uniparental inheritance and mating type mutants, like previous authors, we find that UPI does indeed improve fitness. However, as UPI increases in the population its relative fitness advantage diminishes in a frequency dependent manner. Critically, the fitness benefits of UPI 'leak' into the biparentally reproducing part of the population, limiting the spread of UPI. Only when mating types pre-exist can uniparental mutants become associated with them, leading to a population with strict UPI. We discuss how our findings relate to previous analyses and to the patterns of mitochondrial inheritance actually seen in protists.

3.2 Model outline

The core model is based on a simple life cycle for an infinite population of diploid, unicellular organisms similar to that of the previous Chapter (Fig. 3.1). These undergo clonal expansion during which they are subject to mutation and selection. We do not explicitly model this, but for simplicity impose mutation (step one) followed by selection (step two). Each cell contains a fixed number M of mitochondria that can be wild type or mutant resulting in M+1 possible mitochondrial states. The wild type mitochondria in each cell mutate independently with probability μ (back mutation is initially ignored). After mutation, selection changes the relative frequency of each mitochondrial state. Fitness is defined as a concave function of the number of mitochondrial mutations as in Chapter 2,

$$w(j) = 1 - \left(\frac{j}{M}\right)^2 \tag{3.1}$$

where j is the number of mutant mitochondria in the cell $j \in \{0, 1, ..., M\}$; (Fig.3.2(a)). Since a cell contains many mitochondria, small numbers of mitochondrial mutations are likely to have minor fitness effects, as suggested by the high threshold of mitochondrial mutations required to cause a significant decline in oxidative phosphorylation (Adkins et al., 1996). Fitness decline should then be sharper as the mutation load increases, as is indeed evident in many mitochondrial diseases (Rossignol et al., 2003). This justifies the choice of a quadratic fitness function in Eq. 3.1. We also considered the impact of employing a convex fitness function, although this is naturally unrealistic (see Appendix at the end of this Chapter).

Following selection, surviving cells undergo meiosis (step three). The cell's population of mitochondria is doubled to $2M$ and then reduced through two cell divisions to produce four haploid gametes each with $M/2$ mitochondria. At each meiotic cell division, the mitochondrial genotypes of the parent cell are randomly segregated between the two daughter cells, as is indeed the case in mitotic divisions in eukaryotes (i.e. sampling without replacement) (Birky, 2001). Gametes then randomly fuse with each other to form the next generation of cells (step four).

We explicitly model the evolution of mitochondrial inheritance by assuming nuclear control through a single locus. Gametes with the wild type allele a cause mitochondria to be inherited biparentally (BPI) when they fuse with other a gametes. Gametes with the mutant A allele pass on their mitochondria uniparentally (UPI) when they fuse with an a gamete, by excluding the mitochondria from the a gamete. We define fusions between two A gametes as having BPI of mitochondria. We also modelled the alternative assumptions that AxA fusions are inviable (Hutson and Law, 1993), or result in uniparental inheritance (Hastings, 1992) (see Appendix).

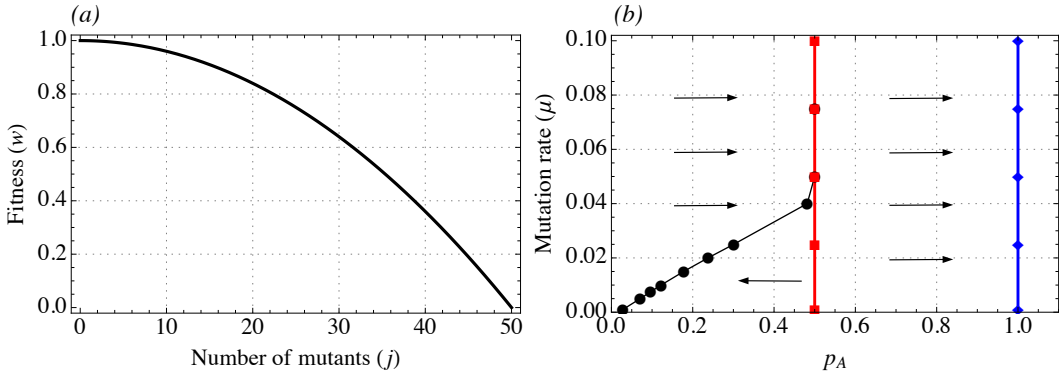

Figure 3.2: (*a*) Concave fitness curve given by Eq.3.1 for $M = 50$. (*b*) Schematic representation of the three equilibria for $M = 50$ and varying μ, showing E_1 (black), E_2 (red) and E_3 (blue).

We consider the invasion of the *A* allele into a BPI population of the *a* allele. The *A* allele is introduced at a frequency of 1%, and then frequency change is tracked through numerical simulations to define equilibria and stability (we assumed no other mutations between *a* and *A* gametes). The rate of UPI is maximized under these assumptions when the frequency of the *A* allele, p_A reaches 0.5. We make small modifications to this basic model, to examine the spread of selfish mutants and the benefits of mitonuclear coadaptation. Note that we assume gamete control of mitochondrial inheritance rather than the diploid parental cell, as this simplifies the dynamics and is true of protists such as *Chlamydomonas* (Goodenough et al., 2007). However, the life cycle is idealized and not intended to replicate any particular protist. A detailed mathematical derivation of the equations used in our simulation is given in the Appendix.

3.3 Results

3.3.1 Mitochondrial mutation pressure

There are three equilibria (p_a^*, p_A^*) : $E_1 = (1\text{-}c, c)$ where $0< c <0.5$, is stable and reached when the *A* mutant is introduced into a population fixed for the *a* allele ($p_a = 1$); $E_2 = (0.5, 0.5)$ is generally not attractive; and $E_3 = (0,1)$ cannot be invaded by the *a* allele (Fig.3.2(*b*)). Hence the uniparental *A* mutatant is favoured and spreads in a population that has biparental inheritance of mitochondria, but only to a polymorphic equilibrium with p_A^* generally < 0.5. The wildtype allele *a* did not invade a population fixed for the UPI allele (i.e. $p_A = 1$) and was eliminated if the initial frequency satisfied $p_A > 0.5$ (Fig.3.2(*b*)).

To understand the forces determining the three equilibria we plotted the normalised mean fitness of each genotype and gene while forcing the population to remain at a specified frequency of *A*, p_A (mitochondria were allowed to evolve; Fig.3.3). When the *A* allele is first introduced ($p_A \approx 0$), its fitness is effectively de-

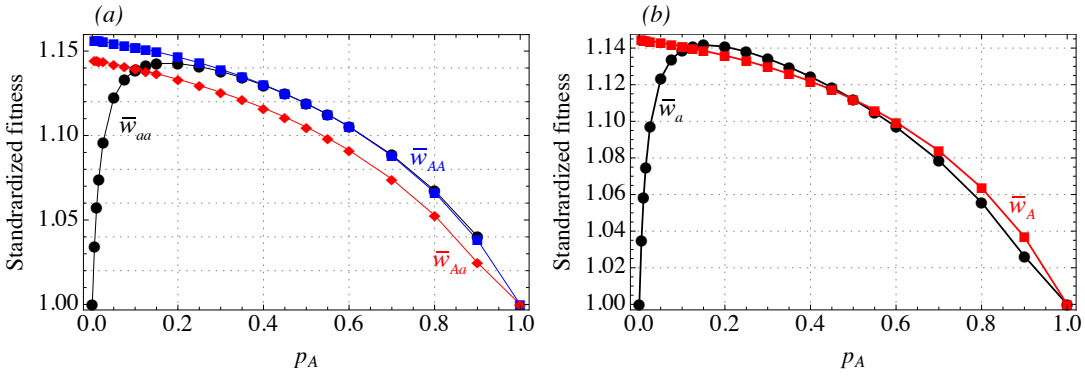

Figure 3.3: Normalised mean fitness (mean fitness divided by mean BPI fitness) of (*a*) genotypes $\bar{w}_{aa}$ (black line), $\bar{w}_{Aa}$ (red line) and $\bar{w}_{AA}$ (blue line), and (*b*) genes $\bar{w}_a$ (black line) and $\bar{w}_A$ (red line), for fixed values of p_A. Parameter values: $M = 50$, $\mu = 0.01$.

termined by matings between *A* and *a* gametes which are always uniparental. Since UPI increases variation in mitochondrial mutation load (Bergstrom and Pritchard, 1998) (also see previous Chapter), the *A* allele is associated with more low and high fitness mitotypes. Given a concave fitness curve (Eq. (3.1) and Fig. 3.2(*a*)), the net effect is an initial decline in fitness of the *A* allele (Fig.3.4 (*b*)). But within a small number of generations (~ 5 when M=50, μ=0.01; Fig. 3.4 (*b*)), the *A* allele accumulates a fitness benefit due to the cumulative removal of mutant mitochondria made possible by heightened mitochondrial variation. We then have $\bar{w_A} >> \bar{w_a}$, so p_A increases (Fig. 3.4(*a*)).

'Leakage' of improved mitochondria is then a key factor that limits the spread of UPI and the *A* allele. When *A* and *a* gametes fuse, the *Aa* zygote inherits the cumulative high fitness mitochondria generated by UPI present in the *A* population. Surviving *Aa* zygotes ultimately produce new gametes, both *A* and *a*. This allows the improved mitochondria to 'leak' into the *a* population (Fig.3.5). Initially this effect is weak, as *a* x *A* fusions are rare compared to *a* x *a* fusions. But as the *A* allele spreads, the leakage becomes more significant and p_{aa} undergoes a sharp rise (Fig.3.4(*a*)). Thus the presence of *A* gametes in a population to some degree 'cleans up' the mitochondria of the population as a whole, which can be seen in the improved fitness distribution of *aa* as well as *Aa* individuals (Fig. 3.6). So as the *A* allele spreads, the relative fitness advantage of UPI declines.

In addition, as p_A increases, *A*x*A* matings become more common. In our model these have BPI, thereby reducing the rate at which mutant mitochondria are removed. The combination of the short-term disadvantage to *A* of increased variance due to UPI and the reduction in cleansing of mutations because of *A*x*A* fusions, result in a reduction of $\bar{w}_A$ as p_A increases (Fig.3.3(*b*)). This, along with the increasing value of $\bar{w}_a$ due to leakage, leads to the polymorphic equilibrium at E_1 (i.e. $\bar{w}_A = \bar{w}_a$). The polymorphic equilibrium E_1 is stable because moving towards E_2 (higher p_A) heightens leakage of improved mitochondria, and

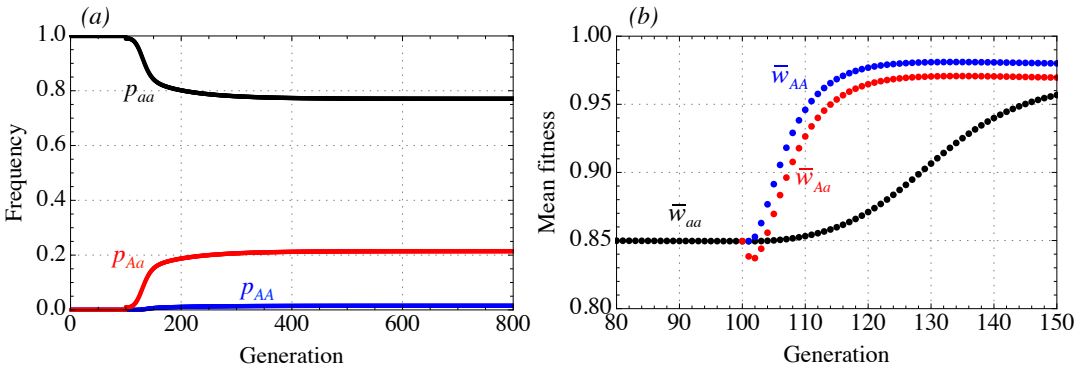

Figure 3.4: (*a*) Trajectories of genotype frequencies through time and (*b*) mean genotype fitness (scaled to maximal fitness of unity for no mitochondrial mutants), from an initial frequency$p_A = 0.01$ at generation 100. Parameter values: $M = 50$, $\mu = 0.01$.

therefore further increases the fitness for $\bar{w}_a$ relative to $\bar{w}_A$ (Fig.3.3 (*b*)).

The second stable equilibrium, E_3, occurs when the *A* mutation is fixed (p_A=1, Fig.3.2(*b*)). It might seem paradoxical that this is not invaded by the *a* allele, as this causes UPI of mitochondria. However, the *a* allele is the alternate uniparental pattern of 'kill your own mitochondria' rather than 'kill your partner's mitochondria' (Randerson and Hurst, 1999). In this case, the *a* allele does not invade because there is no cumulative purging of mitochondrial mutations (as the *a* allele never passes on its mitochondria, so any benefits of UPI are lost). When p_A is large, any improved mitochondria associated with a gametes are lost in the following generation, as mitochondria are only inherited from the *A* gamete, which carries the unimproved biparental mitochondrial state. In addition, as noted above, *A*x*a* fusions increase variation in mitochondria mutation load which in the short-term produces a net fitness disadvantage for the *a* allele (Fig. 3.4 (*b*)). These considerations also explain why the equilibrium at E_2 (i.e. $p_A = p_a$) is unstable. If p_A is slightly lower than p_a, selection drives the population to E_1, and if p_A is slightly higher than p_a, selection drives the population to E_3.

Our results show that alleles causing uniparental inheritance are subject to frequency-dependent selection. They typically invade to reach an intermediate value (p_A = 0.1-0.2, UPI rate 18-32%, given $\mu = 0.01$ and M=50; Fig.3.2 (*b*)). Only when the mitochondrial mutation rate (μ) and number of mitochondria (M) are very large, does $p_A \rightarrow 0.5$ (i.e. E_1 merges with E_2, Fig.3.2 (*b*)). At these high values, fitness is considerably reduced for both uni- and bi-parental zygotes (for example, when M=100 and $\mu = 0.1$ E_1 merges with E_2 and we have ($\bar{w}_{aa}$, $\bar{w}_{Aa}$, $\bar{w}_{AA}$) = (0.447, 0.440, 0.447)) (see Appendix). These high values may seem somewhat implausible but are not unreasonable in certain cases (see Discussion).

We repeated the analysis above using the assumption that *A*x*A* matings are uniparental, with the transmitting role randomly assigned to one of the partners (Hastings, 1992). In this case the fitness of *A* is fixed

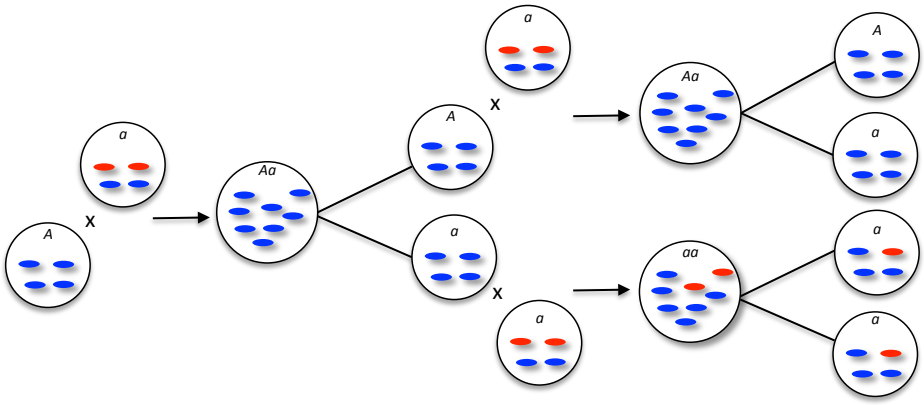

Figure 3.5: Schematic representation of leakage of UPI benefits to the BPI part of the population. Small circles are gametes, big circles are zygotes. Ovals are mitochondria, blue being wildtype and red mutants. Reading from left to right, when the UPI gene A is at low frequency, it becomes associated with fit mitochondria and so A gametes are highly likely to carry no mutants (all blue). When a less fit a gamete fuses with a fit A gamete (first fusion), they produce a mutant-free zygote. In turn, this produces mutant free A and a gametes, that are likely to fuse with other unimproved a gametes as these are common in the population (second fusion). Even though the resulting a x a zygotes have BPI, they have lower mutational load than typical a x a fusions, and produce fitter a gametes because of the leakage of improved mitotypes from A gametes in Aa individuals. Further leakage over many generations leads to the cumulative improvement of mitotypes in the a population.

and independent of p_A as all matings involving A gametes are uniparental. Nevertheless, fitness benefits still leak from A to a gametes and an intermediate frequency of A is sufficient to ensure that $\bar{w}_a$=$\bar{w}_A$ resulting in a polymorphic equilibrium equivalent to E_1. Further complexities related to the existence of E_2 and the stability of $E3$ are discussed in the Appendix.

We repeated the above analysis using a convex fitness curve. A fitness curve of this nature is hard to justify in unicellular organisms as it predicts that cell fitness expected decreases sharply as mutations accumulate. It causes a much greater benefit when moving from BPI to UPI and it follows that E_1 merges with E_2 even with lower values of M and μ (Appendix, Section 3.5.2). The third equilibrium E_3 (i.e. at $p_A = 1$) also exists but is unstable. Further analysis of the complexities of a convex fitness assumption is included in the Appendix.

3.3.2 Mutants with a replicative advantage

The selfish conflict theory (Eberhard, 1980; Hastings, 1992; Hurst and Hamilton, 1992; Hutson and Law, 1993; Partridge and Hurst, 1998; Randerson and Hurst, 1999), predicts that UPI evolved to protect against the spread of mutant mitochondria that are fast replicators. To explicitly implement this in our model, we included a sampling step after mutation and before selection. In this step, we sample with replacement given that mutant mitochondria have a relative advantage $1+k$ (so the probability of sampling a mutant

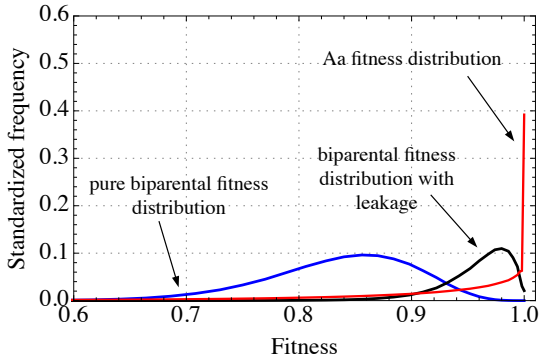

Figure 3.6: Fitness distribution of a population with strict biparental inheritance when $p_A = 0$ (blue) showing a low mean due to the accumulation of mitochondrial mutations. In contrast, at the equilibrium E_1 (p_A = 0.12), the fitness distribution of *Aa* individuals (red) with uniparental inheritance has many individuals having very high fitness (> 0.95) due to repeated cleansing of mitochondrial mutations, although with high variance (*AA* individuals have a similar pattern, not shown). Leakage of this benefit can be seen in the fitness distribution of aa individuals (black), calculated at the equilibrium E_1. Parameter values: $M = 50$, μ = 0.01.

mitochondrion is $\frac{x(1+k)}{M+xk}$ given a cell with x mutant mitochondria, see Fig. 3.11 in Appendix).

In this case, a similar pattern of three equilibria is found. The value of p_A at equilibrium E_1 increases with k (the replicative advantage). Above a threshold value of k, p_A becomes equal to 0.5 and equilibria E_1 and E_2 merge (Fig.3.7(a)). A replicative advantage for mutant mitochondria is equivalent to increasing M or μ, favouring a higher frequency of UPI. Thus, selfish mitochondrial mutants increase the spread of UPI. Once again, the third equilibrium E_3 (i.e. $p_A = 1$) still exists and is stable for the reasons discussed in the previous section.

3.3.3 Mitonuclear coadaptation

We model mitonuclear coadaptation by using the formulation of the previous Chapter, where a gene in the nucleus interacts with a gene in the mitochondria. Both genes have two allelic states, 0 and 1. There are thus three diploid nuclear genotypes (00), (01), (11) and $M + 1$ mitochondrial states. States 0 and 1 in the mitochondria no longer represent wild type and mutant respectively, but are either matched or unmatched mitochondria with respect to the nucleus. The impact of each allele on cell fitness depends on this interaction between the nucleus and mitochondria,

$$w(i,j) = \begin{cases} 1 - \left(\frac{j}{M}\right)^2 & \text{if } i = 1 \\ 1 - \frac{1}{2}\left(\frac{j}{M}\right)^2 - \frac{1}{2}\left(\frac{M-j}{M}\right)^2 & \text{if } i = 2 \\ 1 - \left(\frac{M-j}{M}\right)^2 & \text{if } i = 3 \end{cases} \tag{3.2}$$

where i is the nuclear state and j the mitochondrial state (Hadjivasiliou et al., 2012). We also modify the mutation step in the life cycle so nuclear and mitochondrial genes mutate with probabilities ν and μ respectively (assuming $\nu << \mu$), and equally in both directions, $0 \leftrightarrow 1$.

The complexities of this model were discussed in a non-evolutionary context in the previous Chapter. Apropos the evolutionary discussion here, a similar pattern of three equilibria is found once again. The nucleus converges to one of the homozygote states (00 or 11) which the mitochondria largely match (mainly 0 or 1). At this coadapted equilibrium, mitochondria can again be thought of as wild type (matching the nucleus) or mutant (in disagreement with the nucleus), which explains why the two models yield similar results. In general, the value of p_A at equilibrium E_1 is lower for similar mitochondria mutation rates and E_1 did not merge with E_2 even under increased M or μ. This reflects the symmetry of mutation between the mitochondria states (0 and 1) which mutate between each other with the same probability, generating matched (from unmatched) as well as unmatched (from matched) mutants. Because the nuclear alleles can adapt to mitochondrial mutations, and vice versa, the effective mitochondrial mutation rate μ is lower than in the mutation accumulation model, explaining why p_A is lower at equilibrium.

A further complication is that external factors, such as environmental pressures, could result in periodic switching of the dominant mitonuclear state (0 or 1). For example mitochondria adapt to both temperature and diet, and fluctuations in either might in principle undermine fitness (Willett and Burton, 2003; Wallace, 2013). We implemented this by periodically imposing a cost to the dominant state, forcing it to switch (see Appendix). These switches favoured higher values of p_A, closer to 0.5. Higher levels were favoured as UPI aided faster switching to better-adapted mitonuclear states. However, once a general state of mitonuclear coadaptation had been achieved, p_A returned towards the equilibrium at E_1 (Fig. 3.13 in the Appendix). Only under strong and frequent switching did the frequency of the uniparental allele A rise significantly, but even then never to $p_A = 0.5$.

3.3.4 Mating types

In the analyses above, we consider the spread of a mutation inducing UPI. However, the fact that AxA fusions are still possible means that BPI cannot be eliminated unless A and a become associated with self-incompatible mating types, as is the case in many unicellular eukaryotes (Birky, 1995). We modelled this by considering that a further nuclear locus controls self-compatibility, denoted by the mating type index m ($m \in 1, 2, ...$). Gametes can fuse with anyone but self (e.g. a_1 will not fuse with a_1/A_1 but can fuse with a_2/A_2 and so forth). The presence of mating types potentially allows complete UPI if the A and a alleles are associated with different mating types.

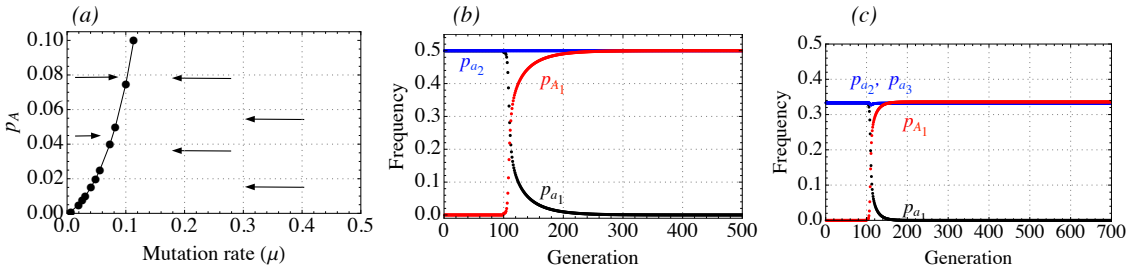

Figure 3.7: Equilibria with mating types. (a) When the uniparental inheritance mutation is linked to a mating type allele (A_1) that controls self-compatibility, there is an equilibrium at E_1 with p_{A_1} ¡ 0.5, but no equilibria at E_2 or E_3 ($\mu = 0.01$ and $M = 50$, for comparison with Fig 3.2 (b)). (b) Change in gene frequency across time (generation), from introduction of the uniparental inheritance mutation A_1 allele (initial frequency 10^{-2} at generation 100) into a population with biparental inheritance but two pre-existing mating types until stability is reached at equilibrium. With high values of $M = 100$ and $\mu = 0.1$, a_1 is replaced by A_1 and the equilibrium population has strict UPI. (c) With more mating types (three in this case), a_1 is again replaced by A_1, but there is no reduction in the number of mating types ($M = 100$, $\mu = 0.1$).

We first introduce A_1 into a population fixed for a (i.e. $p_a = 1$), assuming that the mating type index is linked to the uniparental inheritance modifier. The frequency of A_1 increases but only to an intermediate point equivalent to E_1 (Fig. 3.7(a)). This is for similar reasons that A reaches an intermediate frequency in the previous section; in particular that the benefits of UPI leak into the a population. A notable distinction is that A_1 x A_1 fusions are impossible, so A_1 has a mating rate disadvantage. This results in having a slightly lower value at E_1.

Adding a further mating type allele a_2 could be advantageous, as A_1 x a_2 fusions are exclusively UPI. However, the a_2 allele decreases monotonically in a population at E_1 (or for other non-zero values of $p_{A_1} < E_1$). The a_2 allele does not spread because it does not improve fitness beyond what is already achieved through A_1 x a fusions, but has a slight mating rate disadvantage (as self-incompatible).

We then introduce A_1 into a population with two pre-existing mating types a_1 and a_2 ($p_{a_1} = p_{a_2} = 0.5$). Again A_1 spreads, this time at the expense of a_1 alone, to a stable state corresponding to E_1, with the population at equilibrium made up of A_1a_2 and a_1a_2 individuals. For the same conditions that equilibrium E_1 merged with E_2 without mating types (i.e. high M and μ, selfish mutations or a convex fitness curve), A_1 displaces a_1 altogether leaving a population with two mating types with strict UPI (Fig. 3.7(b)). If the uniparental inheritance mutation (A_1) invades a population with 3, 4 or more mating types, we find that equilibrium frequency is reduced as the reciprocal of the number of mating types (Fig.3.7(c)). There is no collapse to two mating types. All the analyses above were repeated assuming full recombination between the mitochondrial inheritance locus and the mating-type locus. When full recombination was assumed, the mutant imposing UPI (A) spreads to a similar frequency as before. However, in this case each mating type

becomes associated with both uniparental (A) and biparental (a) inheritance alleles. So full UPI is only possible with tight linkage (Appendix).

3.4 Discussion

A number of experimental and theoretical analyses indicate that uniparental inheritance of mitochondria can improve fitness and have led to the suggestion that this force underlies the evolution of two mating types, leading to anisogamy and the evolution of true sexes (Eberhard, 1980; Hurst and Hamilton, 1992; Hastings, 1992; Hutson and Law, 1993; Partridge and Hurst, 1998; Randerson and Hurst, 1999; Hoekstra, 2011; Sharpley et al., 2012). Our modeling confirms that UPI does indeed improve fitness. This holds when UPI reduces mutation load, limits the proliferation of selfish mutants or improves mitonuclear coadaptation. But our explicit consideration of mitochondrial evolution, with uniparental or biparental inheritance and mating types, shows that there are real limits to what is possible.

We show that the fitness benefits arising from UPI are acquired cumulatively, over multiple generations. In the short-term, increased variation is detrimental, as many low fitness variants are generated. It is only after selection has had time to remove these less fit individuals that better mitochondrial adaptation builds up. Crucially, during the spread of a mutant inducing UPI, the fitness benefits of UPI inevitably leak through the population. The BPI part of the population benefits from regular infusions of fit mitochondria from UPI gametes, and leakage increases with the frequency of the UPI mutant. This makes the fitness advantage of UPI frequency dependent, and generally limits the spread and fixation of UPI mutants.

Critically, leakage as defined and illustrated in this work depends upon the possibility in our model that gametes from UPI individuals can mate with both gametes from UPI and BPI individuals in successive generations. This seems like a natural assumption, as there is no obvious reason why the mode of mitochondrial inheritance would also determine mate choice in unicellular species without invoking other mechanisms or adaptive benefits. However, a more complex genetic combination forcing cells with the uniparental mutant to only mate with themselves, or have an increasing preference of doing so, could alter the degree and power of leakage. In that sense, the merits of this work lie in uncovering a previously neglected feature of UPI, without necessarily deeming it universal.

Our work suggests that UPI itself is unlikely to drive the evolution of two distinct mating types, and that the conditions for this to happen can be much more stringent than previously thought. Others have also expressed uncertainty as to whether mating types can be understood as a consequence of UPI, but empirical data are conflicting and do not unambiguously support or refute the UPI hypothesis (Birky, 2001; Billiard et al., 2011; Hoekstra, 2011; Billiard et al., 2012; Perrin, 2012), Nonetheless, it seems likely that mating

types existed before the evolution of uniparental inheritance (Hoekstra, 1982; Iwasa and Sasaki, 1987; Czaran and Hoekstra, 2004; Billiard et al., 2011; Perrin, 2012). Only when we assume the pre-existence of two mating types, did we find conditions that can drive UPI to fixation, in particular, high mutation rates, large mitochondrial numbers or selfish mutants. Very high mtDNA numbers and mutation rates are possible. For example, the amoeba *Pelomyxa carolinensis* has as many as 300,000 mitochondria (Daniels and Breyer, 1968). Likewise, mitochondrial mutation rates can be extremely high - estimates for petite mutants in yeast are orders of magnitude higher than the nuclear rate (Linnane et al., 1989). We also find that for UPI to go to fixation it is necessary that the gene for mitochondrial inheritance occurs in tight linkage with the mating type locus, although some UPI is possible without linkage. We finally observe that the invasion of a UPI mutant cannot reduce the number of mating types in a population that already possesses more than two mating types.

Our results contrast with those reported from previous modelling work (Randerson and Hurst, 1999; Hurst and Hamilton, 1992; Hutson and Law, 1993). In large part this is due to their unrealistic assumption of a fixed cost for BPI caused by cytoplasmic mixing. The assumption that cells suffer the same cost independently of the number of mitochondrial mutants they carry totally alters the dynamics. Our work shows that it is important to consider the frequency-dependent interplay of costs and benefits associated with each mode of inheritance which naturally leads to the emergence of intermediate values of uniparental and biparental inheritance. Our results echo those of Hastings (1992), who explicitly modelled mitochondrial evolution. However, the significance of leakage was not studied or discussed in any detail, leading to (in our view) an inappropriate weight being placed on UPI as the motor force for the evolution of two mating types and anisogamy (Hastings, 1992).

Like previous authors (Hoekstra, 1987; Hastings, 1992; Randerson and Hurst, 1999), we found that a UPI modifier that kills its own mitochondria will not spread. The major problem is that such a modifier cannot become associated with the fit mitotypes and so the potential benefits gained though cleansing of mitochondrial mutants are always lost in the following generation. An exception to this rule is when selection on mitochondrial mutants follows a convex curve. Then uniparental inheritance spreads because higher variance in mitochondrial mutation load is favoured each generation (see Appendix). However, a convex fitness relationship seems unlikely to be a general feature of mitochondria mutants or heteroplasmy, so the relevance of this result may be limited. This is certainly the case in mitochondrial diseases, where the mutant load must be greater than about 40% before any symptoms become apparent, and there is no reason to suppose that single celled organisms are any different in this regard (Rossignol et al., 2003).

A related issue is the possibility of negative epistatic effects arising from the mixing and interaction of

different mitotypes (Lane, 2012; Sharpley et al., 2012). We have not explicitly modelled this here. But our results suggest that hybridization between populations with different, incompatible mitotypes will evolve towards a homoplasmic state, as was found experimentally by Sharpley et al. (2012). This is equivalent to our formulation of mitonuclear co-adaptation, where genes in the nucleus and mitochondria need to match in order for efficient function. However, as mentioned above, we did not find that this qualitatively altered the frequency-dependent outcome of selection for uniparental inheritance. Finally, we should mention that unlike other authors we impose no additional cost for UPI (e.g. related to the need for amplification of a smaller cytoplasm (Randerson and Hurst, 1999)). In that sense our model is conservative and even tighter conditions for the spread of UPI and mating types would be expected had those costs been implemented.

Our findings suggest a continuum of UPI levels is possible depending on the energetic demands (number of mitochondria), mutation rates and nature of mutations (selfish or not). This prediction is consistent with a number of empirical observations - the prevalence of some degree of UPI in unicellular eukaryotes (Xu, 2005); the presence of a mixture of maternal or paternal UPI as well as biparental zygotes in some unicellular organisms, slime molds and plants (Birky, 1995; Silliker et al., 2002); the persistence of BPI in organisms with two mating types such as yeast (Perlman and Birky, 1974); numerous distinct mechanisms of generating UPI (implying multiple origins and fluctuating selection) (Xu, 2005; Billiard et al., 2011); and tight linkage of mitochondrial inheritance and mating type loci associated with apparently strict UPI in some protists such as *Chlamydomonas reinhardtii* and *C. smithii* (Aoyama et al., 2006; Nakamura, 2010). But better investigations of the natural variation in rates of uniparental inheritance and associations with mating types in protists are needed, as are more studies of mitochondrial number and properties. Mitochondrial number, size and behaviour differs between cells of the same (Blank et al., 1980) and closely related species (Visviki, 2000) potentially accounting for differences in the spread of UPI in protists.

Finally, note that in this work we used an infinite population approach. This was significant in reducing computational time, while preliminary results with a finite population model generally converged to the findings presented here. One issue is that the stable equilibrium found here is polymorphic (E_1), while two unstable equilibria were also found (E_2 and E_3). In smaller populations, random fluctuations alone could drive the uniparental inheritance allele to extinction or fixation. In that sense, the work presented here does not explore the effects of random drift. Such explorations would be interesting but are beyond the scope of this chapter.

One matter that remains to be addressed is the importance of UPI and mating types in the evolution of anisogamy. Future work needs to examine the role of mitochondrial fitness in the tight linkage of UPI with the germline/soma distinction of multicellular organisms.

3.5 Appendix

3.5.1 Mathematical Derivations

We present a derivation of the equations modelling the life cycle outlined in the main text and shown in Fig.3.1. We begin by deriving the equations for the simplest of our models, in which wild type mitochondria are subject to deleterious mutation pressure. We define the two dimensional random variable $\mathbf{X}^t = (X_1^t, X_2^t)$ to represent the mitochondrial state and nuclear genotype of the diploid unicellular organisms (referred to from now on as 'cells') in the population at generation t where X_1^t is the number of mutant mitochondria carried by a cell and X_2^t is the mitochondrial inheritance locus of that cell. Hence, X_1^t takes values in $\{0, 1, ..., M\}$ and X_2^t takes values in $\{aa, Aa, AA\}$. It follows that there are $(M + 1)$ possible mitochondrial states. By definition their frequencies over all mitochondrial inheritance genotypes sum up to one,

$$\sum_{i,j} P(\mathbf{X}^t = (i, j)) = 1, \forall\ t.$$

We derive the change in the relative frequency of each genotype following each step of the life cycle (Fig.3.1). We assume an infinite population and so ignore drift in the nuclear locus, but include sampling (i.e. drift) of the mitochondrial population at reproduction (see below). If $P(\mathbf{X}^t = (i, j))$ denotes the population distribution at the onset of the life cycle (generation t) then $P(\mathbf{X}^{t+1} = (i, j))$ denotes the probability distributions at the onset of the next life cycle (generation $t + 1$).

During each generation the population will have gone through five steps as described in the main text (mutation, selection, meiotic step 1, meiotic step 2 and syngamy). So to go from generation t to generation $t+1$, the population undergoes five intermediate steps. We denote the probability distribution after each step by $P(\mathbf{X}^{t,\tau_s})$ where s takes values in $\{1, 2, 3, 4, 5\}$ and $P(\mathbf{X}^{t,\tau_0})$ is the distribution at the onset of generation t. It also follows that $P(\mathbf{X}^{t,\tau_5} = (i, j)) = P(\mathbf{X}^{t+1,\tau_0} = (i, j))$.

Relative frequency calculation

Following mutation

We let Z_k be the number of new mutants that a cell carrying k mutations may accumulate. Z_k is a random variable following a binomial distribution $\mathrm{B}(M - k, \mu)$ and we have,

$$P(Z_k = l) = \binom{M-k}{l} \mu^l (1-\mu)^{M-l}.$$

Hence, we obtain the cell's distribution following mutation,

$$P(\mathbf{X}^{t,\tau_1} = (i, j)) = \sum_{k=0}^{k=i} P(\mathbf{X}^{t,\tau_0} = (k, j)) P(Z_k = i - k)\ ,\ \forall\ j.$$

Following selection

We use a standard population genetic model of generational frequency change in a large population,

$$P(\mathbf{X}^{t,\tau_2} = (i, j)) = \frac{P(\mathbf{X}^{t,\tau_1} = (i, j))w(i)}{\bar{w}}, \forall\ j,$$

where $\bar{w} = \sum_{i,j} P(\mathbf{X}^{t,\tau_1} = (i, j))w(i)$ and $w(i)$ is the fitness of a cell with i mutant mitochondria as defined by Eq. 3.1 in the main text.

Following meiosis

Meiosis takes place in two stages and so this derivation is performed in two steps.

STEP 1: first meiotic subdivision

In the first step, the nuclear mitochondrial inheritance alleles and each mitochondrial gene are first duplicated, and then two diploid daughter cells are formed by random segregation, each with M mitochondria. The population is defined by $\mathbf{X}^{t,\tau_2}$ at the onset of meiosis. We define the random variable $\mathbf{X}^{t,\tau_3}$, which takes values (i, j) where $i \in \{0, 1, ..., M\}$ and $j \in \{aa, Aa, AA\}$ as before, to define the population following the first meiotic step.

We now define the random variable Y_k to be the number of mutant mitochondria sampled from a parent cell with k mutant mitochondria. Sampling takes place without replacement at this stage and so we have,

$$P(Y_k = l) = \frac{\binom{2k}{l}\binom{2(M-k)}{M-l}}{\binom{2M}{M}}.$$

Given that nuclear and mitochondrial genes are independently inherited following cell division we obtain,

$$\begin{aligned}
P(\mathbf{X}^{t,\tau_3} = (i, aa)) &= \sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k, aa)) + \frac{1}{6}\sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k, Aa)),\\
P(\mathbf{X}^{t,\tau_3} = (i, Aa)) &= \frac{2}{3}\sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k, Aa)),\\
P(\mathbf{X}^{t,\tau_3} = (i, AA)) &= \sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k, AA)) + \frac{1}{6}\sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k, Aa)).
\end{aligned}$$

STEP 2: second meiotic subdivision

In this step, each diploid cell resulting from the first meiotic division randomly segregates to produce two haploid gametes, each containing $M/2$ mitochondria. We define the random variable $\mathbf{X}^{t,\tau_4}$ to represent the population of gametes following this second division step. This can take values in (p, q) where p is

the number of mutant mitochondria and q is the mitochondrial inheritance gene. Then, p takes values in $\{0, 1, ..., M/2\}$ and q takes values in $\{a, A\}$ where we assume that M is an even number. This is equivalent to $P(\mathbf{X}^{t,\tau_4} = (i, j))$ =0 for $i > M/2$.

We also define the random variable Z_k to be the number of mutant mitochondria sampled from a parent cell with k mutant mitochondria following the second meiotic step. As before, sampling takes place without replacement and we have,

$$P(Z_k = l) = \frac{\binom{k}{l}\binom{M-k}{M/2-l}}{\binom{M}{M/2}}.$$

Using the fact that nuclear and mitochondrial genes are independently inherited following cell division we obtain the gamete distributions following meiosis,

$$\begin{aligned} P(\mathbf{X}^{t,\tau_4} = (p, a)) &= \sum_{k=p}^{M} P(Z_k = p)P(\mathbf{X}^{t,\tau_3} = (k, aa)) + \frac{1}{2}\sum_{k=p}^{M} P(Z_k = p)P(\mathbf{X}^{t,\tau_3} = (k, Aa)), \\ P(\mathbf{X}^{t,\tau_4} = (p, A)) &= \sum_{k=p}^{M} P(Z_k = p)P(\mathbf{X}^{t,\tau_3} = (k, AA)) + \frac{1}{2}\sum_{k=p}^{M} P(Z_k = p)P(\mathbf{X}^{t,\tau_3} = (k, Aa)). \end{aligned}$$

Following syngamy

We now let $\mathbf{X}^{t,\tau_4}$ be the gametes right before syngamy and we let $\mathbf{X}^{t,\tau_5}$ be the new cells following syngamy. When inheritance of mitochondria is biparental, the number of mutant mitochondria of the new cell is given by the sum of the mutants that the two gametes carry. Using the assumption that fusions between two A or two a gametes are biparental we get,

$$\begin{aligned} P(\mathbf{X}^{t,\tau_5} = (i, aa)) &= \sum_{k=0}^{i} P(\mathbf{X}^{t,\tau_4} = (k, a))P(\mathbf{X}^{t,\tau_4} = (i-k, a)), \\ P(\mathbf{X}^{t,\tau_5} = (i, AA)) &= \sum_{k=0}^{i} P(\mathbf{X}^{t,\tau_4} = (k, A))P(\mathbf{X}^{t,\tau_4} = (i-k, A)). \end{aligned}$$

When inheritance is uniparental, the mitochondria of the passive gamete (not passing on its mitochondria) are discarded and we sample with replacement from the active gamete to obtain M mitochondria for the new zygote (note, this is better than simply doubling the number of mitochondria, otherwise we always have even numbers of mutant mitochondria in UPI zygotes). We define the random variable Q_k to be the number of mutant mitochondria sampled from a gamete which carries k mutants. This follows a Binomial distribution $\mathrm{B}(M, 2k/M)$ from which we have,

$$P(Q_k = l) = \binom{M}{l}\left(\frac{2k}{M}\right)^{l}\left(\frac{M-2k}{M}\right)^{M-l}.$$

Using the assumption that fusions between an a and an A gamete are uniparental we then obtain,

$$P(\mathbf{X}^{t,\tau_5} = (i, Aa)) \quad = \quad \sum_{l=0}^{M/2} P(\mathbf{X}^{t,\tau_4} = (l, a)) \sum_{k=0}^{M/2} P(Q_k = i) P(\mathbf{X}^{t,\tau_4} = (k, A)).$$

Mating types

The derivations above are for the simplest model presented in the main text which only addresses mitochondrial mutational pressure. Also, we only considered nuclear genes a and A. When mating types A_1, a_1, A_2, a_2 are implemented, the calculations are essentially the same but extended to more than three genotypes appropriately. In addition, if there was recombination between the two nuclear loci (inheritance of mitochondria and mating type), this was applied to generate the resulting nuclear genotype frequencies. In the final step (syngamy) the same probabilistic rules are followed and uniparental or biparental inheritance is assumed according to the gamete's mitochondrial inheritance locus.

Simulations

The biological complexity encompassed by this model prevents us from solving analytically for the equilibrium states. So the asymptotic behavior and equilibria of the life cycle were explored using numerical simulation coded in C++. We assumed that equilibrium had been reached when the maximum changes in mitochondrial state frequency and nuclear gene frequency across a generation are smaller than an appropriately small value ϵ, taken to be 10^{-9}. Our results were qualitatively robust to changes in the parameter values and an equilibrium point was reached within approximately 2000 generations in most simulations.

3.5.2 Extensions to the model

In this section we provide results that are supplementary to each of the four result subsections in the main text in Chapter 3.

Mitochondrial mutation pressure

High M and μ

In Section 3.1 in the main text we discuss the impact of M and μ on the E_1 equilibrium value for p_A. As M and μ increase so does p_A and eventually this pushes the first equilibrium E_1 to merge with E_2. The reason for this relates to the capacity of A cells to allow leakage of UPI benefits to a cells.

When M and μ are low, UPI is very effective at keeping a high proportion of cells in the fittest states in the A population. A significant proportion of A cells have nearly perfect fitness (see Fig.3.8). This means that A x a fusions generate highly fit a gametes whose frequency may then be amplified via selection to produce a highly fit population of aa zygotes. However, when M and μ increase, the ability of UPI to maintain such a high proportion of the population at high fitness is impaired (Fig.3.8 second and third columns, for increased μ and M respectively). This in turn means that the leakage of fitness advantage from A to a cells is impaired. So p_A can increase further before an equilibrium is reached (Fig.3.8).

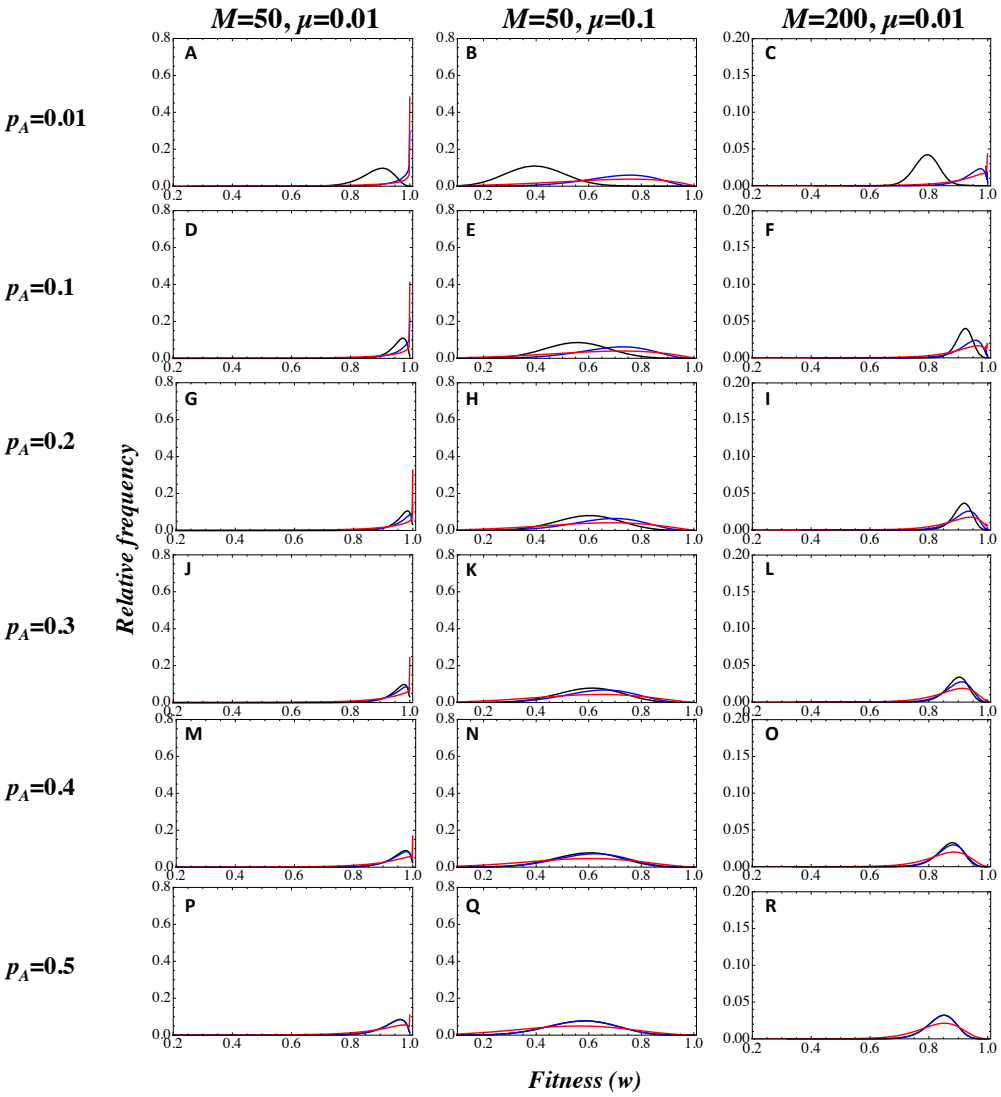

Figure 3.8: Fitness distributions for each genotype (aa:black; Aa:red; AA:blue) for different values of M and μ indicated at the top off each column. The population was held at a fixed value of p_A (indicated at the beginning of each row) and the mitochondria were allowed to evolve. The figures illustrate that depending on the values of M and μ, different values of p_A affect the distribution of mitochondrial fitness resulting from selection and 'leakage'.

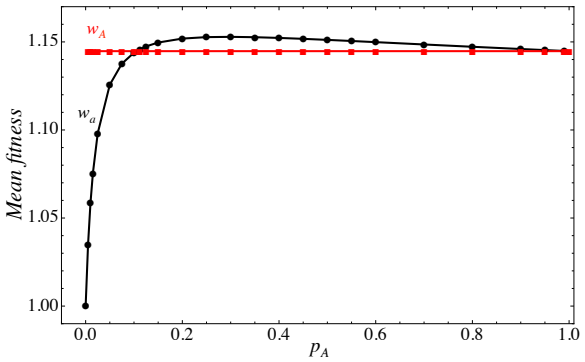

Figure 3.9: Mean fitness of a ($\bar{w}_a$, black line) and A ($\bar{w}_A$, red line) for fixed values of p_A using a concave fitness function and assuming that A x A matings are uniparental. Parameter values: M=50, μ=0.01.

A x A Uniparental

We also considered the case where matings between two A cells are uniparental (like in Hastings (1992)). In this case there is no cost to $\bar{w}_A$ from increasing p_A, as A x A matings are still uniparental, so the average fitness of A is independent of p_A. However, $\bar{w}_a$ increases in a frequency-dependent manner with p_A due to increasing leakage from matings between a and A (Fig. 3.9). This still results in an E_1 equilibrium when $\bar{w}_a = \bar{w}_A$.

Values of p_A above E_1 result in an increase in $\bar{w}_a$. This is because A uniparental matings generate high variance at each generation with more lower fitness individuals. This short term disadvantage is offset by the longer term cleansing of the mitochondrial mutation load, hence $\bar{w}_A > \bar{w}_a$ when p_A is infrequent and leakage is weak. But for higher values of p_A, $\bar{w}_a > \bar{w}_A$ as a enjoys the benefits of leakage while being able to avoid the disadvantage of short-term increased variation and more lower fitness individuals. This explains why E_3 is unstable. At $p_A = 1$, all matings are uniparental and the mitochondrial mutation load is minimised. So the mitotypes of a mutants are equally cleared of mitochondrial mutations. But the a mutant has the additional advantage of a x a matings, that are BPI and have lower variance. Even though these are initially rare, they have higher fitness on average, so the a mutant will invade a population fixed for A.

Note that a biological mechanism that allows A x A matings to have random uniparental inheritance is unlikely to occur without any costs (Randerson and Hurst, 1999). Such costs will decrease the fitness of AA zygotes and therefore the fitness of A cells as p_A increase. This would result in a frequency-dependent decrease in the fitness of A, hindering its spread.

Convex Fitness Curve

We repeated our analysis with the assumption of a convex fitness curve given by,

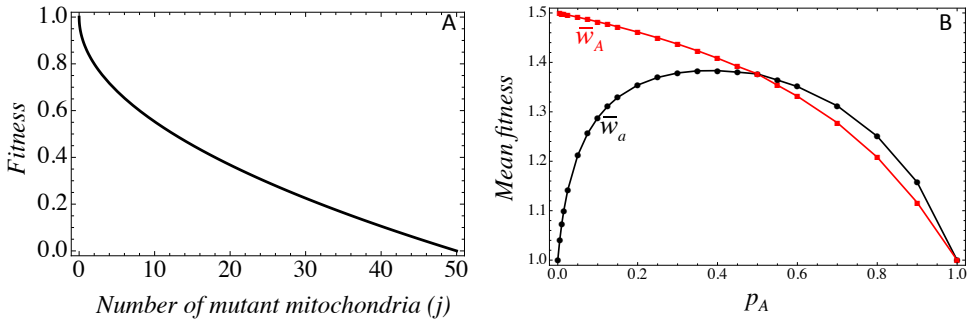

Figure 3.10: (A): Convex fitness curve (B): Mean fitness of a ($\bar{w}_a$, black line) and A ($\bar{w}_A$, red line) for fixed values of p_A using a convex fitness function. Parameter values: $M = 50$, $\mu = 0.01$.

$$w(j) = 1 - \sqrt{\frac{j}{M}},$$

where M is the number of mitochondria in each cell and j is the number of mutants (Fig.3.10A). Here we assume that mitochondrial mutants cause a sharp and increasingly steep fall in oxidative phosphorylation, and hence fitness. This assumption is difficult to justify biologically as it implies that the steepness in fitness decline is higher with fewer mutants and becomes less steep as the number of mutants increases. This is against both intuition and empirical evidence but is included here for completeness (and perhaps there are situations where it might apply).

The assumption of a convex curve causes a much greater benefit when moving from BPI to UPI. For example, the relative advantage at $p_A = 0.1$ ($M = 50$ and $\mu = 0.01$) with a convex curve is ~0.5, (Fig.3.10B), compared to only ~0.15 with a concave curve (Fig.3.2 (c)). This is because there is a short-term advantage to increased variance with a convex curve (Fig.3.10A), and leakage is not fast enough to bring $\bar{w}_a = \bar{w}_A$ before the uniparental inheritance allele (A) suffers from a significant decrease in fitness due to frequent biparental A x A matings. It follows that E_1 merges with E_2 even for lower values of M and μ.

The third equilibrium E_3 with $p_A = 1$ also exists but is unstable. When $p_A \approx 1$, all a matings are uniparental, whereas almost no A matings are. So $\bar{w}_a > \bar{w}_A$ even though there is no cumulative benefit in the a population, because a is associated with higher variance in mitotypes and this confers a short-term fitness advantage. This makes E_3 unstable and drives the population to $p_A = 0.5$. This is an important finding as it shows that even modifiers of the 'kill your own mitochondria' type can spread under some circumstances. Note that leakage take places both with a convex fitness curve as with a concave fitness curve. It results in an increase in $\bar{w}_a$ as p_A increases, reducing the relative fitness difference between UPI and BPI.

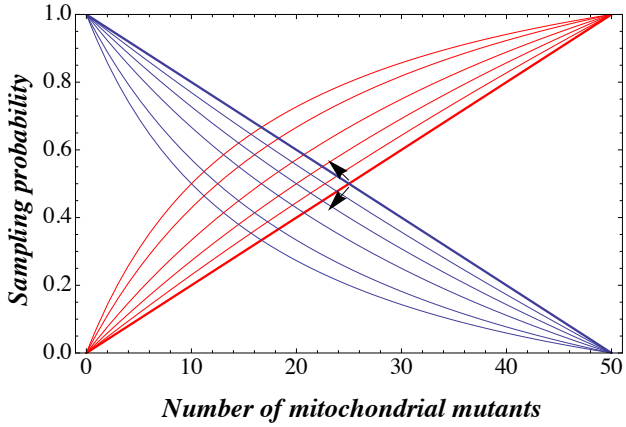

Figure 3.11: Probability of sampling a mutant (red) or wild-type (blue) mitochondrion under the assumption of selfish conflict. Number of mitochondria M=50.The lines are for increasing values of replicative advantage k in the direction of the arrows.

Selfish mitochondrial mutants

For the selfish mutant case, we implement a step after mutation and before selection. At this step mutant mitochondria are given an advantage. So if a cell carries l mutant mitochondria the probability of sampling a mutant mitochondrion should be higher than $\frac{l}{M}$. We defined this probability to be $\frac{l(1+k)}{M+lk}$ where the parameter k determines the mutant advantage. This is equivalent to within cell selection with the fitness of wild type and selfish mutants being equal to 1 and $1+k$ respectively. This is an appropriate function, as the overall advantage of mutant over wild-type mitochondria increases with the ratio of mutant mitochondria, due to the additive effect of the advantage of mutant mitochondria. The sampling probabilities for different values of k can be seen on Fig.3.11.

To derive the equations implementing this step in the life cycle we define Y_r as the number of mutant mitochondria sampled from a cell carrying r mutants. Then, Y_r follows $\mathrm{B}\left(M, \frac{r(1+k)}{M+rk}\right)$ and we have,

$$P(Y_r = l) = \binom{M}{l}\left(\frac{r(1+k)}{M+rk}\right)^{l}\left(1-\frac{r(1+k)}{M+rk}\right)^{M-l}.$$

Letting $\mathbf{X}^{t,\tau_s}$ and $\mathbf{X}^{t,\tau_{s+1}}$ define the population before and after this step takes place we obtain,

$$P(\mathbf{X}^{t,\tau_{s+1}} = (i,j)) = \sum_{r=0}^{r=M} P(\mathbf{X}^{t,\tau_s} = (r,j))P(Y_r = i).$$

The frequency of p_A at equilibrium increased when this step was implemented. This increase was higher for larger values of k (Fig.3.12A).

It is important to note that we only consider a single round of within cell selection when implementing this step. Unicellular eukaryotes may go through several rounds of vegetative growth before they reproduce

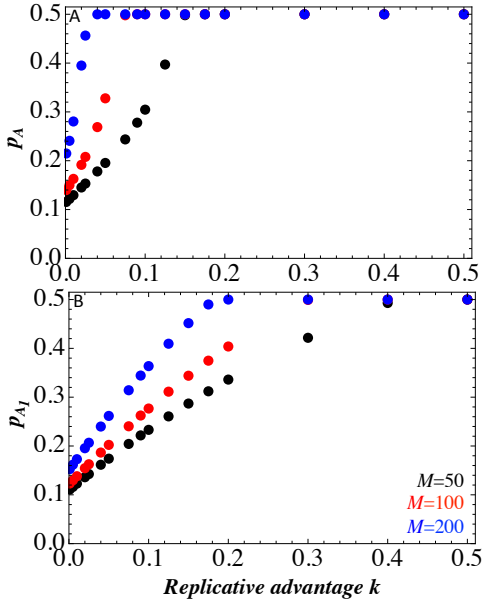

Figure 3.12: Frequencies of A and A_1 at equilibrium for different values for the advantage of mutant mitochondria k. Mutation rate μ=0.01.

sexually, which would require several sampling steps before the sexual step in our model. If such a consideration was implemented, we ought to also implement between-cell-selection during growth, which would presumably diminish the spread of cells that carry many selfish mutations. The trade-off among between-cell-selection during growth (presumably improving fitness) and the multiple rounds of within-cell-selection (giving selfish mutants the chance to spread) needs further examination, however. Additionally, a selfish mutant is unlikely to immediately take over following its appearance in a cell. If this were not true, and given that a selfish mutation can arise with a certain probability in any cell, the benefits of UPI over BPI become less obvious. In fact, a fundamental assumption of previous work (e.g. (Hutson and Law, 1993)) is that UPI is beneficial because it only allows a single (rather than a double) dose of the selfish mutant. Assuming that a spectrum of mutational load is maintained in the population, we anticipate that the main feature our work identified, namely leakage of fit mitochondria from UPI cells to BPI cells, will persist even with several rounds of within cell selection. This may not follow if the vegetative growth is so long that the association between the mode of mitochondrial inheritance and the fitness of the cells is lost, but then the benefits of UPI as opposed to BPI also diminish.

Mitonuclear coadaptation

Mitonuclear coadaptation is somewhat more complex as a nuclear gene interacting with the mitochondria has to be defined. The equations for this case are modified following their definition (Eq. (3.2)), and

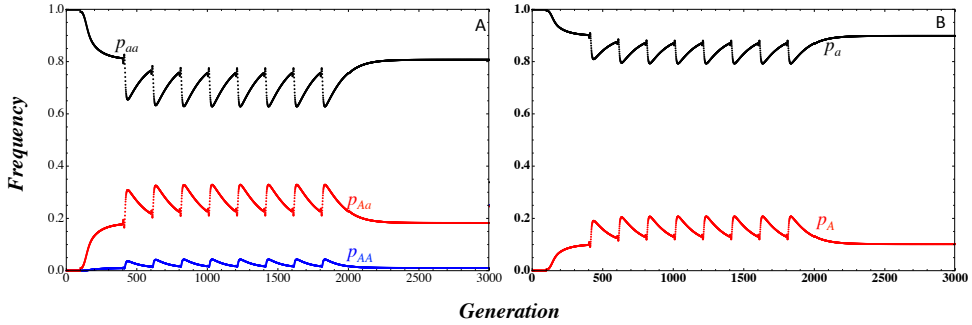

Figure 3.13: Change in (A) genotype (p_{aa}, p_{Aa}, p_{AA}) and (B) gene (p_a, p_A) frequency across time (generation), from introduction of the uniparental inheritance mutation A allele (initial frequency 10^{-2} at generation 100) until stability is reached at equilibrium E_1. Here we assume fluctuating environmental conditions force the dominant mitonuclear state to switch by imposing a cost $q = 0.75$ every $p = 200$ generation for a duration of $d = 20$ generations. Once the fluctuations are removed the population returns to its initial equilibrium. Other parameters: (M, μ, ν)= (50, 0.01, 0.0001).

derivation for the simple mutation model (also refer to Chapter 2)

Fluctuating external factors

In addition, we considered a regularly changing environment. To model this we assumed that the nuclear optimum fluctuates. We imposed a cost (q) at the selection step either on the 00 nuclear state (when at that optimum) or on the 11 nuclear state (when at that optimum). This was imposed on the population periodically (every p generations) for a duration of d generations.

We found that if the cost was high enough (high q) and was imposed on the population for long enough (high d), then the population switched states during the time the cost was imposed. This caused an increase in the frequency of A in the population. During the switch mitochondria inherited uniparentally are more efficient at adapting to a new nuclear background than those inherited biparentally, explaining the increase in the degree of uniparental inheritance. Once the fluctuations were removed however, the population returned to its initial equilibrium (Fig.3.13).

Mating types

In the main text we consider the effect of introducing mating types (A_1) to a population where $p_a = 1$. We saw that this resulted in the spread of A_1 to an equilibrium equivalent to E_1 (Fig.3.7(a)). Once at E_1, we then introduced a second mating type allele p_{a_2} into this polymorphic A_1/a population. However, the a_2 allele did not invade, but simply monotonically decreased in frequency (Fig.3.14). This is a general finding. Namely that once a polymorphic equilibrium is reached (with some degree of uniparental inheritance), further alleles that potentially increase the degree of uniparental inheritance are not favoured.

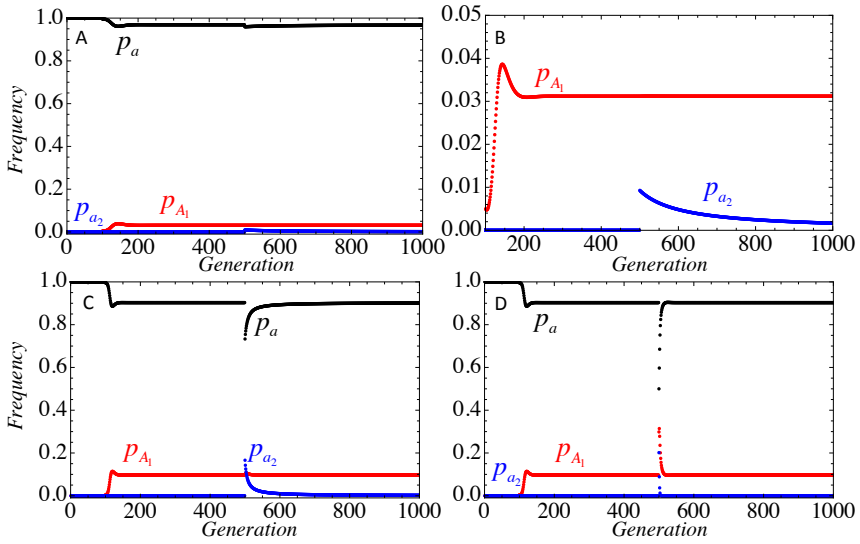

Figure 3.14: Change in genotype frequency (p_{aa}, p_{A_1a}, p_{aa_2}, $p_{A_1a_2}$), from introduction of the uni-parental inheritance allele A_1 at generation 100, and the allele a_2 at generation 500 (initial frequency 10^{-2}). B: Zoomed-in to illustrate the monotonic decrease in p_{a_2}. C: Allele a_2 is introduced at a higher frequency (0.2). D: Allele a_2 is introduced at a higher frequency (0.3) and is only allowed to fuse with A_1 (and not with a). A,B:(M, μ) = (50, 0.01) and C,D:(M, μ) = (100, 0.01).

This was true even when a_2 was introduced at a higher frequency (Fig.3.14C) and when a_2 was allowed to fuse only with A_1 (and so have strict UPI fitness) (Fig. 3.14 D) . This is because a benefits from the presence of A_1 in the population through leakage. When a_2 was subsequently added there was no significant additional fitness benefits from A_1 x a_2 matings that could give $\bar{w}_{a_2} >> \bar{w}_a$. This along with the slight disadvantage a_2 suffers by not being able to fuse with self (very low when the frequency of a_2 is low) result in a monotonic decrease of the frequency of a_2.

Alternatively, we assumed that two mating types a_1 and a_2 pre-exist and then introduced A_1. This causes A_1 to displace a_1 up to a degree equivalent to E_1. For higher M and μ, A_1 displaced a_1 altogether, leading to an equilibrium at which there is complete uniparental inheritance of mitochondria (Fig.3.15).

When recombination was allowed ($R = 0.5$), the frequency of A reached similar levels to those seen without recombination. Recombination allowed both A_1 and A_2 alleles to spread, until both reached E_1-like equilibria with complementary frequencies of the a_1 and a_2 alleles. The frequency of the uniparental inheritance alleles rises with higher values of M and μ. However, in this case complete UPI is not possible because each mating type is equally associated with uniparental (A) and biparental (a) mitochondrial inheritance alleles, so at equilibrium $p_{a_1} = p_{a_2} = p_{A_1} = p_{A_2} = 0.25$ (Fig.3.16). So when mating types pre-exist, strict UPI requires complete linkage between the mating type and mitochondrial inheritance loci.

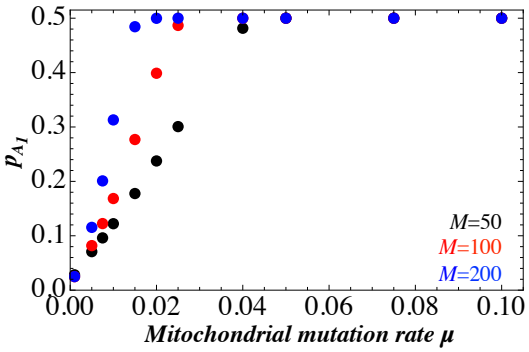

Figure 3.15: Equilibrium frequency of A_1 when introduced into a population with $p_{a_1} = p_{a_2} = 0.5$ for different M and μ

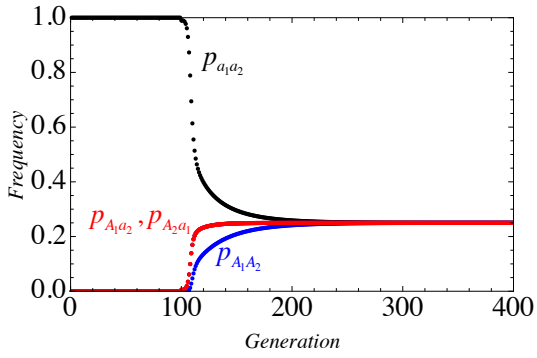

Figure 3.16: Change in genotype frequency ($p_{a_1a_2}$, $p_{A_1a_2}$, $p_{a_1A_2}$, $p_{A_1A_2}$), from introduction of the uniparental inheritance mutation A_1 allele (initial frequency 10^{-2} at generation 100) until stability is reached. Full recombination between the mating type and mitochondrial inheritance loci is assumed. Parameters used: $(M, \mu) = (50, 0.01)$.

Part II

Cell-cell signalling and the evolution of mating types

This part of my thesis examines the role of cell-cell signalling during sex and its potential part in the evolution of mating types. Unicellular organisms typically generate and detect chemical signals to facilitate between and within cell communication. Naturally, one expects that gametes too employ such signals to coordinate mating. The idea that an asymmetry in gamete communication improves partner recognition and attraction was first proposed by Hoekstra (1982), but received little attention thereafter. In Chapter 4 I perform a comprehensive literature review across protist to elucidate the function of mating types, searching especially for mating-type-specific functions throughout mating. In Chapter 5, I develop a biophysical model of gamete interactions in partner attraction (sexual chemotaxis). The aim of my model is to assess whether secreting and detecting the same chemoattractant can impair the ability of gametes to find one another, and if and when two mating types with mating-type-specific roles in chemotactic signalling can improve mating.

Chapter 4

Literature review of mating-type-specific functions

While our understanding of mating type evolution remains limited, a thorough literature review of gamete interactions across isogamous species, and mating-type-specific functions, is currently lacking. In this chapter I review mating type interactions across protists to fill this gap. This serves to aid our understanding of the role, and thereby evolution, of mating types.

It was previously proposed that an asymmetry in the communication between gametes leads to more effective gamete attraction and recognition, and that this underscores the evolution of mating types. But this hypothesis received little attention. I discuss the relevance of my review to the proposition that the adaptive benefit of mating types lie in their capacity to regulate gamete interactions. This survey points at possible future directions for work in this field.

4.1 Introduction

Most prominent hypotheses for the origin of mating types leave a theoretical gap in our understanding of mating type evolution (see Introduction and Chapter 3). Surprisingly, very little theoretical work has considered the contribution of physical and chemical constraints that gametes experience during sex in the evolution of isogamous mating types (Billiard et al., 2011). Although different organisms employ diverse means to achieve sexual reproduction, there is a common underlying process where gametes (or nuclei) from two partners must recognise one another and fuse. Naturally, one expects that some form of communication between fusing gametes (or nuclei) coordinates sexual reproduction.

Communication between unicellular life forms is necessary for processes other than sex (e.g. quorum sensing in bacteria (Waters and Bassler, 2005), aggregate formation in social amoebas (Bonner and Savage, 1947). These interactions are typically manifested through the generation and response to chemical signals (although complex cells within multicellular organisms also employ electrical or mechanical signals (Magee et al., 1998; Reinhart-King et al., 2008). Chemical communication occurs through the release of chemical molecules by the cell, and activation of a cellular response when the pertinent molecules bind to cognate receptors on the cell surface. The presence of such complementary signals enabling sexual partners to interact with one another and so synchronize sex is a natural expectation.

In the early 1980's Hoekstra proposed that mating types evolved as a consequence of selection for an asymmetry in gamete communication (attraction and recognition) (Hoekstra, 1982). This hypothesis proposes that two mating types, one producing a pheromone and one responding to it, improve mating. The principal idea is based on the assumption that gametes employ complementary chemical signals and receptors to recognize and attract one another, and that when gametes secrete and detect the same substance their receptors saturate, leading to less efficient mating. That gametes should use species-specific molecules to coordinate sex is a reasonable expectation. Furthermore, the assumption that secreting and detecting the same signal can lead to receptor saturation and so impede the potential response to an external signal is well-founded as cell-membrane receptors are indeed saturable (e.g. (Klepsch et al., 2011)). Despite the sound premises upon which Hoekstra's work was built, he was himself sceptical of its power to explain the evolution of mating types, perhaps due to the absence of empirical evidence supporting its premises and conclusions (e.g. the lack of evidence that gametes in isogamous species use pheromones) (Hoekstra, 1982; Hoekstra et al., 1984). Hoekstra's hypothesis has received little attention compared to other theories for mating type evolution, and no further theoretical or experimental work pertinent to this model has followed.

Here, in support of Hoekstra's idea, I propose that mating types must typically express complementary but opposite signals and cognate receptors because this asymmetry improves the efficacy of gamete interactions. Asymmetric mating signals and responses further enforce distinct sexual identities and lay the foundation for mating type evolution. This should not only be true during gamete attraction, but throughout the sexual process. I predict that in the absence of an asymmetry in signal transaction, gametes are prone not only to saturation, but more importantly, to self-excitation triggered by the binding of their signal on their own receptors.

In this chapter I carry out a comprehensive literature review on mating type functions across eukaryotes. I particularly review the literature of cell-cell interactions during sex across protists, explicitly looking for mating-type-specific functions in sexual chemotaxis, adhesion and fusion with a focus on isoga-

mous species. I found that notwithstanding a remarkable diversity in modes of sexual reproduction across species, mating types ubiquitously employ mating-type-specific signals and receptors in an asymmetric manner, throughout mating. My survey supports the theoretical expectation that mating types must assume complementary but opposite roles throughout mating, first put forward by Hoekstra (1982).

The primary motivation of this work, was to identify the most basic functions that mating types confer beyond the morphological asymmetry exemplified in oogamous and complex multicellular organisms, justifying the emphasis placed on isogamous protists. I summarize my global findings in Table 4.1 in the main text, and provide more detailed summary Tables in the Appendix at the end of the chapter (Tables 4.2 - 4.4). At the end of this chapter, I discuss the relevance of my review to the evolution of mating types and Hoekstra's assertion that the adaptive benefits of mating types lie in their capacity for asymmetric signalling in partner attraction and recognition. Finally, I develop Hoektra's ideas further by pointing at possible constraints, other than receptor saturation, that could deem mating-type-specific signalling during gamete communications beneficial.

4.2 Method

Koonin (2010) defined five eukaryotic supergroups in his phylogenetic study: Plantae, Chromalveolates, Unikonts, Excavates and Rhizaria. Although eukaryotic phylogeny remains a contentious area, I will follow this structure for my review. Plantae consist of land plants, green algae, red algae and glaucophytes. Land plants are mainly multicellular with complex life cycles, and red algae are mainly oogamous and both groups were excluded from the review. Sexual reproduction is unknown in the glaucophytes (although it probably exists). In contrast, a lot is known about sexual reproduction and mating type behaviour in green algae that I summarize in what follows. In Chromalveolates, I found information mainly on ciliates, diatoms and brown algae. Within the unikonts, we have substantial information on fungi, and some information on amoebozoa. Finally, very little is known about sexual reproduction in the Excavates and Rhizaria and, to my knowledge, no information on mating behaviour in these supergroups is available.

I searched for evidence for sexual reproduction within eukaryotic subgroups. I did that by entering 'subgroup name' and 'sex' or 'sexual reproduction' in Web of Knowledge. I subsequently searched for mating type functions during sexual chemotaxis by entering 'subgroup name' and 'pheromones' and 'chemotaxis'. I initially used reviews on specific groups or species. Those were available for algae and fungi. I then used references within the reviews, references within those references and more recent papers that cited either the reviews or papers of interest. Some reviews on gamete chemotaxis also included information on other mating-type-specific functions. To extend that information, I searched for mating-type-specific inter-

actions during adhesion and fusion in Web of Knowledge by typing ‘subgroup name’ and ‘pheromones’ and ‘mating type’ or ‘subgroup name’ and ‘adhesion’ and ‘mating type’ or ‘subgroup name’ and ‘fusion’ and ‘mating type’. When I recovered partial information for specific species, I followed with more species specific searches.

One seeming difficulty of this review is that mating types by definition are expected to have different recognition mechanisms (how else would they differ from one another?). The aim of this review, however, is to elucidate whether mating-type-specific functions go beyond a mere recognition system (such as that seen in self incompatibility types in multicellular hermaphrodites), thereby suggesting a functional significance tight to mating-type-specific interactions.

4.3 Literature review

4.3.1 Plantae (Green Algae)

There are many studies of sexual reproduction in green algae because of the ease with which the sexual process can be induced in these organisms in the laboratory. The sexual mechanisms observed in unicellular green algae can form a basis for understanding more complex processes present in multicellular algae and plants.

Chlamydomonas

Chlamydomonas are arguably the best-studied species of unicellular algae (Quarmby, 1994; Goodenough et al., 2007). These biflagellate green algae have two mating types mat+ and mat – determined at the haploid level. The vegetative stage of *Chlamydomonas’* life cycle is haploid and mating type is determined by a mat biallelic, non-recombinant locus. Under stressful conditions, minus cells activate the minus and suppress the plus genes. This enables them to enter the mating process as minus cells. The reverse process occurs in the plus cells.

Sexual chemotaxis was reported for different species of *Chlamydomonas*. The mat + gametes (larger or female gametes) secrete compounds that attract the mat – gametes (smaller or male gametes) in the anisogamous species *Chlamydomonas allensworthi*, *Chlamydomonas suboogama* and *Chlamydomonas pseudogigantea* (Maier, 1993; Starr et al., 1995). Anisiogamy in *Chlamydomonas* is not necessarily equivalent to oogamy. For example, in *C.suboogama* the two mating types differ size and their degree of motility but only marginally. Reports of chemoattraction in isogamous species of *Chlamydomonas* are conflicting. One possible reason secretion levels of pheromones tend to be very low resulting in hard to detect concentrations.

Often, the involvement of the putative substances is inferred through the observation of directed migration of one gamete type towards the other. Experiments by Tsubo (1957, 1961) indicate that the minus gamete in the isogamous species *Chlamydomonas rotunda* and *Chlamydomonas eugametos* are attracted to the plus gamete via the release of diffusible pheromones

The two mating types of *Chlamydomonas* are responsible for the production of mating-type-specific agglutinin glycoproteins responsible for the adhesion of complementary gametes. When gametes of the opposite mating type come in contact, they immediately adhere to one another by interlinking their mating-type-specific agglutinins, expressed on the entire length of the flagellar surface (Demets et al., 1990; Goodenough et al., 1995; Pan and Snell, 2000). Flagellar adhesion is a critical step in *Chlamydomonas*' mating triggering a cascade of signalling events that lead to zygote formation. The mating-type-specificity of the agglutinins ensures that the mating process is only initiated in the presence of a partner and that adhesion is robust, while avoiding adhesion of two flagella from the same gamete type.

The agglutinins of the two mating types do not only act as adhesion molecules in *Chlamydomonas*, but are pivotal for cell-cell synchronization throughout the mating process. Gametes prompt responses in the opposite mating type by modulating the expression level of their mating-type-specific agglutinins. Variation in the agglutinins' expression level and geometrical distribution on the flagella during conjugation, drive the two cells to simultaneously develop mating structures and fuse (Goodenough and Weiss, 1975; Demets et al., 1990). Demets et al. (1990) showed that isolated strains of one mating type of *C. eugametos* executed the necessary steps of sexual conjugation in the presence of the opposite mating type agglutinin even in the absence of a partner. Their work suggests that the agglutinin molecules of the opposite mating type are necessary and sufficient to simulate key developmental stages of conjugation, and the authors proposed that synchronous behaviour is only possible through mating-type-specific complementarity.

Desmidiales

Unlike *Chlamydomonas*, mating type is determined at the diploid level in Desmidiales. Most *Closterium* species have two mating types, mat + and mat –. Sexual reproduction in *Closterium* takes place in five steps; differentiation into gametes, sexual pair formation, papillae formation, protoplast release and protoplast fusion to form a zygote where this process.

Mating-type-specific pheromones that induce sexual cell division in the opposite mating type were identified in *Closterium ehrenbergii* (Hogetsu and Yokoyama, 1979; Fukumoto et al., 1997). Experiments in *C. ehrenbergii* indicate the use of complementary chemoattractants from the two mating types stimulating pair formation (Coesel and de Jong, 1986; Fukumoto et al., 1998). Similarly, directed migration via the use

of mating-type-specific pheromones was also demonstrated for *Closterium acerosum* (Maier, 1993).

Mating-type-specific molecules were also shown to participate in protoplast release in *Closterium peracerosum -strigosum-littorale* (Tsuchikane and Fukumoto, 2003). Kato et al. (1981) and Kato et al. (1984) detected substances released by the two mating types that simulate protoplast release from the opposite mating type. Sekimoto and collaborators later confirmed this and identified the putative molecules and their cognate receptors in the two mating types (Sekimoto et al., 1990; Sekimoto and Fujii, 1992; Sekimoto et al., 1993). Mating-type-specific substances and their interactions were also indispensable for cell aggregation, papilla and zygospore formation in *C. ehrenbergii* (Hogetsu and Yokoyama, 1979).

Additionally, mating-type-specific pheromones were inferred in the sexual reproduction of the isogamous desmid *Cosmarium botrytis*. Mating types exemplified asymmetric behaviour in chemotaxis leading to pair formation and during the conjugation process (Brandham, 1967). The identification of the putative substances was not possible at that stage, and no follow up work was carried out to my knowledge.

4.3.2 Chromalveolates

Ciliates

Mating systems in ciliates have been studied for over 70 years (Sonneborn, 1937). Mating type is determined at the diploid level and partners conjugate and then undergo meiosis followed by micronuclei formation and exchange. Ciliates can have multiple mating types and employ mating-type-specific pairs of pheromones and receptors to achieve mating. The molecules that determine mating type are generally related to these pheromones and their cognate receptors. Pheromones and other mating-type-specific molecules in ciliates can be secreted or cell-bound depending on the species (Phadke and Zufall, 2009).

Euplotes use pheromones to attract mating partners and to induce pair formation. Mating type in *Euplotes octocarinatus* is determined through four codominant alleles, possible combinations of which give rise to ten mating types. Each mating type produces a single mating-type-specific pheromone and only develops mating competence when it binds to a non-self pheromone (Heckmann and Kuhlmann, 1986). Mating-type-specific compounds also act as chemoattractants with cells being attracted to all pheromones except the one they secrete themselves (Kuhlmann et al., 1997). Similarly, different mating types in *Euplotes raikovi* grow vegetatively through mitotic division when binding to their own pheromone secreted continuously in the extracellular environment. Only when they bind to a non-self pheromone secreted by another mating type do they arrest growth and develop mating competence (Vallesi et al., 2005). Employment of pheromones to induce gametogenesis and as chemoattarctants in a similar manner was also reported in *Euplotes woodruffi* (Kosaka, 1991) and *Ephelota gemmipara* (Sonneborn, 1978). This mechanism imposes

self-incompatibility at the partner recognition level.

Mating type pheromones in *E. octocarinatus* also cause mating-type-specific responses between complementary gametes and are responsible for pair formation and conjugation (Kuhlmann et al., 1997). The same is true in the ciliates *Euplotes raikovi* (Vallesi et al., 2005), *Euplotes woodruffi* (Kosaka, 1991), *Ephelota gemmipara* (Kuhlmann et al., 1997), *Blepharisma japonicum* (Sugiura et al., 2010) and *Dileptus margaritifer* (Afon'Kin, 1991).

Cell adhesion is mediated through cilia adhesion in *Euplotes*, via mating-type-nonspecific adhesins (Plumper et al., 1995). Even though the agglutinins used for cilia adhesion are not specific to the mating type, mating-type-specific pheromone signals and receptors are used to coordinate adhesion and fusion. For example, the ciliate *Dileptus margaritifer* forms mating pairs due to the expression of mating type nonspecific cell surface molecules (Afon'Kin, 1991). However, the two partners coordinate the expression of their adhesion proteins by secreting and responding to pheromones in a mating-type-specific manner. Experiments with a single mating type strain of *D. margaritifer* found that conjugation is highly unstable between gametes of the same mating type, and striking rates of disintegration followed initial pair formation with nearly no pairs outstanding at the end of the experiment (Afon'Kin, 1991). Afon'Kin (1991) argues that pairs of different mating type continue to stimulate each other using mating-type-specific pheromones resulting in perpetual adhesine production until fusion is completed. Kuhlmann and Heckmann (1991) reported similar results for *E. octocarinatus* where pairs of the same mating type were able to form in laboratory experiments but were unstable and generally separated before entering meiosis.

In contrast to *Euplotes*, most species of *Paramecium* do not secrete their pheromones. They instead retain these molecules on their cell surface to be readily used when they come in contact with potential partners. Interestingly, *Paramecium* is an exception among ciliates in that sexual cells produce mating-type-specific agglutinins and conjugation is initiated with mass cell agglutination following mixture of compatible types (Afon'Kin, 1991). Mating-type-specific substances were reported in the isogamous *Paramecium bursaria* having four mating types. The putative pheromones were responsible for pair formation, conjugation, adhesion and fusion (Cohen and Siegel, 1963). No chemotaxis was observed in this species and partner attraction is not believed to occur amongst Paramecia.

Finally, the ciliate *Tetrahymena thermopkila* also normally uses mating-type-specific pheromones that lead to pair formation, conjugation and adhesion (Kitamura et al., 1986).

Diatoms

Diatoms are unicellular green algae with a unique sexual reproductive system which is nonetheless highly uniform amongst different species. Sex can only occur between gametes that are below a species-specific sexual size threshold (Geitler, 1935).

The involvement of pheromones in partner attraction and cell-cell interactions has been inferred in the diatom *S. robusta*. This pennate diatom has two mating types mat + and mat–. At suitable gamete densities, pairs or clusters of mat + gametes migrate towards attracting mat – gametes (Gillard et al., 2013). Gillard et al. (2013) inferred that these diatoms use multiple reciprocal mating-type-specific signals to ensure the presence of a partner before they engage in mating, and concluded that the use of pheromone signals enhances mate-finding success.

The use of pheromones and their receptors to guide gametes through pair formation and copulation is thought to occur in most diatoms (Chepurnov et al., 2004). The identification of such substances in diatoms is particularly challenging because of the specificity of their life cycles that are challenging to induce in laboratory environments. However mating-type-specific behaviours such as the migration of one mating type towards the other and the necessity of surface recognition between gametes before fusion indicate that mating-type-specific signals such as those seen in other algae also occur in diatoms (Chepurnov et al., 2004).

Golden Algae

Golden algae are a large group of mostly unicellular algae living chiefly in fresh water. Sexual reproduction is thought to be rare in the golden algae but has been documented and studied in some species.

Mating-type-specific behaviour was inferred in *Dinobryon cylinricum* that has two mating types and exhibits slight anisogamy with the female mating type being slightly larger than the male. In this species, mating type + (the female) secretes a substance that induces gamete formation in the – mating type (the male)(Sandgren, 1981). In the isogamous golden algae *Synura petersenii* mixing compatible mating types and contact between them was necessary for efficient sexual reproduction but the substances involved in gamete formation and fusion were not identified (Sandgren and Flanagin, 1986).

Brown Algae

Brown algae are mainly multicellular marine algae. Sexual reproduction in brown algae can be isogamous, anisogamous or oogamous. Gametes are often released in the water where fusion takes place. The life cycle and sexual reproduction vary greatly from one species to the other. Here I will summarize some of the most

general mating-type-specific features known in isogamous species of Brown Algae.

Pheromones in brown algae have been studied extensively and are very well characterized in terms of their function and molecular composition. As a rule, female gametes release pheromones that attract male gametes. This is also true in the isogamous species *Scytosiphon lomentaria*, *Colpomenia bullosa* and *Ectocarpus siliculosus*, where the female equivalent mating type releases the pheromone and the male mating type directs its movement accordingly (Kajiwara et al., 1991; Schmid, 1993). In the families of Laminariaceae, Alariaceae, and Lessoniaceae the same pheromones used for chemoattraction also trigger the release of – mating type gametes from the male organs. (Sekimoto, 2005).

Although the study of mating type and sex specific roles in brown algae has focused on pheromones inducing chemoattraction, the two partners also use specific signals to coordinate cell-cell interactions following chemoattraction. One example is the isogamous *Ectocarpus siliculosus* where mating-type-specific glycoproteins and cognate receptors are responsible for gamete recognition and adhesion (Schmid, 1993; Schmid et al., 1994). While the two mating types of *E. siliculosus* are morphologically the same, their mating behaviour is different. The + (or female) gametes only swim for a short period of time after which they ingest their flagella and secrete 'a bouquet of hydrocarbons as male-attracting pheromones'. They also have species- and mating-type-specific recognition sites on their surfaces. The – (or males) gametes on the other hand, swim for prolonged periods and have pheromone receptor sites for signal processing necessary for their chemotactic response. They recognise the + gametes through their anterior flagellum, attach to it and proceed to fusion (Schmid et al., 1994).

4.3.3 Unikonta

Fungi

The use of pheromones is prominent in the fungal kingdom. Gametes in fungi use pheromones to attract and grow towards partners and to regulate cell-cell recognition and cell fusion. Fungi are usually immobile and gametes direct themselves towards partners through polarisation and growth according to a pheromone gradient, a process known as chemotropism. Here I place more emphasis on yeasts that are unicellular exemplifying simpler life cycles. I then give a less detailed report of mating-type-specific functions in fillamentous ascomycetes and bascidiomycetes.

Yeasts

Saccharomyces cerevisiae, a thoroughly studied organism, has two mating types, **a** and α . Mating type is determined at the haploid level and a single genetic locus, mat, which codes for mating-type-specific

proteins. The pertinent genes are differentially expressed at this locus in the two mating types. During mating, gametes undergo polarization and project tips along pheromonal gradients generated by gametes of the opposite mating type. Mating type **a** gametes secrete **a**-factor pheromones and respond to α-factor gradients via membrane receptors. The reverse is true for mating type α cells. Amongst the genes differentially expressed in a and α cells are those responsible for mating-type-specific pheromone production and their cognate surface receptors (Merlini et al., 2013).

The mating system of fission yeast *Schizosaccharomyces pombe*, a distant relative of *S. cerevisiae*, has many similarities (Merlini et al., 2013). Two mating types, P (plus) and M (minus) exist and mating-type-specific pheromones and surface receptors are also used to induce growth towards gametes of the opposite mating type. This complementary mechanism of mating-type-specific pheromones and their cognate receptors is common to many other yeasts, with the regulating genes being referred to as α- and a-class precursor genes due to structural similarities with the corresponding genes in *S. cerevisiae* (Merlini et al., 2013). Some examples of other yeasts exhibiting such pheromone-receptors pairs and chemotropic behaviour are the Kluyveromyces yeast *Kluyveromyces lactis* (Coria et al., 2006), the oleaginous yeast *Rhodosporidium toruloides* (Abe et al., 1975) and the encapsulated yeast *Cryptococcus neoformans* (Shen et al., 2002).

Dorer et al. (1995) performed experiments with *S. cerevisiae* wild type **a** cells and found that when membrane receptors saturate, **a** cells undergo polarization and growth along a random direction. This slowed the mating process down as much as 15 fold, suggesting that receptor saturation severely impedes directed growth towards the opposite mating type.

Importantly, mating-type-specific pheromones in yeasts also coordinate cell-cell interactions at the adhesion, conjugation and fusion levels. Gametes in *S. cerevisiae* synthesize their mating-type-specific agglutinins that interact with one another during cell adhesion and conjugation (Cappellaro et al., 1991). By comparing wild-type cells to mutants lacking their mating-type-specific agglutinins, Lipke et al. (1989) showed that although the relevant agglutinin proteins are not essential for conjugation, they increase conjugation efficiency in liquid culture by a factor of 10^5 . Similarly, mating types P and M in *S. pombe* are mutually simulated by mating-type-specific P- and M- factors respectively, generating a series of events that lead to mating. Chemotropic growth causes the two cells of the opposite mating type to come in contact at which point they adhere to one another via mating-type-specific agglutinins (Sharifmoghadam et al., 2006; Xue-Franzen et al., 2006). Recent experimental work showed that varying levels of M-factor expression induce appropriate responses in P cells at different stages of mating and holds a key role in synchronising cell adhesion and conjugation (Seike et al., 2013). Similar processes were found in other, less extensively studied, yeasts such as *Kluyveromyces lactis* (Coria et al., 2006), *Hansenula wingei* (Crandall and Brock,

1968) and *Saccharomyces kluyveri* (Mccullough and Herskowitz, 1979; Lasky and Ballou, 1988).

Filamentous ascomycetes

Filamentous ascomycetes have more complex life cycles than yeasts (Coppin et al., 1997). During their vegetative phase, filamentous ascomycetes grow as mycelia that are networks of partially separated hypha containing haploid nuclei. The sexual cycle begins with the differentiation of mycelia into female and male structures containing female and male gametes respectively. Male and female structures of different mating types then fuse and a donor cell from the male structure enters the primary ascogonium (female gamete) cell. Nuclear fusion does not take place at this stage however. Instead, the nuclei proliferate and then migrate in pairs of opposite mating type to specialised cell structures where nuclear fusion takes place. Notably, filamentous ascomycetes have both mating types and sexes.

The main role of mating types in filamentous ascomycetes is to control mechanisms that regulate partner recognition, fertilization and nuclear fusion. For many filamentous ascomycetes mating-type-specific genes were shown to be both necessary and sufficient for mating specificity (Shiu and Glass, 2000). Like the majority of ascomycetes, *Neurospora crassa* has two mating types, mat A and mat a. The mating process involves polarized growth of the male hyphae towards female cells of the opposite mating type. This is mediated by expressing mating-type-specific pheromones and receptors that are indispensible to the sexual development of *N. crassa* (Kim and Borkovich, 2006). Attraction of male cells towards female structures of the opposite mating type was found in many other filamentous ascomycetes (Bistis, 1996, 1998; Coppin and Debuchy, 2000; Shiu and Glass, 2000). Note that growth of male structures towards female structures is mating-type-specific and male hyphae cannot grow towards and fuse with the female cells of the same mating type.

Mutations in mating type genes affect nuclear identity and fusion in the filamentous ascomycetes suggesting that nuclear recognition is also a mating-type-specific trait. When nuclei of the opposite mating type approach one another they release signals that simulate nuclear migration and fusion with the success of this process relying on the proper association between the two nuclei (Thompson-Coffe and Zickler, 1994; Coppin and Debuchy, 2000; Xiang and Glass, 2004).

Basidiomycetes

Basidiomycetes are much more complex and diverse in their appearance and life cycle than ascomycetes. Mating type identity is determined at specific loci having different alleles and fusion normally occurs between individuals of different mating types that are morphologically indentical. Basidiomycetes are notable

for having multiple mating types ranging from two up to several thousand. This has arisen through gene duplication and recombination (Raudaskoski and Kothe, 2010). As in ascomycetes, mating types in basidiomycetes control the recognition between compatible partners and the process that follows, giving rise to progeny. The mechanisms that achieve this are diverse (Coppin et al., 1997; Casselton, 2002). Here I focus on the most general traits of basidiomycetes and the role that mating-type-specific genes hold in nuclear recognition and fusion.

Sexual cell fusion and nuclear fusion in basidiomycetes can be separated by prolonged periods. Basidiomycetes spend most of their life cycle as dikaryons that are filamentous mycelia formed after sexual cell fusion holding the nuclei from compatible mating partners. The dikaryon divides mitotically during the asexual phase with the nuclei from compatible partners remaining closely associated in each mitotic division until the life cycle reaches the nuclear fusion phase. Mating-type-specific proteins hold a key role in synchronised nuclear division and preservation of compatible nuclei in close association, and eventually control nuclear fusion that occurs in specialised fruiting structures (Casselton, 2002).

Heterobasidiomycetes use pheromone signals much like ascomycetes to mediate mating partner choice, with cognate pheromone and receptors initiating the mating process when haploid cells or organs of the opposite mating type come in contact. In the heterobasidiomycetes yeast *Rhodosporidium toruloides* mating is initiated through conjugation tubes directed towards the mating partner in an asymmetric, mating-type-specific manner (Abe et al., 1975). *R. toruloides* has two mating types and a diffusible pheromone secreted form one of the mating types induces tube formation in the other (Kamiya et al., 1978). In *Ustilago maydis*, cells secrete mating pheromones that attract compatible partners (Banuett, 1995). Following pheromone binding to its cognate receptors mating structures are formed leading to fusion and the formation of the dikaryon. Remarkably, this is true despite *U. maydis* having some 50 mating types. In fact, mating type protein-protein interactions in basidiomycetes are highly specific presumably as a result of the evolution of multiple mating types. This indicates that highly robust mechanisms were selected to reinforce coordination during mating.

In homobasidiomycetes (mushrooms) fusion between mycelia can occur independently of mating type. In these fungi partner recognition shifts to the nuclear level. Mating-type-specific pheromones are activated following fusion and are used to form compatible nuclei pairs in the dikaryon and maintain the dikaryophase (Raudaskoski and Kothe, 2010). A notable example is that of *Schizophyllum commune* which has more than 20000 mating types. Molecular analyses found more than 75 different pheromones and several receptors, and each unique receptor-pheromone combination gives rise to a different mating type (Fowler and Vaillancourt, 2007). The pheromones and receptors *S. commune* are used for post cell fusion events such as

nuclear recognition and fusion (Wendland et al., 1995; Kothe, 1999). Remarkably, each mating type in *S. commune* amasses genes for several pheromones, having at least one pheromone to activate the receptors of other mating types but never its own receptor. In addition, a high degree of specificity is required for nuclear communication and the full completion of sexual development (Fowler and Vaillancourt, 2007). Although the mating system of *S. commune* largely restricts sibling matings, these are still possible 25% of the time, suggesting that inbreeding avoidance cannot be the main function of these complex mating interactions. The situation is similar in other mushroom species that were studies such as *Coprinus cinereus* (Olesnicky et al., 1999). These have several thousands of mating types resulting from gene duplications with compatibility between mating types determined by their pheromone and receptor specificities (Casselton, 2002).

There are many variations in basidiomecetes mating (Bolker and Kahmann, 1993; Coppin et al., 1997; Casselton, 2002; Raudaskoski and Kothe, 2010). Apropos to this report is the persistence of underlying mechanisms that are mating-type-specific and ensure successful partner recognition and fusion (at the cell or nuclear level accordingly). The diversity of mating strategies in basidiomycetes renders this generality all the most remarkable and relevant to the determination of mating type identity.

Amoebozoa

The existence of sexual reproduction is not certain in some species of amoebozoa and it is best studied in the slime molds (although see (Lahr et al., 2011)). Slime molds have complex life cycles going through fruiting body formation phases as well as unicellular phases. They are classified as protists, they have isogametes and their sexual phase is mainly unicellular.

Cellular slime molds

In the cellular slime molds, the unicellular phase of the life cycle is initiated following spore release from the fruiting body. The spores then germinate and release haploid amoeboid cells that grow vegetatively while food supplies are abundant. Under stressful conditions the unicellular amoiboids will either aggregate chemotactically to form a fruiting body or will fuse to form a diploid zygote giant cell known as a macrocyst. O'Day and Lewis (1975) and later MacHac and Bonner (1975) showed that macrocyst formation is regulated via pheromones where gametes secrete mating-type-specific pheromones mediating a response in cells of different mating type in *Dictyostelium discoideum*. A similar interaction was found in other species of *Dictocyclium* such as *Dictyostelium purpureum* (Lewis and O'Day, 1976) and *Dictyostelium giganteum* (Lewis and O'Day, 1979) with mating-type-specific pheromones inducing macrocyst formation between cells of opposite mating types.

Table 4.1: This table indicates the presence of mating-type-specific functions during sex initation, sexual chemotaxis, gamete adhesion and gamete conjugation and fusion, across the Phyla explored in this review. The relevant species-specific information and correspnding references can be found on Tables 4.2-4.4.

Phylum	Species	Sex initiation	Chemotaxis	Adhesion	Fusion/Conjugation
Green Algae	*Chlamydomonas*	Yes	Yes	Yes	Yes
	Closterium	Yes	Yes	Unknown	Yes
	Other	Unknown	Yes	Unknown	Unknown
Ciliates	*Euplotes*	Yes	Yes	No	Yes
	Paramecia	Unknown	No	Yes	Yes
	Other	Yes	Yes	Unknown	Yes
Diatoms	*	Unknown	Yes	Unknown	Unknown
Brown Algae	*	Unknown	Yes	Yes	Yes
Yeasts	*	Yes	Yes	Yes	Yes
Filamentous Ascomycetes	*	No	Yes	No	Yes
Basidiomycetes	*	Yes	Yes	No	Yes
Slime Molds	*Dictyostelium*	Yes	Yes	Unknown	Yes

* The information uncovered in Green Algae was mainly on *Chlamydomonas* and *Closterium*; in Ciliates on *Euplotes* and *Paramecia*. In Slime Molds the only species family I found relevant information on was *Dictyostelium*. By including the family name I emphasize the bias towards these species. When no species family is provided, no information related to any particular species more than any other was found. See Tables 4.2-4.4 for further information.

O'Day and Lewis (1981) describe a series of experiments using multiple combinations of different mating types of *D. discoideum*. These confirmed the presence of at least two interacting pheromones that are mating-type-specific, and necessary for mycrocyst development and completion of the sexual phase. More recent work also identified mating-type-specific genes the distruption of which resulted in cell fusion suppression in *D. discoideum* (Muramoto et al., 2003).

4.3.4 Homothallic mating

Notwithstanding the diversity in life cycle and mating responses amongst species, the current survey shows that parallels exist across taxa with mating types resolving the same fundamental problem: how to recognise a potential partner, and synchronize the mating process (Tables 4.2-4.4). The prediction that mating partners express complementary but opposite signals, finds support across eukaryotic protists. Nonetheless, this perspective is not without challenges.

I discussed the role of mating types in species where fusion normally occurs between two gametes of different mating type. These are defined as being heterothallic. In heterothallic species, rare fusions between gametes expressing the same mating type are possible under laboratory conditions. Moreover, certain species do not appear to have distinct mating types and fusion takes place between what seems to be two morphologically, behaviourally *and* genetically identical cells. The fusion of two gametes of the same mating type is termed homothallism. The relationship between heterothallism and homothallism was the focus of various studies. Many of these question whether homothallic cells posses heterothallic traits. In

fact, modern homothallism seems to be derived from heterothallism and pure homothallism *per se* is now rare. In this section I discuss known homothallic occurrences in protists, and relate these findings to the hypothesis that polarised mating functions improve the sexual process.

Homothallism in Plantae

The *Closterium* species *C. psl* exhibits both heterothallic and homothallic mating. In homothallic strains, sexual fusion occurs mainly between two sister gametangial cells derived from a single vegetative cell. Recent experiments with homothallic and heterothallic strains of *C. psl* indicate that the presence of mating type + cells in the homothallic culture alters the mating dynamics and led Tsuchikane et al to conclude that at least one of the sister gametangial cells of the homothallic strain exemplify heterothallic mat – traits (Tsuchikane et al., 2012). Interestingly, in this particular study homothallic cells moved significantly less actively than heterothalic ones. Presumably the ability to generate robust and detectable chemotactic signals and thus migrate longer distances to find a partner is a characteristic lacking in homothallic strains of *C. psl*. Also, in all experiments with homothallic strains of *C. pls*, sexual cell division and pair formation was highly dependent on cell density with very low or high densities rendering mating unlikely (Tsuchikane et al., 2012). Earlier studies by the same group, led them to the conclusion that sexual cell division in homothallic strains of *C. psl* is a segregative process that produces two complementary mating types, equivalent to those in heterothallic strains (Tsuchikane et al., 2010).

The idea that homothallic algae also have mating types is not new and was first put forward by Hartmann. In 1955, Hartmann provided an extensive review of experiments that support this idea (Hartmann, 1955). The conclusions of these experiments are based on the premise that homothallic species do not necessarily produce equal numbers of the two mating types. Following sexual pairing, some gametes were left unmated and could not mate amongst each other but would fuse with gametes from a different culture (Hartmann, 1955). Experiments with homothallic strains of *Chlamydomonas monoica* indicated that gametes derived from the same clone function as mat + or mat – during mating supporting this view (VanWinkle-Swift and Hahn, 1986).

Homothallism in Chromaveolates

Although homothallism is possible under laboratory environments in some ciliate species, it is unstable. In experiments with heterothallic and homothallic strains of *D. margaritifer* homothallic pairs were highly unstable compared to their heterothallic counterparts exemplifying striking rates of disintegration after pair formation, with nearly no pairs outstanding at the end of the experiment (Afon'Kin, 1991). The author

argued that homothallic pairs could initially form because of the mating type nonspecificity of adhesion molecules in most ciliates (see Table 4.3). Heterothallic pairs continue to stimulate each other using mating-type-specific pheromones resulting in perpetual adhesine production until conjugation is concluded, explaining why homothallic pairs could not complete conjugation (Afon'Kin, 1991). Only when the levels of pheromone that induces adhesion is high in the medium, due to the presence of heterothallic pairs in the culture, does homothallic pair formation become possible. The formation of homothallic pairs was limited, not possible or highly unstable in experiments using other ciliate species (Akada, 1985a,b; Dini and Luporini, 1985; Heckmann and Kuhlmann, 1986; Valbonesi et al., 1987)

Homothallism in the Unikonta

Homothallism has been studied and characterised better in fungi than any other taxa, and several different mechanisms of homothallism were identified. These are typically divided into primary and secondary homothallism.

Haploid cells can switch mating type and so two sister cells from the same clone can mate. This is one form of secondary homothallism and has been described in the two model yeasts *S.cervisiae* (Herskowitz and Oshima, 1981; Klar et al., 1982) and *S. pombe* (Dalgaard and Klar, 1999; Vengrova and Dalgaard, 2004), in the fillamentous ascomycetes *Cryphonectria parasitica* (Marra and Milgroom, 2001; Marra et al., 2004), *Ceratocystis coerulescens* (Harrington and McNew, 1997) and *Glomerella cingulata* (Cisar and TeBeest, 1999) and the basidiomycete *Agrocybe aegerita* (Labarere and Noel, 1992). The other form of secondary homothallism, also known as pseudohomothallism, is the maintenance of two nuclei from compatible mating types into the same spore. Following germination the spore gives rise to two distinct and intercompatible mating types. This has also been well documented (Raju, 1992). Secondary homothallism preserves fusions between cells of opposite mating type and so fusions remain asymmetric. This is compatible with the idea that mating-type-specific gamete communication is central to sexual reproduction.

More frequently, homothallic fungi carry genes for both mating types within a single genome - this is primary homothallism (Beatty et al., 1994; Glass et al., 1990; Lin and Heitman, 2007). The function of mating type genes in homothallic strains is not clear, although they are indispensable to the mating process (Poggeler, 2000; Lee et al., 2003; Mayrhofer et al., 2006). For example, in homothallic strains of *Sordaria macrospora*, such genes encode two cognate pheromone-receptor pairs similar to those of *S. cerevisiae* and were necessary for sexual development (Mayrhofer et al., 2006). This suggests that mating type genes in homothallic species may maintain their mating-type-specific roles during mating, and function as different mating types relying on differential gene transcriptions (Lin and Heitman, 2007). In agreement with this,

Coppin et al. (1997) proposed that homothallic mating may depend on the differential expression of genes related to opposite mating types in heterothallic species. If true, this underscores the primacy of asymmetric gamete functions during mating. However, most work on homothallic fungi focused on determining mating-type-specific molecular structures and less is known about the function of these substances, leaving many questions unanswered.

On rare occasions, homothallic species express genes of only one mating type (Glass et al., 1988, 1990; Glass and Smith, 1994). Here too, the exact function of these genes and a direct comparison in mating efficiency with related heterothallic species are lacking. Apropos this, the filamentous ascomycete *Sordaria brevicollis* has two mating types and is usually heterothallic. Nonetheless mat-1 strains have the ability to reproduce sexually which is reminiscent of homothallic fungi with genes from only one mat strain. However, this type of mating in *S. breviollis* is inefficient compared crossings of different mating types in the same species (Robertson et al., 1998). This relative inefficiency suggests that the presence of both mating types improves mating. Most experimental studies on homothallic mating have focused on the possibility of same mating type fusions without a direct comparison between homo- and heterothallic strains in terms of mating robustness and efficiency. Experiments similar to those in *S. borellis* comparing homothallic and related heterothallic species would improve our understanding of homothallic mating and its evolutionary significance.

Theoretical expectations such as the compelling adaptive features of self-mating, the maintenance of heterothallic functions within homothallic strains and phylogenetic evidence, suggest that heterothalism is a precursor to modern homothallism in fungi (Nauta and Hoekstra, 1992; Coppin et al., 1997; Yun et al., 1999; Ni et al., 2011). Because modern hetero- and homothallism are highly derived, the ancestral state early in the evolution of sexual reproduction remains hard to predict.

4.4 Discussion

Despite a remarkable diversity in life cycle and mode of sexual reproduction amongst protists, asymmetric interactions between mating partners are ubiquitous, even in isogamous species with morphologically identical gametes. The findings of this review suggest that mating types hold a crucial functional role in isogamous sex. I further propose that the universal asymmetry in mating-type communication demonstrated here underscores the evolution of mating types. Likewise, Hoekstra (1982) suggested that an asymmetry in gamete communication improves recognition and attraction between gametes, and could explain the evolution of isogamous mating types.

I suggest that the requirement for asymmetric signalling during gamete interactions has its roots in

the physiological constraints encompassed in pairwise cell-cell interactions, crucial for sex. This is for two main reasons. Firstly, without an asymmetry in gamete communication all gametes will generate and respond to the same signal (typically chemical signal). Cell-membrane receptors, however, are saturable, and simultaneous signalling and sensing can compromise the detection of and sharp response to external signals. Hoekstra (1982) has also noted that receptor saturation can impede the ability of cells to attract and recognize one another (this argument formed the premise of his model), and points out that factors such as the geometrical distribution of molecules and receptors on the cell's surface, the binding affinity and diffusivity of the putative molecules will determine the susceptibility to self-binding. Consequently, experimental work focusing on the nature of the chemical signals employed by gametes, the physiology of their receptors and their susceptibility to saturation can guide future theoretical work, for example by defining pertinent costs for an evolutionary model.

A further issue coupled with generating and responding to the same signal is that of self-excitation or self-stimulation. As seen in this review, gametes frequently use chemical signals to indicate their readiness to engage in sex, to attract partners and to coordinate cell adhesion and fusion. In the absence of an asymmetry in these signalling communications the challenge of distinguishing between a self-produced and an external signal emerges. The false perception of a self-induced signal as that of a partner could impede the ability of gametes to respond to external signals and move towards partners in sexual chemotaxis, urge a gamete to initiate cellular fusion at the absence of a potential partner, or impair coordination between gametes during conjugation and fusion. The presence of different mating types, with the capacity of mating-type-specific signalling and sensing overcomes this difficulty as well as the hazards of receptor saturation.

Instances reported in this survey that highlight the significance of mating-type-specific signals and asymmetric pairwise gamete interactions, are plentiful: same-mating-type cultures of the green algae *C. pslc* move significantly slower than gametes in heterothallic cultures (Tsuchikane and Fukumoto, 2003), *Euplotes* grow vegetatively and arrest their life cycle preparing for sex only when they detect non-self pheromones (Vallesi et al., 2005), *Chlamydomonas* coordinate the amount of mating-type-specific agglutinins expresses along their flagella in a pairwise manner thereby coordinating conjugation and fusion (Demets et al., 1990), and yeast gametes vary the expression levels of their mating-type-specific factors to induce appropriate responses in gametes of the opposite mating type during fusion (Seike et al., 2013). The assertion that receptor saturation and self-stimulation can impair mating, however, needs to be rigorously examined through theoretical modeling and further experiments. The impact of simultaneous generation and response to the same chemoattractant during sexual chemotaxis is the focus of the following chapter.

Although mating types with the capacity for mating-type-specific signalling roles can avoid the haz-

ards of receptor saturation and self-stimulation that arise in a single chemical signal-receptor system, the question of whether other mechanisms could achieve this, emerges. A possible solution is that of periodic signalling and sensing between the two partners. This, however, could restrict the complexity of gamete interactions or the speed and efficiency of gamete communication. Alternatively, gametes could 'calculate' their partner's signal by assessing the difference between their own signal and that of the environment. Such an endeavour seems unlikely for simple unicellular organisms, however, as factors such as diffusion and fluid flows are expected to generate considerable complexities. Further work should establish whether protists engage in similar forms of communication for processes other than sex, and if they do, whether they employ alternative mechanisms that could be used to improve mating, without mating types.

A frequently overlooked, but important, distinction is that between mating types and self-incompatibility (SI) types. Typically, SI types are found in multicellular, oogamous and hermaphrodite species, and the SI alleles prohibit fusion between two gametes from the same individual. Systems of SI have been the centre of many experimental and theoretical studies that have located adaptive benefits in inbreeding avoidance (Charlesworth and Charlesworth, 1979; Uyenoyama, 1988a; Iwano and Takayama, 2012). In contrast to SI types, mating types are a feature of isogamous unicellular organisms, where inbreeding depression is not a major consideration (Billiard et al., 2011; Perrin, 2012). In addition, this review indicates that mating types are responsible for the coordination of several key steps leading to sex, and so their functional role goes well beyond that of mere self-incompatibility.

The existence of homothallic species across eukaryotic taxa poses the main challenge to the ideas proposed here, as it seems to suggest that same mating type fusions (lacking any asymmetry in communication) are functional. However, the majority of homothallic species maintain their mating-type-specific functions, activated differentially. Therefore, gamete communication remains essentially asymmetric. Furthermore, I do not claim that homothallism is implausible - its adaptive benefits are certainly compelling. Nonetheless, I expect that purely homothallic fusions, lacking any form of asymmetry in gamete communication will exhibit impaired mating efficiency. This is supported by some experimental work (Robertson et al., 1998), although further experiments that rigorously quantify the efficiency of homothallic matings while directly contrasting it to the efficiency of heterothallic interactions in similar, or the same, species would be valuable.

This review indicates that coordination between gametes throughout mating is achieved via mating-type-specific signals and responses (Tables 4.2-4.4). Although the impact of this mating-type-specificity on successful sexual reproduction was previously recognized (Coppin et al., 1997), its significance in the evolution of mating types has received little attention. Here I propose that the evolution of mating types

has its roots in the adaptive benefits of asymmetric signalling and sensing during mating, which can avoid hazards emerging from a single chemical-receptor system of communication, such as receptor saturation and self-stimulation. A thorough understanding of the physiochemical constraints cells experience during sex is imperative for placing the right weight on an asymmetry in gamete interactions, and evaluate its role in the evolution of mating types. Future experimental and theoretical work will focus on this task.

4.5 Appendix

The information provided in Section 4.3 is summarised in full on Tables 4.2 - 4.4 below.

Table 4.2: Mating-type-specific functions in Plantae (Green Algae)

Species	Phylum	Isogamy*/ anisogamy	Mating type No	Sex initiation	Chemotaxis	Adhesion	Fusion	References
Chalmydomonas allensworthii	Green algae	SA	2	Unknown	Yes	Unknown	Unknown	Starr et al. (1995)
Chalmydomonas suboogama	Green algae	A	2	Unknown	Yes	Unknown	Unknown	Maier (1993)
Chalmydomonas pseudogigantea	Green algae	A	2	Unknown	Yes	Unknown	Unknown	Harris (2008) Vandover (1972)
Chalmydomonas rotunda	Green algae	I	2	Unknown	Yes	Unknown	Yes	Tsubo (1957, 1961)
Chalmydomonas reidendii	Green algae	I	2	Yes	None Detected	Yes	Yes	Goodenough et al. (2007)
Chalmydomonas eugametos	Green algae	I	2	Unknown	Conflicting reports	Yes	Yes	Tomson et al. (1986) Demets et al. (1990)
Closterium ehrenbergii	Green algae	I	2	Yes	Yes	Unknown	Unknown	Fukumoto et al. (1997, 1998) Coesel and de Jong (1986)
Closterium acerosum	Green algae	I	2	Unknown	Yes	Unknown	Unknown	Maier (1993)
Closterium pslc	Green algae	I	2	Yes	Unknown	Unknown	Yes	Kato and Sasaki (1985) Sekimoto et al. (1993) Tsuchikane and Fukumoto (2003)
Hyalotheca dissiliens	Green algae	I	2	Unknown	Yes	Unknown	Unknown	Maier (1993)

Continued on next page

Table 4.2 – continued from previous page

Species	Phylum	Isogamy*/ anisogamy	Mating type No	Sex initiation	Chemotaxis	Adhesion	Fusion	References
Cosmarium botrytis	Green algae	I	2	Unknown	Yes	Unknown	Unknown	Brandham (1967)

* I=Isogamy; A= Anisogamy ; SA=Indications for Slight Anisogamy

Table 4.3: Mating-type-specific functions in the Chromalveolates

Species	Phylum	Isogamy*/ anisogamy	Mating type No	Sex initiation	Chemotaxis	Adhesion	Fusion	References
Euplotes octocarinatus	Ciliates	I	10	Yes	Yes	No	Yes**	Kuhlmann et al. (1997) Heckmann and Kuhlmann (1986)
Euplotes raikovi	Ciliates	I	8	Yes	Yes	No	Yes**	Vallesi et al. (2005)
Euplotes woodruffi	Ciliates	I	13	Unknown	Yes	No	Yes**	Kosaka (1991)
Ephelota gemmipara	Ciliates	I	?	Unknown	Yes	No	Yes**	Kuhlmann et al. (1997)
Blepharisma japonicum	Ciliates	I	2	Yes	Yes	Unknown	Yes**	Sugiura et al. (2005, 2010) Honda and Miyake (1975) Miyake and Rivola (1989)
Dileptus margaritifer	Ciliates	I	3	Unknown	Yes	Unknown	Yes**	Afon'Kin (1991)
Paramecium bursaria	Ciliates	I	4	Unknown	No	Yes	Yes**	Cohen and Siegel (1963) Jennings (1939)
Tetrahymena thermopkila	Ciliates	I	7	Unknown	Unknown	No	Yes**	Kitamura et al. (1986)
Seminavis robusta	Diatoms	I	2	Unknown	Yes	Unknown	Unknown	Gillard et al. (2013)
Dinobryon cylinricum	Chrysophyceae	SA	2	Unknown	Yes	Unknown	Unknown	Sandgren (1981)

Continued on next page

Table 4.3 – continued from previous page

Species	Phylum	Isogamy*/ anisogamy	Mating type No	Sex initiation	Chemotaxis	Adhesion	Fusion	References
Scytosiphon lomentaria	Brown algae	I	2	Unknown	Yes	Unknown	Unknown	Kajiwara et al. (1991)
Colpomenia bullosa	Brown algae	I	2	Unknown	Yes	Unknown	Unknown	Kajiwara et al. (1991)
Ectocarpus siliculosus	Brown algae	I	2	Unknown	Yes	Yes	Yes	Muller (1978) Schmid (1993)

* I=Isogamy; A= Anisogamy; SA=Indication for Slight Anisogamy

** Ciliate gametes do not fuse, they conjugate and exchange nuclei. Yes/No here indicates the presence of mating-type-specificity during conjugation

Table 4.4: Mating-type-specific functions in the Unikonta

Species	Phylum	Isogamy*/ anisogamy	Mating type No	Sex initiation	Chemotaxis	Adhesion	Fusion	References
Saccharomyces cerevisiae	Yeast	I	2	Yes	Yes	Yes	Yes	Merlini et al. (2013) Banuett (1998) Lipke et al. (1989) Cappellaro et al. (1991)
Schizosaccharomyces pombe	Yeast	I	2	Yes	Yes	Yes	Yes	Nielsen et al. (1996) Seike et al. (2013) Kjærulff et al. (1997)
Cryptococcus neoformans	Yeast	I	2	Yes	Yes	Unknown	Yes	Shen et al. (2002)
Rhodosporidium toruloides	Yeast	I	2	Unknown	Yes	Unknown	Yes	Abe et al. (1975)
Kluyveromyces lactis	Yeast	I	2	Unknown	Yes	Unknown	Yes	Coria et al. (2006)
Hansenula wingei	Yeast	I	2	Unknown	Unknown	Yes	Yes	Crandall and Brock (1968)
Saccharomyces kluyveri	Yeast	I	2	Yes	Unknown	Yes	Unknown	Mccullough and Herskowitz (1979) Lasky and Ballou (1988)
Neurospora crassa	*Filamentous ascomycete*	I	2	No	Yes	No	Yes	Fleissner et al. (2008) Kim and Borkovich (2006)
Ustilago maydis	Basidiomycetes	I	50	No	Yes	No	Yes	Banuett (1995) Hsueh and Heitman (2008) Bakkeren et al. (2008)

Continued on next page

Table 4.4 – continued from previous page

Species	Phylum	Isogamy*/ anisogamy	Mating type No	Sex initiation	Chemotaxis	Adhesion	Fusion	References
Schizophyllum commune	Basidiomycetes	I	>20,000	Yes	No	No	Yes**	Kothe (1999)
Coprinus cinereus	Basidiomycetes	I	2	No	No	No	Yes**	Kronstad and Staben (1997)
Dictyostelium discoideum	Slime mold	I	3	Yes	Yes	Unknown	Yes	O'Day and Lewis (1975) MacHac and Bonner (1975) Muramoto et al. (2003)
Dictyostelium giganteum	Slime mold	I	4	Yes	Unknown	Unknown	Unknown	Lewis and O'Day (1979)

* I=Isogamy; A= Anisogamy

** Nuclear, not cellular, fusion

Chapter 5

Cell-cell signaling in sexual chemotaxis and the evolution of mating types [1]

Many sexual organisms indicate their readiness to mate and attract partners using chemical signals. A major challenge to the evolution of such sexual signals is, however, that they can also trigger the secreting cell's own receptors (self-stimulation), thereby impairing its ability to distinguish between its own, and external signals. In this chapter, I argue that mating types pose a solution to the apparent inevitability of self-stimulation during sexual chemotaxis by ensuring that mating partners generate or respond to a chemical signal, but not both. To rigorously assess this hypothesis I develop a biophysical model for signaller-detector dynamics based on chemical diffusion, chemotaxis and individual cell movement. This model serves to quantify the movement impediment that simultaneous secretion and detection of the same chemoattractant (self-secretion) can cause by considering three different movement frameworks: (i) random movement, (ii) chemotactic movement with simultaneous secretion and detection and (iii) chemotactic movement with mutually exclusive secretion and detection. As anticipated, my model indicates that self-secretion impairs the ability of gametes to detect external signals and move towards potential partners. I present a quantitative analysis that specifies the physiological conditions under which self-incompatible mating types with mating-type-specific pheromones and receptors improve partner finding during sex. Furthermore, I discuss the relevance of my findings to the evolution of mating types while pointing at limitations of the current model, and extensions that will strengthen the proposition that mating-type-specific functions improve mating efficacy and that therein lie their adaptive benefits.

[1] Part of thus study was contacted in collaboration with Yoh Iwasa during a three month visit to Kyushu University at Fukuoka, supported by a CoMPLEX study abroad fellowship.

5.1 Introduction

The previous chapter demonstrates that signalling is omnipresent throughout sex with gamete pair formation, adhesion and fusion being orchestrated via complementary chemical signals secreted and sensed by the two mating partners. Furthermore, the literature on cell-cell interactions during sex indicates that communication between gametes occurs in a mating-type-specific manner so that compatible mating types secrete and detect complementary but opposite signals. In the previous chapter I proposed that mating types, carrying the capacity for mating-type-specific sensing and signalling, are an adaptation that improves the efficacy of gamete interactions that are crucial for sex. Although this applies throughout mating, this chapter focuses on the significance of asymmetric signalling during gamete chemotaxis.

Autocrine signalling is the ability of cells to generate chemical signals that bind to their own receptors generating a self-response, and is fundamental to a range of cellular processes both within multicellular organisms (Junger, 2011), and in unicellular organisms (Mann and Firtel, 1991). Nonetheless, secreting and detecting the same cue ('self-secretion') can be problematic when a sharp response to an external signal is desirable. This is particularly so in chemotaxis where cells respond to chemical signals by adjusting their movement. The issue of self-secretion in a single chemoattractant-receptor system features in a number of biological systems (Hofer et al., 1995; Goryachev et al., 2012). Furthermore, theoretical studies of the impact of self-secretion on the movement of a particle in isolation, indicate that the particle's motion will be restricted compared to another, freely diffusing nonchemotactic particle (Tsori and De Gennes, 2004; Sengupta et al., 2009; Taktikos et al., 2011). Self-secretion may be disruptive in a single chemoattractant-receptor system for three reasons. Firstly, the local concentration of the chemoattractant due to self-signalling will always be higher than that of a remote signaller, triggering the cell's own receptors ('self-stimulation'), and impairing the perception of and clear respond to an external signal (Fig. 5.1(*a*)). Secondly, self-secretion during movement causes a tail of high concentration behind the moving cell due to diffusion and accumulation of chemical molecules. A self-induced asymmetry alters the net concentration difference around a moving cell, thereby reducing its ability to respond appropriately to external signals, or worse prompting the cell to reverse its direction of movement (Fig. 5.1(*b*); also see (Taktikos et al., 2011)). Finally, self-secretion can induce receptor saturation. The molecules secreted by the cell occupy its own receptors, preventing a chemotactic response (Fig. 5.1(*c*)).

Numerous studies have focused on mate searching and on the adaptive benefits of asymmetric male and female roles (Hammerstein and Parker, 1987; Puurtinen and Kaitala, 2002; Kokko and Wong, 2007). The significance of asymmetric chemotaxis whereby the size or motility of one sex or gamete type differs from the other, and the impact of this on the efficiency of pair formation have also been studied (Hoekstra et al.,

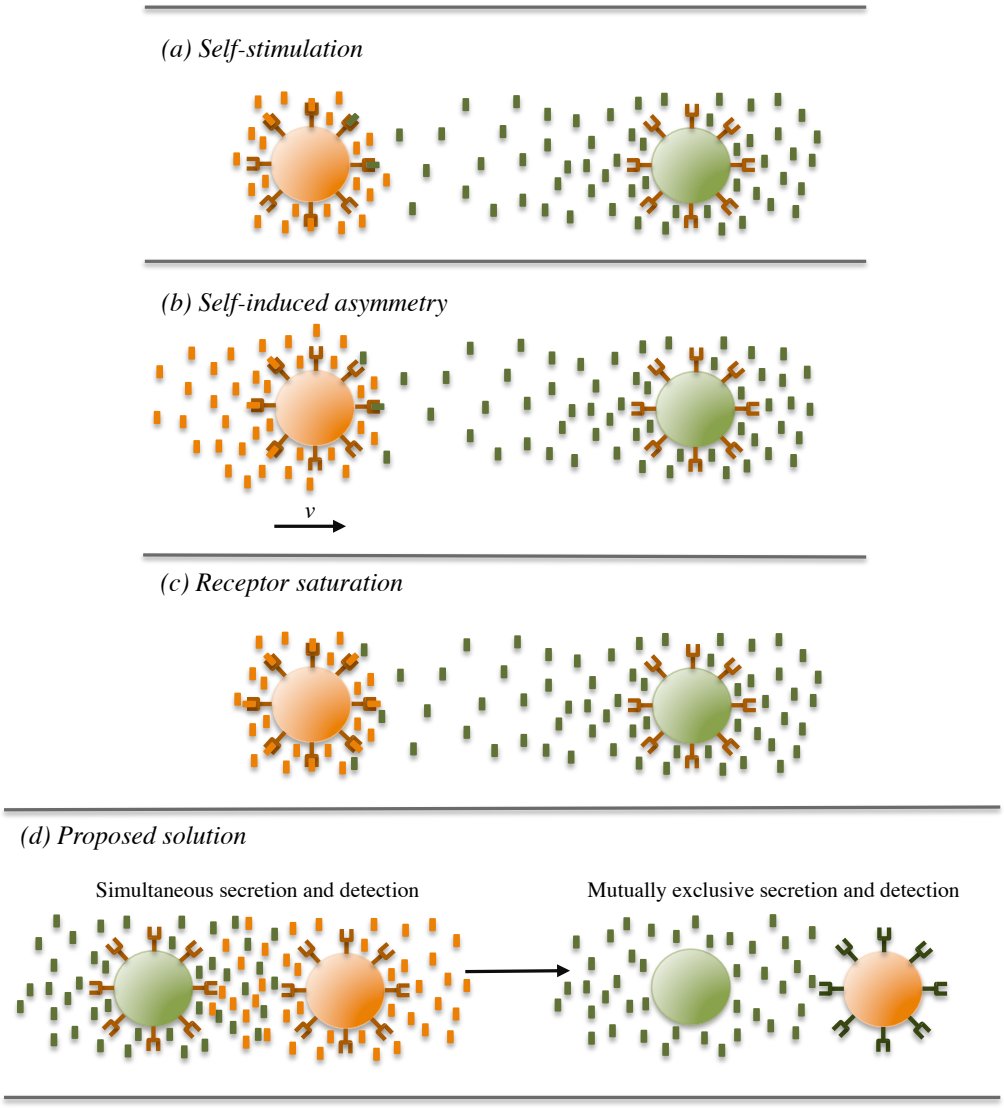

Figure 5.1: The consequences of self-secretion and proposed solution. Cells secrete a diffusible chemical (orange and green rectangles) that binds on membrane receptors thereby inducing a chemotactic signal. Secreting a chemical that can bind on the cell's own receptors is problematic for three reasons. (*a*): Self-secretion can cause self-stimulation. The receptors of the cell on the left are sensitive both to its own signal and to the signal of the remote cell. The remote signal (green rectangles) reaches the cell at a low concentration (due to diffusion). The cell's own secreted molecules bind to its receptors stochastically resulting in a net occupancy that is not indicative of the external signal. (*b*): Self-secretion during movement causes a tail of high concentration behind the moving cell due to diffusion and accumulation of chemical molecules during movement. The left cell is moving to the right. This induces a high chemical concentration behind it. It follows that receptor occupancy is higher at the left than the right side, prompting the cell to change direction and move backwards. (*c*): Self-secretion can cause receptor saturation. The molecules secreted by the cell bind to its own receptors. By the time the external signal diffuses to the detecting cell, all receptors are occupied and a chemotactic response is not possible. (*d*): A transition from a chemotactic system where all potential partners secrete and detect the chemical signal to one where gametes either secrete or detect the signal, but not both, can pose a solution.

1984; Cox and Sethian, 1985; Dusenbery, 2000, 2006). The majority of past work focused, however, on the contribution of chemotaxis to the evolution of anisogamy in a previously isogamous population, reaching the common conclusion that large and immobile gametes secreting sexual pheromones, and small, highly motile searcher gametes improve mate finding (Hoekstra et al., 1984; Cox and Sethian, 1985; Dusenbery, 2000, 2006). While these works highlight the significance of sexual chemotaxis and successfully draw an evolutionary link from isogamy to anisogamy and oogamy, they presuppose the existence of two mating types, without which the conditions for anisogamy to evolve become much more stringent. The role of an asymmetry in chemical signalling in isogamous mating types, which is often presupposed (Dusenbery, 2000), has barely been studied. An exception is work by Hoekstra, proposing that mating types evolved as a consequence of selection for an asymmetry in gamete attraction and recognition (Hoekstra, 1982). His model, much like this work, proposes that two mating types, one producing a pheromone and one responding to it would lead to more efficient mating. This was shown by initially assuming that the population is pansexual (any gamete can mate with any other gamete) and that mating is mediated through a bipolar system of pheromone-receptor interactions so that all gametes secrete and detect a single pheromone. The fate of genes that restrict cells to either secreting or detecting the pertinent pheromone, but not both, was then studied in the context of population genetics by assuming a relative cost for gametes that secrete *and* detect the same pheromone. Hoekstra's model provides an interesting insight into the spread of mating-type-specific genes, yet it does not explicitly consider the impact of self-secretion on gamete chemotaxis. The lack of an appropriate theoretical framework for studying the supposed impediment of self-secretion has led to the assumption a moderate and fixed cost for simultaneous secretion and detection. Furthermore, it limits the extent of physical and quantitative conclusions.

In this chapter I develop a biophysical model to assess the hypothesis that asymmetric signalling improves mating, focusing on partner attraction. My model is based on chemical signalling and diffusion, and individual cell movement and is examined subject to three different assumptions: first, cells execute random walks and pair formation relies on random encounters between them, second, cells generate and respond to the same chemoattractant and move accordingly and third, half of the cells follow random walks while generating a chemical signal and the remaining cells sense the chemical concentration and move chemotactically without themselves secreting the relevant chemoattractant. In this latter case cells that confer the same chemotactic roles cannot mate. This framework allows us to firstly assess if and when chemotaxis improve mate finding, and secondly whether two self-incompatible mating types with specialised chemotactic roles can confer an improvement in mating efficiency. Parameter values are varied within ranges that reflect the physicality of small protists and their environments. Although this study does not currently incorporate

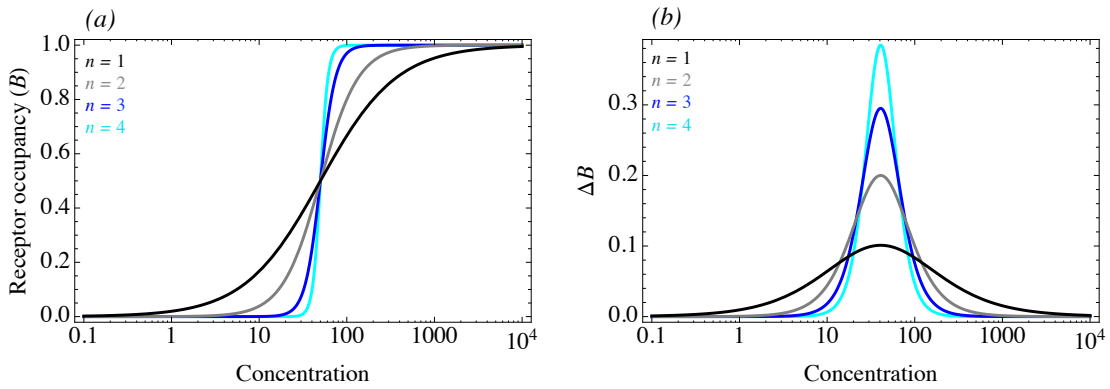

Figure 5.2: Receptor occupancy and difference in receptor occupancy against concentration on a log scale (assuming exponential chemical concentration gradients).(*a*): Log scale plot of the Hill functions describing receptor occupancy against concentration for different values of the Hill coefficient n. (*b*): Log scale plot of the difference in receptor occupancy across a cell's membrane against the average local concentration assuming an exponential concentration field (i.e. fixed $\Delta C/C$) for different values of the Hill coefficient n. The dissociation constant K_d is set equal to 50.

genetics, my results indicate that self-secretion may impose a severe impediment on sexual chemotaxis. I discuss the relevance of my findings to the evolution of mating types, while pointing at limitations entailed in this work and extensions that can tackle these constraints.

5.2 How mating types can avoid self-stimulation: a model

5.2.1 Chemotaxis in eukaryotes

Recent work suggests that eukaryotic cells sense chemical fields around them in a polarized manner, via saturable membrane receptors (Herzmark et al., 2007). At chemical concentrations much lower than the dissociation constant, chemical binding to the receptors is negligible. As the concentration increases towards the receptor's dissociation constant, receptor occupancy rises sharply and beyond a threshold in concentration, receptors saturate and no further binding can occur.

Receptor occupancy can be quantified using Hill functions so that $B = \dfrac{C^n}{K_d^n + C^n}$ where B is the receptor occupancy, C is the chemical concentration at the cell's surface, K_d is the constant of dissociation and n is the Hill coefficient that determines the interaction between the chemical and the receptor (Fig. 5.2(*a*)). This formulation was first proposed by Hill (1910) and has been subsequently applied broadly to pharmacological modelling (Goutelle et al., 2008), and was used to model the ligand occupancy on eukaryotic cell membrane receptors during chemotaxis in experimental studies (Herzmark et al., 2007). Experiments indicate that the difference in receptor occupancy across a cell's polarised front and rear, $\Delta B = B_{front} - B_{rear}$,

dominates its movement (Herzmark et al., 2007). The quantity ΔB is maximised for concentrations that maximise the gradient of the function $B = \frac{C^n}{K_d^n + C^n}$ (Fig. 5.2). Here, we deal with exponential changes in concentration and receptor saturation as chemical diffusion generates exponential chemical concentration fields.

5.2.2 Cell Sensing and Movement

I construct a two dimensional model of chemical diffusion and individual cell movement. The formulation in two dimensions addresses key features of the problem while keeping computational time low. Assume that cells take up a round space of diameter d and have the ability to secrete a diffusible chemical signal while they move. In the absence of chemotaxis cell movement is random (equivalent to an unbiased random walk). This is modelled on a discretized two dimensional grid, with the x and y increments, Δx and Δy respectively, being equal to λ, and assuming periodic boundary conditions (Fig. 5.3). Cell movement is determined by the following set of equations:

$$\begin{aligned} P(X_j^{t+\mu} = X_j^t + \lambda) &= \epsilon \\ P(X_j^{t+\mu} = X_j^t) &= 1 - 2\epsilon \\ P(X_j^{t+\mu} = X_j^t - \lambda) &= \epsilon \end{aligned} \tag{5.1}$$

where X_j^t is the x coordinate of the j^{th} cell at time t, μ is the time step within the simulation and λ is the length of the increments in the two dimensional grid (Fig. 5.3). The equivalent relations follow for movement in the y direction. We assume independence between movement in the x and y direction. It follows that the cell can move to any of its 8 neighbouring points at each time step in the simulation (Fig. 5.3). The quantity 2ϵ is equal to the probability of moving in the x or y directions at any time step within the simulation. The cell speed, v, is equal to $\sqrt{v_x^2 + v_y^2}$, and the expected speed in the x and y directions satisfy:

$$E(v_i) = 2\lambda\epsilon/\mu; i \in \{x, y\} \tag{5.2}$$

Cells may also sense the chemical concentration around them and move accordingly. We model chemotactic movement by assuming that cells assess the difference in receptor occupancy at their front and rear in the x and y directions, which determines their next step. In that sense, we decompose chemical sensing into the x and y axes and approximate receptor occupancy by the occupancy at $x_0 \pm d/2$ and $y_0 \pm d/2$ where (x_0, y_0) is the centre of the cell (Fig. 5.3). We quantify receptor occupancy assuming that receptors

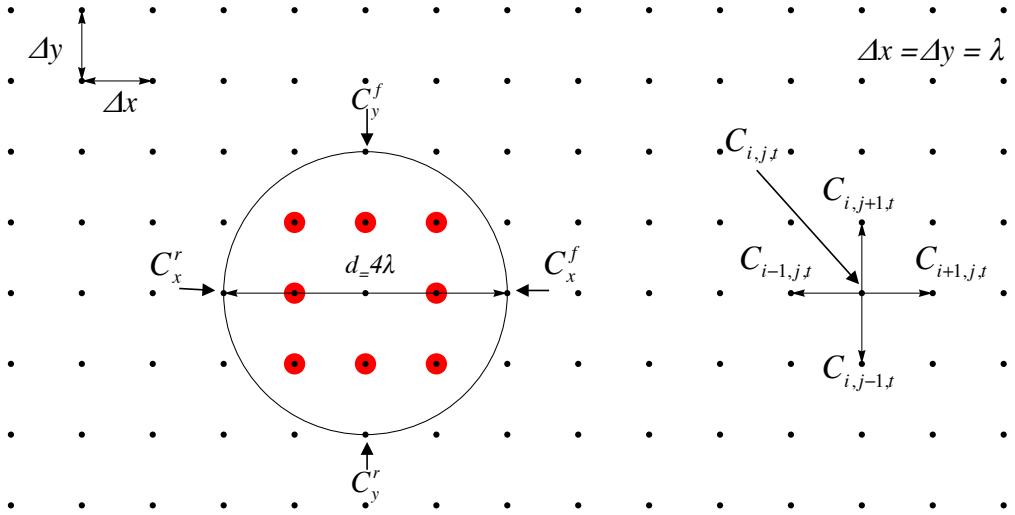

Figure 5.3: Two dimensional grid forming the basis for numerical solutions to our model. Increments in the x and y directions, Δx and Δy respectively, are equal to λ. Cells occupy a circular space of diameter d, where d must be equal to an even factor of λ (here, $d = 4\lambda$). At each time step in the simulation, cells may move a distance equal to λ in the x and/or y directions. The red dots indicate the possible positions of the cell's centre time $t + 1$. The concentration at the front (f) and rear (r) of the cell in the x and y directions, C^r_x, C^f_x, C^r_y, C^f_y are shown across the cell. The chemical concentration at position $(x, y) = (i, j)$ and time t is defined at $C_{i,j,t}$. We assume diffusion occurs in the x and y directions at each time step (RHS diagram); this is implemented using a finite difference approximation (see Appendix).

have a simple binding interaction with the chemical substance setting $n = 1$ in the Hill function above as in Herzmark et al. (2007). We let B^f_i, B^r_i, C^f_i and C^r_i with $i \in \{x, y\}$, be the receptor occupancy and mean concentration at the front and rear of the cell in the x and y directions. It follows that the receptor occupancy at the front of the cell satisfies, $B^f_i = \dfrac{C^f_i}{K_d + C^f_i}$. Similarly, the fraction of occupied receptors at the rear of the cell is $B^r_i = \dfrac{C^r_i}{K_d + C^r_i}$. Cells respond to ΔB_i with $i \in \{x, y\}$, which now satisfies,

$$\Delta B_i = \frac{K_d(C^f_i - C^r_i)}{(K_d + C^f_i)(K_d + C^r_i)} = \frac{K_d \Delta C_i}{(K_d + C^r_i)^2 + \Delta C_i(K_d + C^r_i)} \tag{5.3}$$

where $\Delta C_i = C^f_i - C^r_i$.

Experimental work indicates that a linear relationship between ΔB and the chemotactic prowess of eukaryotic cells is a reasonable assumption (Herzmark et al., 2007; Fuller et al., 2010). Although this may vary between organisms and under different conditions, we adopt this simple assumption and leave the study of more complex relationships between ΔB and the cell's response for future work. We define a_i as the chemotactic index, which determines the cells' chemotactic responsiveness and set it equal to:

Table 5.1: Parameters, variables and symbols

Physiological Parameter	**Range of values considered**	**Description**
d	20μm	Cell diameter
v	$20\mu ms^{-1}$ - $500\mu ms^{-1}$	Cell speed
K_d	[a]	Receptor dissociation constant
γ	0 - 300	Chemotactic sensitivity
S	[a]	Secretion rate per cell
D	$5\ 10^{-11}$ - 10^{-9} m^2s^{-1}	Chemical diffusivity
u	$10^{-12}s^{-1}$	Chemical degradation rate
ρ_0	$\sim 10^8$ cells m^{-2}	Initial cell density
Simulation Parameter		
μ	0.001	Time step within the simulation
λ	0.05	Displacement within simulation
N	75-200	Size of two dimensional grid
ϵ	[b]	A function of μ and k; determines the cell speed
M	2-40	Initial number of cells in grid
Variable		
$\mathbf{x}$	-	Position in two dimensions
t	-	Time in seconds (s)
$C(\mathbf{x}, t)$	-	Chemical concentration at $\mathbf{x}$ at time t measured in mol m^{-2}
C_{front}, C_{rear}	-	Average concentration at the front or rear of cell
B_{front}, B_{rear}	-	Receptor occupancy at the front or rear of cell
a	-	Chemotactic index; determined by B_{front} and B_{rear}
ρ	-	Cell density

[a] This relative values of S and K_d are important rather than the corresponding absolute values. We consider values of S/K_d from 10^{-4} to 10^4

[b] The value of ϵ is determined by v, μ and λ.

$$a_i = \gamma \frac{K_d \Delta C_i}{(K_d + C_i^r)^2 + \Delta C_i (K_d + C_i^r)} \tag{5.4}$$

We model chemotactic movement of individual cells by adding a bias term to equations (5.1), a_i, equivalent to the chemotactic index derived in Eq.(5.4) so that,

$$\begin{aligned} P(X_j^{t+\mu} = X_j^t + \lambda) &= \epsilon(1 + a_x) \\ P(X_j^{t+\mu} = X_j^t) &= 1 - 2\epsilon \\ P(X_j^{t+\mu} = X_j^t - \lambda) &= \epsilon(1 - a_x) \end{aligned} \tag{5.5}$$

The equivalent equations hold for movement in the y direction. The chemotactic index a_i takes values in $[0, 1]$ (if $a_i > 1$, we set a_i=1); in the absence of a chemical signal, $a_i = 0$ and cell movement reduces to a random walk (Eq. (5.1)). On the other hand, when the cell's next step is determined by the local chemical concentration without any stochastic effects or noise, $a_i = 1$. Note that this is a generalised formulation for cell movement that is not an exact representation of the movement of any specific organism.

We assume that when two cells come in contact, they mate with probability 1. This could be modified, for example to reflect that cells carrying complementary agglutinins are more likely to adhere following contact (see Chapter 4). Although this is an important consideration, the primary interest of this work is the impact of self-secretion on chemotactic movement and we leave the investigation of this interesting feature for future work.

5.2.3 Chemical Diffusion

Chemical diffusion occurs in the presence of a concentration gradient and it results in transport of chemical mass. In two dimensions, this process is described by the diffusion equation,

$$\frac{\partial C(\mathbf{x}, t)}{\partial t} = D \bigtriangledown^2 C(\mathbf{x}, t) \tag{5.6}$$

where $C(\mathbf{x}, t)$ is the chemical concentration at time t and coordinates $\mathbf{x} = (x, y)$, and D is the chemical diffusivity in the medium. Given an initial concentration field, the concentration at later times changes according to Eq. (5.6).

Here we assume that cells have the capacity to secrete a specific chemoattractant and so may contribute to the chemical concentration field as they move. We modify the classical diffusion equation in Eq. (5.6) to obtain,

$$\frac{\partial C(\mathbf{x},t)}{\partial t} = D \bigtriangledown^2 C(\mathbf{x},t) - uC(\mathbf{x},t) + S \sum_{j=0}^{j=M} I_j \delta(x - X_j^t)\delta(y - Y_j^t) \tag{5.7}$$

where u is the chemical degradation rate (due to chemical disintegration and receptor binding), S measures the pheromone secretion rate per cell, I_j is the secretion index of cell j (equal to 1 if the cell is a signaller and 0 otherwise), M is the number of cells present in the grid, X_j^t and Y_j^t are the coordinates of the j^{th} cell at time t, and $\delta(.)$ is the Dirac delta function so that,

$$\delta(z) = \begin{cases} 1, & \text{if } z = 0. \\ 0, & \text{otherwise.} \end{cases} \tag{5.8}$$

We assume that the degradation rate, u, is orders of magnitude smaller than the diffusivity throughout this work (exact value specified throughout).

Using the formulation of chemical sensing and secreting, and of random and chemotactic movement defined thus far, four types of cells are possible: those that move randomly (movement determined by Eq. (5.1)) and do not secrete the chemoattractant ($I_j = 0$), those that move randomly (movement determined by Eq. (5.1)) and secrete the chemoattractant ($I_j = 1$), those that move chemotactically (movement determined by Eq. (5.5)) and do not secrete the chemoattractant ($I_j = 0$) and those that move chemotactically (movement determined by Eq. (5.5)) and secrete the chemoattractant ($I_j = 1$). By comparing mating efficiency for different combinations of these four types of cells, this work assesses if and when chemotaxis improve mate finding, quantifies the movement impediment suffered by cells secreting and detecting the same chemoattractant and evaluates if and when self-incompatible mating types with mating-type-specific chemotactic roles can improve mating.

We use a finite difference approximation to solve Eq. (5.7), assuming periodic boundary conditions (see Chapter 19 in (Press et al., 2007)). For the derivation of the numerical method, boundary effects, and stability conditions see the Appendix at the end of this chapter. The parameters and terms used throughout this work are defined in Table 5.1.

Before proceeding to the results section, I present a series of numerical solutions for Eq. (5.7), for a single cell moving at constant velocity. This it merely to gain an understanding of some of the key model parameters before we proceed with the analysis. Fig. 5.4 shows the concentration around the moving cell after it travels a fixed distance, for different parameter choices. It is evident from the figure that the cell movement generates a local asymmetry in chemical concentration as anticipated.

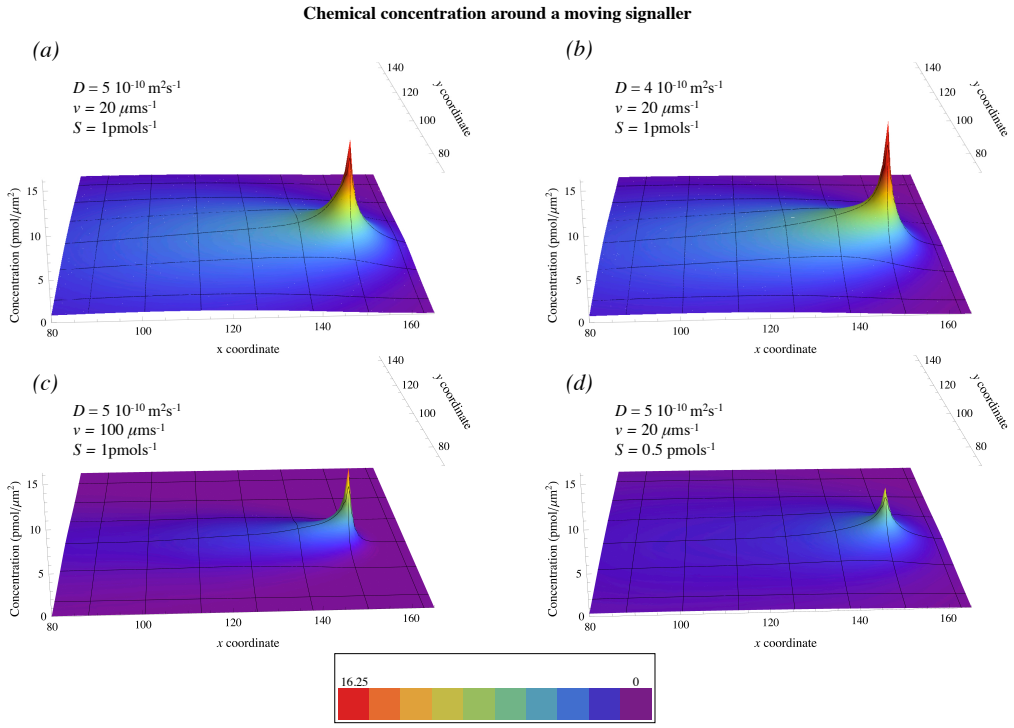

Figure 5.4: Chemical concentration around moving cell. Chemical concentration around a secreting cell of diameter 20μm for varying diffusivity, D, cell speed, v, and secretion rate S. The cell is located at (x, y)=(150, 100) and its diameter spans 4 increments on the two dimensional grid. Cell movement generates an asymmetry in concentration around the moving cell, which is intensified at higher cells speed (b) and lower diffusivity (c). The secretion rate determines the maximum concentration.

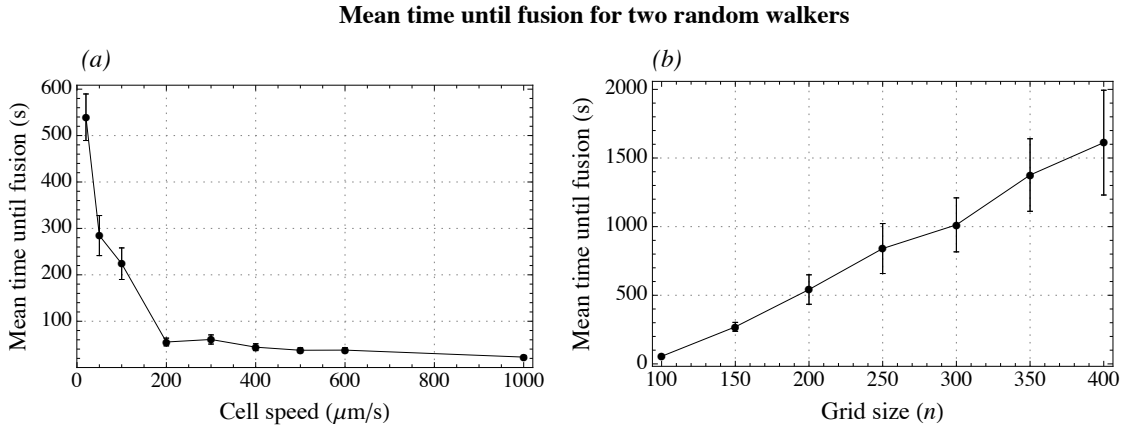

Figure 5.5: Mean time until fusion for two cells following unbiased random walks, at initial separation distance of 40μm. (*a*): Mean time until fusion against cell speed. (*b*): Mean time until fusion against cell density. Results are averaged over 40 simulations. Bars indicate the standard error of the mean. Other parameters, d =10μm, v=100μms^{-1}.

5.3 Results

5.3.1 Two cells

We begin by studying the dynamics of pair formation in an idealized two-cell case; we place two cells at fixed distance from one another and measure the time until they meet. We investigate the dynamics of pair formation when: 1) movement is random (this is equivalent to a random walk), 2) movement is chemotactic and both cells secrete and respond to the same chemical and 3) one cell secretes a chemical but moves randomly while the second cell moves chemotactically responding to the chemical without secreting. Because the movement dynamics change when certain parameters change (e.g. cell speed) even without chemotaxis, we use the outcome of the random walks as a baseline to compare cases 2 and 3. Parameter values are varied within ranges that reflect the physicality of small protists and their environments.

This is a highly idealised case that does not reflect the environments of unicellular protists. For example, the cell density is defined as the number of cells in the grid over the area of the grid, $M/N^2 = 2/N^2$, where N is the grid size and $M = 2$ signifies that we are considering only two cells. It follows that 'cell density' decreases as N increases even though the number of cells is kept fixed at $M = 2$. In addition, the disadvantage of self-incompatibility that mating types confer disappears when we only consider two cells. Despite these constraints, the two cell formulation allows us to examine cells in isolation, and evaluate the impact of different physiological parameters before we move on to study the interaction of multiple cells in the next section.

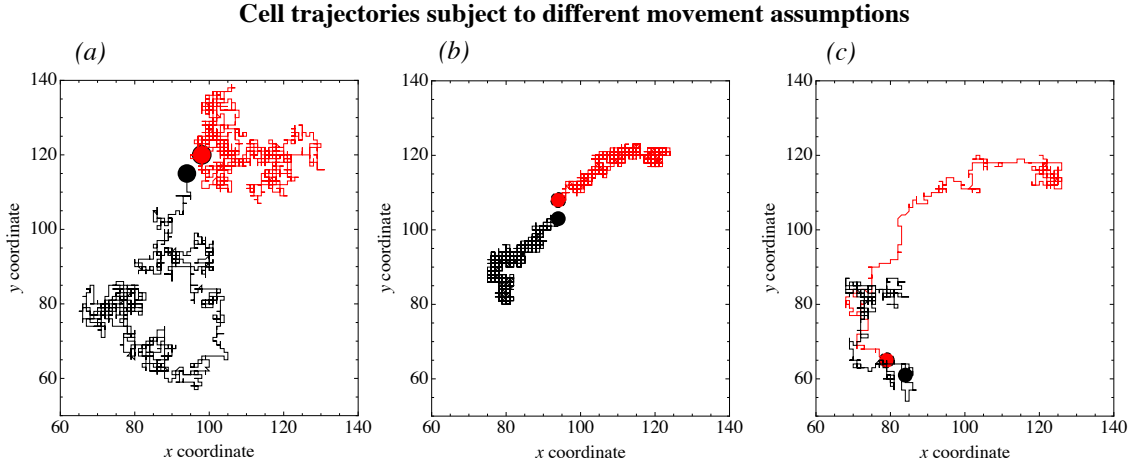

Figure 5.6: Cell trajectories subject to different cell movement assumptions. (*a*): Both cells move at random; (*b*): both cells move chemotactically secreting and responding to the same chemoatractant; (*c*): One cell secretes the chemoatractant but moves at random (black) while the other moves chemotactically (red). Parameters used: d =20μm, v=100μms^{-1}, $S/K_d = 10^{-1}$, $\gamma = 100$, $D = 5.5\ 10^{-10}$m^{2}s^{-1}, $u = 10^{-8}$s^{-1}.

Random Walk

With no chemotaxis the time until the cells meet depends on their speed and the cell density. The higher the cell speed and the smaller the two dimensional grid (i.e. the higher the cell density), the faster the two cells meet (Fig. 5.5). As the mean time until the cells meet increases, so does its variance (Fig 5.5).

Chemotactic movement

Isolated instances of the cell trajectories subject to the three different movement assumptions are plotted in Fig. 5.6 (*a*-*c*). The first plot shows the cell trajectories without chemotaxis; these are unbiased random walks (Fig. 5.6 (*a*)). For the second plot, both cells secrete and detect the chemoattractant (Fig. 5.6(*b*)). Here, both cells migrate towards one another, but movement is subdiffusive and the cells move around their local trail while moving towards one another, resulting in a lower approaching speed. This finding is similar to that of Taktikos et al. (2011), where self-secreting cells were shown to repeatedly move around themselves while they diffused away from their initial position. In the third plot, one cell secretes the chemoattractant but moves randomly (black trajectory) while the other cell moves chemotactically (red trajectory). Here, the responding cell migrates sharply towards the signaller (Fig. 5.6(*c*)). Note that there is a time delay between the onset of movement/secretion and the diffusion/detection of the signal to the attracted cell, explaining why the attracted cell initially appears to be moving randomly.

The mean square displacement (MSD) [2] of a random walker will typically have a linear relationship

[2] MSD = $\Sigma_{i=1}^{N}$ ($\mathbf{x}_i$ - $\mathbf{x}_0$)^2/ N, where $\mathbf{x}_0$= cell's initial position, $\mathbf{x}_i$ = current position of i^{th} instance, N=number of repetitions.

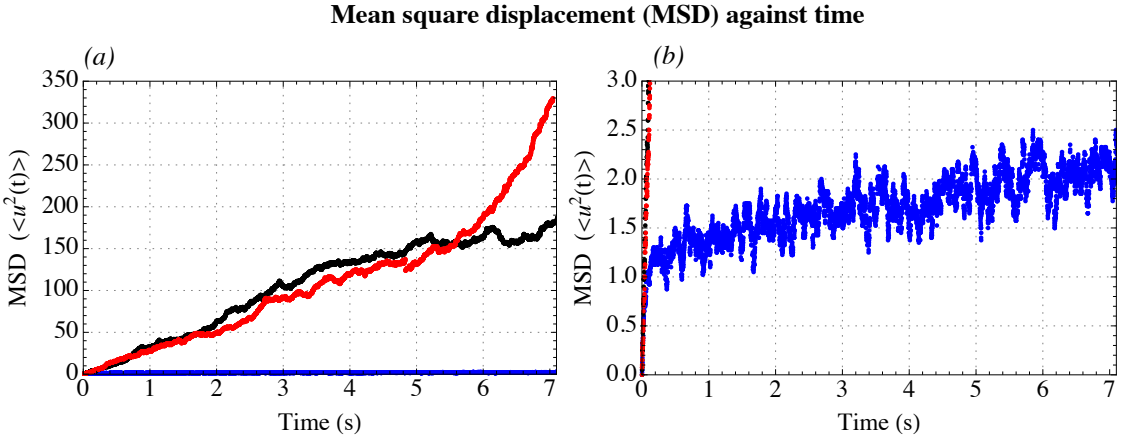

Figure 5.7: Diffusive behaviour subject to different cell movement assumptions. (*a*): The mean square distance (MSD) against time for cell moving randomly (black), a cell responding to an external signal without secreting (red) and a cell responding to an external signal while secreting (blue), (*b*): zoomed in picture to illustrate behaviour of simultaneous signaller-detector. Results were averaged over 40 simulations. Parameters used: d =20μm, $S/K_d = 10^{-1}$, $\gamma = 100$, $D = 5.5\ 10^{-10}\text{m}^2\text{s}^{-1}$, $u = 10^{-8}\text{s}^{-1}$.

with time (Metzler and Klafter, 2000). On Fig. 5.7 the MSD is plotted as a function of time for the three types of movement studied here. As expected, the MSD is approximately linear with time when movement is random (black). For a detector that does not secrete, the MSD is initially linear but becomes superdiffusive (i.e. MSD increases faster than linearly with time) once the chemical signal of the remote signaller becomes detectable. The opposite pattern is true when the detector also secretes the chemical. Now, the MSD increases slowly with time, i.e. in a subdiffusive manner due to interference from self-secretion (Fig. 5.7(*b*)) . These results are a formal representation of Fig. 5.6(*a-c*).

Before we move on to examine the impact of they key model parameters on the general trends illustrated in Fig. 5.6, we note that the relationship between the dissociation constant, K_d, and secretion rate, S, rather than the respective absolute values, dictates the cell's response. This follows from Eq.(5.3) and is confirmed using simulation (Fig. 5.8). In that sense the impact of S and K_d, is best understood by studying the relationship between them (also see Table 5.2).

Simultaneous secretion and detection

In order to compare the time until fusion subject to different movement scenarios, we define the ratio t_s/t_{rw} where t_s and t_{rw} are the time until the two cells meet when secretion and detection are simultaneous and when cells follow random walks respectively. We investigate the value of t_s/t_{rw} varying the key model parameters. A ratio measures the factor by which the time until fusion changes under different scenarios. This is a better measure than a difference as changes in cell speed will inevitably affect t_{rw} and t_s and so the

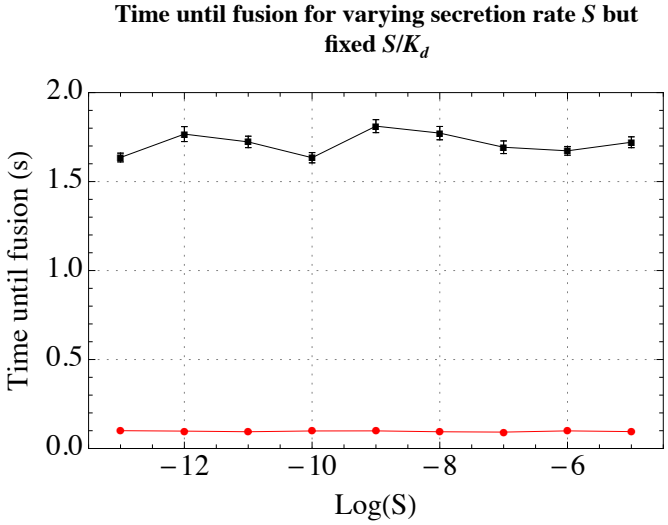

Figure 5.8: Mean time until fusion for two cells for two cells at initial separation distance of 40μm for varying secretion rate, S, but fixed S/K_d. Black is simultaneous secretion and detection; red for mutually exclusive secretion and detection. Results are averaged over 40 simulations. Bars indicate the standard error of the mean. Other parameters, d=20μm, $S/K_d = 10^{-1}$, $\gamma = 100$, $D = 5.5\ 10^{-10}\mathrm{m}^2\mathrm{s}^{-1}$, $u = 10^{-8}\mathrm{s}^{-1}$.

corresponding time differences. Results are shown on Fig. 5.9(a-c), and are summarized in Table 5.3 which can be found in the Appendix at the end of this chapter.

The ratio of the time until two cells meet when secretion and detection are simultaneous over that for cells following random walks, t_s/t_{rw}, is plotted against the logarithm of the ratio of the secretion rate, S, to the dissociation constant, K_d, $\log(S/K_d)$ on Fig. 5.9(a) for varying cell speed, v. A chemotactic response is possible only for a range of values for S/K_d. This follows from the definition of ΔB in Eq. 5.3 (also see Table 5.2).

The value of t_s/t_{rw} then depends on the cell speed. At high v ($v = 500\mu\mathrm{ms}^{-1}$), t_s/t_{rw} raises well above 1, whereas at lower values of v, ($v = 100\mu\mathrm{ms}^{-1}$ and $v = 200\mu\mathrm{ms}^{-1}$), t_s/t_{rw} is below 1 indicating a positive chemotactic response. This can be explained as follows. Firstly, when both cells secrete the chemoattractant the net concentration around them increases, and cells are prone to receptor saturation (Fig. 5.1). More importantly, a moving signaller generates an asymmetry in chemical concentration around it, which alters the net difference in receptor binding across the cell, compromising external signals (Fig. 5.1, Fig. 5.4 and Eq. 5.4). The asymmetry around a moving signaller is intensified at higher speeds, further impairing its ability to respond to an external signal (Fig. 5.4). When the asymmetry is very high (at higher speeds), it can even lead to a net receptor occupancy that points backwards, prompting the cell to change its direction while it moves towards a potential partner (Fig. 5.1). This is evident when one looks at the trajectories of simultaneous signallers-detectors on Fig. 5.6 (b), and explains why t_s/t_{rw} increases sharply

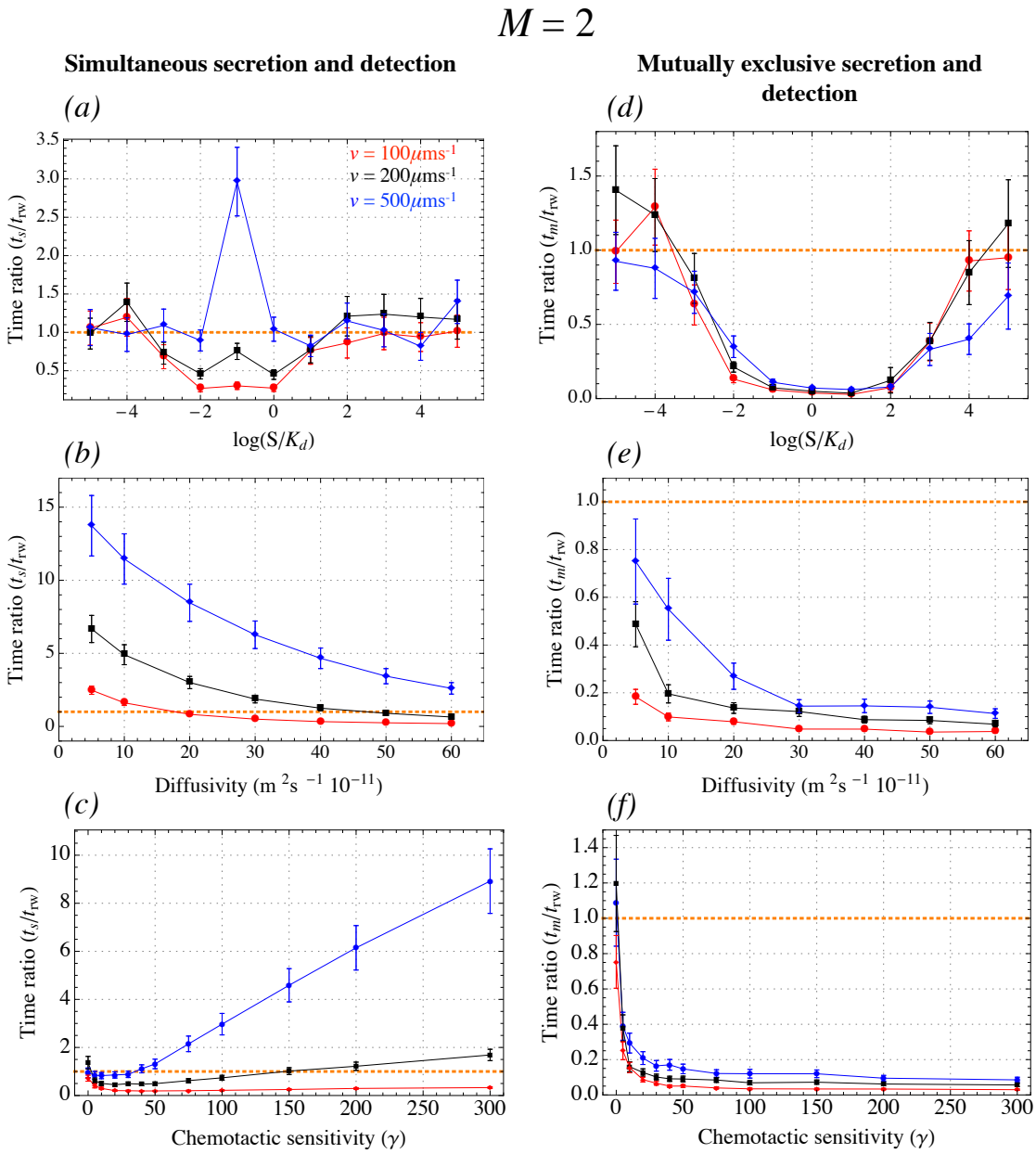

Figure 5.9: The ratios t_s/t_{rw} and t_m/t_{rw} against the key model parameters for different cell speed, v. Red: v=100 μms^{-1}, black v=200 μms^{-1}, blue: v=500 μms^{-1}. The ratio t_s/t_{rw} is always higher than t_m/t_{rw}, indicating that self-secretion impedes pair formation. (a): The ratio t_s/t_{rw} against the log ratio of the secretion rate, S, to the dissociation constant, K_d, log(S/K_d), (b): The ratio t_s/t_{rw} against the chemical diffusivity D, (c): The ratio t_s/t_{rw} against the chemotactive sensitivity constant γ, (d): The ratio t_m/t_{rw} against the log ratio of the secretion rate, S, to the dissociation constant, K_d, log(S/K_d), (e): The ratio t_m/t_{rw} against the chemical diffusivity D, (f): The ratio t_m/t_{rw} against the chemotactive sensitivity constant γ. Results were averaged over 40 simulations. The bars indicate the standard error of the mean. Baseline parameters: d= 20 μm, v=100μms^{-1}, S/K_d = 10^{-1}, γ=100, D=5.5 10^{-10}m^{2}s^{-1}, u=10^{-8}s^{-1}. Because the range in the ratio values varies greatly, the results are not plotted on the same scale. For a direct comparison of values see Table 5.3 in the Appendix at the end of this chapter.

for $v = 500\mu\text{ms}^{-1}$. For slower moving cells, the asymmetry around the moving signaller is less severe, $t_s/t_{rw} < 1$, and a positive chemotactic response follows (Fig. 5.9(a)). These observations suggest that interference caused by self-signalling is not due to receptor saturation alone; the cell movement itself holds a significant role, and cell speed dominates a qualitative shift in the relative efficiency of sexual chemotaxis.

We also plotted the ratio t_s/t_{rw} against the chemical diffusivity D for varying cell speed. Coordination between cell speed and chemical diffusivity is critical as it defines, 1) the correlation between the signal and the position of the signaller, 2) the steepness of the concentration gradient and 3) the asymmetry around a moving signaller (and so the effect of self-secretion) (recall Fig. 5.4 and refer to Table 5.2).

The ratio t_s/t_{rw} is above 1 as the chemical diffusivity, D, varies (Fig. 5.9(b)) and increases further for higher cell speed and lower diffusivity, D. Only when the cell speed becomes low relative to diffusion, does this ratio decrease below 1 implying a positive chemotactic response. This can be understood in the following way. As D increases with respect to v, the chemical signal spreads more readily conveying a clearer cue. When D is low relative to v, the association between the cell's location and the chemical signal is lost and chemoattraction is less effective (i.e. signalling cells have already moved before others receive their signal). In addition, the asymmetry around moving signallers is intensified at high speeds and low diffusivity (Fig. 5.4). This interferes with the net concentration around the cell and further impairs movement when secretion and detection are simultaneous. Note that reasonable values for D for a small molecule in water are around $10^{-10}\text{m}^2\text{s}^{-1}$.

Finally, we plotted t_s/t_{rw} against the chemotactic constant γ (Fig. 5.9 (c)). For values of $\gamma < 50$, $t_s/t_{RW} < 1$, indicating a decrease in time until pair formation. As γ increases there is an increase t_s/t_{rw} , particularly for higher cell speed (v=500 μms^{-1}). For very high values of γ, the movement stochasticity diminishes and cells follow the chemical signal deterministically. Although this can be beneficial when the chemical signal is a clear indication of a partner's position, when the signal is noise due to self-secretion, maintaining a certain degree of stochasticity is desirable.

Mutually exclusive secretion and detection

We repeated the above analysis assuming that secretion and detection are mutually exclusive so that one cell secretes the signal but moves randomly while the other cell moves chemotactically without secreting. We let t_m be the time until the two cells meet when secretion and detection are mutually exclusive.

The ratio t_m/t_{rw} is plotted against $\log(S/K_d)$ on Fig. 5.9(d), for varying speed. Like t_s/t_{rw}, t_m/t_{rw} diverges from 1 only for values of S/K_d for which a chemotactic response is possible (Table 5.2). Then, the time until fusion decreases relative to that for two random walkers, and $t_m/t_{rw} < 1$ (Fig. 5.9 (d)). This

Table 5.2: The impact of the key model parameters explained

S **and** K_d**:** The secretion rate S determines the chemical concentration contribution from each secreting cell. Given a specific value for the dissociation constant, K_d, binding to membrane receptors depends on the concentration around the cell (recall Sections 5.2.2 and 5.2.3), that itself relies on the secretion rate. Depending on the Hill constant and K_d, there are thresholds in concentration, C_{max} and C_{min} beyond and below which binding is suboptimal (Fig. 5.2). This is true for fixed chemical diffusivity D and cell speed v.
D **and** v**:** The relationship between the chemical diffusivity, D, and the cell speed, v, determines the correlation between the chemical signal and the position of the cell. When cells move too fast relative to diffusion, the association between the chemical signal reaching a detecting cell and the secreting cell's position is lost thereby impairing chemotaxis. In addition, when cells secrete and detect the same signal, high v relative to D intensifies the asymmetry in the chemical concentration around the moving signaller (Fig. 5.4).
γ**:** The value of γ determines the degree to which cells respond to chemical signals or equivalently, the degree of noise in the cell's response. Very high values of γ can result in a chemotactic index equal to 1, meaning that cells follow the chemical concentration deterministically. This may not necessarily be optimal, as signallers are motile and so the chemical concentration is only indicative of their position. When γ is close to zero on the other hand, cell movement reduces to a random walk. Depending on whether chemotaxis confers an improvement or an impairement to mate finding, higher values of γ are beneficial or disadvantageous.

decrease is initially small, reaches an optimal value as S/K_d increases, and decreases beyond that point to become negligible as receptors saturate at high concentrations. The drop in t_m/t_{rw} is more steep than that seen for t_s/t_{rw}, and is independent of cell speed (also see Table 5.11 in the Appendix at the end of this chapter). Additionally, the thresholds of S/K_d below and above which no significant chemical signal is detectable, are lower for mutually exclusive secretion and detection than for simultaneous secretion and detection. This comes as no surprise; when both cells are signallers two chemical fields rather than one are juxtaposed leading to high concentrations and receptor saturation for smaller secretion rates.

In Fig. 5.9 (*e*), the ratio t_m/t_{rw} is plotted against diffusivity, D, for varying cell speed, v. The value of t_m/t_{rw} was always below 1 but increases to values closer to 1 for lower D and higher v. Recall from the previous section that when D is very low relative to v, the association between the signalling cell's position and the signal itself is lost (i.e. the cell has already moved by the time its signal reaches a potential partner).

The chemotactic constant γ in Eq. (5.4) determines the sensitivity of the chemical response. When physiological parameters are so that chemotactic movement is possible, the value of γ holds a key role in the mating kinetics by defining the strength of the chemotaxis. It follows that t_s/t_{rw} decreases as γ increases (Fig. 5.9 (*f*)).

Finally, note that t_m/t_{rw} is always smaller than t_s/t_{rw}, indicating that adaptations that render secretion

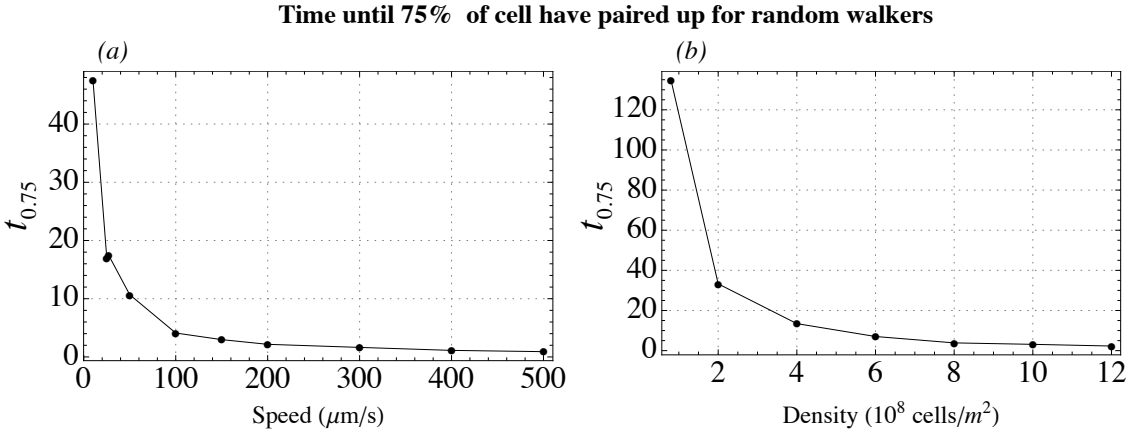

Figure 5.10: The time in seconds until initial cell density reduces by 75%, $t_{0.75}$, when cells perform random walks. (*a*): $t_{0.75}$ against cell speed. (*b*): $t_{0.75}$ against initial cell density. Results are averaged over 40 simulations. Baseline parameters: d= 20 μm, v=100μms^{-1}, $S/K_d = 10^{-1}$, γ=100, D=5.5 10^{-10}m^{2}s^{-1}, u=10^{-8}s^{-1}, ρ_0=1.6 10^8 cells m^{-2}.

and detection mutually exclusive can be beneficial as they substantially reduce the time until gametes meet.

5.3.2 Multiple cells

We proceed by considering an environment where many cells are present, seeking a mate. This resembles the most common sexual phase of protists' life cycles. Vegetative cells enter the sexual phase producing isogametes that must form mating pairs. We expect that our findings from the simple two-cell case to change due to complex dynamics emerging from the presence of many cells. For example, the simultaneous presence of many secreting cells impacts the overall concentration field. Also, the drawbacks following self-incompatibility now become apparent; cells with mutually exclusive secretion and detection can only mate with half of the population.

The relative advantage of sexual chemotaxis is assessed by contrasting three cases: 1) all cells in the population can mate with one another and there is no chemotaxis (movement is equivalent to a random walk); 2) all cells in the population can mate with one another and all cells secrete and chemotactically respond to the same substance; 3) half of the cells secrete a chemoattractant but move randomly and the remaining cells do not secrete the putative substance but move chemotactically. In this latter scenario cells from different groups may not fuse, i.e. we assume mating types with mating-type-specific roles in chemotactic signalling. Each simulation begins by randomly placing 40 cells, $M = 40$, across a two dimensional grid with $N = 100$. More details on the simulations are provided in the Appendix.

Random Walk

Chemotaxis is beneficial in environments where finding a sexual partner through random movement is difficult. For example, cells meet less frequently when cell speed and/or cell density are lower. To illustrate this, Fig. 5.10 shows the time until 75% of the cells have paired up, $t_{0.75}$, against initial cell density and cell speed.

The measure $t_{0.75}$ is employed throughout this section as a means to contrast different scenarios. This provides a better description of mating efficiency than the mean time until pair formation; the latter is heavily influenced by the long time taken until the final pairs fuse at low cell densities.

Chemotactic movement

We repeat the analysis performed for the two cell case in the previous section by varying the key model parameters $(S/K_d, v, D, \gamma)$ subject to the two different types of chemotactic movement. The results are plotted on Fig. 5.11, and summarized on Table 5.4 which can be found in the Appendix at the end of this chapter. The general trends uncovered in the two cell case also appear here.

Simultaneous secretion and detection

We define the ratio $t^s_{0.75}/t^{rw}_{0.75}$ where $t^s_{0.75}$ and $t^{rw}_{0.75}$ are the time until 75% of the cells have paired up for simultaneous chemical secretion and detection and for random movement respectively.

In Fig. 5.11(*a-c*), $t^s_{0.75}/t^{rw}_{0.75}$ is plotted against $\log(S/K_d)$, D and γ. As any cell is free to mate with any other cell, when the relationship between S and K_d is so that no chemical sensing is possible (or if $\gamma = 0$), the cell pairing dynamics subject to simultaneous secretion and detection are equivalent to those for random movement and $t^s_{0.75}/t^{rw}_{0.75}$ is equal to 1.

When chemical sensing is possible, the ratio $t^s_{0.75}/t^{rw}_{0.75}$ is larger than 1 for $v = 200\ \mu\text{ms}^{-1}$ and $v = 500\ \mu\text{ms}^{-1}$ (Fig. 5.11(*a-c*)). Recall that when cells move at high speeds, the asymmetry in chemical concentration around them is large impairing their ability to move towards potential partners (Fig. 5.11 and Fig. 5.4). Likewise, recall that the association between a signalling cell's position and the the signal itself is lost when cells move very fast relative to diffusion. Only when cell speed is equal to $100\mu\text{ms}^{-1}$, does $t^s_{0.75}/t^{rw}_{0.75}$ decreases below 1; this is only true for values of S/K_d that permit chemotactic sensing and high diffusivity, D, relative to cell speed, v (Fig. 5.9(*a-c*)).

The impact of γ, determining the sensitivity of a cell to the chemical signal, depends on whether physiological parameters such as the secretion rate, S, the dissociation constant, K_d, the cell speed, v, and the chemical diffusivity D, induce an improvement in the efficiency of pair formation. Higher values of γ

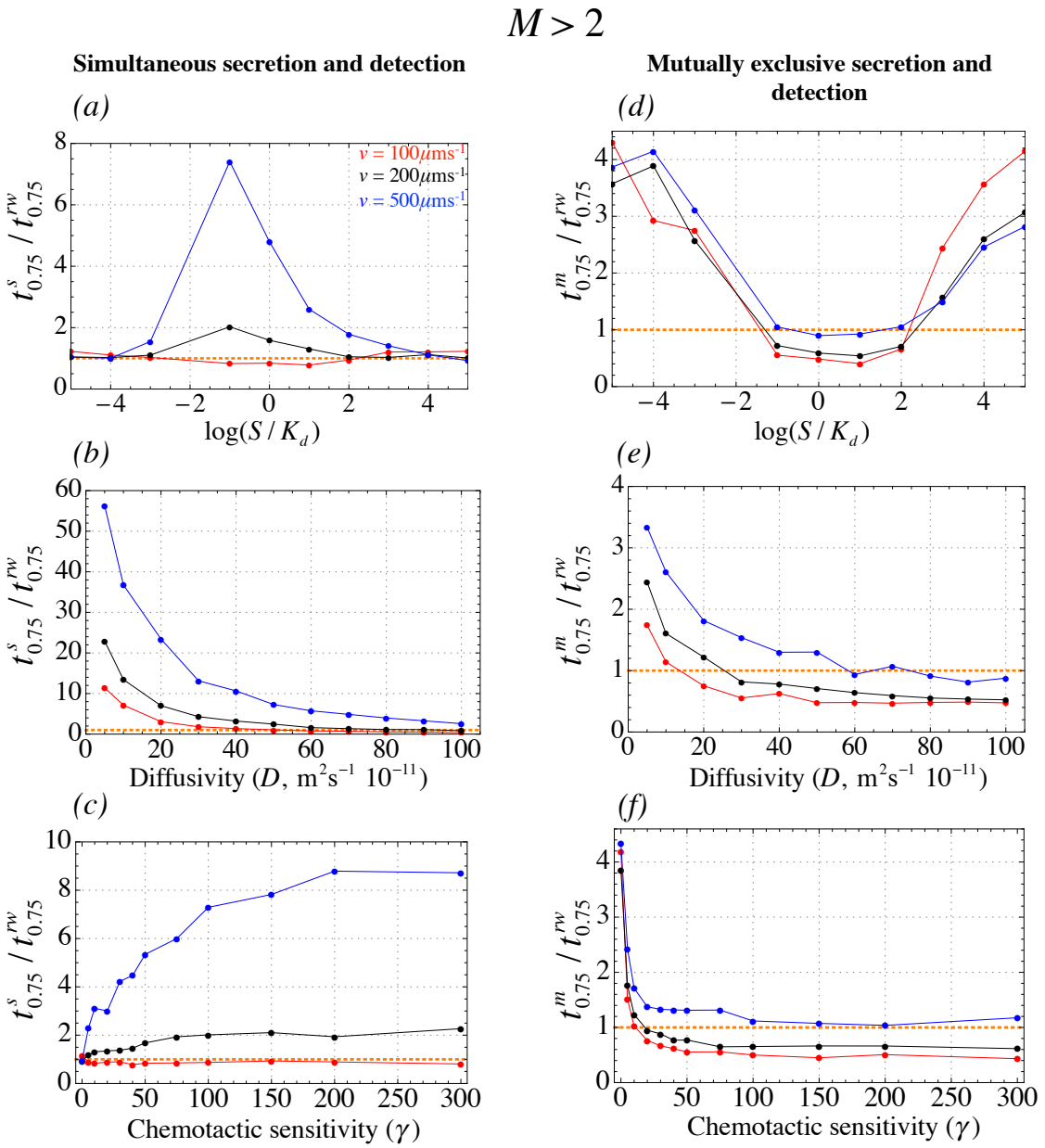

Figure 5.11: The ratios $t^s_{0.75}/t^{rw}_{0.75}$ and $t^m_{0.75}/t^{rw}_{0.75}$ against the key model parameters for different cell speed, v. Red: v=100 μms^{-1}, black v=200 μms^{-1}, blue: v=500 μms^{-1}. (a): The ratio $t^s_{0.75}/t^{rw}_{0.75}$ against the log ratio of the secretion rate, S, to the dissociation constant, K_d, log(S/K_d), (b): The ratio $t^s_{0.75}/t^{rw}_{0.75}$ against the chemical diffusivity D, (c): The ratio $t^s_{0.75}/t^{rw}_{0.75}$ against the chemotactive sensitivity constant γ, (d): The ratio $t^m_{0.75}/t^{rw}_{0.75}$ against the log ratio of the secretion rate, S, to the dissociation constant, K_d, log(S/K_d), (e): The ratio $t^m_{0.75}/t^{rw}_{0.75}$ against the chemical diffusivity D, (f): The ratio $t^m_{0.75}/t^{rw}_{0.75}$ against the chemotactive sensitivity constant γ. Baseline parameters: d= 20 μm, S/K_d = 10^{-1}, γ=100, D=5.510^{-10}m^{2}s^{-1}, u=10^{-8}s^{-1}, ρ_0 =1.6 10^8 cells m^{-2}. Results were averaged over 40 simulations. Because the range in the ratio values varies greatly, the results are not plotted on the same scale. For a direct comparison of values see Table 5.4 in the Appendix at the end of this chapter.

then lead to further improvement or deterioration accordingly (Fig. 5.11(*c*)). These findings are qualitatively equivalent to those uncovered in the two-cell case (see Table 5.2 for a summary of each parameter's impact).

Mutually exclusive secretion and detection

We define the ratio $t^m_{0.75}/t^{rw}_{0.75}$ where $t^m_{0.75}$ is the time until 75% of the cells have paired up for mutually exclusive chemical secretion and detection and for random movement respectively.

When the relationship between S and K_d is so that no chemical sensing is possible (or if $\gamma = 0$) the value of $t^m_{0.75}$ increases to approximately 4 times that of $t^{rw}_{0.75}$, reflecting the disadvantage that self-incompatible mating types confer (Fig. 5.11(*d-f*)).

When the relationship between S and K_d is such that chemical sensing is possible, this disadvantage is countered by the efficacy of chemotaxis with mating-type-specific functions. For fast moving cells (v = 500 μms^{-1}) this matches the efficacy of pair formation without chemotaxis for appropriate values of D and γ, and $t^m_{0.75}/t^{rw}_{0.75}$ is approximately 1 (Fig. 5.11(*d-f*)). Cells move quickly enough to encounter one another by chance within short enough time at these relatively high densities, explaining why $t^m_{0.75}/t^{rw}_{0.75}$ does not decrease below 1.

For slower moving cells (v = 200 μms^{-1} and v = 100 μms^{-1}), $t^m_{0.75}/t^{rw}_{0.75}$ does decrease below 1. The reduction in $t^m_{0.75}/t^{rw}_{0.75}$ depends on the relationship between S and K_d, and D and v as well as the chemotactic sensitivity γ. When S/K_d is so that chemical sensing can follow, the decrease in $t^m_{0.75}/t^{rw}_{0.75}$ depends on the balance between the chemical diffusivity, D and cell speed, v. As in the two cell case, when v is too high relative to D the association between the chemical signal and the cell's position is weakened hindering pair formation (Fig. 5.11(*d*), also see Table 5.4 in the Appendix). Because chemotaxis is always beneficial when secretion and detection are mutually exclusive, the larger the value of γ, the more efficient is pair formation.

Finally, note that in the cases when both simultaneous and mutually exclusive secretion and detection confer an advantage ($t^s_{0.75}/t^{rw}_{0.75}$ <1 and $t^m_{0.75}/t^{rw}_{0.75}$ <1), we always have $t^m_{0.75} < t^s_{0.75}$, indicating that mating types with mating-type-specific chemotactic roles can improve mating efficiency despite the anticipated impediment of self-incompatibility preventing cells to fusing with 50% of the population (Fig. 5.11 and Table 5.4 in Appendix).

5.4 Discussion

The primary purpose of this chapter was to quantify the efficiency of pair formation between sexual cells subject to different assumptions of chemoatraction and mating compatibility, and to assess whether self-

incompatible mating types with mating-type-specific chemotactic roles improve partner finding. Throughout this work, parameters that reflect the physicality of small protists and their environments were employed. My results indicate that simultaneous secretion and detection of a single chemoattractant (self-secretion) may cause a severe movement impediment and so hinder the ability of gametes to find a partner. Mutually exclusive roles in signal generation and detection, on the other hand, resulted in faster pair formation, even when cells conferring the same roles could not pair with one another. The findings of this chapter suggest that asymmetric roles in sexual chemotaxis matter, even without anisogamy. Variation in parameters such as the cell speed, the secretion rate, the receptor dissociation constant, chemical diffusivity and cell density determine the movement impairment experienced by cells secreting and detecting the same chemical. Importantly, my conclusions become meaningful only when chemotaxis provides an advantage in partner finding, which is true unless cell density or speed are very high.

Measuring cell density in natural populations of protists is challenging, and not many reports providing appropriate values for this parameter are available. Work by Sheridan et al. (2002) indicates that the population densities of many aquatic protists can be significantly lower than the values I employed. The relatively high initial density assumed here permits measurements of the cell density time decay without increasing the underlying grid size and so keeping computational time tractable (see Appendix). Here I focused on small protists (diameter $\simeq 20\ \mu$m) with speed in the range of $100 - 500 \mu\text{ms}^{-1}$. This is indicative of small algae such as *Chlamydomonas*, but unicellular eukaryotes can be several times larger and faster. An exhaustive phase-space analysis where lower cell densities, and a wide range of cell sizes and speeds are considered, is necessary to relate my findings across species.

Although this is not an evolutionary model, it serves to dynamically quantify drawbacks when cells secrete and detect the same chemoattractant in sexual chemotaxis. Simultaneous secretion and detection of a single chemical signal causes receptor saturation, but most importantly, it leads to self-stimulation and induces an asymmetry around moving signallers that can trigger a reversal in their direction as they move towards potential partners. The movement impediment that follows suggests that the assumption of a moderate and fixed fitness coefficient in an evolutionary model may be inappropriate. Crucially, gamete density changes throughout mating as gametes turn into zygotes. I expect this to impact the relative advantage of genes pertinent to signalling and mating compatibility during sex.

Hoekstra (1982) developed an evolutionary model to assess whether two mating types, one producing a chemoatractant and one responding to it, would lead to more efficient mating. This work provided an interesting insight into the spread of mating type modifiers. Yet, it did not consider gamete interactions and chemotaxis, limiting its physical conclusions. Furthermore, Hoekstra's model requires the pre-existence

of a bipolar system of secretion and detection for the evolution of two mating types (referred to as pseudo mating types). My findings complement those of Hoekstra by rigorously assessing the costs of self-secretion for a range of physiological parameters. In addition, this work suggests that a bipolar system comprising a single chemical-receptor pair can be less efficient than no chemotaxis at all, questioning the significance of this presumption; my results suggest that the ability to generate and respond to a signal should evolve separately.

To my knowledge the theoretical results of this chapter have not been tested experimentally. However, experiments with homothallic (same mating type) and heterothallic (opposite mating type) gametes of *Closterium peracerosum-strigosum-littorale* indicate exactly what our model predicts: in contrast to heterothallic gametes, homothallic gametes are confined to perpetual movement around their initial position, unable to migrate through longer distances (see SI videos in (Tsuchikane et al., 2012)). Further experiments explicitly focusing on this question would be valuable.

5.5 Limitations and further work

This work provides grounds for an explicit evolutionary model and can be extended to incorporate genetics without the need of implicit fitness assumptions; the physiochemical constraints encountered by gametes directly define mating success. The degree of movement impairment due to self-secretion illustrated here suggests that genes conferring the ability to either secrete or detect a specific chemoattractant (but not both) would evolve, at least to some extent. The simplest evolutionary scenario involves two alternative alleles in tight linkage each encoding for the production of a pheromone, or the production of a receptor that generates a chemotactic response to the putative pheromone. If the loci are in tight linkage, a gamete can be a secretor, a detector or none of the two (for example consider the following genes/phenotypes a: wild-type (no secretion or detection), S: gene for secretion, D: gene for detection). I predict, given the findings of this chapter, that secretors and detectors will form mating pairs faster than gametes that do not assume any of these properties. Moreover, it is natural to expect that the relative advantage of being a secretor increases as the frequency of detectors increases in the population and vice versa. I expect the relevant evolutionary dynamics to result in a population with 50% secretors (S gene) and 50% detectors (D gene) where fusions between secretors or between detectors would be very rare (if they are permitted at all). Despite this seemingly straightforward analysis, an evolutionary model may encompass further complexities. For example, if the loci for secretion and detection are not in close linkage, inevitably, some individuals would end up secreting and detecting the chemoattractant. The evolution of linkage between the two loci must also be considered explicitly. My prediction is that tight linkage between the two genes would

evolve. Interestingly, this is the case in modern eukaryotes that utilise sexual chemotaxis (see Chapter 4).

The majority of isogamous protists utilize pheromones for gamete chemoattraction in an asymmetric manner, supporting the predictions of this work. Typically, all gametes secrete a mating-type-specific pheromone but only respond to non-self pheromones (see Chapter 4). Here, I didn't consider multiple pheromones, but I expect that the use of mating-type-specific pheromones and receptors by both mating types will confer an even greater advantage for mating types than that observed for a single chemoattractant and mutually exclusive secretion and detection. Interestingly, it has been suggested that if two mating types are restricted to either secreting or detecting a pheromone, but not both, the evolution of anisogamy follows through disruptive selection (Kochert, 1978; Hoekstra et al., 1984; Hoekstra, 1984). Theoretical work on the evolution of anisogamy in an initially isogamous population with mating types and chemotaxis, unequivocally supports this prediction (Hoekstra et al., 1984; Hoekstra, 1984; Cox and Sethian, 1985; Dusenbery, 2000, 2006).

A critical limitation to this model is that it only applies to motile organisms, or organisms that live in environments favouring the use of sexual chemotaxis. Other important factors, however, like the probability of successful fertilization once two cells come in contact or efficient communication leading to gamete formation, both of which are nearly ubiquitous in unicellular eukaryotes (Chapter 4), may benefit from asymmetric gamete signalling. The problem of self-stimulation is omnipresent throughout gamete interactions, and an asymmetry in chemoattraction is unlikely to be the sole adaptive mating-type-specific trait. For example, recent work using yeast cells by Youk and Lim (2014) has shown that generating and detecting the same signal can diminish the capacity of cells to respond to external signals even without cell movement. Although the physiochemical costs and benefits of self-stimulation during gamete recognition and fusion are not easy to quantify theoretically, experimental work could elucidate this matter. An evolutionary model may incorporate such costs by modulating the probability of successful fusion following pair formation. The real challenge to this theory is the presence of organisms that appear to use no diffusible signals for gamete communication (e.g. Paramecia). Such organisms typically use membrane bound molecules and receptors that act for gamete recognition and are mating type specific. Does a mating-type-specific asymmetry improve the strength and robustness of gamete adhesion and fusion? This is a question that deserves further examination. Apropos this, Euplotes use mating-type-specific pheromones but non-mating-type adhesines to mate (Chapter 4; Table 4.2). Initially, adhesion occurs between gametes from any mating type but the pairs formed are robust and can go through full conjugation and fusion only when the two gametes are of opposite mating type. It was suggested that same-mating-type conjugation is coordinated through mating-type-specific pheromones released by the mating gametes, which are necessary for stable adhesion

and conjugation Euplotes (Afon'Kin, 1991).

A final issue that remains to be examined is that of alternative mechanisms that avoid self-stimulation. For example, *Dictyostelium* amoebae secrete cyclic adenosine monophosphate (cAMP) inducing a chemical gradient that they then follow to assemble into an aggregate. These cells avoid self-stimulation by secreting their chemoattractant periodically, allowing for synchronization between the periodicity of the external signal and intracellular dynamics (Hofer et al., 1995). Why wouldn't this also apply to gamete interactions? One explanation is that periodicity compromises the strength of the signal, and the sharpness of the response which can be particularly problematic when a highly specific pairwise communication is required (rather than aggregate to single cell communication).

This study improves our understanding of the role that self-secretion and the self-stimulation following from it, hold during sexual chemotaxis and suggests that the costs previously alluded to are justifiable and can be severe. It also provides grounds for an explicit evolutionary model that can be founded upon the physiochemical constraints gametes experience during sex. An exhaustive phase-space analysis (e.g. considering larger cells and lower cell densities) and consideration of alternative mechanisms that could avoid self-stimulation will strengthen our conclusions. Finally, the incorporation of genetics will place our findings in an evolutionary context. Further work will focus on these tasks.

5.6 Appendix

5.6.1 Numerical solution

Consider Eq.(5.7),

$$\frac{\partial C(\mathbf{x},t)}{\partial t} = D \bigtriangledown^2 C(\mathbf{x},t) - uC(\mathbf{x},t) + s \sum_{j=0}^{j=M} I_i \delta(x - X_j^t)\delta(y - Y_j^t)$$

In order to obtain a numerical approximation to the solution of Eq. (5.7), we introduce a cubic mesh consisting of nodes $(x_k, y_l, t_j) \in \mathbb{R}^3$ with 0= $t_0 < t_1 < t_2 <...$, 0= $x_0 < x_1 < .... < x_N = L$ and 0= $y_0 < y_1 < .... < y_N = L$. For simplicity, we maintain a uniform mesh spacing in all three dimensions, so that

$$\Delta t = t_{j+1} - t_j, \qquad \Delta x = x_{k+1} - x_k = \frac{L}{N}, \qquad \Delta y = y_{l+1} - y_l = \frac{L}{N}$$

represent the time step and the spatial mesh size in the x and y spatial directions respectively. It is essential for the stability of the solutions that we do not a priori require the spatial and time steps to be equal. This is developed further in the next section. We use the following notation,

$$C_{k,l,j} \approx C(x_k, y_l, t_j,) \qquad \text{where} \qquad t_j = j\Delta t, \qquad x_k = k\Delta x, \qquad y_l = l\Delta y \qquad \text{and} \qquad \Delta x = \Delta y = \lambda$$

to denote the numerical approximation to the solution at each node.

We construct a numerical solution scheme using finite difference approximations to the derivatives appearing in Eq.(5.7). We approximate the second order derivative using the central difference formula, and hence obtain

$$\nabla^2 C(x_k, y_l, t_j) \approx \frac{C_{k+1,l,j} - 2C_{k,l,j} + C_{k-1,l,j}}{\lambda^2} + \frac{C_{k,l+1,j} - 2C_{k,l,j} + C_{k,l-1,j}}{\lambda^2} + O(\lambda^2) \tag{5.9}$$

The time derivative is approximated by the one-sided difference approximation so that,

$$\frac{\partial C(x_k, y_l, t_j)}{\partial t} \approx \frac{C_{k,l,j+1} - C_{k,l,j}}{\Delta t} + O(\Delta t) \tag{5.10}$$

Substituting Eq. (5.6.1) and Eq. (5.6.1) into Eq.(5.7) we obtain the following expression for the finite difference approximation,

$$C(x_k, y_l, t_j) \approx \frac{D\Delta t}{\lambda^2}\left(C_{k+1,l,j} + C_{k-1,l,j} + C_{k,l+1,j} + C_{k,l-1,j} - 4C_{k,l,j}\right) - u\Delta t C_{k,l,j} + s\Delta t \sum_{w=0}^{w=M} I_i \delta(k - X_w^j)\delta(l - Y_w^j) \tag{5.11}$$

where X_w^j and Y_w^j are the x and y coordinates of the w^{th} cell in the simulation at time $t = j$, and M cells are initially placed in the grid.

We assume that the solution behaves periodically at the boundaries, that is:

$$C_{N,y_l,t} = C_{0,y_l,t} \qquad \text{and} \qquad C_{x_k,N,t} = C_{x_l,0,t}, \qquad \forall t \tag{5.12}$$

This is equivalent to solving for a mesh of fixed size at the centre of a large cell population (so away from the boundaries).

Periodic boundaries are also imposed on cell movement so that cells can move from position (x_N, y_l) to either of (x_{N-1}, y_l), (x_{N-1}, y_{l-1}), (x_{N-1}, y_{l+1}), (x_0, y_l), (x_0, y_{l-1}), (x_0, y_{l+1}), (x_N, y_{l-1}), (x_N, y_{l+1}), and similarly

for changes in the y direction near the edges.

5.6.2 Stability Conditions

We derive the conditions that need to be imposed on the time and space steps (Δt and λ respectively) for the error in the numerical solution to remain bounded. We begin by considering the one dimensional version of Eq.(5.7):

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2} - uC(x,t) + s\sum_{j=0}^{j=M} I_i \delta(x - X_j^t) \tag{5.13}$$

The stability conditions for the homogeneous equation,

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2} - uC(x,t)$$

are sufficient to ensure stability for the non-homogeneous case. The discrete form of the equation assumes solutions that are Fourier nodes of the form, $C_{j,t} = UG^n e^{ikj}$. For the numerical error to remain bounded we need to satisfy the condition $|G| < 1$. Substituting in the discrete form of the equation we obtain,

$$\frac{UG^{t+\Delta t}e^{ikj} - UG^t e^{ikj}}{\Delta t} = D\frac{UG^t e^{ik(j+\lambda)} - 2UG^t e^{ikj} + UG^t e^{ik(j-\lambda)}}{\lambda^2} - uUG^t e^{ikj}$$

Dividing through by $G^t e^{ikj}$ and solving for G we obtain,

$$G = 1 + \frac{\Delta t D}{\lambda^2}(e^{ik\lambda} + e^{-ik\lambda} - 2) - u\Delta t$$

Using cosine identities this can be reduced to,

$$G = 1 + \frac{\Delta t D}{\lambda^2}(2cos(k\lambda) - 2) - u\Delta t$$

which is equivalent to,

$$G = 1 - 4\frac{\Delta t D}{\lambda^2}sin^2(k\lambda/2) - u\Delta t.$$

Given that Δt, D, λ, u and $sin^2(.)$ are all positive, for $|G| < 1$ we need,

$$1 - 4\frac{\Delta t D}{\lambda^2}sin^2(k\lambda/2) - u\Delta t < -1$$

and hence that,

$$\Delta t < \frac{2\lambda^2}{u\lambda^2 + 4D}.$$

In a similar manner we can obtain the stability condition in the two dimensional problem to be,

$$\Delta t < \frac{2\lambda^2}{u\lambda^2 + 8D}$$

assuming that the step in the x and y direction is of the same length λ.

5.6.3 Grid size

Solutions to a problem using periodic boundary conditions should not depend on the size of the grid we choose. Hence, the behaviour of our system should be independent of the value of N. To choose an appropriate value for N we plotted the cell density over time, starting from the same initial density ρ_0 used throughout the main text, for the upper and lower speed limits used, assuming random movement and the two types of chemotactic movement. The baseline parameters used throughout Section 5.3.2 are used here. It is evident from Fig. 5.12 that $N = 100$ is an appropriate value for the purposes of this work as we only consider densities so that $M/N^2 \geq 0.001$. The grid size only appears to have an impact when secretion and detection are mutually exclusive because then fusions are quick and the density declines faster. Even then, $N = 100$ provides an acceptable h size for densities above 0.001 (Fig. 5.12 (e) and (f)).

These results indicate why a larger value for n is necessary if we were to examine lower cell densities. For $N \leq 100$, the grid size effect becomes evident just after a cell density equivalent to $M/N^2 \approx 0.001$.

5.6.4 Tables

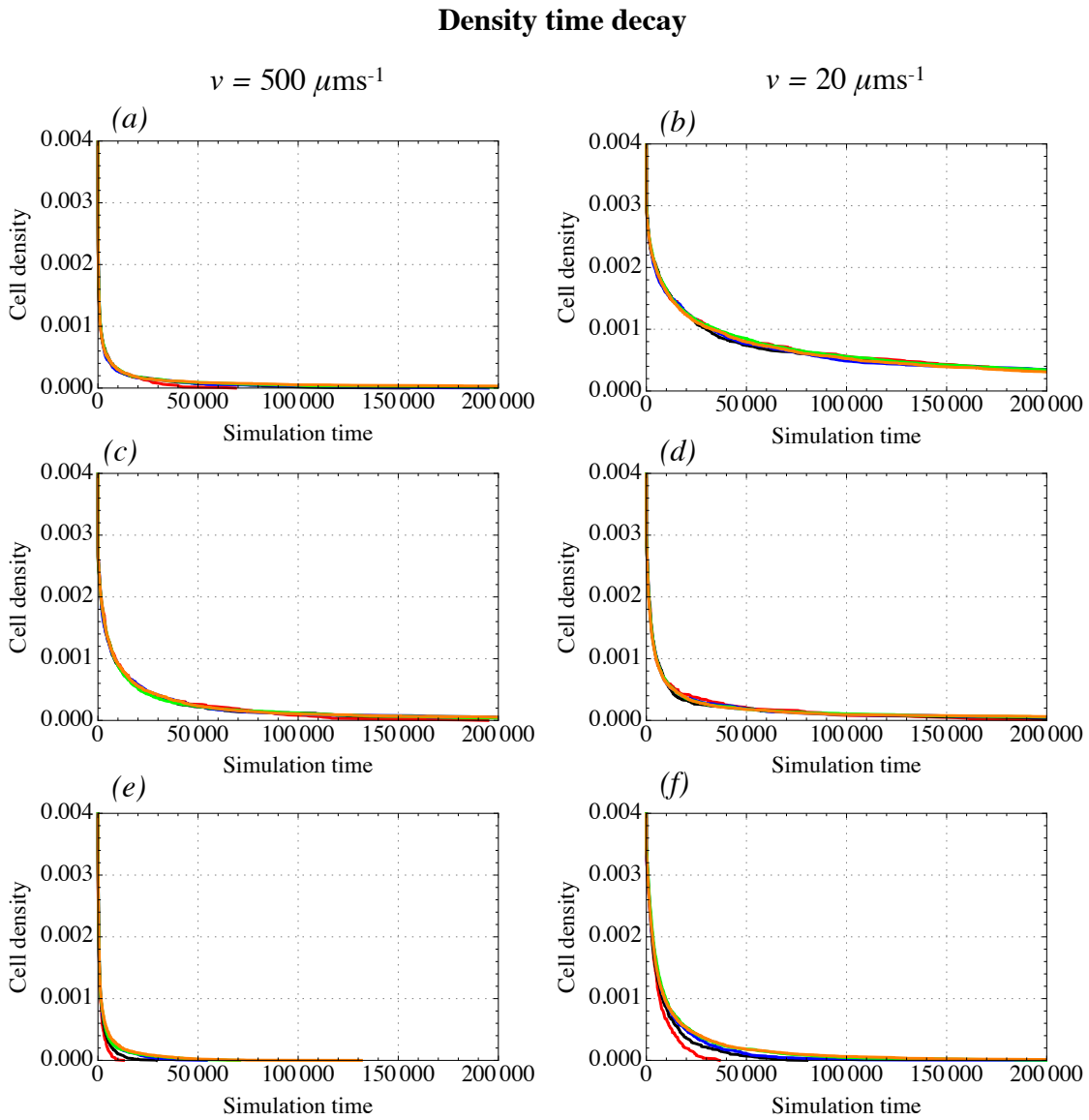

Figure 5.12: Grid size effects. Relative cell density (M/N^2) change over time for different grid size, N, for cell speed equal to $20\mu ms^{-1}$ (left hand side) and $500\mu ms^{-1}$ (right hand side).(a), (b): Random movement, (c), (d): secretion and detection simultaneous, (e), (f): mutually exclusive secretion and detection. Red: $N = 75$, Black: $N = 100$, Blue: $N = 125$, Orange: $N = 150$, Green: $N = 175$. Other parameters: d=20μm, $\gamma = 100$, $K_d = 10s$,$u = 10^{-6}$. Results are averaged over 40 simulations.

Table 5.3: Mean time until fusion for $M = 2$ for different parameter values. The mean time until fusion for two cells placed at initial distance of 40μm from one another assuming cells follow random walks (t_{rw}), both cells secrete and detect the same chemoattactant (t_s) and one cell secretes the chemoattractant but moves randomly while the second cell detects but does not secrete the chemoattactant (t_m). The mean time is listed for three different cell speeds (v_1 = 100μm s^{-1} , v_2 = 200μm s^{-1}, v_3 = 500μm s^{-1}). Baseline parameters: d= 20 μm, S/K_d = 10^{-1}, γ=100, D=5.5 10^{-10}m^{2}s^{-1}, u=10^{-8}s^{-1}, ρ_0=1.6 10^8 cells m^{-2}. Results were averages over 40 simulations.

		$(t_{rw,v_1}, t_{rw,v_2}, t_{rw,v_3})$ = (14.43, 8.20, 4.11)	
	S/K_d	$(t_{s,v_1}, t_{s,v_2}, t_{s,v_3})$	$(t_{m,v_1}, t_{m,v_2}, t_{m,v_3})$
D=5.5 10^{-10} m^2 s^{-1}	10^{-4}	(17.25, 11.41, 3.99)	(16.62, 10.14, 3.60)
γ = 100	10^{-1}	(4.37, 6.16, 12.17)	(0.85, 0.59,0.45)
	1	(3.39,3.69, 4.27)	(0.53, 0.40, 0.29)
	10	(10.96, 6.34,3.38)	(0.40, 0.30,0.25)
	10^4	(13.55, 9.82, 3.36)	(13.39, 6.96, 1.64)
	D		
S/K_d=10^{-1}	5	(49.67, 54.67, 56.38)	(3.69, 3.99, 3.08)
γ =100	20	(16.67, 24.61, 34.74)	(1.59, 1.12, 1.11)
	40	(6.62, 10.22, 19.14)	(0.97, 0.72, 0.60)
	100	(2.12, 2.37, 4.068)	(0.60, 0.48, 0.42)
	γ		
S/K_d=10^{-1}	5	(81.30, 50.29, 34.56)	(50.56, 31.09,15.97)
D =5.5 10^{-10} m^2 s^{-1}	20	(41.06, 36.86, 35.15)	(17.54,10.42,8.56)
	50	(38.60 , 40.41,53.98)	(10.16 , 7.39, 6.04)
	100	(43.47, 60.35, 121.94)	(31.22, 5.62, 4.92)
	300	(66.39, 138.36, 366.34)	(5.96 , 4.62, 3.50)

Table 5.4: The time until 75% of the cells have paired up, $t_{0.75}$, for different parameter values. We define $t_{0.75}^{rw,v_i}$, $t_{0.75}^{s,v_i}$, $t_{0.75}^{m,v_i}$ as in the main text with the v_i index indicating the cell speed: (v_1 = 100μm s^{-1} , v_2 = 200μm s^{-1}, v_3 = 500μm s^{-1}). Baseline parameters used: d= 20 μm, S/K_d = 10^{-1}, γ=100, D=5.5 10^{-10}m^{2}s^{-1}, u=10^{-8}s^{-1}, ρ_0=1.6 10^8 cells m^{-2}. Results were averaged over 40 simulations.

		$(t_{0.75}^{rw,v_1}, t_{0.75}^{rw,v_2}, t_{0.75}^{rw,v_3})$	(5.61, 3.11, 1.22)
	S/K_d	$(t_{0.75}^{s,v_1}, t_{0.75}^{s,v_2}, t_{0.75}^{s,v_3})$	$(t_{0.75}^{m,v_1}, t_{0.75}^{m,v_2}, t_{0.75}^{m,v_3})$
D=5.5 $10^{-10} m^2 s^{-1}$	10^{-4}	(6.22, 3.19, 1.22)	(16.41, 12.09, 5.05)
γ = 100	10^{-1}	(4.66, 6.31, 9.03)	(3.105, 2.25, 1.28)
	1	(4.73, 4.94, 5.85)	(2.72, 1.84,1.09)
	10	(4.39, 4.07,3.17)	(2.26,1.68, 1.13)
	10^4	(6.79, 3.49, 1.36)	(20.02, 8.09, 3.00)
	D		
S/K_d=10^{-1}	5	(63.90, 71.29, 68.60)	(9.77, 7.60, 4.07)
γ =100	20	(17.26, 21.80, 28.56)	(4.22, 3.80, 2.12)
	40	(7.68, 10.01, 12.99)	(3.52,2.43, 1.58)
	100	(2.15, 2.66, 3.14)	(2.67, 1.63, 1.07)
	γ		
S/K_d=10^{-1}	5	(4.98, 3.67, 2.79)	(8.45, 5.48, 2.96)
D =5.5 $10^{-10} m^2 s^{-1}$	20	(4.99, 4.19,3.67)	(4.29, 2.95, 1.68)
	50	(4.70, 5.22, 6.52)	(3.11, 2.40, 1.60)
	100	(4.89, 6.26, 8.89)	(2.81, 2.04, 1.36)
	300	(4.54, 7.06, 10.63)	(2.43, 1.92, 1.43)

Chapter 6

Summary, further work and conclusions

The aim of this work was to explicate the role and thereby evolution of asymmetric partner fusions that are nearly universal in sexual organisms. This is essentially the question of the evolution of sexual asymmetry, yet the evolution of male and female gametes (i.e. the evolution of anisogamy) has already received ample attention. In this thesis I focused on the evolution of morphologically identical mating types – arguably the evolution of sexual asymmetry in its most rudimentary form. Although the evolution of mating types received less consideration than that of anisogamous sexes, it is a substantial field and cannot be addressed exhaustively in a single piece of work. This thesis addressed and expanded two specific hypotheses that deal with the evolution of mating types. Naturally, I split my work into two parts. The first part dealt with the impact of mitochondrial evolution and inheritance on the evolution of mating types and sexes. The second part addressed the evolution of mating types by considering the functional role of an asymmetry in gamete-to-gamete interactions in achieving sexual reproduction. In this Chapter I summarize my findings and point towards directions for future work.

6.1 Summary and further work

6.1.1 Mitochondria in the evolution of mating types and sexes

Summary

A large body of theoretical work along with the prevalence of uniparental inheritance (UPI) of mitochondria endowed ample support to the proposition that the adaptive benefits of mating types and two sexes lie in their capacity to regulate UPI. However, *why* and *how* exactly UPI improves mitochondrial fitness was not clearly understood. Virtually all theoretical studies on the matter assumed that the merits of UPI are rooted in its capacity to restrict the spread of selfish cytoplasmic elements that are harmful to the host cell, the most common example being mutant mitochondria that are fast replicators but impair cell fitness. Some instances of such selfish mitochondrial mutations are known justifying this assumption, but are limited (Taylor et al., 2002; Hintz et al., 1988). In addition, UPI and two sexes can only evolve given the premise of restricting selfish cytoplasmic elements if the nuclear modifiers that impose UPI appear while the selfish element is at an intermediate frequency (but not at fixation), which poses a restriction (Hoekstra, 2011; Billiard et al., 2011).

In Chapter 2, I presented a model investigating an alternative hypothesis for the virtues of UPI. This new hypothesis, proposed by Lane (2006), asserts that UPI is advantageous because it helps maintain coadaptation between the nuclear and mitochondrial genomes. In fact oxidative phosphorylation, the most crucial mitochondrial function, relies on the interaction of proteins encoded by the nucleus and the mitochondria. Ample evidence across species suggests that the two genomes coevolved over time (Blier et al., 2001; Dowling et al., 2008; Burton and Barreto, 2012). Given the different tempi of evolution in the nucleus and mitochondria, how is this coevolved state to be maintained? Lane (2006) proposed that UPI is an adaptation that facilitates the evolution and maintenance of mitonuclear coadaptation. The mathematical model introduced in Chapter 2 supports this proposition. Specifically, I found that UPI in combination with tight mitochondrial bottlenecks leads to a better coadapted state, more rapidly. This is significant as it suggests that tighter control of coadpatation, and so oxidative phosphorylation, is possible under UPI. Importantly, the faster rates of evolution with UPI indicate that reaching novel coadapted mitonuclear states, for example due to environmental shifts or other external factors, is another adaptive benefit of UPI. However, at mitonuclear equilibrium the nucleus is generally stable and the mitochondria remain largely homoplasmic and so match the nucleus through continuous mutation and selection. It follows that at the coadapted equilibrium, mitochondria can be thought of as wild type (matching the nucleus) or mutant (in disagreement with the nucleus). In that sense, the coadaptation model reduces to a model of mitochondrial mutation

accumulation, albeit rooted in a different conceptual premise. The explicit consideration of mitochondrial evolution in our model indicates that UPI and bottlenecking decrease mitochondrial heteroplasmy (and so decrease within cell variation) but increase between cell variation thereby facilitating selection. Although similar conclusions were reached by Bergstrom and Pritchard (1998), most models of UPI and mating type evolution simply assumed a fixed cost for biparental inheritance without further theoretical justification.

If UPI does improve fitness, how is it to be regulated? This is where the evolution of mating types and sexes come in: self-incompatible mating types and sexes can assume distinct roles in mitochondrial inheritance in a pairwise manner, so that one partner passes on its mitochondria, and the other does not. In Chapter 3, I showed that this seemingly straightforward assertion entails considerable problems. By developing an explicit model of mitochondrial evolution and the parallel spread of UPI mutants in a biparental population, I found that although UPI improves fitness under mitochondrial mutation accumulation, selfish conflict and mitonuclear coadaptation, its spread in the population is limited. As UPI increases in the population its relative fitness advantage diminishes in a frequency dependent manner. This is because the fitness benefits of UPI 'leak' into the biparentally reproducing part of the population through successive matings, favouring some degree of UPI, but not leading to the establishment of linked mating types. This is an important finding as it demonstrates the UPI benefits can spread in a population without the necessity of strict UPI regulation. In fact, my model predicts a continuum of UPI levels depending on energetic demands, mitochondrial mutation rates and the nature of the mutants (selfish or not). In agreement with this prediction, some plants and many unicellular protists have both mating types and UPI but the two are not linked leading to a mixture of uniparental and biparental individuals (Birky, 1995; Xu, 2005).

The work presented in Chapter 3 contradicts several theoretical studies which suggested that mating types and sexes can readily spread on the premise of selection for UPI. The striking divergence of our findings to that of previous workers is due to their assumption of a fixed cost for cells carrying selfish mitochondrial mutants, independently of the residual mutational load. This assumption is not well founded, as the number of mitochonrial mutations is likely to dominate cell fitness. In fact, there is a relatively high threshold of mitochondrial mutations within a cell required to cause a significant decline in oxidative phosphorylation in mitochondrial diseases (Adkins et al., 1996). We explicitly implemented this in our model by employing a fitness curve that is a concave function of the number of mitochondria in a cell, and allowed mitochondria to evolve independently of the nucleus. The assumption of fixed fitness costs is particularly problematic in this context because mutation and adaptation occur at two levels: that of the nucleus and that of the mitochondria. Treating the latter as static fails to represent the actual population dynamics and interaction between two different levels of evolution - that of the mitochondria and that of the

nucleus.

The new perspective on the fitness benefits of UPI as a dynamic and frequency dependent feature helps us understand how mitochondrial fitness can be maintained without strict UPI, and explain the complex patterns of mitochondrial inheritance seen in unicellular protists and some plants, whereby a mixture of uniparental and biparental offspring or UPI without strict linkage to mating types is often the case (Birky, 1995; Xu, 2005). In addition, preexisting mating types and high mutation rates or high energetic demands in our model could lead to strict UPI. In contradicting previous work, our findings challenge the postulation that the evolution of mating types and sexes can be understood solely as a consequence for selection for UPI.

Further work

The work presented in Chapters 2 and 3 deepens our understanding of UPI, its evolution and its relevance to the evolution of mating types. It was possible to relate this work to a range of natural observations, and to achieve a solid understanding of the models in Chapters 2 and 3, more can be done.

The mitonuclear co-adaptation model developed in Chapter 2 assumed that nuclear genes are additive in their effect on mitochondria and that both alleles considered are equally active. Naturally, one expects that the mitochondrial population interacts with both nuclear genes. One possibility not considered, however, is that of imprinting or epistatic effects but no evidence supporting either of these is available. Further experimental work examining this question would be valuable. Theoretically, different possibilities can be examined by defining fitness as a function of the degree of mitochondrial matching against one of the nuclear genes chosen at random, the nuclear gene that better matches the mitochondria or the nuclear gene that matches the mitochondria the least. I do not expect these modifications to qualitatively change our findings. The capacity of UPI to decrease heteroplasmy while increasing between cell variation remains, and would presumably promote faster evolution towards a fitter mitonuclear state for uniparental rather than biparental zygotes, independent of the different fitness definitions. An additional issue appears when dealing with multiple loci in the mitochondria, interacting with multiple loci in the nucleus. Given our results in Chapter 2, I anticipate that the nuclear loci converge towards a homozygous state with the mitochondria loci in agreement with the corresponding nuclear loci. The dynamics of such a system could encompass unforeseen complexities, however, which can only be examined directly.

Further work pertinent to Chapter 3 needs to examine the significance of UPI and its evolutionary dynamics in multicellular organisms, where maternal inheritance of mitochondria is the rule. Indeed, a different picture emerges when one considers complex multicellular organisms, where the impact of con-

tinuous mitochondrial segregation during developmental growth can generate somatic tissues with a high mutational load. It follows that our findings do not simply map onto complex multicellular organisms. Vegetative segregation entails stochasticity, and mitochondria are not equally divided between the two daughter cells during mitosis. It follows that a small number of mutations in the zygote can lead to a large mutational load in certain tissues, which could be detrimental for the organism if the tissues with compromised mitochondrial performance have high energetic demands (Jenuth et al., 1997; Lane, 2012). UPI in conjunction with a tight bottleneck has been shown to reduce heteroplasmy in the zygote (Bergstrom and Pritchard, 1998; Hadjivasiliou et al., 2012) and could presumably maintain highly fit mitochondria throughout developmental growth. Even this seemingly simple prediction is not so straightforward, however. For example, the association between the zygote's initial fitness and the fitness of the mature organism is expected to change in a non-trivial manner, and the interaction between different sources of variation (vegetative segregation, UPI, bottleneck) is likely to be multiplex. In addition, 'leakage' of improved mitochondria while UPI mutants spread, like that identified in unicellular organisms, is likely to introduce further intricacies changing the relative adaptive benefits of UPI and bottleneck mutants during their evolution. The focus of future work on this matter should be the evolution of UPI in a multicellular population with pre-existing mating types. The following issues can then be addressed:

1. Assuming two mating types with mitochondria inherited biparentally, do UPI mutants spread and do they become linked to genes that determine mating type?

2. Assuming that UPI and two mating types already exist but are not linked, does linkage (and so strict UPI) evolve?

3. Is there a link between the evolution of UPI and the evolution of anisogamy? For example, is a tight bottleneck linked with UPI favourable and when does it spread to fixation?

4. How does the mitonuclear coadaptation model change in the context of multicellularity? Can mitotic divisions and mitochondrial segregation during development lead to badly coadapted states given a relatively fit zygote?

Some of these questions are being addressed in ongoing work, the initial stages of which I was involved in. Current findings suggest that UPI with a tight bottleneck, pointing towards anisogamy, can evolve more readily in multicellular organisms that have high mitochondrial mutation rates and high energetic demands (article in preparation).

6.1.2 Signalling in the evolution of mating types and sexes

Summary

Rolf Hoekstra is arguably the most prolific of all workers who addressed the evolution of sexes and mating types (Hoekstra, 1982; Hoekstra et al., 1984; Hoekstra, 1984, 1987, 1990a; Hoekstra et al., 1991; Hoekstra, 2000; Czaran and Hoekstra, 2004). In 1982 he developed an evolutionary model proposing that an asymmetry in the communication between gametes leads to more effective mating, underscoring the evolution of mating types (Hoekstra, 1982). This theory asserts that two mating types, producing mating-type-specific pheromones and receptors, improve gamete recognition and attraction. This proposition received little recognition and no further theoretical or experimental work was carried out to address it. Hoekstra himself was sceptical about his theory. In later work (Hoekstra et al., 1984), he says:

Particularly intriguing is the problem of the evolution of two mating types from an original situation in which all (iso-) gametes are alike functionally. This problem has been analyzed by Hoekstra (1982), who shows that it is difficult to explain. Evolution towards unipolarity in gamete recognition or adhesion (implying the existence of two mating types) requires a more than twofold disadvantage for bipolarity, However, the evolution of mating types is much more likely when a pheromonal attraction mechanism is already present, but such pheromonal systems seem to be rare among isogamous algae.

In the second part of this thesis I re-examine the role of cell-cell communication during sex and its potential impact on the evolution of mating types, focusing on the effect of self-signalling (generating and responding to the same signal) throughout gamete interactions that are crucial for sex. My work shows that pheromonal systems of attraction, not only are not rare in isogamous species, but are nearly universal. I further developed an explicit theoretical model that quantified the disadvantage for bipolarity, showing that it can be more severe than initially thought, and that 'a more than twofold disadvantage' is not unlikely.

In Chapter 4, I reviewed the literature of cell-cell interactions during sex across protists from all eukaryotic groups. My review indicated that different mating types employ complementary but different signals and receptors in a mating-type-specific manner. The ubiquity of this finding across eukaryotic taxa despite a remarkable diversity in life cycle and mode of sexual reproduction, suggests that there is a functional significance to an asymmetry in gamete communication, and support Hoekstra's proposition and predictions as well as my ideas (Hoekstra, 1982). Hoekstra focused on receptor saturation as the primary cause of the evolution of mating types. In Chapter 4, I expanded his ideas by proposing that generating and detecting the same signal not only causes receptor saturation, compromising external signals from potential partners, but more importantly, it can cause self-excitation. The capacity of self-released molecules to bind to the

secreting cell's own receptors prohibit a clear distinction between the cell's own and an external signal, thereby impairing the ability of mating cells to attract one another (sexual chemotaxis), to detect the presence of a potential partner and to coordinate adhesion and fusion. The employment of complementary but opposite signals by different mating types throughout mating, and the obstruction of sexual reproduction in the absence of an asymmetry in signalling reported in experiments (e.g. Afon'Kin (1991)), support these suggestions. These considerations led me to endorse the proposition that the evolution of mating types has its roots in the adaptive benefits of asymmetric gamete interactions. Nonetheless, further study of the physiochemical constraints cells experience during sex is necessary before placing this postulation in an evolutionary context.

In Chapter 5 I developed a biophysical model to assess the hypothesis that mating types with the capacity for asymmetric signalling improves mating, focusing on partner attraction. My model was based on chemical secretion and diffusion, explicitly considered individual cell movement and was examined subject to three different assumptions. First, random cell movement, second, chemotactic cell movement with all cells generating and responding to the same chemoattractant, and third, half of the cells following random walks while generating a chemical signal and the remaining cells moving chemotactically without themselves secreting the relevant chemoattractant. By measuring the time until a certain number of cell-pairs form in the three different scenarios I assessed first, when and to what degree chemotaxis improves mate finding, and second whether two self-incompatible mating types with specialised chemotactic roles can confer an improvement in mating efficiency. My findings indicated that unless cells move at very high speeds or live at very high densities, chemotaxis confers an advantage in partner finding. Secretion and detection of the same chemoattractant, however, can cause a severe movement impediment, hindering the efficiency of chemotaxis. On the other hand, mutually exclusive roles in signal generation and detection resulted in faster pair formation even when cells conferring the same roles could not pair with one another. This was true for a range of physiological parameters reflecting the physicality of small protists. My findings led me to the conclusion that the movement impediment due to self-secretion, first alluded to by Hoekstra (1982), is justifiable and can be severe. More importantly, the improvement in pair formation efficiency subject to two mating types secreting or detecting the chemoattractant, but not both, was often significant enough to overcome the disadvantage that emerges when gametes can only mate with half of the population. These findings are important as they identify and evaluate what causes a movement impediment when gametes simultaneously secrete and sense the same chemical, beyond receptor saturation. Furthermore, one of the main limitations in Hoektra's work was the lack of an explicit quantification of the costs that self-secretion may cause. Although the findings of Chapter 5 were not placed in an evolutionary context,

they provide grounds for a detailed evolutionary model where the physiochemical constraints encountered by gametes directly define mating success (rather than an abstract fitness coefficient). Admittedly, however, an evolutionary model will encompass unforeseen complexities. I discuss extensions to this work and the development of an evolutionary model below.

Future work

The work presented in Chapters 4 and 5 opens up new directions for understanding the function and evolution of mating types, both experimental and theoretical. The assertion that mating types resolve the apparent inevitability of receptor saturation and self-stimulation in a single signal-receptor system, and that therein lie their adaptive advantages, requires some further theoretical and empirical justification. For example, an obvious question that emerges is that of other possible mechanisms that could avoid receptor saturation and self-stimulation in a single signal-receptor system, without the need for mating types. A further look at the literature, searching for possible means employed by eukaryotic cells to distinguish their own chemical signal from external ones, is important. For example, somatic cells of the filamentous ascomycete *Neurosporra crassa*, use a single chemoattractant and cognate receptor pair to grow towards one another and form a network of cells. Somatic cells avoid self-stimulation by periodically sending and receiving the chemoattractant in a pairwise manner (Goryachev et al., 2012). Why couldn't gametes adopt a similar strategy, where they generate their signals periodically? One possible problem is that periodicity impairs the strength of the signal and the sharpness of the response, particularly so in motile species (*N. crassa* are non-motile). In addition, when the primary purpose of the signal is to indicate readiness to mate or coordinate fusion, it is not clear how periodicity could resolve the issue of self-stimulation and receptor saturation without compromising gamete interactions (e.g. opposite mating types often coordinate fusion by regulating the amount of pheromone they release). These issues can be addressed theoretically using mathematical modeling that quantifies the efficiency of gamete interactions under different scenarios, or experimentally by directly contrasting the efficacy of homothallic and heterothallic mating or using organisms such as yeast or *Chalmydomonas* where the mating type and pheromone/receptor loci can be easily manipulated.

The work in Chapter 5, provides rigorous justification for the assumption that secreting and detecting the same chemoattractant impairs partner attraction. Furthermore, it quantifies the pertinent costs and provides grounds for an explicit evolutionary model. Nonetheless, further work is necessary. Firstly, an extensive phase-space analysis considering all parameter ranges relevant to microorganisms is important to relate my findings across unicellular protist. More importantly, an evolutionary aspect needs to be incorporated in the model. This can be done by assuming that secretion and detection of a chemoattractant are genetically

determined. Consider for example two loci so that aa and bb indicate a non-signaller and non-searcher respectively, Aa or AA and Bb or BB indicate a signaller and a searcher (assuming that signalling and searching are both dominant genes for the sake of simplicity). Given the findings of Chapter 5, I anticipate that the evolution of tight linkage between the two loci so that the population ends at a state with 50% AAbb and 50% aaBB, would be favoured. However, several issues are likely to emerge in an evolutionary model. As discussed in Chapter 5, the adaptive benefits of being a searcher will likely depend on the frequency of signallers in the population and vice versa. In addition, in a situation where the population consists of nearly 50% searchers (aaBB) and 50% signallers (AAbb), it is not obvious why should some signallers not evolve the capacity to also search (favour AABB mutants). Although the findings in Chapter 5 indicate that such cells would be bad searchers, it is not obvious why their signalling capacity should be compromised should they also 'search' (by which I mean, become able to fuse with other signalers). If the ability to search comes at any cost (e.g. energetic), however, a significant advantage would be necessary for a signaller to also evolve the ability to search, something that the model in Chapter 5 disputes. These trade-offs will be examined in an evolutionary model in follow-up work.

6.2 Some further issues

My focus in this thesis has been the evolution of mating types in isogamous species. Questions that naturally arise from this work, but that I have not explicitly addressed, primarily concern the determination of the number of mating types, and the link between isogamous mating types and anisogamous sexes. These are complex matters that deserve a substantial attention and work in their own right. Here I restrict myself to briefly discussing the relevance of my findings and conclusions regarding the evolution of mating types to the highly variable number of mating types across protists, and to the evolution of anisogamy.

6.2.1 The number of mating types

The number of mating types varies greatly in isogamous protists, ranging from two in most species, up to several thousand in some basidiomycetes. What determines the number of mating types is perhaps as great a conundrum as the forces that drove the evolution of mating types in the first place. If the primary function of mating types is indeed to coordinate partner attraction, recognition and fusion as postulated in the second part of this thesis, then the mating system itself is likely to determine the number of mating types. This is because the mating system defines the essential degree of specificity in gamete communication throughout sex. For example, a very large number of mating types is most common in basidiomycetes

where only nuclear, and not cellular, fusion is mating-type-specific. The most extensively studied case is arguably that of *S. commune* having more than 20000 mating types. Specific pairs of multiple pheromones and receptors determine mating type identity and coordinate pairwise nuclear migration and fusion, following hyphae fusion that is itself non-mating-type-specific (Fowler and Vaillancourt, 2007). The shift of mating-type-function from the cellular to the nuclear level could reduce the degree of specificity required for the communication between mating partners, thereby tolerating more variability in mating-type-specific mechanisms. Likewise, multiple mating types are often the case in ciliates where gametes conjugate and exchange nuclei, but do not fuse (Phadke and Zufall, 2009). In contrast, algae that are highly motile, utilize chemotaxis and undergo pairwise cellular fusions prior to nuclear fusion have, as a rule, only two mating types.

The possible connection between the number of mating types and the requirement for asymmetric gamete communications calls for further examination however, and the work carried out in this thesis does not appreciably cover this matter. Other factors such as genetic constraints could regulate the number of mating types and there is a considerable body of work dealing with the determination of the number of mating types (Billiard et al., 2011).

6.2.2 From mating types to sexes

Throughout this work, it became increasingly apparent to me that a distinction between mating types and sexes is not easy to assert. The definition of sexes itself becomes bleary when one is dealing with unicellular organisms that are not necessarily oogamous. In the case of oogamy, the egg and sperm define the female and male gametes respectively. Furthermore, in anisogamous species the female is defined to be the larger or less motile gamete, whereas the male is the smaller and more motile gamete. There exists, however, a continuum in anisogamous species and the degree of anisogamy can be significantly reduced to the point where only a slight size difference between the two ‘sexes’ is present. This apparent continuity is perhaps an indication that, at least in some species, anisogamy is derived from isogamy. As I briefly discussed in Chapter 5, many authors have examined the evolution of anisogamy from an initially isogamous population with two mating types. The a priori presence of chemotaxis utilized for partner attraction was shown to facilitate the evolution of anisogamy in multiple different modeling contexts, whereby the mating type that secretes the chemoattractant becomes large and highly immotile, whereas the mating type that responds to the chemoattractant benefits from being small and highly motile (Hoekstra, 1984; Cox and Sethian, 1985; Dusenbery, 2000, 2006). In most isogamous species, however, both (or all) mating types possess a mating-type-specific pheromone *and* a mating-type-specific receptor. In that sense, mating-type-specific functions

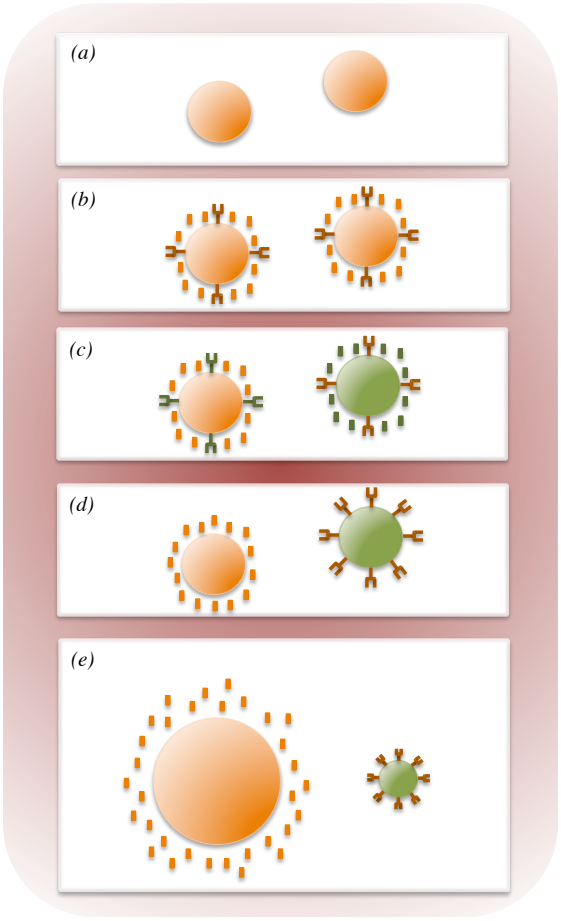

Figure 6.1: Possible evolutionary transitions leading to mating types and sexes. Circles represent gametes, shown with their secreted pheromones and receptors. (*a*): In the absence of a species-specific signal/receptor pair, communication between gametes and fusion is not feasible. (*b*): The use of a single pheromone-receptor pair for gamete communications can facilitate gamete communication, but is problematic as it leads to receptor saturation and can cause self-excitation (Chapters 4 and 5). (*c*): The evolution of mating-type-specific pheromones and receptors so that mating gametes generate and respond to different signals, prevents receptor saturation and the risk of self-excitation. (*d*): Different mating types are restricted to either secreting of detecting a pheromone, but not both, through disruptive selection. This can increase the degree of specificity in gamete interactions as more resources can be used for only generating or responding to a signal. (*e*): The pheromone-producing mating type becomes larger (and less motile), maximizing its probability of being found and optimizing the resources put into secreting. The pheromone-searcher mating type becomes smaller (and more motile), optimizing its ability to search.

are present but in a somewhat 'symmetric' manner. This does not provide the necessary grounds for the evolution of anisogamy as in the models of Hoekstra (1984); Cox and Sethian (1985); Dusenbery (2000, 2006) and could perhaps justify the persistence of isogamous mating types. Only when the chemotactic functions of opposite mating types become polarized so that one type solely secretes and one responds to a pertinent chemoattractant do the conclusions drawn by Hoekstra (1984); Cox and Sethian (1985); Dusenbery (2000, 2006) readily follow (Fig. 6.1).

A further complexity is that some protists maintain both mating types and 'sexes'. To my knowledge, this is only true in some filamentous fungi and ciliates where fertilization only entails nuclear, but not cellular, fusions. In these cases, 'female' and 'male' signify a distinction in the size and behaviour of the fusing nuclei so that female nuclei are larger and immotile and male nuclei are smaller and motile, migrating towards the female nucleus. Fusions only occur between female and male nuclei of different mating type, and mating-type-specific functions are still in operation, for example coordinating conjugation in ciliates (Phadke and Zufall, 2009). Why 'sexes' and mating types are not linked in these cases remains puzzling. The presence of both sexes and mating types, however, suggests that the role of 'sexes' is not necessarily the same as that of mating types, despite the recurrent concurrence of the two. We can speculate that in these instances the benefits of sexes lie in the division of the two mating nuclei into stationary/migratory or larger/smaller although this requires further examination. Finally, note that when mating types and sexes become linked, inevitably so do their functions. Therefore, functions that were primarily mating-type-related may appear to be sex-related (the reverse argument is unlikely to hold as the evolution from anisogamy back to isogamy is highly improbable).

6.3 Conclusion

The nearly ubiquitous asymmetry in gamete fusions across sexual species, i.e. the existence of mating types and sexes, was the motivation of this thesis. Unlike previous research, my work indicated that uniparental inheritance of mitochondria, although a powerful mechanism, cannot alone explain the evolution of mating types and sexes. Furthermore, my survey of the literature and theoretical investigation led me to assert that the primary function and adaptive benefits of mating types lie in their capacity to regulate asymmetric communication throughout gamete interactions that are fundamental to sexual reproduction.

The existence of females and males across complex organisms encompasses a range of remarkable consequences, from the evolution of sex-specific roles in insects (Blum, 2012), to the complex interactions and behavioural distinctions between males and females in animals and humans. Where do the origins of this profound division lie? Once one looks closer, she finds that this distinction has deep roots, and that

what appear to be the simplest of eukaryotic organisms also necessitate a sexual asymmetry. To answer the question of the evolution of the sexes is an immense task that a single piece of work could not possibly resolve. I hope, however, that the work carried out towards this thesis forms a step forward in our attempts to understand the evolution of mating types and sexes, pointing towards new challenges and directions for thought and study in this field.

Bibliography

Abe, K., Kusaka, I., and Fukui, S. (1975). Morphological change in the early stages of the mating process of *Rhodosporidium toruloides*. *J. Bacteriol.*, 122(2):710–718.

Adkins, R. M., Honeycutt, R. L., and Disotell, T. R. (1996). Evolution of eutherian cytochrome C oxidase subunit II: Heterogeneous rates of protein evolution and altered interaction with cytochrome C. *Mol. Biol. Evol.*, 13(10):1393–1404.

Afon'Kin, S. (1991). Cell-cell recognition in *Dileptus*. The dynamics of homo- and heterotypic pair formation during conjugation. *Acta Protozool.*, 30:93–98.

Akada, R. (1985a). Mating types and mating-inducing factors (gamones) in the ciliate *Euplotes patella* syngen 2. *Genet. Res.*, 46(2):125–132.

Akada, R. (1985b). Role of soluble factors (gamones) and calcium ions in conjugation of the ciliate *Euplotes patella* syngen 2. *J. Exp. Zool.*, 233(2):169–173.

Aoyama, H., Hagiwara, Y., Misumi, O., Kuroiwa, T., and Nakamura, S. (2006). Complete elimination of maternal mitochondrial DNA during meiosis resulting in the paternal inheritance of the mitochondrial genome in *Chlamydomonas* species. *Protoplasma*, 228(4):231–242.

Bakkeren, G., Kamper, J., and Schirawski, J. (2008). Sex in smut fungi: Structure, function and evolution of mating-type complexes. *Fungal Genet. Biol.*, 45(Suppl 1):S15–21.

Banuett, F. (1995). Genetics of *Ustilago maydis*, a fungal pathogen that induces tumors in maize. *Annu. Rev. Genet.*, 29:179–208.

Banuett, F. (1998). Signalling in the yeasts: an informational cascade with links to the filamentous fungi. *Microbiol. Mol. Biol. Rev.*, 62(2):249–274.

Beatty, N. P., Smith, M. L., and Louise Glass, N. (1994). Molecular characterization of mating-type loci in selected homothallic species of *Neurospora*, *Gelasinospora* and *Anixiella*. *Mycol. Res.*, 98(11):1309–1316.

Bell, G. (1978). The evolution of anisogamy. *J. Theor. Biol.*, 73(2):247–270.

Bell, G. (1982). *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. Univ of California Press, Berkeley.

Bell, G. (1993). The sexual nature of the eukaryote genome. *J. Hered.*, 84(5):351–359.

Bergstrom, C. T. and Pritchard, J. (1998). Germline bottlenecks and the evolutionary maintenance of mitochondrial genomes. *Genetics*, 149:2135–2146.

Billiard, S., López-Villavicencio, M., Hood, M. E., and Giraud, T. (2012). Sex, outcrossing and mating types: unsolved questions in fungi and beyond. *J. Evol. Biol.*, 25(6):1020–1038.

Billiard, S., Villavicencio, M. L., and Devier, B. (2011). Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. *Biol. Rev.*, 86(2):421–442.

Birky, C. W. (1995). Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proc. Natl. Acad. Sci. USA*, 92(25):11331–11338.

Birky, C. W. (2001). The inheritance of genes in mitochondria and chloroplasts : Laws, mechanisms and models. *Annu. Rev. Genet.*, 35:125–158.

Bistis, G. N. (1996). Trichogynes and fertilization in uni- and bimating type colonies of *Neurospora tetrasperma*. *Fungal Genet. Biol.*, 20(1):93–98.

Bistis, G. N. (1998). Physiological heterothallism and sexuality in Euascomycetes: A partial history. *Fungal Genet. Biol.*, 23(3):213–222.

Blakeslee, A. F. (1904). Sexual reproduction in the Mucorineae. *Proc. Natl. Acad. Sci. USA*, 40:205–319.

Blank, R., Hauptmann, E., and Arnold, C. G. (1980). Variability of mitochondrial population in *Chlamydomonas reinhardii*. *Planta*, 150(3):236–241.

Blier, P. U., Dufresne, F., and Burton, R. S. (2001). Natural selection and the evolution of mtDNA-encoded peptides: evidence for intergenomic co-adaptation. *Trends Genet.*, 17(7):400–406.

Blum, M. (2012). *Sexual Selection and Reproductive Competition in Insects*. Elsevier, London.

Bolker, M. and Kahmann, R. (1993). Sexual pheromones and mating responses in fungi. *Plant Cell*, 5(10):1461–1469.

Bonner, J. T. and Savage, L. J. (1947). Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold *Dictyostelium discoideum*. *J. Exp. Zool.*, 106(1):1–26.

Brandham, P. E. (1967). Time-lapse studies of conjugation in *Cosmarium botrytis* . *Can. J. Bot.*, 45(4):483–497.

Brawley, S. H. and Johnson, L. E. (1992). Gametogenesis, gametes and zygotes: An ecological perspective on sexual reproduction in the algae. *Br. Phycol. J.*, 27(3):233–252.

Bulmer, M. G. and Parker, G. A. (2002). The evolution of anisogamy: a game-theoretic approach. *Proc. Biol. Sci.*, 269(1507):2381–2388.

Burton, R. S. and Barreto, F. S. (2012). A disproportionate role for mtDNA in Dobzhansky-Muller incompatibilities? *Mol. Ecol.*, 21(20):4942–4957.

Cappellaro, C., Hauser, K., Mrsa, V., Watzele, M., Watzele, G., Gruber, C., and Tanner, W. (1991). *Saccharomyces cerevisiae* a-and alpha-agglutinin: characterization of their molecular interaction. *EMBO J.*, 10(13):4081–4088.

Casselton, L. A. (2002). Mate recognition in fungi. *Heredity*, 88(2):142–147.

Charlesworth, D. and Charlesworth, B. (1979). The evolution and breakdown of S-allele systems. *Heredity*, 43(1):41–55.

Chepurnov, V. a., Mann, D. G., Sabbe, K., and Vyverman, W. (2004). Experimental studies on sexual reproduction in diatoms. *Int. Rev. Cytol.*, 237:91–154.

Cisar, C. R. and TeBeest, D. O. (1999). Mating system of the filamentous ascomycete, *Glomerella cingulata*. *Curr. Genet.*, 35(2):127–133.

Cockerham, C. C., Burrows, P. M., Young, S. S., and Prout, T. (1972). Frequency-dependent selection in randomly mating populations. *Am. Nat.*, 106(950):493–515.

Coesel, P. F. M. and de Jong, W. (1986). Vigorous chemotactic attraction as a sexual response in *Closterium ehrenbergii* Meneghini (Desmidiaceae, Chlorophyta). *Phycologia*, 25(3):405–408.

Cohen, L. W. and Siegel, R. W. (1963). The mating-type substances of *Paramecium bursaria*. *Genet. Res.*, 4(01):143.

Coppin, E. and Debuchy, R. (2000). Co-expression of the mating-type genes involved in internuclear recognition is lethal in *Podospora anserina. Genetics*, 155(2):657–669.

Coppin, E., Debuchy, R., Arnaise, S., and Picard, M. (1997). Mating types and sexual development in filamentous ascomycetes. *Microbiol. Mol. Biol. Rev.*, 61(4):411–428.

Coria, R., Kawasaki, L., Torres-Quiroz, F., Ongay-Larios, L., Sánchez Paredes, E., Velázquez Zavala, N., Navarro-Olmos, R., Rodríguez González, M., Aguilar Corachán, R., and Coello, G. (2006). The pheromone response pathway of *Kluyveromyces lactis*. *FEMS Yeast Res.*, 6(3):336–344.

Cosmides, L. M. and Tooby, J. (1981). Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.*, 89:83–129.

Cox, P. A. and Sethian, J. A. (1985). Gamete motion, search, and the evolution of anisogamy, oogamy, and chemotaxis. *Am. Nat.*, 125(1):74–101.

Crandall, M. a. and Brock, T. D. (1968). Molecular basis of mating in the yeast *Hansenula wingei*. *Bacteriol. Rev.*, 32(3):139–163.

Crnokrak, P. and Roff, D. A. (1999). Inbreeding depression in the wild. *Heredity*, 83(3):260–270.

Czaran, T. L. and Hoekstra, R. F. (2004). Evolution of sexual asymmetry. *BMC Evol. Biol.*, 4(1):34.

Dalgaard, J. Z. and Klar, A. J. (1999). Orientation of DNA replication establishes mating-type switching pattern in *S. pombe*. *Nature*, 400(6740):181–184.

Daniels, E. W. and Breyer, E. P. (1968). Starvation effects on the ultrastructure of amoeba mitochondria. *Zeitschrift fr Zellforsch. und Mikroskopische Anat.*, 91(2):159–169.

Demets, R., Tomson, A. M., Stegwee, D., and Ende, H. V. D. (1990). Cell-cell coordination in conjugating *Chlamydomonas* gametes. *Protoplasma*, 155:188–199.

Dini, F. and Luporini, P. (1985). Mating-type polymorphic variation in *Euplotes minuta* (Ciliophora: Hypotrichida) . *J. Euk. Microbiol.*, 32(1):111–117.

Dorer, R., Pryciak, P. M., and Hartwell, L. H. (1995). *Saccharomyces cerevisiae* cells execute a default pathway to select a mate in the absence of pheromone gradients. *J. Cell Biol.*, 131(4):845–861.

Dowling, D. K., Friberg, U., and Lindell, J. (2008). Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends Ecol Evol*, 23(10):546–554.

Dusenbery, D. B. (2000). Selection for high gamete encounter rates explains the success of male and female mating types. *J. Theor. Biol.*, 202(1):1–10.

Dusenbery, D. B. (2006). Selection for high gamete encounter rates explains the evolution of anisogamy using plausible assumptions about size relationships of swimming speed and duration. *J. Theor. Biol.*, 241(1):33–38.

Eberhard, W. G. (1980). Evolutionary consequences of intracellular organelle competition. *Q. Rev. Biol.*, 55(3):231–249.

Ebersold, W. T. (1967). *Chlamydomonas reinhardi*: Heterozygous diploid strains. *Science*, 157(3787):447–449.

Edwards, A. (2008). GH Hardy (1908) and Hardy–Weinberg Equilibrium. *Genetics*, 179(3):1143–1150.

Esser, C., Ahmadinejad, N., Wiegand, C., Rotte, C., Sebastiani, F., Gelius-Dietrich, G., Henze, K., Kretschmann, E., Richly, E., Leister, D., Bryant, D., Steel, M. A., Lockhart, P. J., Penny, D., and Martin, W. (2004). A genome phylogeny for mitochondria among alpha-proteobacteria and a predominantly eubacterial ancestry of yeast nuclear genes. *Mol. Biol. Evol.*, 21(9):1643–1660.

Fleissner, A. e., Simonin, A. R., and Glass, N. L. (2008). Cell fusion in the filamentous fungus, *Neurospora crassa*. *Methods Mol. Biol.*, 475:21–38.

Fowler, T. J. and Vaillancourt, L. J. (2007). Pheromones and pheromone receptors in *Schizophyllum commune* mate recognition: Retrospective of a half-century of progress and a look ahead. In Heitman, J., Kronstad, J. W., and Casselton, L. A., editors, *Sex in Fungi: Molecular Determination and Evolutionary Implications*, pages 301–315. ASM Press, Washington D.C.

Fukumoto, R., Fujii, T., and Sekimoto, H. (1998). A newly identified chemotactic sexual pheromone from *Closterium ehrenbergii*. *Sex. Plant Reprod.*, 11(2):81–85.

Fukumoto, R.-h., Fujii, T., and Sekimoto, H. (1997). Detection and evaluation of a novel sexual pheromone that induces sexual cell division of *Closterium ehrenbergii* (Chlorophyta). *J. Phycol.*, 33(3):441–445.

Fuller, D., Chen, W., Adler, M., Groisman, A., Levine, H., Rappel, W.-J., and Loomis, W. F. (2010). External and internal constraints on eukaryotic chemotaxis. *Proc. Natl. Acad. Sci. USA*, 107(21):9656–9659.

Galloway, R. E. and Goodenough, U. W. (1985). Genetic analysis of mating locus linked mutations in *Chlamydomonas reinhardii*. *Genetics*, 111(3):447–461.

Geitler, L. (1935). Reproduction and life history in diatoms. *Bot. Rev.*, 1(5):149–161.

Gillard, J., Frenkel, J., Devos, V., Sabbe, K., Paul, C., Rempt, M., Inz e, D., Pohnert, G., Vuylsteke, M., and Vyverman, W. (2013). Metabolomics enables the structure elucidation of a diatom sex pheromone. *Angew. Chem. Int. Ed. Engl.*, 52(3):854–857.

Glass, N. L., Metzenberg, R. L., and Raju, N. B. (1990). Homothallic *Sordariaceae* from nature: The absence of strains containing only the a mating type sequence. *Exp. Mycol.*, 14(3):274–289.

Glass, N. L. and Smith, M. L. (1994). Structure and function of a mating-type gene from the homothallic species *Neurospora africana*. *Mol. Gen. Genet.*, 244(4):401–409.

Glass, N. L., Vollmer, S. J., Staben, C., Grotelueschen, J., Metzenberg, R. L., and Yanofsky, C. (1988). DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. *Science*, 241(4865):570–573.

Goodenough, U., Lin, H., and Lee, J.-H. (2007). Sex determination in Chlamydomonas. *Semin. Cell Dev. Biol.*, 18(3):350–361.

Goodenough, U. W., Armbrust, E. V., Campbell, A. M., and Ferris, P. J. (1995). Molecular genetics of sexuality in Chlamydomonas. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 46(1):21–44.

Goodenough, U. W. and Weiss, R. L. (1975). Gametic differentiation in *Chlamydomonas reinhardtii*. III. Cell wall lysis and microfilament-associated mating structure activation in wild-type and mutant strains. *J. Cell Biol.*, 67(3):623–637.

Goryachev, A. B., Lichius, A., Wright, G. D., and Read, N. D. (2012). Excitable behavior can explain the 'ping-pong' mode of communication between cells using the same chemoattractant. *BioEssays*, 34(4):259–266.

Goutelle, S., Maurin, M., and Rougier, F. (2008). The Hill equation: a review of its capabilities in pharmacological modelling. *Fundam. Clin. Pharmacol.*, 22(3):633–648.

Gray, M. W., Burger, G., and Lang, B. F. (1999). Mitochondrial evolution. *Science*, 283(5407):1476–1481.

Grell, K. G. (1973). *Protozoology.* Springer, Berlin.

Haag, E. S. (2007). Why two sexes? Sex determination in multicellular organisms and protistan mating types. *Semin. Cell Dev. Biol.*, 18(3):348–349.

Hadjivasiliou, Z., Lane, N., Seymour, R. M., and Pomiankowski, A. (2013). Dynamics of mitochondrial inheritance in the evolution of binary mating types and two sexes. *Proc. Biol. Sci.*, 280(1769):20131920.

Hadjivasiliou, Z., Pomiankowski, A., Seymour, R. M., and Lane, N. (2012). Selection for mitonuclear co-adaptation could favour the evolution of two sexes. *Proc. Biol. Sci.*, 279(1734):1865–1872.

Hammerstein, P. and Parker, G. A. (1987). Sexual selection: games between the sexes. In Bradbury, J. W. and Anderson, M. B., editors, *Sexual Selection: Testing the Alternatives*, pages 119–142. Wiley, Chichester, UK.

Harrington, T. C. and McNew, D. L. (1997). Self-fertility and uni-directional mating-type switching in *Ceratocystis coerulescens*, a filamentous ascomycete. *Curr. Genet.*, 32(1):52–59.

Harris, E. H. (2008). *The Chlamydomonas Sourcebook, Vol. 1: Introduction to Chlamydomonas and Its Laboratory Use*. Academic Press, San Diego.

Hartl, D. L. and Clark, a. G. (1997). *Principles of Population Genetics*. Sinauer Associates Incorporated, Sunderland.

Hartmann, M. (1955). Sex problems in algae, fungi and protozoa: A critical account following the review of R. A. Lewin. *Am. Nat.*, 89(849):321–346.

Hastings, I. M. (1992). Population genetic aspects of deleterious cytoplasmic genomes and their effect on the evolution of sexual reproduction. *Genet. Res.*, 59(3):215–225.

Hauswirth, W. W. and Laipis, P. J. (1985). Transmission genetics of mammalian mitochondria: a model and experimental evidence. In Quagliariello, E., Slater, E. C., Palmieri, F., Saccone, C., and Kroon, A. M., editors, *Achievements and perspectives of mitochondrial research*, pages 49–59. Elsevier Science, Amsterdam.

Heckmann, K. and Kuhlmann, H. W. (1986). Mating types and mating inducing substances in *Euplotes octocarinatus*. *J. Exp. Zool.*, 96(1):87–96.

Hedrick, P. W. and Kalinowski, S. T. (2000). Inbreeding depression in conservation biology. *Annu. Rev. Ecol. Syst.*, 31(1):139–162.

Hermann, G. J. and Shaw, J. M. (1998). Mitochondrial dynamics in yeast. *Annu. Rev. Cell Dev. Biol.*, 14:265–303.

Herskowitz, I. (1985). Master regulatory loci in yeast and lambda. *Cold Spring Harb. Symp. Quant. Biol.*, 50(0):565–574.

Herskowitz, I. and Oshima, Y. (1981). Control of cell type in *Saccharomyces cerevisiae*: Mating type and mating-type interconversion. *Cold Spring Harb. Monogr. Arch.*, 11:181–209.

Herzmark, P., Campbell, K., Wang, F., Wong, K., El-Samad, H., Groisman, A., and Bourne, H. R. (2007). Bound attractant at the leading vs. the trailing edge determines chemotactic prowess. *Proc. Natl. Acad. Sci. USA*, 104(33):13349–13354.

Hill, A. V. (1910). The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *J physiol*, 40(4):iv–vii.

Hillen, T. and Painter, K. J. (2009). A user's guide to PDE models for chemotaxis. *J. Math. Biol.*, 58(1-2):183–217.

Hintz, W., Anderson, J. B., and Horgen, P. A. (1988). Nuclear migration and mitochondrial inheritance in the mushroom *Agaricus bitorquis*. *Genetics*, 119(1):35–41.

Hoekstra, R. F. (1982). On the asymmetry of sex: evolution of mating types in isogamous populations. *J. Theor. Biol.*, 98(3):427–451.

Hoekstra, R. F. (1984). Evolution of gamete motility differences II. Interaction with the evolution of anisogamy. *J. Theor. Biol.*, 107(1):71–83.

Hoekstra, R. F. (1987). The evolution of sexes. In Stearns, S. C., editor, *The Evolution of Sex and Its Consequences*, pages 59–91. Birkhauser, Basel.

Hoekstra, R. F. (1990a). Evolution of uniparental inheritance of cytoplasmic DNA. In Smith, J. M. and Vida, G., editors, *Organisational Constraints on the Dynamics of Evolution*, pages 269–278. Manchester University Press, Manchester.

Hoekstra, R. F. (1990b). The evolution of male-female dimorphism: Older than sex? *J. Genet.*, 69(1):11–15.

Hoekstra, R. F. (2000). Evolutionary origin and consequences of uniparental mitochondrial inheritance. *Hum. Reprod.*, 15(Suppl 2):102–111.

Hoekstra, R. F. (2011). Nucleo-cytoplasmic conflict and the evolution of gamete dimorphism. In Togashi, T. and Cox, P. A., editors, *Evolution of Anisogamy*, pages 111–130. Cambridge University Press, Cambridge.

Hoekstra, R. F., Iwasa, Y., and Weissing, F. J. (1991). The origin of isogamous sexual differentiation. In Selten, I. R., editor, *Game Equilibrium Models I*, pages 155–180. Springer Berlin Heidelberg, Berlin.

Hoekstra, R. F., Janz, R. F., and Schilstra, A. J. (1984). Evolution of gamete motility differences I. Relation between swimming speed and pheromonal attraction. *J. Theor. Biol.*, 107(1):57–70.

Hofer, T., Maini, P. K., Sherratt, J. A., Chaplain, M. A. J., and Murray, J. D. (1995). Resolving the chemotactic wave paradox: a mathematical model for chemotaxis of *Dictyostelium* amoebae. *Journal of Biological Systems*, 3(4):967–973.

Hogetsu, T. and Yokoyama, M. (1979). Light, a nitrogen-depleted medium and cell-cell interaction in the conjugation process of *Closterium ehrenbergii* Meneghini. *Plant Cell Physiol.*, 20(4):811–817.

Honda, H. and Miyake, A. (1975). Taxis to a conjugation-inducing substance in the ciliate *Blepharisma*. *Nature*, 257(5528):678–680.

Hsueh, Y.-P. and Heitman, J. (2008). Orchestration of sexual reproduction and virulence by the fungal mating-type locus. *Curr. Opin. Microbiol.*, 11(6):517–524.

Hurst, L. D. and Hamilton, W. D. (1992). Cytoplasmic fusion and the nature of sexes. *Proc. Biol. Sci.*, 247(1320):189–194.

Hutson, V. and Law, R. (1993). Four steps to two sexes. *Proc. Biol. Sci.*, 253(1336):43–51.

Iwano, M. and Takayama, S. (2012). Self/non-self discrimination in angiosperm self-incompatibility. *Current Opinion in Plant Biology*, 15(1):78–83.

Iwasa, Y. and Sasaki, A. (1987). Evolution of the number of sexes. *Evolution*, 41(1):49–65.

Jansen, R. P. and de Boer, K. (1998). The bottleneck: mitochondrial imperatives in oogenesis and ovarian follicular fate. *Mol. Cell. Endocrinol.*, 145(1-2):81–88.

Jennings, H. S. (1939). Genetics of *Paramecium bursaria*. I. Mating types and groups, their interrelations and distribution; mating behavior and self sterility. *Genetics*, 24:202–233.

Jensen, R. E. and Hobbs, A. A. (2000). Yeast mitochondrial dynamics: fusion, division, segregation, and shape. *Microsc. Res. Tech.*, 51(6):573–583.

Jenuth, J. P., Peterson, A. C., and Shoubridge, E. A. (1997). Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. *Nature Genetics*, 16(1):93–95.

Junger, W. G. (2011). Immune cell regulation by autocrine purinergic signalling. *Nat. Rev. Immunol.*, 11(3):201–212.

Kajiwara, T., Hatanaka, A., Kodama, K., Ochi, S., and Fujimura, T. (1991). Dictyopterenes from three Japanese brown algae. *Phytochemistry*, 30(6):1805–1807.

Kamiya, Y., Sakurai, A., Tamura, S., Takahashi, N., Abe, K., Tsuchiya, E., Fukui, S., Kitada, C., and Fujino, M. (1978). Structure of rhodotorucine A, a novel lipopeptide, inducing mating tube formation in *Rhodosporidium toruloides*. *Biochem. Biophysic. Res. Commun.*, 83(3):1077–1083.

Kato, A., Obokata, J., and Sasaki, K. (1981). Mating type interaction in *Closterium peracerosum-strigosum-littorale*: Mating induced protoplast release. *Plant Cell Physiol.*, 22(7):1215–1222.

Kato, A. and Sasaki, K. (1985). Sexual interaction in heterothallic strains of *Closterium peracerosum-strigosum-littorale*. *Plant Physiol.*, 77:556–559.

Kato, A., Yamazaki, T., and Sasaki, K. (1984). Differences in physiological properties and sexual substances between heterothallic strains of *Closterium*. *Journ. Fac. Sci. Hokido Univ. Ser.V (Botany)*, 13(3, 4):267–280.

Kawano, S., Anderson, R. W., Nanba, T., and Kuroiwa, T. (1987). Polymorphism and uniparental inheritance of mitochondrial DNA in *Physarum polycephalum*. *J. Gen. Microbiol.*, 133(11):3175–3182.

Keightley, P. D. and Otto, S. P. (2006). Interference among deleterious mutations favours sex and recombination in finite populations. *Nature*, 443(7107):89–92.

Kim, G. H. (1997). Gamete recognition and signal transduction during fertilization in Red Algae. *Kor. J. Phycol.*, 12(4):263–268.

Kim, H. and Borkovich, K. A. (2006). Pheromones are essential for male fertility and sufficient to direct chemotropic polarized growth of trichogynes during mating in *Neurospora crassa*. *Eukaryot. Cell*, 5(3):544–554.

Kirkpatrick, M., Johnson, T., and Barton, N. (2002). General models of multilocus evolution. *Genetics*, 161:1727–1750.

Kitamura, A., Sugai, T., and Kitamura, Y. (1986). Homotypic pair formation during conjugation in *Tetrahymena thermophila* . *J. Cell Sci.*, 234:223–234.

Kjærulff, S., Dooijes, D., Clevers, H., and Nielsen, O. (1997). Cell differentiation by interaction of two HMG-box proteins: Mat1-Mc activates M cell-specific genes in *S. pombe* by recruiting the ubiquitous transcription factor Ste11 to weak binding sites. *EMBO J.*, 16(13):4021–4033.

Klar, A. J., Hicks, J. B., and Strathern, J. N. (1982). Directionality of yeast mating-type interconversion. *Cell*, 28(3):551–561.

Klepsch, M. M., Persson, J. O., and de Gier, J.-W. L. (2011). Consequences of the overexpression of a eukaryotic membrane protein, the human KDEL receptor, in *Escherichia coli*. *J. Mol. Biol.*, 407(4):532–542.

Kochert, G. (1978). Sexual pheromones in algae and fungi. *Ann. Rev. Plant Physiol.*, 29:461–486.

Kokko, H. and Wong, B. (2007). What determines sex roles in mate searching? *Evolution*, 61(5):1162–1175.

Koonin, E. V. (2010). The origin and early evolution of eukaryotes in the light of phylogenomics. *Genome Biol.*, 11(5):209.

Kosaka, T. (1991). Mature cells attracting cells of the complementary mating type in *Euplotes woodruffi* syngen 3 (Ciliophora, Hypotrichida). *Zool. Sci.*, 8(4):681–692.

Kothe, E. (1999). Mating types and pheromone recognition in the homobasidiomycete *Schizophyllum commune*. *Fungal Genet. Biol.*, 27(2-3):146–152.

Krakauer, D. C. and Mira, A. (1999). Mitochondria and germ-cell death. *Nature*, 400(6740):125–126.

Kronstad, J. W. and Staben, C. (1997). Mating type in filamentous fungi. *Annu. Rev. Genet.*, 31:245–276.

Kubo, K.-i., Entani, T., Takara, A., Wang, N., Fields, A. M., Hua, Z., Toyoda, M., Kawashima, S.-i., Ando, T., Isogai, A., Kao, T.-h., and Takayama, S. (2010). Collaborative non-self recognition system in S-RNase-based self-incompatibility. *Science*, 330(6005):796–799.

Kuhlmann, H. W., Br u nen Nieweler, C., and Heckmann, K. (1997). Pheromones of the ciliate *Euplotes octocarinatus* not only induce conjugation but also function as chemoattractants. *J. Exp. Zool.*, 277(1):38–48.

Kuhlmann, H. W. and Heckmann, K. (1991). Nuclear processes in *Euplotes octocarinatus* during conjugation. *Eur. J. Protistol.*, 26(3):370–386.

Labarere, J. and Noel, T. (1992). Mating type switching in the tetrapolar basidiomycete *Agrocybe aegerita*. *Genetics*, 131(2):307–319.

Lahr, D. J. G., Parfrey, L. W., Mitchell, E. a. D., Katz, L. a., and Lara, E. (2011). The chastity of amoebae: re-evaluating evidence for sex in amoeboid organisms. *Proc. Biol. Sci.*, 278(1715):2081–2090.

Lane, N. (2006). *Power, Sex, Suicide: Mitochondria and the Meaning of Life*. Oxford University Press, Oxford.

Lane, N. (2008). Mitochondria and the W chromosome: low variability on the W chromosome in birds is more likely to indicate selection on mitochondrial genes. *Heredity*, 100(5):444–445.

Lane, N. (2011a). Energetics and genetics across the prokaryote-eukaryote divide. *Biol. Direct*, 6(1):35.

Lane, N. (2011b). Evolution. The costs of breathing. *Science*, 334(6053):184–185.

Lane, N. (2011c). Mitonuclear match: Optimizing fitness and fertility over generations drives ageing within generations. *BioEssays*, 33(11):860–869.

Lane, N. (2012). The problem with mixing mitochondria. *Cell*, 151(2):246–248.

Lasky, R. D. and Ballou, C. E. (1988). Cell-cell recognition in yeast: Isolation of intact alpha-agglutinin from S*accharomyces kluyveri. Proc. Natl. Acad. Sci. USA*, 85(2):349–353.

Lee, J., Lee, T., Lee, Y.-W., Yun, S.-H., and Turgeon, B. G. (2003). Shifting fungal reproductive mode by manipulation of mating type genes: obligatory heterothallism of *Gibberella zeae*. *Mol. Microbiol.*, 50(1):145–152.

Lewis, K. E. and O'Day, D. H. (1976). Sexual hormone in the cellular slime mould *Dictyostelium purpureum. Can. J. Microbiol.*, 22(9):1269–1273.

Lewis, K. E. and O'Day, D. H. (1979). Evidence for a hierarchical mating system operating via pheromones in *Dictyostelium giganteum. J. Bacteriol.*, 138(1):251–253.

Lin, X. and Heitman, J. (2007). Mechanisms of homothallism in fungi and transitions between heterothallism and homothallism. In Heitman, J., Kronstad, J. W., Taylor, J. W., and Casselton, L. A., editors, *Sex in Fungi: Molecular Determination and Evolutionary Implications*, pages 35–57. ASM Press, Washington, D.C.

Linnane, A. W., Marzuki, S., Ozawa, T., and Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet*, 1(8639):642–645.

Lipke, P. N., Wojciechowicz, D., and Kurjan, J. (1989). AG alpha 1 is the structural gene for the *Saccharomyces cerevisiae* alpha-agglutinin, a cell surface glycoprotein involved in cell-cell interactions during mating. *Mol. Cell Biol.*, 9(8):3155–3165.

Lynch, M., Koskella, B., and Schaack, S. (2006). Mutation pressure and the evolution of organelle genomic architecture. *Science*, 311(5768):1727–1730.

MacHac, M. A. and Bonner, J. T. (1975). Evidence for a sex hormone in *Dictyostelium discoideum*. *J. Bacteriol.*, 124(3):1624–1625.

Magee, J., Hoffman, D., Colbert, C., and Johnston, D. (1998). Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. *Annu. Rev. Physiol.*, 60:327–346.

Maier, I. (1993). Gamete orientation and induction of gametogenesis by pheromones in algae and plants. *Plant. Cell Environ.*, 16:891–907.

Mann, S. and Firtel, R. A. (1991). A developmentally regulated, putative serine threonine protein-kinase is essential for development in *Dictyostelium*. *Mech. Dev.*, 35(2):89–101.

Marra, R. E., Cortesi, P., Bissegger, M., and Milgroom, M. G. (2004). Mixed mating in natural populations of the chestnut blight fungus, *Cryphonectria parasitica. Heredity*, 93(2):189–195.

Marra, R. E. and Milgroom, M. G. (2001). The mating system of the fungus *Cryphonectria parasitica*: selfing and self-incompatibility. *Heredity*, 86(2):134–143.

Maynard Smith, J. (1978). *The Evolution of Sex*. Cambridge University Press, Cambridge.

Mayrhofer, S., Weber, J. M., and P o ggeler, S. (2006). Pheromones and pheromone receptors are required for proper sexual development in the homothallic ascomycete *Sordaria macrospora*. *Genetics*, 172(3):1521–1533.

Mccullough, J. and Herskowitz, I. (1979). Mating pheromones of *Saccharomyces kluyveri* : pheromone interactions between *Saccharomyces kluyveri* and *Saccharomyces cerevisiae* . *J. Bacteriol.*, 138(1):146–154.

Merlini, L., Dudin, O., and Martin, S. G. (2013). Mate and fuse: how yeast cells do it. *Open Biol.*, 3(3):130008.

Metzler, R. and Klafter, J. (2000). The random walk's guide to anomalous diffusion: a fractional dynamics approach. *Phys. Rep.*, 339(1):77.

Mishmar, D., Ruiz-pesini, E., and Mondragon-palomino, M. (2006). Adaptive selection of mitochondrial complex I subunits during primate radiation. *Gene*, 378:11–18.

Miyake, A. (1974). Cell interaction in conjugation of ciliates. *Curr. Top. Microbiol. Immunol.*, 64:49–77.

Miyake, A. (1996). Fertilization and sexuality in ciliates. In Hausmann, K. and Bradbury, P., editors, *Ciliates: Cells as Organisms*, pages 243–290. Gustav Fischer, Stuttgart.

Miyake, A. and Rivola, V. (1989). Chemoattraction by gamones in *Blepharisma japonicum*. *J. Protozool.*, 36:24A.

Muller, D. G. (1978). Locomotive responses of male gametes to the species specific sex attractant in *Ectocarpus siliculosus* (Phaeophyta). *Arch fur Protistenkd*, 120(4):371–377.

Muramoto, T., Suzuki, K., Shimizu, H., Kohara, Y., Kohriki, E., Obara, S., Tanaka, Y., and Urushihara, H. (2003). Construction of a gamete-enriched gene pool and RNAi-mediated functional analysis in *Dictyostelium discoideum*. *Mech. Dev.*, 120(8):965–975.

Nabholz, B., Glemin, S., and Galtier, N. (2008). Strong variations of mitochondrial mutation rate across mammals–the longevity hypothesis. *Mol. Biol. Evol.*, 25(1):120–130.

Nakamura, S. (2010). Paternal inheritance of mitochondria in Chlamydomonas. *J. Plant Res.*, 123(2):163–170.

Nauta, M. J. and Hoekstra, R. F. (1992). Evolution of reproductive systems in filamentous ascomycetes. 1. Evolution of mating types. *Heredity*, 68:405–410.

Ni, M., Feretzaki, M., Sun, S., Wang, X., and Heitman, J. (2011). Sex in fungi. *Annu. Rev. Genet.*, 45:405–430.

Nielsen, O., Friis, T., and Kjaerulff, S. (1996). The S*chizosaccharomyces pombe* map1 gene encodes an SRF/MCM1-related protein required for P-cell specific gene expression. *Mol. Gen. Genet.*, 253(3):387–392.

Nozaki, H., Mori, T., Misumi, O., Matsunaga, S., and Kuroiwa, T. (2006). Males evolved from the dominant isogametic mating type. *Curr. Biol.*, 16(24):R1018–R1020.

O Morgan, D. (2007). *The Cell Cycle*. Principles of Control. New Science Press.

O'Day, D. H. and Lewis, K. E. (1975). Diffusible mating-type factors induce macrocyst development in *Dictyostelium discoideum*. *Nature*, 254(5499):431–432.

O'Day, D. H. and Lewis, K. E. (1981). Pheromonal interactions during mating in Dictyostelium. In O'Day, D. H. and Horgen, P. A., editors, *Sexual Interactions in Eukaryotic Microbes*. Accademic Press, New York.

Olesnicky, N. S., Brown, A. J., Dowell, S. J., and Casselton, L. A. (1999). A constitutively active G-protein-coupled receptor causes mating self-compatibility in the mushroom Coprinus. *EMBO J.*, 18(10):2756–2763.

Otto, S. P. (2009). The evolutionary enigma of sex. *Am. Nat.*, 174(Suppl 1):S1–S14.

Palmer, J. D. and Herbon, L. A. (1988). Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. *J. Mol. Evol.*, 28(1-2):87–97.

Pan, J. and Snell, W. J. (2000). Signal transduction during fertilization in the unicellular green alga, *Chlamydomonas*. *Curr. Opin. Microbiol.*, 3(6):596–602.

Parker, G. A., Baker, R. R., and Smith, V. (1972). Origin and evolution of gamete dimorphism and male-female phenomenon. *J. Theor. Biol.*, 9:529–553.

Partridge, L. and Hurst, L. D. (1998). Sex and conflic. *Science*, 281(5385):2003–2008.

Perlman, P. S. and Birky, C. W. (1974). Mitochondrial genetics in Bakers' yeast: a molecular mechanism for recombinational polarity and suppressiveness. *Proc. Natl. Acad. Sci. USA*, 71(11):4612–4616.

Perrin, N. (2012). What uses are mating types? The 'developmental switch' model. *Evolution*, 66(4):947–956.

Phadke, S. S. and Zufall, R. a. (2009). Rapid diversification of mating systems in ciliates. *Biol. J. Linn. Soc.*, 98(1):187–197.

Plumper, E., Freiburg, M., and Heckmann, K. (1995). Inhibition of pair formation by concanavalin-a and concanavalin-a-binding glycoconjugates in *Euplotes octocarinatus*. *J. Eukaryot. Microbiol.*, 42(2):166–173.

Poggeler, S. (2000). Two pheromone precursor genes are transcriptionally expressed in the homothallic ascomycete *Sordaria macrospora*. *Curr. Genet.*, 37(6):403–411.

Press, W. H., Teukolsky, S. A., Vetterling, W. T., and Flannery, B. P. (2007). *Numerical Recipes in C++: The Art of Scientific Computing*. Cambridge University Press, Cambridge.

Puurtinen, M. and Kaitala, V. (2002). Mate-search efficiency can determine the evolution of separate sexes and the stability of hermaphroditism in animals. *Am. Nat.*, 160(5):645–660.

Quarmby, L. M. (1994). Signal transduction in the sexual life of *Chlamydomonas*. *Plant Mol. Biol.*, 26:1271–1287.

Raju, N. B. (1992). Functional heterothallism resulting from homokaryotic conidia and ascospores in *Neurospora tetrasperma*. *Mycol. Res.*, 96(2):103–116.

Ramesh, M. A., Malik, S. B., and Logsdon, Jr, J. M. (2005). A phylogenomic inventory of meiotic genes: evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. *Curr. Biol.*, 15(2):185–191.

Rand, D. M. (2001). The units of selection on mitochondrial DNA. *Annu. Rev. Ecol. Syst.*, 32(1):415–448.

Randerson, J. P. and Hurst, L. D. (1999). Small sperm, uniparental inheritance and selfish cytoplasmic elements: a comparison of two models. *J. Evol. Biol.*, 12:1110–1124.

Raper, J. (1966). *Genetics of Sexuality in Higher Fungi*. The Ronald Press, New York.

Raudaskoski, M. and Kothe, E. (2010). Basidiomycete mating type genes and pheromone signaling. *Eukaryot. Cell*, 9(6):847–859.

Reinhart-King, C. A., Dembo, M., and Hammer, D. A. (2008). Cell-cell mechanical communication through compliant substrates. *Biophys. J.*, 95(12):6044–6051.

Robertson, S. J., Bond, D. J., and Read, N. D. (1998). Homothallism and heterothallism in *Sordaria brevicollis*. *Mycol. Res.*, 102(10):1215–1223.

Rossignol, R., Faustin, B., Rocher, C., Malgat, M., Mazat, J.-P., and Letellier, T. (2003). Mitochondrial threshold effects. *Biochem. J.*, 370:751–762.

Roze, D., Rousset, F., and Michalakis, Y. (2005). Germline bottlenecks, biparental inheritance and selection on mitochondrial variants: a two-level selection model. *Genetics*, 170(3):1385–1399.

Sandgren, C. D. (1981). Characteristics of sexual and asexual resting cyst (statospore) formation in dinobryon *Cylindricum imhof* (Chrysophyta). *J. Phycol.*, 17(2):199–210.

Sandgren, C. D. and Flanagin, J. (1986). Heterothallic sexuality and density dependent encystment in the Chrysophycean alga *Synura petersenii Korsh*. *J. Phycol.*, 22:206–216.

Schmid, C. E. (1993). Cell-cell-recognition during fertilization in *Ectocarpus siliculosus* (Phaeophyceae). *Hydrobiologia*, 260-261(1):437–443.

Schmid, C. E., Schroer, N., and Muller, D. G. (1994). Female gamete membrane glycoproteins potentially involved in gamete recognition in *Ectocarpus siliculosus* (Phaeophyceae). *Plant Sci.*, 102:61–67.

Seike, T., Nakamura, T., and Shimoda, C. (2013). Distal and proximal actions of peptide pheromone M-factor control different conjugation steps in fission yeast. *PLoS One*, 8(7):e69491.

Sekimoto, H. (2005). Plant sex pheromones. *Vitam. Horm.*, 72:457–478.

Sekimoto, H. and Fujii, T. (1992). Analysis of gametic protoplast release in the *Closterium peracerosum-strigosum-littorale* (Chlorophyta). *J. Phycol.*, 28(5):615–619.

Sekimoto, H., Inoki, Y., and Fujii, T. (1993). Detection and evaluation of an inducer of a diffusible mating pheromone of the heterothallic *Closterium peracerosum-strigosum-littorale* complex. *Plant Cell Physiol.*, 34(7):991–996.

Sekimoto, H., Satoh, S., and Fujii, T. (1990). Biochemical and physiological properties of a protein inducing protoplast release during conjugation in the *Closterium peracerosum-strigosum-littorale* complex. *Planta*, 182(3):348–354.

Sengupta, A., van Teeffelen, S., and Loewen, H. (2009). Dynamics of a microorganism moving by chemotaxis in its own secretion. *Phys. Rev. E*, 80(3):1122–1128.

Sharifmoghadam, M. R., Bustos-Sanmamed, P., and Valdivieso, M.-H. (2006). The fission yeast Map4 protein is a novel adhesin required for mating. *FEBS Lett.*, 580(18):4457–4462.

Sharpley, M. S., Marciniak, C., Eckel-Mahan, K., McManus, M., Crimi, M., Waymire, K., Lin, C. S., Masubuchi, S., Friend, N., Koike, M., Chalkia, D., MacGregor, G., Sassone-Corsi, P., and Wallace, D. C. (2012). Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. *Cell*, 151(2):333–343.

Shen, W. C., Davidson, R. C., Cox, G. M., and Heitman, J. (2002). Pheromones stimulate mating and differentiation via paracrine and autocrine signaling in *Cryptococcus neoformans*. *Eukaryot. Cell*, 1(3):366–377.

Sheridan, C. C., Steinberg, D. K., and Kling, G. W. (2002). The microbial and metazoan community associated with colonies of *Trichodesmium spp.*: a quantitative survey. *J. Plankton Res.*, 24(9):913–922.

Shiu, P. and Glass, N. L. (2000). Cell and nuclear recognition mechanisms mediated by mating type in filamentous ascomycetes. *Curr. Opin. Microbiol.*, 3:183–188.

Silliker, M. E., Liles, J. L., and Monroe, J. A. (2002). Patterns of mitochondrial inheritance in the myxogastrid *Didymium iridis*. *Mycologia*, 94(6):939–946.

Smith, E. F. and Lefebvre, P. A. (1996). PF16 encodes a protein with armadillo repeats and localizes to a single microtubule of the central apparatus in Chlamydomonas flagella. *J. Cell Biol.*, 132(3):359–370.

Smith, L. C., Bordignon, V., Couto, M. M., Garcia, S. M., Yamazaki, W., and Meirelles, F. a. v. V. (2002). Mitochondrial genotype segregation and the bottleneck. *Reprod. Biomed. Online*, 4(3):248–255.

Sonneborn, T. M. (1937). Sex, sex inheritance and sex determination in *Paramecium aurelia*. *Proc. Natl. Acad. Sci. USA*, 23(7):378–385.

Sonneborn, T. M. (1978). Genetics of cell-cell interactions in ciliates. *Birth Defects Orig. Artic. Ser.*, 14(2):417–427.

Starr, R. C., Marner, F. J., and Jaenicke, L. (1995). Chemoattraction of male gametes by a pheromone produced by female gametes of Chlamydomonas. *Proc. Natl. Acad. Sci. USA*, 92:641–645.

Sugiura, M., Kawahara, S., Iio, H., and Harumoto, T. (2005). Developmentally and environmentally regulated expression of gamone 1: The trigger molecule for sexual reproduction in *Blepharisma japonicum. J. Cell Sci.*, 118(12):2735–2741.

Sugiura, M., Shiotani, H., Suzaki, T., and Harumoto, T. (2010). Behavioural changes induced by the conjugation-inducing pheromones, gamone 1 and 2, in the ciliate *Blepharisma japonicum. Eur. J. Protistol.*, 46(2):143–149.

Takayama, S. and Isogai, A. (2005). Self-incompatibility in plants. *Annu Rev Plant Biol*, 56:467–489.

Taktikos, J., Zaburdaev, V., and Stark, H. (2011). Modeling a self-propelled autochemotactic walker. *Phys. Rev. E*, 84(4):1924–1935.

Taylor, D. R., Zeyl, C., and Cooke, E. (2002). Conflicting levels of selection in the accumulation of mitochondrial defects in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA*, 99(6):3690–3694.

Thompson-Coffe, C. and Zickler, D. (1994). How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle in filamentous ascomycetes. *Dev. Biol.*, 165(1):257–271.

Thrailkill, K. M. and Birky, C. W. (1980). Intracellular population genetics: evidence for random drift of mitochondrial allele frequencies in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Genetics*, 96(1):237–262.

Tomson, A. M., Demets, R., Sigon, C. A., Stegwee, D., and van den Ende, H. (1986). Cellular interactions during the mating process in *Chlamydomonas eugametos*. *Plant Physiol.*, 81(2):522–526.

Tsori, Y. and De Gennes, P. G. (2004). Self-trapping of a single bacterium in its own chemoattractant. *Europhys. Lett.*, 66(4):599–602.

Tsubo, Y. (1957). On the mating reaction of a Chlamydomonas, with special references to clumping and chemotaxis. *Bot. Mag.*, 70(832):327–334.

Tsubo, Y. (1961). Chemotaxis and sexual behavior in Chlamydomonas. *J. Protozool.*, 8(2):114–121.

Tsuchikane, Y. and Fukumoto, R. (2003). Sex pheromones that induce sexual cell division in the *Closterium peracerosum–strigosum– littorale complex* (Charophyta). *J. Phycol.*, 39:303–309.

Tsuchikane, Y., Kokubun, Y., and Sekimoto, H. (2010). Characterization and molecular cloning of conjugation-regulating sex pheromones in homothallic Closterium. *Plant Cell Physiol.*, 51(9):1515–1523.

Tsuchikane, Y., Tsuchiya, M., Hindak, F., Nozaki, H., and Sekimoto, H. (2012). Zygospore formation between homothallic and heterothallic strains of Closterium. *Sex. Plant Reprod.*, 25(1):1–9.

Uyenoyama, M. K. (1988a). On the evolution of genetic incompatibility systems. II. Initial increase of strong gametophytic self-incompatibility under partial selfing and half-sib mating. *Am. Nat.*, 131(5):700–722.

Uyenoyama, M. K. (1988b). On the evolution of genetic incompatibility systems. III. Introduction of weak gametophytic self-incompatibility under partial inbreeding. *Theor. Popul. Biol.*, 34(1):47–91.

Valbonesi, A., Ortenzi, C., and Luporini, P. (1987). Observations on the biology of *Euplotes charon* (Hypotrichida, Ciliophora). *Bolletino di Zool.*, 54(2):111–118.

Vallesi, A., Ballarini, P., and Pretoro, B. D. (2005). Autocrine, mitogenic pheromone receptor loop of the ciliate *Euplotes raikovi*: pheromone-induced receptor internalization. *Eukaryot. Cell*, 4(7):1221–1227.

van der Meer, J. P. and Todd, E. R. (1977). Genetics of *Gracilaria sp*. (Rhodophyceae, Gigartinales). IV. Mitotic recombination and its relationship to mixed phases in the life history. *Can. J. Bot.*, 55(22):2810–2817.

Vandover, B. (1972). *Studies of the Ultrastructure and Reproduction of Several Species of Chlamydomonas and Carteria*. University of Texas at Austin, Austin.

VanWinkle-Swift, K. P. and Hahn, J. H. (1986). The search for mating-type-limited genes in the homothallic alga *Chlamydomonas monoica*. *Genetics*, 113:601–619.

Vengrova, S. and Dalgaard, J. Z. (2004). RNase-sensitive DNA modification (s) initiates *S. pombe* mating-type switching. *Genes Dev.*, 18(7):794–804.

Visviki, I. (2000). A cytological study of the green alga *Chlamydomonas applanata Pringsheim* (Chlamydomonadaceae). *J. Torrey Bot. Soc.*, 127(1):1–8.

Wallace, D. C. (2013). Bioenergetics in human evolution and disease: implications for the origins of biological complexity and the missing genetic variation of common diseases. *Phil. Trans. R. Soc. B*, 368(1622):20120267.

Wang, X. (2001). The expanding role of mitochondria in apoptosis. *Genes Dev.*, 15(22):2922–2933.

Waters, C. M. and Bassler, B. L. (2005). Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.*, 21:319–346.

Wendland, J., Vaillancourt, L. J., Hegner, J., Lengeler, K. B., Laddison, K. J., Specht, C. A., Raper, C. A., and Kothe, E. (1995). The mating-type locus B alpha 1 of *Schizophyllum commune* contains a pheromone receptor gene and putative pheromone genes. *EMBO J.*, 14(21):5271–5278.

Willett, C. S. and Burton, R. S. (2003). Environmental influences on epistatic interactions: viabilities of cytochrome C genotypes in interpopulation crosses. *Evolution*, 57(10):2286–2292.

Wolfe, J. (1982). The conjugation junction of Tetrahymena: its structure and development. *J. Morphol.*, 172:159–178.

Xiang, Q. and Glass, N. L. (2004). The control of mating type heterokaryon incompatibility by vib-1, a locus involved in het-c heterokaryon incompatibility in *Neurospora crassa. Fungal Genet. Biol.*, 41(12):1063–1076.

Xu, J. (2005). The inheritance of organelle genes and genomes: patterns and mechanisms. *Genome*, 958:951–958.

Xue-Franzen, Y., Kjærulff, S., Holmberg, C., Wright, A., and Nielsen, O. (2006). Genomewide identification of pheromone-targeted transcription in fission yeast. *BMC Genomics*, 7(1):303.

Youk, H. and Lim, W. A. (2014). Secreting and sensing the same molecule allows cells to achieve versatile social behaviors. *Science*, 343:1242782.

Yun, S. H., Berbee, M. L., and Yoder, O. C. (1999). Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. *Proc Natl Acad Sci USA*, 96(10):5592–5597.

Zeviani, M. and Di Donato, S. (2004). Mitochondrial disorders. *Brain*, 127(Pt 10):2153–2172.

Zhang, Q. (2003). Examination of the cytoplasmic DNA in male reproductive cells to determine the potential for cytoplasmic inheritance in 295 angiosperm species. *Plant Cell Physiol.*, 44(9):941–951.

Zhao, H., Lu, M., Singh, R., and Snell, W. J. (2001). Ectopic expression of a *Chlamydomonas* mt+-specific homeodomain protein in mt- gametes initiates zygote development without gamete fusion. *Genes Dev.*, 15:2767–2777.

Zhaxybayeva, O. and Doolittle, W. F. (2011). Lateral gene transfer. *Curr. Biol.*, 21(7):R242–R246.

Zouros, E. (2013). Biparental inheritance through uniparental transmission: The doubly uniparental inheritance (DUI) of mitochondrial DNA. *Evol. Biol.*, 40(1):1–31.

Zouros, E., Oberhauser Ball, A., Saavedra, C., and Freeman, K. R. (1994). An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. *Proc. Natl. Acad. Sci. USA*, 91(16):7463–7467.