LUND UNIVERSITY

Sex Chromosome Evolution in a Hermaphrodite

Genetic and phenotypic relationships between sex roles

Nordén, Anna

2017

Link to publication

Citation for published version (APA):
Nordén, A. (2017). *Sex Chromosome Evolution in a Hermaphrodite: Genetic and phenotypic relationships between sex roles*. [Doctoral Thesis (compilation), Department of Biology]. Lund University, Faculty of Science, Department of Biology.

Total number of authors:
1

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Sex Chromosome Evolution in a Hermaphrodite

Genetic and phenotypic relationships between sex roles

ANNA K. NORDÉN
FACULTY OF SCIENCE | DEPARTMENT OF BIOLOGY | LUND UNIVERSITY

Sex Chromosome Evolution in a Hermaphrodite

Genetic and phenotypic relationships between sex roles

Anna K. Nordén

LUND UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Science, Lund University, Sweden.
To be defended in the Blue Hall, Ecology Building, Sölvegatan 37, Lund, Sweden
on the 24th of November at 9.30.

Faculty opponent
Prof. Mike Ritchie
University of St Andrews

Organization
LUND UNIVERSITY

Department of Biology, Evolutionary Ecology, Sölvegatan 37, 223 62, Lund Sweden

Author(s) Anna Nordén

Document name
DOCTORAL DISSERTATION

Date of issue
10/10 2017

Sponsoring organization

Title and subtitle

Sex chromosome evolution in a hermaphrodite: Genetic and phenotypic relationships between sex roles

Abstract

Sex chromosome evolution in a hermaphrodite ancestor starts with the establishment of a sex-determining region (SDR). Over time, sex-specific genes, and/or sexually antagonistic alleles will become linked to the SDR. Sexually antagonistic alleles are a type of genetic variation that increases fitness for one sex while being detrimental for the other sex. Recombination arrest around the SDR and genes linked to it will evolve, effectively preventing breaking up of advantageous gene combinations. Eventually, the region of recombination arrest will increase as more sex-specific genes migrate to the proto-sex chromosome, and this will in the end lead to degenerated sex chromosomes where deleterious mutations accumulate and genes are lost. However, most research to date has focused on old, already degenerated sex chromosomes, and relatively few have looked at the very initial phases of sex chromosome evolution. In this thesis, I aimed at producing a deeper understanding of the very beginning of sex chromosome evolution in a hermaphrodite ancestor. I do this both with sex-limited experimental evolution, simulating the evolution of a sex chromosome in a hermaphrodite (the simultaneous hermaphroditic flatworm *Macrostomum lignano*), and by examining the relationships between male and female fitness components in the study species. When mimicking the evolution of a sex chromosome, a genetic marker (GFP, green fluorescent protein) acted as a sex-determining gene. In the male-limited selection, the marker was passed through sperm (fitness through male sex role), and in the female-limited selection, it passed through eggs (i.e. fitness though female sex role). There were 4 replicate populations per treatment (male-limited, female-limited and control treatment).

Here, I show that additive genetic variance for female fitness is three times larger than male fitness in stock populations of *M. lignano*. I also found that additive genetic variance was environment-specific, and the difference depended on the sex-role. The relationship between male and female fitness was weak both on the genetic and phenotypic level, and it did not seem to change across environments. This indicates that male and female fitness function can evolve independently from each other, and that there was no sexual antagonism between sex roles. Despite this, we could show evidence of a genetically-based trade-off in the sex-limited experimental evolution, indicating that we might have reinforced a negative intersexual genetic correlation between sex roles during the course of the experiment. Gene expression analysis also revealed that the largest number of differentially expressed genes was between the male- and female-limited selection treatments, but there was no expression difference between treatments in the sex-specific organs (antrum and prostate gland). In any case, we could show proof of concept that the early stages of sex chromosome evolution are observable in real time.

Key words Sex chromosome evolution, Hermaphrodite, Inter-sexual genetic correlation, Quantitative genetics, Sexual conflict, *Macrostomum lignano*, Experimental evolution

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language
English

ISSN and key title

ISBN
Print: 978-91-7753-473-0
PDF: 978-91-7753-474-7

Recipient's notes

Number of pages

Price

Security classification

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Date 2017-10-10

Sex Chromosome Evolution in a Hermaphrodite

Genetic and phenotypic relationships between sex roles

Anna Katarina Nordén

LUND UNIVERSITY

Copyright Anna K. Nordén

Cover image by © Elsa Nordén

Faculty of Science
Department of Biology

ISBN (print) 978-91-7753-473-0
ISBN (pdf) 978-91-7753-474-7

Printed in Sweden by Media-Tryck, Lund University
Lund 2017

"We like to feel that we are living in a stable world. The more we learn about the universe, the more we learn about its instability. The more we learn about any science, the more we learn about its endless complexity"

Prof. Walter Mischel in the podcast Invisibilia (NPR)

Contents

List of papers 8
Author contributions 9
Abstract 10
Svensk sammanfattning 11

Introduction and background 13

Why sex? 13
The many costs and few benefits of meiotic sex 13
Sexual conflict over mating rate gives insight into the paradox 14

Evolution of anisogamy 14
Two gamete types 14
Did anisogamy result in gonochorism or hermaphrodites? 15

Hermaphroditism 15
Hermaphroditic plants 15
Simultaneous hermaphroditism – common among invertebrates 17
Sequential hermaphroditism – common among hermaphroditic fish 17
Why be a hermaphrodite? 18

Sex roles, sexual conflict and sexual antagonism 20
Sex roles and interlocus sexual conflict 20
Sexual antagonism 20
Measuring sexual antagonism 22

Evolution of sex chromosomes 24
Sex determination 24
Transitions between hermaphroditism and gonochorism 24
Evolution of heteromorphic sex chromosomes 25
Sex chromosome turnovers 27
Sex reversals 27

Thesis aims 29

General methodology **31**
Study species & laboratory lines 31
Discovery and habitat 31
Anatomy and behavior 31
Worm culturing 32
Experimental evolution 34
Quantitative genetics 35
The components of phenotypic variance 35
Heredity, heritability and genetic correlation 36
The animal model 36
Gene expression and RNA extraction 37
Sex-specific gene expression 37
Results and discussion **39**
Paper I. Sex chromosome evolution: historical insights and future perspectives 39
Paper II. Sex-specific genetic variance for fitness and inheritance of a genetic marker in a simultaneous hermaphrodite 39
Paper III. Environmental variation in sex-specific fitness in a simultaneous hermaphrodite 41
Paper IV. Sex-limited selection in a hermaphrodite leads to changes in sex-role related traits 42
Paper V. Gene-expression differences in a simultaneous hermaphrodite subjected to sex-limited selection 43
Conclusions and future perspectives **45**
Acknowledgements 49
Referenses **51**
Paper I **63**
Paper II **75**
Paper III **95**
Paper IV **117**
Paper V **139**

List of papers

I. Abbott, J. K., **Nordén, A. K.**, and Hansson, B. Sex chromosome evolution: historical insights and future perspectives. *Proc. R. Soc. B 284: 20162806*.[1]

II. **Nordén, A. K.** and Abbott, J. K. Sex-specific genetic variance for fitness and inheritance of a genetic marker in a simultaneous hermaphrodite. *Manuscript*.

III. **Nordén, A. K.**, Moosmann, M. and Abbott, J. K. Environmental variation in sex-specific fitness in a simultaneous hermaphrodite. *Submitted*.

IV. **Nordén, A. K.** and Abbott, J. K. Sex-limited selection in a hermaphrodite leads to changes in sex-role related traits. *Manuscript*.

V. **Nordén, A. K.** and Abbott, J. K. Gene-expression differences in a simultaneous hermaphrodite subjected to sex-limited selection. *Manuscript*.

[1] Paper 1 is Open Access. This article is distributed under the terms of the Creative Commons license (creativecommons.org/licenses/by/4.0/) and is made freely and re-usable and available to all, provided you give appropriate credit to original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

Author contributions

I. JKA, BH and **AKN** conceived the study. All authors drafted the initial manuscript, and JKA merged the parts together. All authors contributed to and gave approval of the submission for publication of the final manuscript.

II. JKA conceived the study, and JKA and **AKN** designed the study. **AKN** performed the study and analysed the results with input from JKA. **AKN** wrote the manuscript with input from JKA.

III. JKA and **AKN** conceived and designed the study. MM and **AKN** performed the study and analysed the results with input from JKA. **AKN** wrote the manuscript with input from the other authors.

IV. JKA conceived the study and **AKN** designed the lab work with input from JKA. **AKN** performed the study and analysed the results with input from JKA. **AKN** wrote the manuscript with input from JKA.

V. JKA conceived the study and **AKN** designed and performed the lab work with input from JKA. JKA and AKN gave input to the analysis that was done by Genevia Technologies. **AKN** wrote the manuscript with input from JKA.

Authors: Anna K. Nordén (AKN), Jessica K. Abbott (JKA), Bengt Hansson (BH), Marvin Moosmann (MM).

Abstract

Sex chromosome evolution in a hermaphrodite ancestor starts with the establishment of a sex-determining region (SDR). Over time, sex-specific genes, and/or sexually antagonistic alleles will become linked to the SDR. Sexually antagonistic alleles are a type of genetic variation that increases fitness for one sex while being detrimental for the other sex. Recombination arrest around the SDR and genes linked to it will evolve, effectively preventing breaking up of advantageous gene combinations. Eventually, the region of recombination arrest will increase as more sex-specific genes migrate to the proto-sex chromosome, and this will in the end lead to degenerated sex chromosomes where deleterious mutations accumulate and genes are lost. However, most research to date has focused on old, already degenerated sex chromosomes, and relatively few have looked at the very initial phases of sex chromosome evolution. In this thesis, I aimed at producing a deeper understanding of the very beginning of sex chromosome evolution in a hermaphrodite ancestor. I do this both with sex-limited experimental evolution, simulating the evolution of a sex chromosome in a hermaphrodite (the simultaneous hermaphroditic flatworm *Macrostomum lignano*), and by examining the relationships between male and female fitness components in the study species. When mimicking the evolution of a sex chromosome, a genetic marker (GFP, green fluorescent protein) acted as a sex-determining gene. In the male-limited selection, the marker was passed through sperm (fitness through male sex role), and in the female-limited selection, it passed through eggs (i.e. fitness though female sex role). There were 4 replicate populations per treatment (male-limited, female-limited and control treatment).

Here, I show that additive genetic variance for female fitness is three times larger than male fitness in stock populations of *M. lignano*. I also found that additive genetic variance was environment-specific, and the difference depended on the sex-role. The relationship between male and female fitness was weak both on the genetic and phenotypic level, and it did not seem to change across environments. This indicates that male and female fitness function can evolve independently from each other, and that there was no sexual antagonism between sex roles. Despite this, we could show evidence of a genetically-based trade-off in the sex-limited experimental evolution, indicating that we might have reinforced a negative intersexual genetic correlation between sex roles during the course of the experiment. Gene expression analysis also revealed that the largest number of differentially expressed genes was between the male- and female-limited selection treatments, but there was no expression difference between treatments in the sex-specific organs (antrum and prostate gland). In any case, we could show proof of concept that the early stages of sex chromosome evolution are observable in real time.

Svensk sammanfattning

Avhandlingens syfte har varit att studera utvecklingen av en könskromosom i *realtid* genom att simulera dess framväxt i en hermafroditisk plattmask i laboratoriet. Jag gjorde detta genom att selektera på dels honlig fitness (fitness via ägg), dels hanlig fitness (fitness via spermier) med hjälp av en genetisk markör, som agerade som en könsbestämmande gen. Detta ska efterlikna den process som borde ha skett då utvecklingen av könskromosomer började för flera miljoner år sedan.

Man tror att hanar och honor utvecklades först och att detta ledde till utvecklingen av könskromosomer. Det började med en urpopulation där alla var hermafroditer, det vill säga individer som kunde reproducera sig både som hane och hona. Två olika mutationer behövdes sedan för att utveckla hanar och honor: en som steriliserar han-funktionerna och en annan som steriliserar hon-funktionerna. Utan de mutationerna blir resultatet en population av hermafroditer och hanar, eller det mer vanliga bland växter, hermafroditer och honor. När väl uppdelningen av könen har skett, kunde utvecklingen av könskromosomer ske.

Individerna hade då en könsbestämmande region på en så kallad proto-könskromosom, det vill säga en kromosom som kommer utvecklas till en könskromosom. Intill den könsbestämmande regionen på proto-könskromosomen migrerar gärna gener och gentyper (alleler) som är gynnsamma för just det könet. Vissa alleler som migrerar är sexuellt antagonistiska, vilket betyder att de till exempel är fördelaktiga för hanars fitness samtidigt som de har negativa effekter på honors fitness. På proto-könskromosomen finns nu den könsbestämmande regionen, samt gener som är fördelaktiga för utvecklingen av just det könet. Dock kan den fördelaktiga kombinationen av gener brytas ner genom att kromosompar rekombinerar (även kallat överkorsning). Därför utvecklas en hämning mot överkorsningen precis runt området där de könsspecifika generna och den könsbestämmande regionen sitter. Under evolutionens gång migrerar fler och fler könsspecifika gener till proto-könskromosomen, och området som hämmats mot överkorsning ökar sakta men säkert tills det eventuellt kan täcka hela kromosomen. Det är precis vad som har hänt i människans Y-kromosom. Forskning har också visat att hämningen av rekombination har resulterat i att Y-kromosomen innehåller få gener och många skadliga mutationer. Teorierna är svåra att bevisa genom att studera könskromosomer som har funnits i flera miljoner år hos till exempel bananflugan eller människan.

Min avhandling kretsar kring teorin om könskromosomers uppkomst i en hermafroditisk urpopulation, och speciellt kring vad som händer i de första utvecklingsfaserna. Jag har undersökt detta både på fenotypisk och genetisk nivå. Hypotesen var att de han-selekterade populationerna efter flera generationer skulle bli bättre på att vara hanar (få fler avkommor via spermier), medan de hon-selekterade populationerna skulle bli bättre på att vara honor (få fler avkommor via ägg). På gen-

nivå är min hypotes att gener som gynnade hanar eller honor skulle koncentreras nära den genetiska markören, och att uttrycket av hon-specifika gener skulle vara högre i de hon-selekterade populationerna, medan uttrycket av han-specifika gener skulle vara högre i de han-selekterade populationerna.

Jag undersökte också relationen mellan hanlig och honlig fitness. För att hermafroditer ska utvecklas till hanar och honor måste det finnas en fördel med det. Det är då antagonistiska gener kommer in i bilden. Jag undersökte på fenotypisk och genetisk nivå om de hermafroditiska maskarna fick kompromissa mellan att vara framgångsrika som hanar eller honor. Vad fanns det för korrelation mellan hanlig och honlig fitness? Svaret är att i populationer som inte var utsatta för könsspecifik selektion fanns det ingen korrelation alls, vilket betyder att honlig och hanlig fitness fungerar oberoende av varandra. Däremot, efter 14 generationer av evolution, kunde jag se en genetisk trade-off mellan hanlig och honlig fitness. Kanske var detta tydligare i den experimentella evolutionen. Denna trade-off är beviset på att evolutionen i laboratoriet har fungerat och att vi faktiskt kan undersöka hur en könskromosom utvecklas i realtid. Dessutom undersökte jag om selektionen ledde till en genetisk respons i populationerna. Det fanns flest signifikant uttryckta gener mellan de han-selekterade och hon-selekterade populationerna, vilket betyder att något definitivt har hänt.

Slutligen fördjupade jag mig i förhållandet mellan den hanliga och honliga könsrollen i hermafroditer. Dels undersökte jag hur stor den genetiska variationen var för hanlig och honlig fitness, dels om den påverkades av ändringar i livsmiljön. Det visade sig att det var större genetisk variation för honlig fitness än hanlig fitness, att den genetiska variationen för fitness ändrades i stressiga miljöer för maskarna (ökad salthalt och lite föda) och ändringen var könsspecifik. Detta tyder på att genetisk variation skiftar och beror på många faktorer. Att det finns genetisk variation i fitness betyder också att det finns något för evolutionen att selektera på.

Jag har kunnat visa att hermafroditer kan svara på könsspecifik selektion och därför att det går att studera hur könskromosomer utvecklas i realtid. Det är jättespännande! I framtiden skulle det vara intressant att titta på kromosomerna i de selekterade populationerna och se om det, i enlighet med evolutionsteorier om könskromosomer, ligger könsspecifika gener runt den genetiska markören som imiterar en könsbestämmande gen. Först då kanske vi kan bekräfta att teorierna om hur könskromosomer utvecklas verkligen stämmer.

Introduction and background

Why sex?

If we want to understand why many animals and some plants have evolved into two distinct mating types, we need to start from the beginning, with the definition of sex. A generally accepted definition of sex is the occurrence of meiosis, i.e. meiotic sex. This involves the fusion of two haploid cells to form a diploid zygote, and ends with the formation of new haploid cells, through the process of meiosis when interacting genomes can recombine (Beukeboom & Perrin 2014). In most cases this is linked with sexual reproduction (Mirzaghaderi & Hoerandl 2016).

The many costs and few benefits of meiotic sex

Sex can be costly; both because it is time consuming on a genomic level, and the act of recombination can also break down successful gene combinations built up by selection (Beukeboom & Perrin 2014, Lehtonen & Kokko 2011, Lewis 1987). Other disadvantages are more related to sexual reproduction, such as the energy spent on development of sex-specific organs and increase in predation risk due to complicated behaviors and morphological displays prior to or during the act of mating (Beukeboom & Perrin 2014, Lewis 1987). The last disadvantage is called the "two-fold cost of sex" and refers to males, who only transfer genes to their offspring, and cannot efficiently convert energy to the production of offspring directly. Sexual reproduction also results in something called "genome dilution", which refers to the fact that only half of the genome of an individual is transferred to the offspring (Lehtonen & Kokko 2011). Note, however, that for hermaphrodites the two-fold cost of sex does not apply, and for hermaphrodites that self fertilize genome dilution does not apply either.

Since sex has evolved, it has to come with some advantages. One of the advantages of meiotic sex could be the repair mechanism of recombination (Mirzaghaderi & Hoerandl 2016). Recombination itself could also be advantageous, especially in variable environments or when there are temporal changes in selection pressures. The mixing of different genomes can reveal hidden genetic variation through recombination, and also break up old associations between genes and combine them into new ones that are beneficial for the novel environment (Beukeboom and Perrin 2014, Otto 2009).

Sexual conflict over mating rate gives insight into the paradox

Because of the many disadvantages of sex, individuals should mostly be asexual with some occasions of sex, since this would minimize the cost. Species with these strategies do exist, but they are few, and this still does not explain why there are so many animals that have evolved sex. A recent study looked into if a facultative reproductive mutant could invade a sexual system (Burke & Bonduriansky 2017). They argue that the conflict over mating rate makes it hard for a facultative reproductive mutant to invade, because they can be selected to avoid all matings since they gain fitness even as virgins. Therefore, it is easier for an asexual mutant to invade a sexual population than a facultative sexual one.

Evolution of anisogamy

The evolution of sex and separate sexes is tightly linked and traces back to the evolution of anisogamy (Lehtonen *et al.* 2016). Anisogamy, meaning gamete dimorphism, is found in metazoans, and defines the sexes; males have small gametes and females have large gametes (Beukeboom & Perrin 2014).

Two gamete types

Anisogamy is very old, and may very well have arisen more than a billion years ago (Lehtonen & Parker 2014). The evolution of gamete dimorphism has happened independently several times in the course of history, and often evolved in conjunction with the complexity of organisms (Beukeboom & Perrin 2014, Charlesworth & Charlesworth 2010). Key aspects are the survival of the zygote, which increases with larger size. A trade-off emerges here: if parents make smaller gametes they can make more of them but larger gametes increase zygote survival (Lehtonen & Parker 2014). It is thought that opposing selective pressures to maximize the total number of gametes led to disruptive selection towards two gamete-size strategies (Beukeboom & Perrin 2014). Models show that given all these circumstances, the fitness of both partners is maximized if one partner evolves small and mobile gametes and the other larger and immobile gametes (Beukeboom & Perrin 2014, Bulmer & Parker 2002).

Once anisogamy has evolved, it can easily be maintained, especially when there is a large size difference between sperm and eggs (Lehtonen & Kokko 2011, Lehtonen & Parker 2014). The explanation for this lies in gamete competition, fertilization success and nutrition provisioning per gamete. Producing many small gametes does not assure that every gamete gets fertilized, and in fact, many gametes (i.e. sperm) will end up unfertilized. Therefore it makes little sense to increase the gamete size (hence provisioning per gamete), especially before it is known which of all gametes will lead to increased fitness. It is simply not worth it energy-wise (Lehtonen & Kokko 2011, Lehtonen & Parker 2014). So in many ways anisogamy and differentiated sexes is an

evolutionary one-way street, which is almost impossible to reverse (Lehtonen & Parker 2014).

Did anisogamy result in gonochorism or hermaphrodites?

Once anisogamy evolved, it created a firm base from which the evolution of separate sexes could develop. But the question still remains if gonochorism evolved directly, meaning males and females as separate individuals, or hermaphrodites first, where the male and female gametes are found in the same individual. The green algae Volvox is extremely anisogamous but includes both hermaphroditic and separate sexed species (Charlesworth & Charlesworth 2010, Isaka *et al.* 2012). In animals however, hermaphroditism is probably the derived state, which is favored under certain ecological conditions, whereas hermaphroditism probably is the ancestral state in flowering plants (Avise 2011, Charlesworth & Charlesworth 1978, Charnov *et al.* 1976). A "gamete-view"-explanation of why separate sexed organisms might be the ancestral state is that the easiest way of altering size of gametes would be to increase cell division (and/or rate), which might be harder if the same individual must produce two kinds of gametes (Lehtonen and Parker 2014).

Hermaphroditism

Organisms that can reproduce as both females and males within their lifetime are called hermaphrodites. They occur in more than 90 % of the plant phyla and 70% of all animal phyla (Rice & Gavrilets 2014). Among vertebrates only around 1% are hermaphroditic, which is essentially fishes, and in invertebrates the frequency is 6%. The latter number increases quite dramatically to 30% if insects are excluded (Avise 2011, Schärer 2009).

Hermaphroditic plants

Although I will focus on hermaphroditic animals, hermaphroditism in plants cannot be neglected since it is very widespread. Most of the theory around the evolution from hermaphroditism to gonochorism has also been developed in plants (Avise 2011).

Botanists have structured the major plant sex conditions in six categories as shown in figure 1. It can be thought of as a continuum with strict dioecy in one end (plants with flowers containing strictly male parts, stamen, or female parts, carpel, on separate individual plants) and strict hermaphroditism on the other end (either as monoecy with individuals having flowers containing only male or female parts on the same individual plant or narrow-sense hermaphroditism with individuals having "perfect" bisexual flowers containing female and male parts). Narrow-sense hermaphroditism

occurs in 72% of all angiosperms and is by far the most common sexual system (Avise 2011). However, most of the Pine trees (*Pinaceae*) and Cypresses and allies

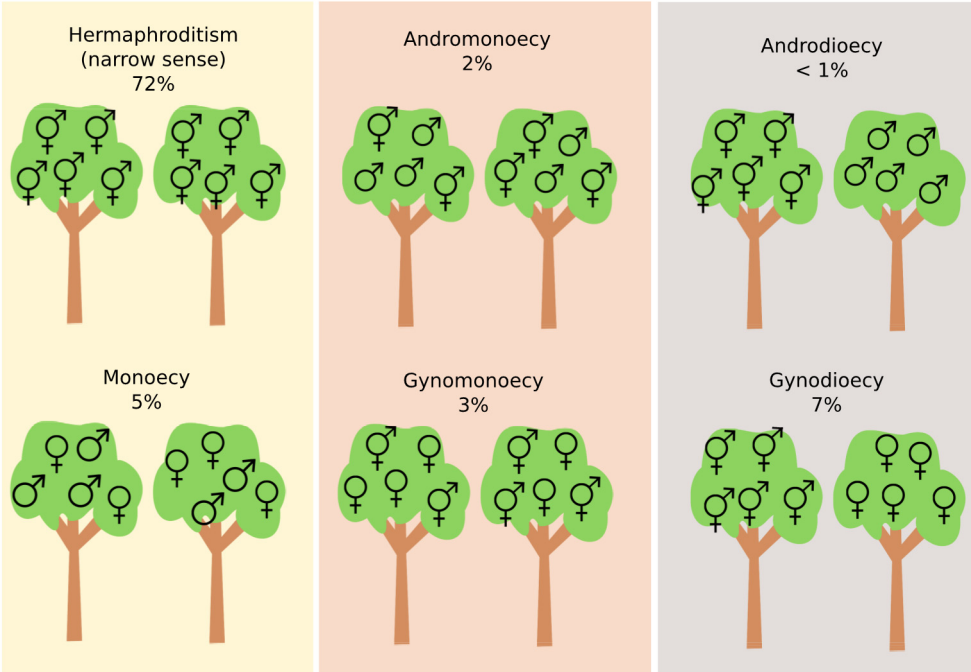


Figure 1. Plant sexual categories can be divided in six categories (excluding dual-sex plants), where narrow sense hermaphroditism and monoecy counts as hermaphrodites and the other categories are a mix of bisexual flowers and male or female flowers. Percentages indicate the approximate percentages of angiosperms that display each sexual category. Adapted after Avise (2011).

(*Cupressaceae*) for example, are monoecious (Avise 2011). The intermediate sexual categories in this sexual system continuum are plants that are either andromonoecious, with "perfect" bisexual flowers and male flowers on same individual or gynomonoecious, with bisexual flowers and female flowers on the same individual. These sexual systems are rather rare, occurring in 2% and 3% of angiosperms, respectively. Horse nettle (*Solanum carolinense*) is an example of an andromonoecious species. The male flowers are smaller than the bisexual flowers, and have a reduced nonfunctional carpel. Gynomonoecy occurs in the Aster genus (*Asteraceae*) among others (Bertin & Kerwin 1998).

The two last sexual systems in plants are androdioecy, with individuals having either only male flowers or only bisexual flowers on individuals or gynodioecy, with individuals having only female flowers or only bisexual flowers on individuals (Avise 2011, Lloyd 1980). Androdioecy is the rarest sexual system in both plants and animals, occurring in less than 1% of angiosperms (Avise 2011). This might be

because it is harder for pure males to achieve a fitness advantage against hermaphrodites than pure females (Charlesworth 1984). Theoretically, pure males can persist in a population with hermaphrodites only if their fertilization success is twice that of hermaphrodites. This could only work if selfing is highly disadvantageous in the species (Charlesworth 1984). Durango root (Datisca glomerata) is androdioecious and just as predicted, this plant has high outcrossing rates (Fritsch & Rieseberg 1992). Gynodioecy occurs in 7% of angiosperms, and is the second most common sexual system described in more than 40 plant taxonomic groups (Avise 2011). Thyme (*Thymus vulgaris*) is one of them, and according to theory again, (Couvet *et al*. 1985) found that hermaphroditic plants had a lower percentage of seed set than female plants, suggesting that there was a selective pressure towards male sexual function.

In a sense, hermaphroditism is more complicated within plants compared to animals, because there is a higher degree of variation in the structural arrangement of the flowers on each plant (Avise 2011). It is also worth noting that the sexual categories of plants are far less rigid than they seem. Some plants have different sexual systems between and sometimes even within populations. Therefore, as mentioned before, plant sexual categories should be seen more as a biological continuum (Avise 2011, Lloyd 1980).

Simultaneous hermaphroditism – common among invertebrates

Hermaphrodites can be either sequential, meaning that there is a temporal separation between being a male and being a female, or simultaneous, which are hermaphrodites that produce sperm and eggs during their whole lifetime (Avise 2011). Among invertebrates, simultaneous hermaphroditism is most common, even though sequential hermaphroditism is represented as well (Avise 2011, Eppley & Jesson 2008).

Jarne & Auld (2006) reviewed the distribution of simultaneous hermaphroditism in invertebrates, occurring in 22 out of 32 phyla. Some examples are corals (*Cnidaria*), love-dart-snails (*Mollusca*), earthworms (*Annelida*) and flatworms (*Platyhelminthes*).

Sequential hermaphroditism – common among hermaphroditic fish

Fishes are the only vertebrates that are hermaphroditic, and most of them are sequential, and few being simultaneous (Avise 2011). The sex change appears in adulthood, and can be either protoandrous, meaning that they change sex from male to female, or protogynous, which means changing sex from female to male. The latter is most common, and beyond this bi-directional sex changers also exist.

Protoandry is not as common among fishes, but exist in anemone fishes (Miura *et al*. 2003). Many anemone fish live in communities with one dominant female and

several smaller males and juveniles. That protoandry is more rare than protogyny in fish might come as a surprise because large females often have a reproductive advantage, since they can produce more eggs and small males can produces lots of sperm relatively cheaply. However, protoandrous species usually result in a male-biased sex ratio, thereby increasing the sperm competition to a degree that it is not advantageous for individual fitness anymore (Avise 2011).

Why be a hermaphrodite?

There are many theories on why hermaphroditism could be advantageous. One factor could be that it provides mating assurance for organisms with low mate search ability, for example sessile or nearly sessile animals, because they can mate with any individual they encounter, or self fertilize if they do not find any partner (Eppley & Jesson 2008). It can also be advantageous to be hermaphroditic for any species with low population density (Charnov 1979, Puurtinen & Kaitala 2002, Tomlinson 1966). Charnov (1979) argued that hermaphroditism will be favored if male reproductive success is limiting, for example when there is a small mating group size so that the number of eggs to be fertilized are small. Another theory is that, in the light of sex allocation theory, hermaphrodites can allocate resources to either male or female gametes depending on the mating group size, which could vary. If the mating group size is for example too small, local sperm competition (i. e. competition among related sperm) will increase, which will decrease male fitness gains (Schärer 2009).

Being a sequential hermaphrodite can have advantages too. Usually there is a reproductive advantage to mating as male or female when being small or large. In protogynous species, such as many wrasses and parrotfish (Avise 2011, Warner 1988), males have a size-advantage, which means that their reproductive output increases with size and age. These fish usually have a sexual system where large males monopolize mates (Warner 1988).

When can a gene for hermaphroditism invade a population with separate sexed individuals? Charnov *et al.* (1976) answered this question theoretically. If one assumes that males and females have equal reproductive success, an outcrossing hermaphrodite can then invade if its reproductive success exceeds the average reproductive success for the individuals that reproduce only as female or male. However the outcome can change depending on the trade-off function between male and female fitness for hermaphrodites (figure 2 after Avise 2011, Charnov *et al.* 1976). If there is a convex fitness curve, i. e. a positive correlation between male and female fitness, the hermaphrodite is the evolutionarily stable outcome. If the fitness curve is concave, which means a negative phenotypic correlation (i.e. a trade-off in sex roles) between male and female fitness, separate sexed individuals are the evolutionary stable outcome. If the fitness curve is mixed between individuals, the outcome is a stable mixed population of the two strategies (Charnov *et al.* 1976).

Selfing versus outcrossing

Indeed, fertilization success is an important factor for the evolution of hermaphroditism (Avise 2011). But with that, comes the question of self-fertilization (i.e. mating with oneself) versus outcrossing. Simultaneous hermaphrodites, those producing female and male gametes during their whole lifetime, are usually capable of self-fertilizing (Avise 2011). If selfing is high in a population of hermaphrodites, genetic variation will drop and homozygosity for any allele will increase. This also means that recessive deleterious alleles become expressed, thus affecting fitness, a phenomenon known as inbreeding depression (Avise 2011).

Although selfing may lead to inbreeding depression, many organisms do self-fertilize, and the fact that it is not immediately replaced entirely by outcrossing probably means that it has some evolutionary advantage (Avise 2011). One of the advantages with selfing is the guarantee of reproduction (Darwin 1876). When no mating partner is around it is better to self than to not reproduce at all. Therefore, selfing will be promoted, as long as there are no other disadvantages, such as inbreeding depression. During stable habitat conditions for example, selfing creates individuals with similar co-adapted gene complexes, which would have been broken apart by outcrossing and recombination. However, as soon as the habitat is changing over time or is heterogeneous, outcrossing should be advantageous.

Jarne & Auld (2006) examined selfing among invertebrates and found that most phyla had an intermediate selfing rate. They suggested that a mixed-mating system with intermediate selfing rates was the most evolutionary stable, taking advantage of the benefits from both selfing and outcrossing in a best-of-both-worlds strategy.

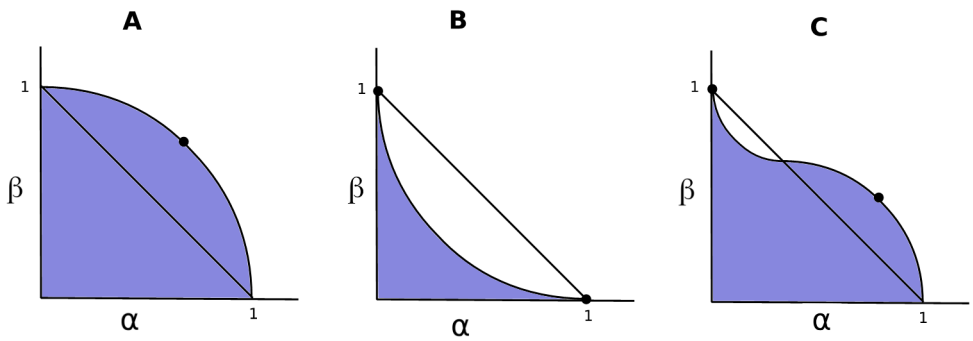


Figure 2. Convex (A), concave (B) and mixed (C) fitness curves for hermaphrodites, where α and β indicates the fitness for male and female sex roles, respectively, for the hermaphrodites relative to the males and females. The filled circle(s) indicate the evolutionary stable outcome of each scenario, where A is hermaphroditism, B is separate sexes and C is a mixture of the two. Adapted after Charnov et al. (1976) and Avise (2011).

Sex roles, sexual conflict and sexual antagonism

The maintenance of anisogamy consequently lead to the evolution of males and females and all that this entails, and these sex differences are usually driven by sexual selection and sexual conflict. This conflict is thought to arise already on the gamete-level, where in females, alleles coding for large gametes are favored, while alleles coding for small gametes are favored in males (Beukeboom & Perrin 2014). Sexual conflict can be broadly divided into inter-locus sexual conflict and intra-locus sexual conflict (Arnqvist & Rowe 2005), depending on the expected genetic basis of the trait.

Sex roles and interlocus sexual conflict

Inter-locus sexual conflict is mediated by opposing behavioral interests among females and males, and the genetic basis of these traits are different for females and males (Arnqvist & Rowe 2005). Inter-locus sexual conflict can happen over any behavior that concerns both sexes, such as fertilization efficiency, mating effort, parental care etc., and adaptations in these traits are expected to be located on different loci in males and females (Arnqvist & Rowe 2005). For example if mating rate is determined at locus A in females and locus B in males, the interaction between these opposing loci is under inter-sexual conflict (Arnqvist & Rowe 2005).

Sexual antagonism

Intra-locus sexual conflict, is when a shared trait for the sexes has opposing fitness optima in males and females (Arnqvist & Rowe 2005). Therefore, intra-locus sexual conflict prevents the sexes from reaching their maximum fitness, and this creates opposite selection pressures, leading to antagonistic selection (figure 3)(Jordan & Charlesworth 2012, Pennell & Morrow 2013, Rice & Gavrilets 2014). The opposite selection pressures on the two sexes can result in the maintenance of polymorphisms, where different alleles are favored in each sex (Connallon & Clark 2012). In fact, polymorphisms at sexually antagonistic loci are estimated to explain a large proportion of the fitness variation within populations (Jordan & Charlesworth 2012). (Intralocus) sexual antagonism has been shown in several animals, such as *Drosophila melanogaster*, red deer (*Cervis elaphus*, Foerster *et al.* 2007), collared flycatchers (*Ficedula albicollis*, Merila *et al.* 1997), Zebra finches (*Taeniopygia gutta*, Price & Burley 1993) and crickets (*Allonemobious socius*, Fedorka & Mousseau 2004) among others.

However, sexual antagonism can sometimes be resolved at a given locus, by decoupling the female and male phenotype, for example via sex-limited mutations or genes located on sex chromosomes (Bonduriansky & Chenoweth 2009a, Mank 2009). In fact, genetic variation that has been characterized as sexually antagonistic

has been shown to be linked to or located on sex chromosomes, such as in red deer (Foerster *et al.* 2007) and *Drosophila melanogaster* (e. g. Connallon & Jakubowski 2009, Gibson *et al.* 2002), although it is worth noting that sexually antagonistic loci are found on autosomes as well, for example in the cricket *allonemobius socius* (Fedorka & Mousseau 2004), *Drosophila serrata* (Delcourt *et al.* 2009) and the side-blotched lizard *Uta stansburiana* (Calsbeek & Sinervo 2004).

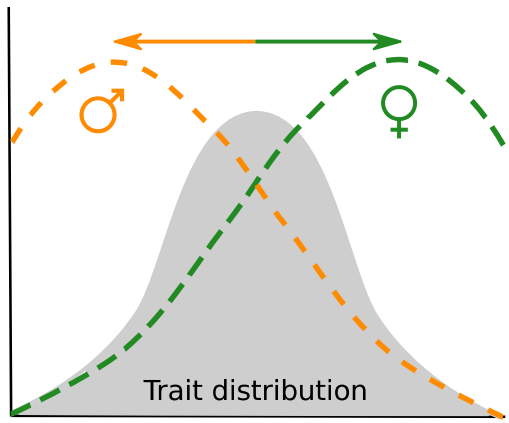


Figure 3. Intralocus sexual conflict for a quantitative trait (grey area) shared between males and females. Dashed lines indicate fitness functions for males (purple) and females (orange). The fitness optima differ between sexes, and the selection is in opposite direction for the sexes.

Sexual antagonism in simultaneous hermaphrodites

Even though sexual antagonism, i. e. opposing selection pressures for males and females, traditionally has been studied in separate-sexed organisms, it is starting to get more attention within hermaphrodites (Abbott 2011, Arnqvist & Rowe 2005, Rice & Gavrilets 2014).

Behaviorally mediated conflict over fertilization success (i. e. inter-locus sexual conflict) has been studied quite extensively. For example, love-dart snails and earthworms mate simultaneously, meaning that each individual functions as male and female during mating, each partner receiving and donating sperm (Avise 2011). This reciprocal exchange of gametes can cause conflict of interest between the two partners and their female and male sex role, since the fitness outcome might be unequal for the different sex roles (Leonard 1993). This might lead to a so-called evolutionary "arms race" (Leonard 1993). The brown garden snail (*Cantareus aspersus*) has shown to be involved in such an arms race (Koene & Chase 1998). The snails pierce the skin of their partner with a calcareous structure that resembles a dart. The dart is covered in mucus, and has been shown to manipulate the female organ of the other partner, and facilitate fertilization success (Koene & Chase 1998). Similarly, earthworms have special bristles called setae, which pierce the skin of their partner during mating and

release an allohormone, which facilitates sperm transfer (Koene *et al.* 2002). An allohormone is a substance, produced by one individual and transferred to the other, that induces a direct behavioral or physiological response (Koene & ter Maat 2001).

If the conflict is occurring in a shared trait (i. e. intra-locus sexual conflict) it can lead to opposite selection pressures for males and females in separate-sexed organisms (Arnqvist & Rowe 2005, Chapman *et al.* 2003). As opposed to inter-locus sexual conflict, it has not been examined in hermaphroditic animals.

Intra-locus conflict in hermaphrodites can rather be seen as opposing selection pressures for female and male sex roles within an individual (Avise 2011, Schärer *et al.* 2014). This complicates things a little, especially for simultaneous hermaphrodites, because one has to consider the consequences for both male and female fitness, and which sex role each partner assumes during mating as well as sex allocation for each sex role (Abbott 2011, Schärer *et al.* 2014). Schärer *et al.* (2014) argued that intra-locus sexual conflict in the strict sense cannot exist in hermaphrodites. Rather, it should be thought of as antagonistic pleiotropy, where the opposing fitness effects in the male and female sex role is traded-off as a life history trait within the lifetime of an individual. That means that sexual antagonism in simultaneous hermaphrodites is more immediate compared to separate-sexed organisms (Schärer *et al.* 2014).

Plant studies have found opposing selection for female and male sex function (Campbell 1989, Morgan & Schoen 1997) but no such studies have yet been performed in a hermaphroditic animal (Abbott 2011). However, male and female fitness is probably not equally distributed in each individual, because usually there is size-dependent sex allocation (Schärer *et al.* 2014). This could lead to linkage between sexually antagonistic alleles and sex allocation alleles (Schärer *et al.* 2014). Sex allocation has been shown to be influenced by the environment such mating group size (Schärer 2009), but also contribute to the standing genetic variation (Schärer *et al.* 2014).

Measuring sexual antagonism

Heritability of fitness and the intersexual genetic correlation

Quantitative genetics (see methodology section) is used to determine the genetic variance and heritability of the traits of interest, and also the genetic correlation rG between a pair of traits (i.e. the proportion of phenotypic co-variance that can be explained by the covariance of the additive genetic variance, COV_A, see methodology section). The heritability measure of fitness traits is generally smaller than for other traits (such as morphology traits), especially in wild populations (Kruuk *et al.* 2000, Mousseau & Roff 1987). This is thought to be because natural selection depletes the genetic variance for fitness, and populations in equilibrium would have no heritable variation for fitness because all alleles linked to fitness will have reached to fixation

(Kruuk *et al.* 2000). However, processes maintaining genetic variation for fitness could be for example sexual antagonism (Connallon & Clark 2012). The potential for sexual antagonism can be estimated with the intersexual genetic correlation, r_{MF}, which is a special case of r_G and describes the proportion of phenotypic covariance that can be explained by the genetic covariance between a shared trait for males and females (Bonduriansky & Chenoweth 2009a). For fitness traits, $r_{MF} < 0$ is evidence for sexual antagonism, whereas a value of 1 is absence of sexual antagonism. It is however worth keeping in mind that r_{MF} assumes variances in male and female fitness do not differ, and that all the genetic variance is additive.

When the intersexual genetic correlation has a value between 0 and < 1, it is hard to draw any conclusions of absence or presence of sexual antagonism, since sex-limited alleles rather than sexually antagonistic alleles could have affected the outcome (Bonduriansky & Chenoweth 2009a). Fitness traits have been found to have a negative r_{MF} in several studies (Berger *et al.* 2014, Brommer *et al.* 2007, Chippindale *et al.* 2001, Foerster *et al.* 2007a). Poissant *et al.* (2010) reviewed the inter-sexual genetic correlation across different populations and between fitness components and other traits. They found that r_{MF} is most often large and positive for most traits except fitness traits, which in general are smaller, and sometimes negative. In case of a positive intersexual genetic correlation it could also be consistent with sexual antagonism, assuming that selection on the trait in question is opposite between the sexes (Poissant *et al.* 2010).

Influence of the environment

The intersexual genetic correlation is expected to differ in environments, due to genotype by environment interactions (Cheng & Kirkpatrick, Punzalan *et al.* 2014). Despite this, fairly few studies have measured the role of sexually antagonistic genetic variation in different environments (Poissant *et al.* 2010, Punzalan *et al.* 2014). Punzalan *et al.* (2014) measured male and female lifetime fitness in *Drosophila serrata* in different lab environments, and found that r_{MF} differed substantially across environments, form negative to positive in some cases. Similarly, other studies have also found environmental variability for r_{MF} in longevity in *Drosophila melanogaster* for different temperatures (Vieira *et al.* 2000), branch number at low and high density in white campion (Lyons *et al.* 1994), and stressful versus benign temperatures in seed beetles (Berger *et al.* 2014). Poissant *et al.* (2010) also looked at r_{MF} in different environments and found it to be very variable, suggesting that only measuring r_{MF} in one environment may not always be sufficient to understand the evolutionary dynamics of the intersexual genetic correlation.

Evolution of sex chromosomes

Sex determination

There is a remarkable diversity in how sex is determined among species. Generally, sex determination mechanisms in animals are divided into environmental sex determination (ESD) and genetic sex determination (GSD) (Bull, 1983).

In animals with ESD, sex is determined by an environmental cue such as photoperiod, habitat, pH or temperature (Bachtrog *et al.* 2014, McCabe & Dunn 1997). This includes marine amphipods (phototperiod-dependent), reptiles (temperature-dependent) and marine spoon worms (substrate-dependent) (Berec *et al.* 2005, Guler *et al.* 2012, Janzen & Paukstis 1991). It is worth keeping in mind that even though genes in ESD do not influence the determination of sex per se, they are still responsible for sexual development in these organisms.

The most common sex determination system known is GSD and there is a wide variety of different types, for example male or female heterogameity and polygenic sex determination. Many mammals and birds have two morphologically different sex chromosomes, called heteromorphic sex chromosomes. Male heterogamety (male XY and female XX system) is found in mammals including humans, and female heterogamety (female ZW and male ZZ system) is found primarily in birds and butterflies (Bull 1983). The X(Z) is large and gene-rich and the Y(W) small and gene-poor (Berset-Brändli *et al.* 2007). This heteromorphism has built up over millions of years over evolutionary time, because of gene loss on the Y(W) chromosome due to restricted recombination between the sex chromosomes. This recombination arrest efficiently preserves the sex-determining loci and sex-linked genes nearby (Charlesworth & Charlesworth 2000).

When several independent loci determine sex, this is called polygenic sex determination (PSD). These systems can have chromosomes modified into a third sex chromosome or have sex determining loci at autosomes that interact with contemporary sex chromosomes (Charlesworth & Charlesworth 2000). The latter is found in the housefly (*Musca domestica*), and several rodents (Veyrunes *et al.*, 2010, Bull and Bulmer, 1981, Gileva and Fedorov 1991, Winking *et al.*, 1981). Since some PSD systems have several sex chromosomes but only two sexes, it means that females and males in the same species can have different genotypic sex. This may result in different classes of females and males with different fitness (Moore & Roberts 2013).

Transitions between hermaphroditism and gonochorism

In animals, contrary to plants, hermaphroditism seems to be the derived state (Avise 2011). With that being said, animal sex determination differs a great deal between taxa, from birds and mammals where the XY/ZW systems are very ancient (Marshall

2016), to fish where sex determination systems seem to be very flexible and have a higher turnover (Avise 2011, Beukeboom & Perrin 2014). However, separate sexes have evolved independently many times in animals and plants (Bachtrog *et al.* 2014). Most of the research on evolutionary transitions from hermaphroditism to gonochorism has been on plants, and that is also where theories of sex chromosome evolution have originated (Avise 2011, Charlesworth *et al.* 2005a).

Dioecy may evolve in two different ways; either by a gradual change of the investment in either sex role, or a rapid change with female- or male-sterility mutation, the latter has been developed in plants (Bachtrog *et al.* 2014).

There are two proposed theoretical pathways to dioecy, one through gynodioecy and the other through androdioecy (Avise 2011). They are both predicted to happen rather rapidly through the appearance of a sterility-mutation. The first path starts with a male-sterility mutation, making some individuals female. Hermaphrodites then decrease female allocation as a response to selection, and eventually lose the female function, thus making them male. This pathway is most common and has been found in plants such as papaya and strawberry (Bachtrog *et al.* 2014). The other evolutionary pathway, through androdioecy, starts with a female-sterile mutant, creating males, which would select for decreased investment in the male function in hermaphrodites. Eventually hermaphrodites lose male function and become entirely female. The gynodioecy pathway to dioecy is far more common, probably because it is easier to have a reproductive advantage compared to hermaphrodites as a pure female for reasons already discussed. Male-sterility mutations are also far more common in plants than female-sterility mutations, although it is impossible to tell if that is a consequence or a cause of the gynodiecy pathway being the most common in plants (Avise 2011, Bachtrog *et al.* 2014).

Can separate-sexed organisms evolve back to being hermaphrodites? In plants, such as the bitter cord family *Momordica*, monoecy evolved from dioecy several times independently (Schaefer & Renner 2010). This reversal can sometimes be impossible for animals with highly differentiated sex chromosomes and complex phenotypes, as mentioned before (Bull & Charnov 1985). But there are cases in which animals have evolved from dioecy to androdioecy, males and hermaphrodites, in branchiopod crustaceans for example (Pannell 2002).

Evolution of heteromorphic sex chromosomes

Once separate sexes have been established from a hermaphrodite ancestor through the possible pathways described in the previous section, recombination in the sex-determining region (female and male determining region respectively) is suppressed, effectively preventing separation of sex-determining gene(s) (figure 4) (Beukeboom & Perrin 2014, Charlesworth & Charlesworth 2000).

There are two ways in which recombination between the proto-X and proto-Y sex chromosomes can be hindered. Either recombination rates can be gradually reduced through genetic modifiers, or inversion of large parts of the chromosome can restrict recombination of these regions (Charlesworth *et al.* 2005a). As sexually antagonistic alleles appear, the recombination suppression region of the proto-Y starts to increase (Charlesworth & Charlesworth 2000, Charlesworth *et al.* 2005a). A sexually antagonistic male-benefit/female-detrimental allele located close to the male sex-determining region on proto-Y will increase when rare, even though the fitness increase is smaller than the fitness decrease in daughters, since it will be transmitted to sons more often than daughters (Beukeboom & Perrin 2014).

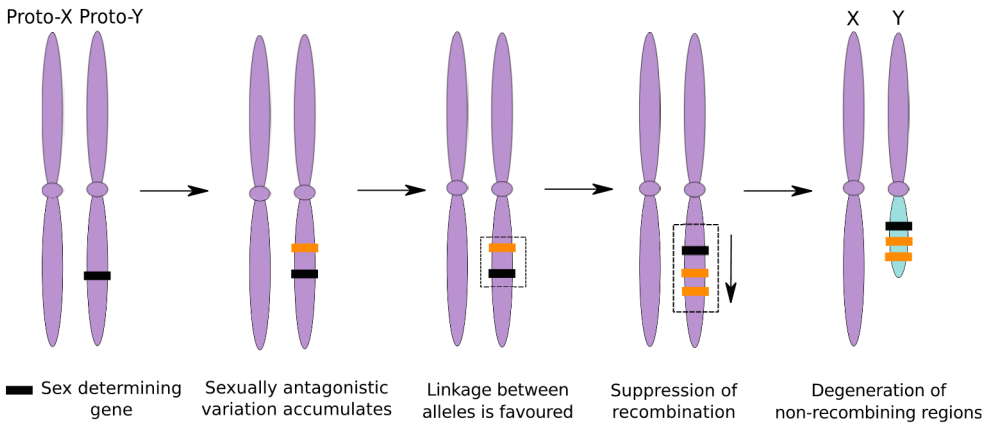


Figure 4. Sex chromosome evolution theory starts with an ordinary pair of autosomes that gain a sex determining function. Accumulation of sexually antagonistic alleles around the sex-determining region favors recombination arrest (e. g. an inversion), suppressing separation of the genes in the sex-determining region. Eventually the non-recombining region accumulates deleterious mutations and gene loss.

However, this does not hold true if the sexually antagonistic locus is located on an autosome, unless the fitness increase in males exceeds the fitness decrease in females (Beukeboom & Perrin 2014, Rice 1984). In this way, the non-recombining region of the proto-Y chromosome will increase and be reinforced by more sexually antagonistic alleles accumulating at its border. Note that the accumulation of sexually antagonistic alleles also happens in the proto-X chromosome, but in a more complicated fashion, since the X spends 1/3 of the time in males (Beukeboom & Perrin 2014). The expansion of the non-recombining area will ultimately involve the whole Y-chromosome except for a small region in the end called PAR (= pseudo-autosomal region) (Beukeboom & Perrin 2014, Charlesworth *et al.* 2005).

This evolution results in strongly differentiated sex chromosomes, with a continuous decay of genetic material and accumulation of deleterious mutations sheltered by the chromosome wide linkage on the Y chromosome (Charlesworth & Charlesworth 2000). The step-wise cessation of recombination is referred to as evolutionary strata,

which are clusters of gametologs (gene orthologs located on each sex chromosome) spatially structured on the sex chromosomes (Wright *et al.* 2014). The avian Z chromosome has formed four strata during approximately 130 million years (Wright *et al.* 2012), and (Lahn & Page 1999) identified at least four strata in the human sex chromosomes.

Degeneration of Y?

With the evolving Y chromosome inevitably accumulating mutations and slowly being more and more genetically eroded, it was previously thought that the Y chromosome was 'born to be destroyed' (Steinemann & Steinemann 2005). In line with this argument are organisms that completely lack the Y chromosome, such as crickets and dragonflies (Beukeboom & Perrin 2014). Whole-genome sequencing of *Drosophila* spp. and primate Y-chromosomes revealed that most of the ancestral genes indeed have been lost, and that genes situated in the Y chromosome are mostly male-specific (Bachtrog 2013). However, predictions of the extinction of the Y chromosome have been exaggerated. Evidence against the disappearance of the Y chromosome comes from studies showing that the erosion of genes is not linear and some genes are protected from decay (Bachtrog 2013, Beukeboom & Perrin 2014). Also, the repetitive DNA sequences located on the Y chromosome have been shown to be important in *D. melanogaster* for regulating gene expression (Beukeboom & Perrin 2014, Francisco & Lemos 2014, Rice & Friberg 2008).

Sex chromosome turnovers

Some sex chromosomes are strongly heteromorphic, such as in most birds and mammals, whereas fishes and amphibians often have homomorphic (i. e. morphologically undifferentiated) sex chromosomes, even though some of them are as evolutionarily old (Beukeboom & Perrin 2014). There are two hypotheses to explain this; occasional recombination due to sex-reversals and high turnover events that can result in a neo-sex chromosome.

Sex reversals

In many species of amphibians, lizards and fish that have female or male heterogametic sex chromosomes, extreme temperatures can override the genetic sex determination mechanism (Bull 1983). This phenomenon, known as sex reversal, is found in for example the common frog (*Rana temporaria*) (Quinn *et al.* 2007), the central bearded dragon (*Pogona vitticeps*) (Holleley *et al.* 2015) and the Nile tilapia (Schartl 2004). Interestingly, recombination rate between all chromosomes - autosomes and sex chromosomes - is dependent on the phenotypic sex in most species, and males usually have lower recombination rates than females (Perrin 2009). Therefore, sex-reversed XY-females generally show the same recombination patterns

as normal XX-females, which have been confirmed by sex-reversal experiments in mice, medaka fish *Oryzias latipes* and the crested newt *Triturus cristatus* (Perrin 2009). Sex-reversed XY-females provide an opportunity for the X and Y to recombine, introducing new genetic variance for male fitness (Perrin 2009). It is worth noting however, that this only works for species with relatively undifferentiated sex chromosomes, since strongly differentiated sex chromosomes are too different to be able to recombine successfully (Perrin 2009).

Turnovers and neo-sex chromosomes

New sex determining loci can invade easily if they are linked to higher fitness, and a chromosome bearing a novel sex-determining locus can later evolve to become a new sex chromosome, resulting in a sex chromosomes turnover (Bachtrog *et al.* 2014). Sex chromosome turnover can happen due to a new sex-determining gene on an autosome, transposition of a sex-determination locus to an autosome, and fusions between autosomes and existing sex chromosomes (Kitano & Peichel 2012). The latter results in the formation of a neo-sex chromosome. Neo-sex chromosomes (in contrast to proto-sex chromosomes) occur by fusions of parts of or whole autosomes to an existing sex chromosome (Beukeboom & Perrin 2014, Francisco & Lemos 2014, Kitano & Peichel 2012). It often results in a multiple sex chromosome system, such as X1X2Y, which is common in fishes (Kitano *et al.* 2009). Other organisms with neo-sex chromosomes include *Drosophila* spp. (*D. miranda*, *D. pseudoobscura* and *D. albomicans* have been studied in detail) that carry a neo-Y chromosome, and a neo-Z chromosome that has been found in the *Sylvioidea* bird family (Pala *et al.* 2012).

Thesis aims

In this thesis, I aim to produce a deeper understanding of the dynamics and trade-offs between male and female sex roles in simultaneous hermaphrodites, and the initial processes required to evolve sex chromosomes in a hermaphrodite ancestor.

In **paper I** we summarize what has been done in the field of sex chromosome research, both theoretical and experimental progress over the years. We then aim at finding gaps in the knowledge where we suggest emphasis can be placed for further research in this field.

In **paper II**, our aim was to estimate the heritability for male and female fitness in the simultaneous hermaphroditic flatworm *Macrostomum lignano*. We also wanted to investigate if the penetrance of a genetic marker (GFP, green fluorescent protein) in individuals of the transgenic line BAS1 differed for the male fitness (sperm) estimate and the female fitness (eggs) estimate. This was interesting, because we wanted to select on that fitness in the selection experiment (in papers IV and V).

In **paper III** we examined how changes in environmental conditions, such as food-restriction and salt stress, affect the genetic variance for fitness components (male and female fitness) in the hermaphroditic flatworm *M. lignano*. We also looked at the relationship between genetic and phenotypic correlations for fitness components in these conditions.

In **paper IV**, we aimed at studying the start of the evolution of a sex chromosome "in action". We subjected a simultaneous hermaphrodite to sex-limited selection, both on the male and female fitness, with the help of a genetic marker (GFP). After some generations, we evaluated the response of the experimental evolution on the male and female fitness in treatments, and morphologically by looking at overall size and size of gonads.

Paper V follows the earlier (paper IV), and the aim was to look at the genomic response to the sex-limited experimental evolution. Here, we looked at levels of RNA expression patterns of sex-specific genes in the selection treatments (male-limited selection, female-limited selection and control treatment) after 21 and 22 generations of sex-limited selection.

General methodology

Study species & laboratory lines

Macrostomum lignano is a small marine free-living flatworm (Macrostomorpha, Platyhelminthes), which is easily cultured in the laboratory. This and its transparent body make it ideal as a model organism for studying everything from developmental biology to evolutionary biology and reproductive biology (Ladurner *et al.* 2005, Marie-Orleach *et al.* 2014).

Discovery and habitat

M. lignano was first found 1995 in samples from a lagoon area called Laguna di Marano, close to a village called Lignano on the Adriatic Sea in Northern Italy (Ladurner *et al.* 2005). *M. lignano* occurs between sand grains, in the uppermost sediment of the intertidal zones on sheltered beaches (Ladurner *et al.* 2005). Salinity preference for *M. lignano* lies between 32 ‰ and 20 ‰ and it is primarily feeding on diatoms (Ladurner *et al.* 2005). It can occur both at high and low population densities in nature (Janicke *et al* 2016).

Anatomy and behavior

M. lignano is 1-2 mm in length, and has a generation time of 3 weeks (Ladurner *et al.* 2005). The animal is colorless, but can appear brownish because of the gut contents, which are usually diatoms. It has two pigment cup eyes, and the tail plate consists of adhesive glands with the ability to stick to sand grains in its natural habitat (Ladurner *et al.* 2000, 2005). The animals are negatively phototactic and actively swim away from any light source. It lacks an anus, and undigested food is regurgitated as sausage-like food pellets (Ladurner *et al.* 2005).

M. lignano has, together with many other macrostomorph flatworms, the capacity to regenerate. It can regenerate missing body parts anteriorly, posteriorly and laterally, except for the brain and the pharynx (Egger *et al.* 2007, Nimeth *et al.* 2007).

Being a simultaneous hermaphrodite, *M. lignano* has paired testes and ovaries on each side of the gut, at about mid-body (Ladurner *et al.* 2005). The female genital opening is located anterior to the male genital pore and it also has a sclerotized male copulatory organ, called a stylet (figure 5) (Ladurner *et al.* 2000). Animals starved for a long time break down their gonads, and the entire body shrinks. After feeding

however, the organs grow back and they regain their ability to reproduce (Egger *et al.* 2007).

Mating in *M. lignano* is reciprocal, meaning that each partner donates and receives sperm during a single copulation (Schärer *et al.* 2004, Vizoso *et al.* 2010). Although it is a simultaneous hermaphrodite, *M. lignano* never self-fertilize (Vizoso *et al.* 2010). Precopulatory behavior includes circling movements around the partner, after which they copulate by simultaneously inserting the stylet in the partner's female opening (Schärer *et al.* 2004, Vizoso *et al.* 2010). Copulation time is rather short, on average 9 seconds (Schärer *et al.* 2004). In some cases a mated worm performs a postcopulatory 'sucking behavior' by positioning its pharynx over its female opening, and (Schärer *et al.* 2004) observed sperm shafts sticking out of the female genital opening after this behavior. It might be an attempt to manipulate the received ejaculate, by removing seminal fluid and possibly sperm (Vizoso *et al.* 2010). *M. lignano* sperm have two long bristles, which are hypothesized to aid in anchoring themselves in the epithelium of the female antrum and preventing them to be easily pulled out by the sucking behavior (Vizoso *et al.* 2010).

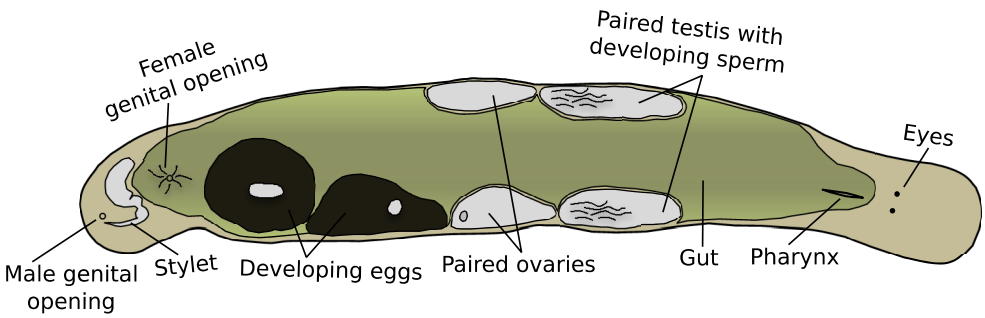


Figure 5. The anatomy of *Macrostomum lignano.*

Worm culturing

Worms are kept in petri dishes with a population size of 100 individuals. They are fed with algae (Nitzchia curvilineata), which are grown in petri dishes for about 2 weeks and then ready to use. An algae plate can sustain a population of 100 worms for about 4 – 5 weeks. Every generation there is a 10% migration between populations. For more detailed information about lab populations see below.

Laboratory populations

Several *M. lignano* culture lines have been established, and below is a short summary of origin and population structure of the different laboratory lines related to my thesis.

LS1 and LS2 – wild type lines

LS1 was created from collections of worms at two field sites; PS in Isola di Martignano, and UV in Bibione near Lignano Sabbiadoro, Italy (Ladurner *et al.* 2005). Crosses between worms from each location were carried out to keep a high genetic variation in the population. Due to an infection by a parasite, the population experienced a bottleneck, but is now stable. It is kept as a metapopulation of 6 paired populations with 100 worms per population (i. e 1200 worms in total). At every generation there is 5% migration between population pairs and once per year all the populations are mixed. (L. Schärer, *personal communication*)

LS2 was collected at the field sites UV in Bibione and P1 near Isola di Martignano, Italy in 2011 (see Ladurner *et al.* 2005). The population originates from 300 individual worms per site, which have been crossed with one parent from each site. The line is now kept as a metapopulation and is maintained in the same way as the LS1 population, except for the yearly mixing. (Zadesenets *et al.* 2016).

DV1 (inbred line) and HUB1 (transgenic line)

DV1 is an inbred line which was initiated by crossing two virgin worms from an outbred laboratory mass culture originally collected in the field 2003 near Bibione (site UV, see Ladurner *et al.* 2005). The offspring were then inbred via full-sib and half-sib mating for 24 generations and the line has since been kept in small populations to maintain inbreeding. DV1 was used to create HUB1, a transgenic line expressing GFP (green fluorescent protein)(Zadesenets *et al.* 2016).

More specifically, when creating HUB1, a DNA-construct was injected into a single cell stage embryo. The DNA-construct contained a transposon (Minos), a promoter region of a *M. lignano* housekeeping gene called elongation-factor-1-alpha (Efalpha), and the coding region for the GFP protein (egfp) (Demircan 2013, Marie-Orleach *et al.* 2014). Minos is a transposable element that was first identified in the fruit fly Drosophila hydei (Pavlopoulos *et al.* 2007). Transposons can move into genomes with a cut-and-paste mechanism that has been widely used in genetic manipulation in research and medicine (Pavlopoulos *et al.* 2007). Thanks to its housekeeping promoter, GFP expression in *M. lignano* is ubiquitous, including in sperm and eggs (Demircan 2013, Marie-Orleach *et al.* 2014).

Since HUB1 originates from DIV1, the two lines are expected to be almost genetically identical (Marie-Orleach *et al.* 2014). Marie-Orleach *et al.* (2014) compared the reproductive performance of DV1 line and the HUB1 line and found no difference in terms of morphological traits, reproductive success or mating rate.

The GFP marker is usually inherited in a Mendelian fashion with a dominant allele on a single locus (Demircan 2013, Marie-Orleach *et al.* 2014). Sometimes however, the proportion does not follow a Mendelian segregation pattern, and this could be either due to phenotypic loss of expression in worms (due to silencing of the GFP or

some kind of developmental problem) or that the GFP gene is inserted several times in the genome, as (Demircan 2013) suggested.

BAS1 – transgenic line

BAS1 is a GFP marked outbred population, which was created by crossing LS1 with the GFP expressing HUB1 line. 8 generations of backcrosses were carried out, with 20 families from each of the 12 LS1 metapopulations. BAS1 is now kept in a metapopulation structure, in the same way as LS1 and LS2.

Worm karyotypes

Egger & Ishida (2005) found the karyotype of *M. lignano* to be 2n=8, with one pair of chromosomes being significantly larger than the other chromosomes. However, it has been shown that *M. lignano* displays karyotype polymorphism (Zadesenets *et al.* 2016). Out of around 130 worms in the DV1 line, 58% had a duplication of the largest chromosome (2n=9), 27% had two extra copies of the large chromosomes (2n = 10), 12 % had the 'normal' karyotype (2n=8), and around 4% had abnormal karyotypes. The same pattern of karyotypes was found in HUB1. The outbred lines (LS1 and LS2) mostly consisted of 2n=8 individuals (97% and 100% respectively)(Zadesenets *et al.* 2016).

Experimental evolution

The study of the process of evolution in experimental populations is called experimental evolution (Kawecki *et al.* 2012). Kawecki *et al.* (2012) distinguish between experimental evolution and artificial selection where the first is studying population changes when experimentally changing conditions such as environment, demography, genetics etc., and the latter is when the researchers select for individuals with special phenotypes, much as in breeding. The main difference between these two approaches is that during experimental evolution, selection can act on any trait related to fitness. The limitations with experimental evolution are that it is usually performed in laboratories with a relatively small effective population size and by using well-known study species such as *Drosophila melanogaster* or *Escherichia coli* (Kawecki *et al.* 2012). Despite that, experimental evolution has still shed some light on important questions such as adaptation to specific environments (e.g. temperature: Lenski & Bennett 1993, nutrition: Kolss *et al.* 2009, parasites: Zbinden *et al.* 2008 and trade-offs: e. g. Lenski 1988) and estimating the impact on genetic variables such as mutation accumulation (Hegreness *et al.* 2006, Perfeito *et al.* 2007). It can also reveal how standing genetic variation and mutations contribute to the selection response (Edward *et al.* 2010). Experimental evolution is a useful tool and a promising method

when studying sex chromosome evolution, because it allows for experimental manipulation and disentangling of cause and effect.

Quantitative genetics

Individuals in populations are often phenotypically diverse, and evolutionary biologists have long tried to tease apart if these differences are caused by genes or the environment. A useful tool to answer questions such as these is the field of quantitative genetics, the genetics of complex traits (Hill 2010). It was founded independently by Ronald Fisher and Sewall Wright in the 1920s and has played a role in both breeding programs but also served as a tool to understand inheritance of complex diseases (Lynch & Walsh 1998). For evolutionary biologists, it can be used to study multi-locus traits and ultimately, to predict if natural and/or sexual selection can act on and make phenotypic changes in the population, or the response to experimental evolution in the laboratory (Hill 2010, Kruuk 2004). Traits need to be heritable in order to evolve, meaning having an underlying genetic basis. If there is no genetic variance in populations, evolution cannot change phenotypes (Kruuk 2004).

The general idea in quantitative genetics is that phenotypic similarity between relatives is due to shared alleles, and is therefore a part of the genetic variation, in contrast to noise due to environmental variation. For example, if related individuals (which share a lot of alleles) are phenotypically more similar to each other than unrelated individuals (which share none or very few alleles), we can assume that genes make an important contribution to the phenotypic variation (Lynch & Walsh 1998, Wilson *et al.* 2010).

The components of phenotypic variance

The total phenotypic variance can be divided into different components, for example $V_P = V_G + V_E$ where V_G is the genetic variance, and V_E is the environmental variance (Falconer & Mackay 1996, Wilson *et al.* 2010). The environmental variance is all non-genetic variance, and the genetic variance can further be divided into three other components: $V_P = V_A + V_D + V_I + V_E$, where V_A is the additive genetic variance, V_D is the variance due to dominance effects, and V_I is the variance from interaction effects (epistasis) (Falconer & Mackay 1996). Dominance (V_D) and interaction (V_I) effects are very hard to measure in populations, which is why most studies focus on estimating how much of the shared phenotypic variance seen in relatives is made up of additive genetic variance (Falconer & Mackay 1996, Kruuk *et al.* 2008, Wilson *et al.* 2010). Additive genetic variance is also expected to contribute the most to the response to selection (Falconer & Mackay 1996, Hill 2010, Hill *et al.* 2008). A simplified version of the equation then becomes $V_P = V_A + V_R$ where the phenotypic

variance is partitioned into additive genetic variance, and residual variance, which is interpreted as mostly coming from environmental effects, but may also include non-additive genetic effects, i.e. epistasis and dominance effects. However, these effects are usually assumed to be negligible (Wilson *et al.* 2010).

The easiest way to estimate the additive genetic variance is through a pedigree, where the relationships between individuals are known and we can measure the phenotypic value (Falconer & Mackay 1996, Wilson *et al.* 2010). Some common pedigree designs are parent-offspring, half-sib and full-sib families. The potential drawback of full-sib analysis is the influence of common environmental effects, but a way to avoid this problem is to use a paternal half-sib design instead, which minimizes maternal effects (Lynch & Walsh 1998).

Heredity, heritability and genetic correlation

Heredity, a parameter that describes the genetic resemblance between parents and offspring (i.e. relatedness), is not the same thing as heritability (Lynch & Walsh 1998). The heritability of a trait is a ratio that describes how much of the phenotypic variance that is composed of additive genetic variance in a population, $h^2 = V_A/V_P$. This heritability, denoted h^2, is called the narrow-sense heritability because it only takes into account the additive genetic effects. The higher relatedness between relatives, the higher the additive genetic variance is (Lynch & Walsh 1998). The broad-sense heritability, H^2, is the ratio between the total genetic variance (including dominance and epistatic effects) and the phenotypic variance $H^2 = V_G/V_P$ (Falconer & Mackay 1996, Lynch & Walsh 1998).

If a pair of traits co-vary, we can estimate the genetic correlation, r_G, which is how much of the total phenotypic covariance, COV_P can be explained by the covariance of the additive genetic variance, COV_A (Wilson *et al.* 2010).

The animal model

Most evolutionary quantitative genetic studies use a mixed effects model known as "the animal model" to decompose phenotypic variance into genetic and environmental components, as well as estimate genetic correlations between traits (Wilson *et al.* 2010). A mixed effects model is a linear regression model using both fixed and random factors as explanatory terms (Kruuk 2004). In the simplest form of animal model, a single trait (y_i) is modeled in an individual, where part of the independent variable is the so-called "breeding value" (or genetic merit) of that individual. In the simplest animal model, the observation for individual i is:

$$y_i = \mu + a_i + e_i$$

Where y_i is the phenotypic trait value of individual i, μ is the phenotypic mean in the population, a_i is the breeding value, and ei is the random residuals error. The breeding value (a_i) is the expressed additive genetic effects on an individual's genotype that is contributing to the mean phenotype in the population (μ). The phenotypic mean μ is a fixed factor and has a known value, whereas the breeding value a_i is not known, and is treated as a random effect in the animal model. This means that it is an estimate of non-independence (i.e. shared genes between individuals) of the individual phenotypic variance (y_i). (Wilson *et al.* 2010).

Gene expression and RNA extraction

In recent years with the arrival of next generation sequencing, it has become easier to examine the genetic changes that underlie the phenotypic change due to selection (Romero *et al.* 2012). RNA-expression, or rather the mRNA in a cell, reflects the activity and hence the protein production activity of a gene (Cooper 2000). A higher expression of mRNA therefore means a higher level of protein production, although many proteins in eukaryotic cells have regulatory functions of other proteins, which makes for greater flexibility of gene expression control (Cooper 2000). Changes in gene regulation (i.e. gene expression levels) are also important contributors to phenotypic diversity, compared to coding sequence, which is often decoupled from gene expression (Connallon & Knowles 2005).

Gene expression has helped to shed light on important questions in evolutionary biology such as speciation (Dunning *et al.* 2016, Parkinson *et al.* 2016), plasticity (Ghalambor *et al.* 2015, Morris *et al.* 2014) and responses to artificial selection (Huang & Agrawal 2016, Yampolsky *et al.* 2012).

Sex-specific gene expression

Genes that show a difference in expression between males and females are said to be sex-biased genes. These include genes that are only expressed in one sex (sex-specific) and genes that show a higher expression in one sex than the other (sex enriched) (Ellegren & Parsch 2007). Studies have revealed that the proportion of sex biased expression differ between organisms, from 2% in the transcriptome of the marine snail *Littorina saxatilis* to 90% in *Drosophila melanogaster* (Ingleby *et al.* 2015, Innocenti & Morrow 2010, Martínez-Fernández *et al.* 2010). Some sex-biased genes are sexually antagonistic, but by far not all, and by considering sex-specific fitness together with gene expression one can disentangle the genes that are involved in sexual conflict (Ingleby *et al.* 2015, Parsch & Ellegren 2013). Sex-biased genes might be regulated by alternative splicing of gene transcripts, microRNAs that bind to the mRNA to regulate expression or translocation of sex-biased genes to sex chromosomes

(Ingleby *et al*. 2015). Meisel (2011) examined the rate of evolution for sex-biased genes in *D. melanogaster* and found that they evolved faster that unbiased genes in reproductive tissues with similar expression breadth. This is probably due to the link to reproductive success (Meisel 2011). Therefore, sex-limited selection might change the expression of sex-biased genes.

Results and discussion

Paper I. Sex chromosome evolution: historical insights and future perspectives

The field of sex chromosome evolution has to a large extent been shaped by findings in model organisms with highly diverged sex chromosomes such as Drosophila melanogaster and humans, even though this is changing, mainly due to the advent of next-generation sequencing. We suggest new approaches integrating the role of ecology and demography in sex chromosome evolution, for example how sex-specific fitness in different ecological conditions affects selection for recombination suppression. The use of experimental evolution could increase understanding of the initial phases of the evolution of sex chromosomes, for example if it is possible to demonstrate a build-up of sexually antagonistic variation around the sex determining region. New sequencing technologies make it possible to investigate phenomena that were not possible to study before, such as gene interactions, expression networks between sex chromosomes and autosomes, and alternative splicing. New theory in this field is definitely needed, since most major advances were in the 70s and the 80s, and with a rather narrow focus. We suggest integrating ecology into theory and focusing on multi-locus models, since outcomes could differ fundamentally from single-locus models (Flaxman *et al.* 2014). Finally, the field could receive new ideas and inspiration by applying approaches developed in the speciation literature, where research has been more successful in integrating ecological and demographic processes.

Paper II. Sex-specific genetic variance for fitness and inheritance of a genetic marker in a simultaneous hermaphrodite

We found that additive genetic variance (V_A) and heritability (h^2) were significantly different from zero in both female fitness and male fitness (figure 6). The V_A estimate for female fitness was three times larger than for male fitness (V_A = 0.33 in female fitness and V_A = 0.08 for male fitness). This is in line with another hermaphrodite study, and studies on separate sexed animals have also found male fitness to have a lower heritability (Foerster *et al.* 2007, Innocenti & Morrow 2010, Merilä & Sheldon

2000). This phenomenon could be because male fitness is influenced by environmental factors to a higher degree than female fitness (and therefore has a higher residual variance, V_R), or because males experience stronger sexual selection, depleting genetic variance in male fitness. Higher residual variance in male fitness could also be linked to higher stochasticity from processes such as sperm competition (Foerster *et al.* 2007).

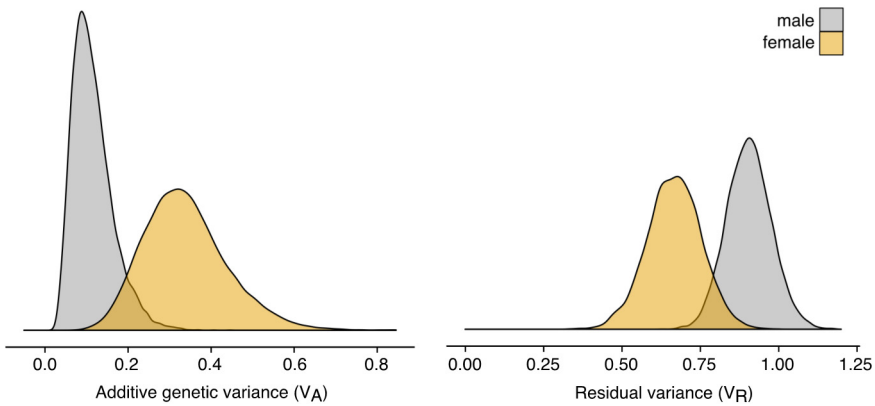


Figure 6. Addititve genetic variance and residual variance for male and female fitness in the stock population.

The phenotypic correlation between the fitness components was weak and only explained 10% of the variation. Similarly, the intersexual genetic correlation for these fitness components was low and not different from zero (r_{MF} = 0.007). This means that male and female fitness components seem to function independently from each other, and there does not seem to be a trade-off or any sexually antagonistic genetic variation in male and female fitness. Yund *et al.* (1997) found a trade-off between male and female reproduction and growth in a hermaphrodite Ascidian, and so this might be something to examine in the future in *M. lignano*.

Finally, we could show that inheritance of the genetic marker (GFP) was positively correlated in male and female fitness measurements, which means that the chance of passing on the marker to the next generation is the same for sperm and eggs. Deviations from the Mendelian inheritance seen in Marie-Orleach *et al.* (2014) can therefore not be explained by variations in the inheritance between gametes (e.g. maternal effects).

Paper III. Environmental variation in sex-specific fitness in a simultaneous hermaphrodite

We found that environmental variation did not change the phenotypic variance in fitness components, but it did change the additive genetic variance (V_A) for male and female fitness. There was a significant interaction between the environment and sex-specific genetic variance, where additive genetic variance (V_A) for female fitness was increased in the hyper-salinity condition and additive genetic variance for male fitness was increased in the food-limited condition (figure 7).

This is interesting, because it means that male and female fitness can experience different outcomes in local adaptation. We hypothesize that female fitness alleles might have a larger impact on population demography (Harts *et al.* 2014), and female-beneficial alleles could therefore increase in the population in high salinity environments. On the other hand, in low-resource habitats where additive genetic variance for male fitness increased, this might favor a response to sexual selection, since sexual selection is generally stronger in males (or the male sex-role in hermaphrodites). Competition for fertilizations - sperm competition - could potentially lead to sexual conflict if the traits increasing male fertilization success are harmful to females. Since we found no strong intersexual genetic correlation between male and female fitness components, and the phenotypic correlation was weak as well, this means that male and female fitness are evolutionarily independent of each other. Therefore, we argue that these results may be applicable to separate sexed organisms as well.

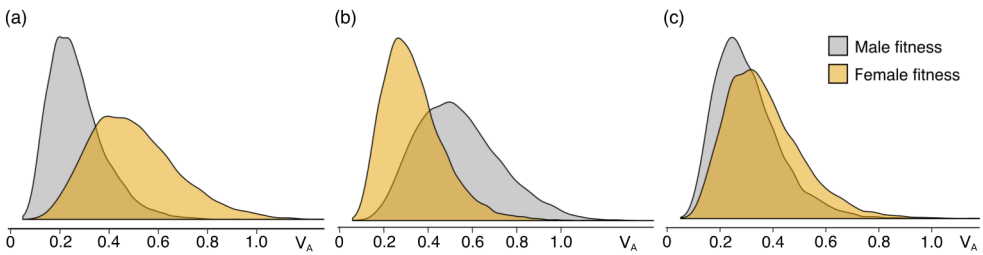


Figure 7. Density plots showing the posterior distribution of the additive genetic variance for male (grey) and female (orange) fitness in respective treatments; salt (a), food-restriction (b) and control (c).

Paper IV. Sex-limited selection in a hermaphrodite leads to changes in sex-role related traits

We found that selection on one sex-role leads to an increase in the sex-specific fitness for that sex-role in a hermaphrodite, which is the first step towards the evolution of a new sex chromosome system (figure 8). Interestingly, quantitative genetic studies (see paper II and III) have found no strong intersexual genetic correlation between fitness components (positive or negative). This can be explained by two possible factors; either because it is easier to detect a genetic correlation in experimental evolution designs is compared to breeding experiments, or because the genetic correlation has been built up de novo during the course of the sex-limited selection (Fuller *et al.* 2005).

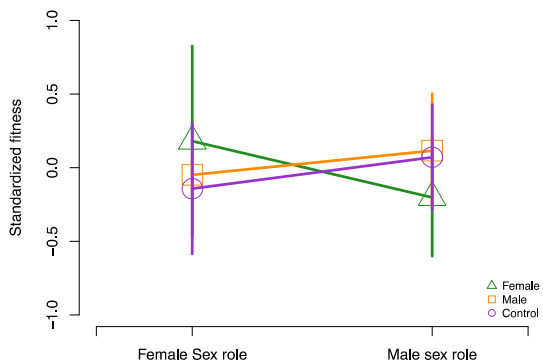


Figure 8. Interaction plot showing the standardized relative fitness in respective sex role (male or female) in each treatment. There was a significant sex role by treatment interaction. The symbols represent each selection treatment (triangle for female selected treatment, square for male selected treatment and circle for control treatment).

The female-selected lines had a stronger response to the selection treatment than the male-selected lines in the fitness assay. That could have been because male fitness is harder to select on through the experimental set-up. Focal worms are mated in a controlled setting of two mating partners, which is smaller than what lab-adapted worms normally are exposed to. Another explanation for a weaker response in the male fitness could be that the additive genetic variation (V_A) for male fitness was lower than female V_A (as shown in paper II), so there was simply less variation to select on.

There was a significant treatment by replicate population interaction for overall body size, but treatment alone could not explain the body size differences. This was probably mostly driven by the large variation in body sizes in the female treatment,

possibly indicating that populations have developed different strategies to increase female fitness.

Neither ovary size nor testis size were different between treatments alone, but increased male fitness was correlated with increased testes size, and decreased body size (irrespective of selection treatment), and this was not seen in female fitness. This is concordant with previous studies, where female fitness does not seem to be linked to morphological traits (i.e. ovary size and overall size), whereas male fitness is linked to larger testes (Janicke *et al.* 2016).

Paper V. Gene-expression differences in a simultaneous hermaphrodite subjected to sex-limited selection

The largest amount of differentially expressed genes could be found between the male- and female-selected treatments, which could indicate a response to the selection (figure 9). However, no clear pattern was seen in the significant GO-terms between treatments, suggesting that the genes that responded to selection might not be functionally related. There were no differences in expression between antrum-specific and prostate-specific genes, and this was interpreted to be a result of either the low annotation quality of the transcriptome, or that *M. lignano* has, in line with hermaphroditic *Caenohabditis elegans*, a reduced number of sex-biased genes compared to gonochorists (Thomas *et al.* 2012), making sex-specific differences harder to detect. Alternatively, since sex-biased genes are known to be tissue-specific and the pattern is often strongest in the gonads (Grath & Parsch 2016), we suggest investigating expression in the testes and ovaries in the future, since they might reflect changes in sex-biased genes better than the prostate gland and the antrum.

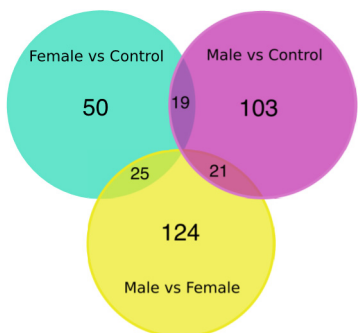


Figure 9. Number of significant differentially expressed genes between each selection treatment (female-limited selection, male-limited selection and control treatment).

Conclusions and future perspectives

In this thesis, I have gained a deeper understanding of sex chromosome evolution in a hermaphrodite ancestor. Mainly, I have asked if it is possible to experimentally simulate sex chromosome evolution in a hermaphrodite and see a response, similar to what we would expect given sex chromosome theory. In addition, I have also examined the components necessary for sex chromosome evolution, such as genetic variance in male and female fitness and the relationship between them. Finally, I looked at how these fitness components interact with the environment. In this section, I will connect results from the different papers in my thesis and draw some general conclusions. Additionally, I will discuss new exciting questions and future research ideas that my thesis has laid the groundwork for.

I have shown that the additive genetic variance is larger for female fitness than male fitness in the simultaneous hermaphrodite *M. lignano*, in line with studies on separate sexed organisms and a hermaphrodite (Foerster *et al.* 2007, Innocenti & Morrow 2010, Merilä & Sheldon 2000, Yund *et al.* 1997). Furthermore, I found a sex-specific additive genetic variance by environment interaction, where the environment affected female and male additive genetic variance in different ways. Environmental effects on quantitative genetic parameters have been shown before, especially in stressful environments (Hoffmann & Merilä 1999, Martinossi-Allibert *et al.* 2017).

According to generally-accepted theories of sex chromosome evolution, the presence of sexual antagonism is an important part in the initial phase (Charlesworth *et al.* 2005). However, we found a very weak intersexual genetic correlation between male and female fitness, and the phenotypic trade-off between them was weak as well. Furthermore, the correlations did not change across environments, suggesting that male and female sex role function independently from each other.

In the sex-limited experimental evolution, however, where we selected on either male or female fitness in *M. lignano*, we could show a fitness response, especially in the female-selected lines, where worms had a higher female fitness and a lower male fitness. This is evidence of a genetically-based trade-off between sex roles. This contradictive result (compared to the other measures of the genetic correlation between sex roles) could either be because experimental evolution is better at estimating genetic correlations (Fuller *et al.* 2005), or because we have reinforced or built up a genetic correlation during selection (Delph *et al.* 2011). In either case, it is

intriguing that the experimental evolution showed a response, since this is the first time a sex-limited selection experiment has been carried out in a simultaneous hermaphrodite. This also demonstrates that it is possible to recreate the first steps towards the evolution of a new sex chromosome system in the lab, where a (pseudo)sterility mutation in combination with a sex-specific fitness locus (or loci) leads to increased fitness in that particular sex. Gene expression data in the same selected lines after generation 21 and 22 showed the largest change in differentially expressed genes between the female-selected and male-selected lines, but there was no difference in antrum- or prostate-specific genes.

The results from my thesis give us hints and ideas of where to go next. It would be exciting to evaluate the additive genetic variance in the evolved populations. It would be interesting to see if the genetic variance for fitness has decreased and if the female and male fitness components have changed in different ways. Given that we have selected on fitness, we expect that the additive genetic variance has decreased (Kruuk 2004), and since we have seen the strongest response in the female-selected lines, female additive genetic variance might be more depleted than male additive genetic variance. It would also be of interest to evaluate the intersexual genetic correlation in the evolved populations, since we have evidence of a genetically-based trade-off. Has it changed during the course of the experiment, and if so how? We would expect to see a $r_{MF} < 0$, if there is sexual antagonism between male and female fitness (Bonduriansky & Chenoweth 2009).

There are many possible responses to look for in the evolved populations on the genomic level. Since sex-specific genes are highly sex-specific and show highest expression in the gonads (Grath & Parsch 2016), a future direction would be to examine the expression profiles of testes and ovaries between treatments. Another exciting approach is to sequence the DNA around the (sex-determining) GFP-locus and assess whether there is a build-up of sex-specific genes around the locus, as predicted by sex chromosome evolution theory, and if different alleles have gone to fixation between treatments.

There are still many more phenotypic traits whose response are also interesting to measure in the evolved populations, for example development and mating time. Sexually antagonistic selection is likely to be strongest at the point of development when male and female organs or structures are starting to develop (Ingleby *et al.* 2015), and therefore it is of interest to measure if there is any difference in development time of male- and female-selected lines. We expect that worms in the male-selected treatment develop faster than the female-selected ones, since testes develop before ovaries (Vizoso *et al.* 2010).

M. lignano has fairly elaborate mating behavior, the most intriguing aspect being the sucking behavior, where worms are thought to remove sperm or seminal fluid form their own antrum after mating (Schärer *et al.* 2004). Seminal fluid proteins are

components in the seminal fluid known to influence sperm competitiveness and can be harmful to females (Edward *et al.* 2015, Lodi & Koene 2017). If the sucking behavior is connected to removal of harmful seminal fluid proteins, there might be more instances of "sucking" after matings with worms having more harmful seminal fluid proteins. We suggest that worms from the male-selected treatment could possibly evolve altered seminal fluid proteins, to increase sperm fertilization success. We also expect the male-selected worms to mate more frequently as opposed to worms in the female-selected treatments, also to increase fertilization success.

In short, as discussed in (Abbott *et al.* 2017), the field of sex chromosome evolution is very active and will probably remain to be so, due to the many exciting new avenues that are waiting to be explored. This thesis demonstrated clearly that despite the many calls for increased production of genomic data from multiple sex chromosome systems (Abbott *et al.* 2017, Bachtrog *et al.* 2014), manipulative experiments are also a potentially valuable way forward.

Acknowledgements

This thesis is not solely the work of one person and I could not have finished this thesis without you:

Jessica, my supervisor, thank you for being the great support and giving me the guidance I needed. Every time I was stuck or panicked, you had a solution, which left me inspired and motivated to continue.

Bengt, min biträdande handledare, tack för att du är så kunnig och omtänksam! Har också varit skönt att kunna förlita mig på din kunskap i sekvenseringsdjungeln.

Min mentor **Åsa**. Våra möten fyllde mig alltid med energi, och gjorde mig på så bra humör. Tack för att du alltid fanns där, vilken fråga jag än hade.

Thanks to the worm community, particularly **Schärer lab** in Basel and **Ramm lab** in Bielefeld. Special thanks to **Lukas Schärer** and **Dita Vizoso** for indispensable support and help with worm culturing and lab methods. I am grateful to **Bahar, Niko, Michael** and **Jeremias** for the tips and good discussions during meetings.

I am happy to have shared ideas and experiences through the STINT collaboration with **Molly Morris** and **Melissa Liotta** at Ohio University and **Oscar Ríos-Cárdenas** at INECOL, Mexico.

Melissa, my favorite Italian-American of all times, thanks for being such a great travelling buddy and friend!

Special thanks to members in Jessica's group: **Katrine, Qinyang**, and the newer ones **Yesbol** and **Aivars**.

Katrine, I don't think I would have survived the long hours in the lab without you. Thank you for making lab work more fun!

Thanks to all members of former **EXEB** for mind-opening and challenging research discussions.

I am grateful for having had the opportunity to be a part of **GENECO** research school. I learned a lot and met so many inspiring and talented people.

Thank you to all **colleagues** in the ecology building for contributing to a great atmosphere in the workplace, during coffee breaks and at the Friday pub. Being a super extrovert myself, it has meant a lot to me.

Special mention to: **Elin, Pablo, Lokesh, Utku, Beatriz, Marco, Tom, Suzan, Linus, John W, John K, Fredrik, Martin, Sissel, Cecilia, Hanna L, Suvi, Arne, Jacob, Reinder, Hannah, Johan, Jannie, Maria, Xi, Jane, Dafne, Julian, Hanna S, Jothi, Juan Pablo**….and others who may read this and get a little bit upset that you haven't been mentioned. Yes **you**! Thanks for making the ecology building a great place to be.

Some have given extra help and support:

Dimi, **Michael** and **Hannah** thanks for giving helpful tips when I was extracting RNA.

Juli, you have been my most consistent office mate. I enjoyed listening to stories about your fieldwork and thank you for caring about how everything was coming along for me.

Anne, du har en skön kombination av humor och jävlar anamma. Utan dig hade jag inte förstått ett dyft av kostnadskalkyler eller OH. Tack för att du bryr dig.

Anna D, tack för att du alltid frågar hur jag (och maskarna) mår och för att du blev min RNA-guru. Och att du lugnt förklarade att det heter SciLife Lab och inte SciFi Lab. Hjälp liksom.

Kajsa, vad skulle jag gjort om jag inte hade en cool vän som kunde skriva kod? Och åka inlines? Antagligen drabbats av panik.

Thank you for always keeping me in a good mood: my lovely 3rd-floor friends **Lovisa, Terese, Jasmine, William** and **Sofia**. Tack för hjälpen i slutet med layouten finaste Lovisa, du är en pärla!

Mina **närmaste vänner**, tack för att ni outtröttligt lyssnar, stöttar, kramar och hjälper mig.

Stort tack min **älskade, härliga familj**, för att ni alltid är där för mig, och ställer upp i vått och torrt. Tack så mycket min söta syster **Elsa** för att du gjorde framsidan och bilderna till kapitlen – de är så underbart fina!

David, min älskling, thank you for being by my side. Te amo.

Referenses

Abbott JK. 2011. Intra-locus sexual conflict and sexually antagonistic genetic variation in hermaphroditic animals. Proc. R. Soc. B Biol. Sci. 278(1703):161–69

Abbott JK, Nordén AK, Hansson B. 2017. Sex chromosome evolution: historical insights and future perspectives. Proc. R. Soc. B Biol. Sci. 284(1854):20162806

Arnqvist G, Rowe L. 2005. Sexual Conflict. Princeton University Press

Avise J. 2011. Hermaphroditism: A Primer on the Biology, Ecology, and Evolution of Dual Sexuality. Columbia University Press

Bachtrog D. 2013. Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. Nat. Rev. Genet. 14(2):113–24

Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, et al. 2014. Sex Determination: Why So Many Ways of Doing It? PLOS Biol. 12(7):e1001899

Berec L, Schembri PJ, Boukal DS. 2005. Sex determination in Bonellia viridis (Echiura: Bonelliidae): population dynamics and evolution. Oikos. 108(3):473–84

Berger D, Grieshop K, Lind MI, Goenaga J, Maklakov AA, Arnqvist G. 2014. Intalocus sexual conflict and environmental stress: sex, genes and conflict in stressful environments. Evolution. 2184–96

Berset-Brändli L, JaquiéRy J, Perrin N. 2007. Recombination is suppressed and variability reduced in a nascent Y chromosome. J. Evol. Biol. 20(3):1182–88

Bertin R, Kerwin M. 1998. Floral sex ratios and gynomonoecy in Aster (Asteraceae). Am. J. Bot. 85(2):235–235

Beukeboom LW, Perrin N. 2014. The Evolution of Sex Determination. Oxford University Press

Bonduriansky R, Chenoweth SF. 2009. Intralocus sexual conflict. Trends Ecol. Evol. 24(5):280–88

Brommer JE, Kirkpatrick M, Qvarnström A, Gustafsson L. 2007. The Intersexual Genetic Correlation for Lifetime Fitness in the Wild and Its Implications for Sexual Selection. PLOS ONE. 2(8):e744

Bull J, Charnov E. 1985. On Irreversible Evolution. Evolution. 39(5):1149–55

Bull JJ. 1983. Evolution of Sex Determining Mechanisms. Benjamin/Cummings Pub. Co.

Bulmer MG, Parker GA. 2002. The evolution of anisogamy: a game-theoretic approach. Proc. R. Soc. Lond. B Biol. Sci. 269(1507):2381–88

Burke NW, Bonduriansky R. 2017. Sexual Conflict, Facultative Asexuality, and the True Paradox of Sex. Trends Ecol. Evol. 32(9):646–52

Calsbeek R, Sinervo B. 2004. Within-clutch variation in offspring sex determined by differences in sire body size: cryptic mate choice in the wild. J. Evol. Biol. 17(2):464–70

Campbell D. 1989. Measurements of Selection in a Hermaphroditic Plant - Variation in Male and Female Pollination Success. Evolution. 43(2):318–34

Chapman T, Arnqvist G, Bangham J, Rowe L. 2003. Sexual conflict. Trends Ecol. Evol. 18(1):41–47

Charlesworth B, Charlesworth D. 1978. A model for the evolution of dioecy and gynodioecy. Am. Nat. 112(988):975–97

Charlesworth B, Charlesworth D. 2000. The degeneration of Y chromosomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355(1403):1563–72

Charlesworth D. 1984. Androdioecy and the evolution of dioecy. Biol. J. Linn. Soc. 22(4):333–48

Charlesworth D, Charlesworth B. 2010. Evolutionary Biology: The Origins of Two Sexes. Curr. Biol. 20(12):R519–21

Charlesworth D, Charlesworth B, Marais G. 2005. Steps in the evolution of heteromorphic sex chromosomes. Heredity. 95(2):118–28

Charnov E. 1979. Simultaneous Hermaphroditism and Sexual Selection. Proc. Natl. Acad. Sci. U. S. A. 76(5):2480–84

Charnov EL, Bull JJ, Maynard Smith J. 1976. Why be an hermaphrodite? Nature. 263(5573):125–26

Cheng C, Kirkpatrick M. 2017. Environmental Plasticity in the Intersexual Correlation and Sex Bias of Gene Expression. J. Hered.

Chippindale AK, Gibson JR, Rice WR. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in Drosophila. Proc. Natl. Acad. Sci. 98(4):1671–1675

Connallon T, Clark AG. 2012. A General Population Genetic Framework for Antagonistic Selection That Accounts for Demography and Recurrent Mutation. Genetics. 190(4):1477

Connallon T, Jakubowski E. 2009. Association Between Sex Ratio Distortion and Sexually Antagonistic Fitness Consequences of Female Choice. Evolution. 63(8):2179–83

Connallon T, Knowles LL. 2005. Intergenomic conflict revealed by patterns of sex-biased gene expression. Trends Genet. TIG. 21(9):495–99

Cooper G. 2000. The Cell: A Molecular Approach. Washington, DC: Sinauer Associates Inc. 2nd edition ed.

Couvet D, Gouyon P, Kjellberg F, Valdeyron G. 1985. Nucleocytoplasmic Differentiation Between Populations - a Cause of the Occurrence of Male Steriles in Natural-Populations of Thyme. Comptes Rendus Acad. Sci. Ser. Iii-Sci. Vie-Life Sci. 300(18):665–68

Darwin CR. 1876. The Effects of Cross and Self Fertilisation in the Vegetable Kingdom. London: John Murray

Delcourt M, Blows MW, Rundle HD. 2009. Sexually antagonistic genetic variance for fitness in an ancestral and a novel environment. Proc. R. Soc. B-Biol. Sci. 276(1664):2009–14

Delph LF, Steven JC, Anderson IA, Herlihy CR, Brodie ED. 2011. Elimination of a Genetic Correlation Between the Sexes Via Artificial Correlational Selection. Evolution. 65(10):2872–80

Demircan TD. 2013. Advancing the flatworm *Macrostomum lignano* as a versatile model organism for stem cell research. Utrecht University Repository

Dunning LT, Hipperson H, Baker WJ, Butlin RK, Devaux C, et al. 2016. Ecological speciation in sympatric palms: 1. Gene expression, selection and pleiotropy. J. Evol. Biol. 29(8):1472–87

Edward DA, Fricke C, Chapman T. 2010. Adaptations to sexual selection and sexual conflict: insights from experimental evolution and artificial selection. Philos. Trans. R. Soc. B Biol. Sci. 365(1552):2541–48

Edward DA, Stockley P, Hosken DJ. 2015. Sexual Conflict and Sperm Competition. Cold Spring Harb. Perspect. Biol. 7(4):

Egger B, Gschwentner R, Rieger R. 2007. Free-living flatworms under the knife: past and present. Dev. Genes Evol. 217(2):89–104

Egger B, Ishida S. 2005. Chromosome fission or duplication in *Macrostomum lignano* (Macrostomorpha, Plathelminthes)–remarks on chromosome numbers in "archoophoran turbellarians." J. Zool. Syst. Evol. Res. 43(2):127–132

Ellegren H, Parsch J. 2007. The evolution of sex-biased genes and sex-biased gene expression. Nat. Rev. Genet. 8(9):689–98

Eppley SM, Jesson LK. 2008. Moving to mate: the evolution of separate and combined sexes in multicellular organisms. J. Evol. Biol. 21(3):727–36

Falconer DS, Mackay TFC. 1996. Introduction to Quantitative Genetics. Harlow: Pearson. 4 edition ed.

Fedorka KM, Mousseau TA. 2004. Female mating bias results in conflicting sex-specific offspring fitness. Nature. 429(6987):65–67

Flaxman SM, Wacholder AC, Feder JL, Nosil P. 2014. Theoretical models of the influence of genomic architecture on the dynamics of speciation. Mol. Ecol. 23(16):4074–88

Foerster K, Coulson T, Sheldon BC, Pemberton JM, Clutton-Brock TH, Kruuk LEB. 2007. Sexually antagonistic genetic variation for fitness in red deer. Nature. 447(7148):1107-U9

Francisco FO, Lemos B. 2014. How Do Y-Chromosomes Modulate Genome-Wide Epigenetic States: Genome Folding, Chromatin Sinks, and Gene Expression. J. Genomics. 2:94–103

Fritsch P, Rieseberg LH. 1992. High outcrossing rates maintain male and hermaphrodite individuals in populations of the flowering plant Datisca glomerata. Nature. 359(6396):633–36

Fuller RC, Baer CF, Travis J. 2005. How and When Selection Experiments Might Actually be Useful. Integr. Comp. Biol. 45(3):391–404

Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. Nature. 525(7569):372–75

Gibson JR, Chippindale AK, Rice WR. 2002. The X chromosome is a hot spot for sexually antagonistic fitness variation. Proc. R. Soc. B-Biol. Sci. 269(1490):499–505

Grath S, Parsch J. 2016. Sex-Biased Gene Expression. Annu. Rev. Genet. 50:29–44

Guler Y, Short S, Kile P, Ford AT. 2012. Integrating field and laboratory evidence for environmental sex determination in the amphipod, Echinogammarus marinus. Mar. Biol. 159(12):2885–90

Harts AMF, Schwanz LE, Kokko H. 2014. Demography can favour female-advantageous alleles. Proc. R. Soc. B Biol. Sci. 281(1790):20140005–20140005

Hegreness M, Shoresh N, Hartl D, Kishony R. 2006. An Equivalence Principle for the Incorporation of Favorable Mutations in Asexual Populations. Science. 311(5767):1615–17

Hill WG. 2010. Understanding and using quantitative genetic variation. Philos. Trans. R. Soc. B Biol. Sci. 365(1537):73–85

Hill WG, Goddard ME, Visscher PM. 2008. Data and Theory Point to Mainly Additive Genetic Variance for Complex Traits. PLoS Genet. 4(2):

Hoffmann AA, Merilä J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol. Evol. 14(3):

Holleley CE, O'Meally D, Sarre SD, Marshall Graves JA, Ezaz T, et al. 2015. Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. Nature. 523(7558):79–82

Huang Y, Agrawal AF. 2016. Experimental Evolution of Gene Expression and Plasticity in Alternative Selective Regimes. PLOS Genet. 12(9):e1006336

Ingleby FC, Flis I, Morrow EH. 2015. Sex-Biased Gene Expression and Sexual Conflict throughout Development. Cold Spring Harb. Perspect. Biol. 7(1):

Innocenti P, Morrow EH. 2010. The Sexually Antagonistic Genes of Drosophila melanogaster. PLoS Biol. 8(3):e1000335

Isaka N, Kawai-Toyooka H, Matsuzaki R, Nakada T, Nozaki H. 2012. Description of Two New Monoecious Species of Volvox Sect. Volvox (volvocaceae, Chlorophyceae), Based on Comparative Morphology and Molecular Phylogeny of Cultured Material1. J. Phycol. 48(3):759–67

Janicke T, Sandner P, Ramm SA, Vizoso DB, Schärer L. 2016. Experimentally evolved and phenotypically plastic responses to enforced monogamy in a hermaphroditic flatworm. J. Evol. Biol. 29(9):1713–27

Janzen FJ, Paukstis GL. 1991. Environmental Sex Determination in Reptiles: Ecology, Evolution, and Experimental Design. Q. Rev. Biol. 66(2):149–79

Jarne P, Auld JR. 2006. Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. Evolution. 60(9):1816–24

Jordan CY, Charlesworth D. 2012. The Potential for Sexually Antagonistic Polymorphism in Different Genome Regions. Evolution. 66(2):505–16

Kawecki TJ, Lenski RE, Ebert D, Hollis B, Olivieri I, Whitlock MC. 2012. Experimental evolution. Trends Ecol. Evol. 27(10):547–60

Kitano J, Peichel CL. 2012. Turnover of sex chromosomes and speciation in fishes. Environ. Biol. Fishes. 94(3):549–58

Kitano J, Ross JA, Mori S, Kume M, Jones FC, et al. 2009. A role for a neo-sex chromosome in stickleback speciation. Nature. 461(7267):1079–83

Koene JM, Chase R. 1998. Changes in the reproductive system of the snail Helix aspersa caused by mucus from the love dart. J. Exp. Biol. 201(15):2313–19

Koene JM, Sundermann G, Michiels NK. 2002. On the function of body piercing during copulation in earthworms. Invertebr. Reprod. Dev. 41(1–3):35–40

Koene JM, ter Maat A. 2001. "Allohormones": a class of bioactive substances favoured by sexual selection. J. Comp. Physiol. [A]. 187(5):323–26

Kolss M, Vijendravarma RK, Schwaller G, Kawecki TJ. 2009. Life-History Consequences of Adaptation to Larval Nutritional Stress in Drosophila. Evolution. 63(9):2389–2401

Kruuk LEB. 2004. Estimating genetic parameters in natural populations using the "animal model." Philos. Trans. R. Soc. B Biol. Sci. 359(1446):873–90

Kruuk LEB, Clutton-Brock TH, Slate J, Pemberton JM, Brotherstone S, Guinness FE. 2000. Heritability of fitness in a wild mammal population. Proc. Natl. Acad. Sci. 97(2):698–703

Kruuk LEB, Slate J, Wilson AJ. 2008. New Answers for Old Questions: The Evolutionary Quantitative Genetics of Wild Animal Populations. Annu. Rev. Ecol. Evol. Syst. 39:525–48

Ladurner P, Rieger R, Baguna J. 2000. Spatial distribution and differentiation potential of stem cells in hatchlings and adults in the marine platyhelminth Macrostomum sp.: A bromodeoxyuridine analysis. Dev. Biol. 226(2):231–41

Ladurner P, Schärer L, Salvenmoser W, Rieger RM. 2005. A new model organism among the lower Bilateria and the use of digital microscopy in taxonomy of meiobenthic Platyhelminthes: *Macrostomum lignano*, n. sp. (Rhabditophora, Macrostomorpha). J. Zool. Syst. Evol. Res. 43(2):114–26

Lahn BT, Page DC. 1999. Four evolutionary strata on the human X chromosome. Science. 286(5441):964–67

Lehtonen J, Kokko H. 2011. Two roads to two sexes: unifying gamete competition and gamete limitation in a single model of anisogamy evolution. Behav. Ecol. Sociobiol. 65(3):445–59

Lehtonen J, Parker GA. 2014. Gamete competition, gamete limitation, and the evolution of the two sexes. MHR Basic Sci. Reprod. Med. 20(12):1161–68

Lehtonen J, Parker GA, Schärer L. 2016. Why anisogamy drives ancestral sex roles. Evolution. 70:

Lenski RE. 1988. Experimental Studies of Pleiotropy and Epistasis in Escherichia Coli. II. Compensation for Maladaptive Effects Associated with Resistance to Virus T4. Evolution. 42(3):433–40

Lenski RE, Bennett AF. 1993. Evolutionary Response of Escherichia coli to Thermal Stress. Am. Nat. 142:S47–64

Leonard JL. 1993. Sexual conflict in simultaneous hermaphrodites: evidence from serranid fishes. Environ. Biol. Fishes. 36(2):135–48

Lewis WM. 1987. The cost of sex. Experientia. Suppl. 55:33–57

Lloyd D. 1980. Sexual Strategies in Plants .3. a Quantitative Method for Describing the Gender of Plants. N. Z. J. Bot. 18(1):103–8

Lodi M, Koene JM. 2017. Hidden female physiological resistance to male accessory gland substances in a simultaneous hermaphrodite. J. Exp. Biol. 220(6):1026–31

Lynch M, Walsh B. 1998. Genetics and Analysis of Quantitative Traits. Sinauer

Lyons EE, Miller D, Meagher TR. 1994. Evolutionary Dynamics of Sex Ratio and Gender Dimorphism in Silene latifolia: I. Environmental Effects. J. Hered. 85(3):196–203

Mank JE. 2009. Sex Chromosomes and the Evolution of Sexual Dimorphism: Lessons from the Genome. Am. Nat. 173(2):141–50

Marie-Orleach L, Janicke T, Vizoso DB, Eichmann M, Schärer L. 2014. Fluorescent sperm in a transparent worm: validation of a GFP marker to study sexual selection. BMC Evol. Biol. 14:148

Marshall K. 2016. Primed to fight: mom's meals make baby stronger. J. Exp. Biol. 219(7):910–910

Martínez-Fernández M, Bernatchez L, Rolán-Alvarez E, Quesada H. 2010. Insights into the role of differential gene expression on the ecological adaptation of the snail Littorina saxatilis. BMC Evol. Biol. 10:356

Martinossi-Allibert I, Arnqvist G, Berger D. 2017. Sex-specific selection under environmental stress in seed beetles. J. Evol. Biol. 30(1):161–73

McCabe J, Dunn AM. 1997. Adaptive significance of environmental sex determination in an amphipod. J. Evol. Biol. 10(4):515–27

Meisel RP. 2011. Towards a more nuanced understanding of the relationship between sex-biased gene expression and rates of protein-coding sequence evolution. Mol. Biol. Evol. 28(6):1893–1900

Merilä J, Sheldon BC. 2000. Lifetime reproductive success and heritability in nature. Am. Nat. 155(3):301–310

Merilä J, Sheldon BC, Ellegren H. 1997. Antagonistic natural selection revealed by molecular sex identification of nestling collared flycatchers. Mol. Ecol. 6(12):1167–75

Mirzaghaderi G, Hoerandl E. 2016. The evolution of meiotic sex and its alternatives. Proc. R. Soc. B-Biol. Sci. 283(1838):20161221

Miura S, Komatsu T, Higa M, Bhandari RK, Nakamura S, Nakamura M. 2003. Gonadal sex differentiation in protandrous anemone fish, Amphiprion clarkii. Fish Physiol. Biochem. 28(1–4):165–66

Moore EC, Roberts RB. 2013. Polygenic sex determination. Curr. Biol. 23(12):R510–12

Morgan M, Schoen D. 1997. Heredity - Abstract of article: Selection on reproductive characters: floral morphology in Asclepias syriaca. Heredity. 79(4):433–41

Morris MRJ, Richard R, Leder EH, Barrett RDH, Aubin-Horth N, Rogers SM. 2014. Gene expression plasticity evolves in response to colonization of freshwater lakes in threespine stickleback. Mol. Ecol. 23(13):3226–40

Mousseau T, Roff D. 1987. Natural-Selection and the Heritability of Fitness Components. Heredity. 59:181–97

Nimeth KT, Egger B, Rieger R, Salvenmoser W, Peter R, Gschwentner R. 2007. Regeneration in *Macrostomum lignano* (Platyhelminthes): cellular dynamics in the neoblast stem cell system. Cell Tissue Res. 327(3):637–46

Pala I, Naurin S, Stervander M, Hasselquist D, Bensch S, Hansson B. 2012. Evidence of a neo-sex chromosome in birds. Heredity. 108(3):264–272

Pannell JR. 2002. The evolution and maintenance of androdioecy. Annu. Rev. Ecol. Syst. 33:397–425

Parkinson JE, Baumgarten S, Michell CT, Baums IB, LaJeunesse TC, Voolstra CR. 2016. Gene Expression Variation Resolves Species and Individual Strains among Coral-Associated Dinoflagellates within the Genus Symbiodinium. Genome Biol. Evol. 8(3):665–80

Parsch J, Ellegren H. 2013. The evolutionary causes and consequences of sex-biased gene expression. Nat. Rev. Genet. 14(2):83–87

Pavlopoulos A, Oehler S, Kapetanaki MG, Savakis C. 2007. The DNA transposon Minos as a tool for transgenesis and functional genomic analysis in vertebrates and invertebrates. Genome Biol. 8 Suppl 1:S2

Pennell TM, Morrow EH. 2013. Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. Ecol. Evol. 3(6):1819–34

Perfeito L, Fernandes L, Mota C, Gordo I. 2007. Adaptive Mutations in Bacteria: High Rate and Small Effects. Science. 317(5839):813–15

Perrin N. 2009. Sex Reversal: A Fountain of Youth for Sex Chromosomes? Evolution. 63(12):3043–49

Poissant J, Wilson AJ, Coltman DW. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: A systematic review of cross-sex genetic correlations. Evolution. 64(1):97–107

Price D, Burley N. 1993. Constraints on the Evolution of Attractive Traits - Genetic (co)variance. Heredity. 71:405–12

Punzalan D, Delcourt M, Rundle HD. 2014. Comparing the intersex genetic correlation for fitness across novel environments in the fruit fly, Drosophila serrata. Heredity. 112(2):143–48

Puurtinen M, Kaitala V. 2002. Mate-search efficiency can determine the evolution of separate sexes and the stability of hermaphroditism in animals. Am. Nat. 160(5):645–60

Quinn AE, Georges A, Sarre SD, Guarino F, Ezaz T, Graves JAM. 2007. Temperature Sex Reversal Implies Sex Gene Dosage in a Reptile. Science. 316(5823):411–411

Rice WR. 1984. Sex Chromosomes and the Evolution of Sexual Dimorphism. Evolution. 38(4):735

Rice WR, Friberg U. 2008. Genetics - Functionally degenerate - Y not so? Science. 319(5859):42–43

Rice WR, Gavrilets S. 2014. The Genetics and Biology of Sexual Conflict. New York: Cold Spring Harbor

Romero IG, Ruvinsky I, Gilad Y. 2012. Comparative studies of gene expression and the evolution of gene regulation. Nat. Rev. Genet. 13(7):505–16

Schaefer H, Renner SS. 2010. A three-genome phylogeny of Momordica (Cucurbitaceae) suggests seven returns from dioecy to monoecy and recent long-distance dispersal to Asia. Mol. Phylogenet. Evol. 54(2):553–60

Schärer L, Janicke T, Ramm SA. Sexual Conflict in Hermaphrodites. 2014. The Genetics and Biology of Sexual Conflict. Rice W., Gavrilets, S. (eds) New York: Cold Spring Harbor

Schärer L. 2009. Tests of Sex Allocation Theory in Simultaneously Hermaphroditic Animals. Evolution. 63(6):1377–1405

Schärer L, Joss G, Sandner P. 2004. Mating behaviour of the marine turbellarian Macrostomum sp.: these worms suck. Mar. Biol. 145(2):

Schartl M. 2004. A comparative view on sex determination in medaka. Mech. Dev. 121(7–8):639–45

Steinemann S, Steinemann M. 2005. Y chromosomes: born to be destroyed. Bioessays. 27(10):1076–83

Thomas CG, Li R, Smith HE, Woodruff GC, Oliver B, Haag ES. 2012. Simplification and desexualization of gene expression in self-fertile nematodes. Curr. Biol. CB. 22(22):2167–72

Tomlinson J. 1966. The advantages of hermaphroditism and parthenogenesis. J. Theor. Biol. 11(1):54–58

Vieira C, Pasyukova EG, Zeng Z-B, Hackett JB, Lyman RF, Mackay TFC. 2000. Genotype-Environment Interaction for Quantitative Trait Loci Affecting Life Span in Drosophila melanogaster. Genetics. 154(1):213–27

Vizoso DB, Rieger G, Schärer L. 2010. Goings-on inside a worm: functional hypotheses derived from sexual conflict thinking. Biol. J. Linn. Soc. 99(2):370–383

Warner RR. 1988. Sex change in fishes: hypotheses, evidence, and objections. Environ. Biol. Fishes. 22(2):81–90

Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, et al. 2010. An ecologist's guide to the animal model. J. Anim. Ecol. 79(1):13–26

Wright AE, Harrison PW, Montgomery SH, Pointer MA, Mank JE. 2014. Independent Stratum Formation on the Avian Sex Chromosomes Reveals Inter-Chromosomal Gene Conversion and Predominance of Purifying Selection on the W Chromosome. Evolution. 68(11):3281–95

Wright AE, Moghadam HK, Mank JE. 2012. Trade-off Between Selection for Dosage Compensation and Masculinization on the Avian Z Chromosome. Genetics. 192(4):1433

Yampolsky LY, Glazko GV, Fry JD. 2012. Evolution of gene expression and expression plasticity in long-term experimental populations of Drosophila melanogaster maintained under constant and variable ethanol stress. Mol. Ecol. 21(17):4287–99

Yund PO, Marcum Y, Stewart-Savage J. 1997. Life-History Variation in a Colonial Ascidian: Broad-Sence Heritabilities and Tradeoffs in Allocation to Asexual Growth and Male and Female Reproduction. Biol. Bull. 192(2):290–99

Zadesenets KS, Vizoso DB, Schlatter A, Konopatskaia ID, Berezikov E, et al. 2016. Evidence for Karyotype Polymorphism in the Free-Living Flatworm, *Macrostomum lignano*, a Model Organism for Evolutionary and Developmental Biology. PLOS ONE. 11(10):e0164915

Zbinden M, Haag CR, Ebert D. 2008. Experimental evolution of field populations of Daphnia magna in response to parasite treatment. J. Evol. Biol. 21(4):1068–78

List of papers

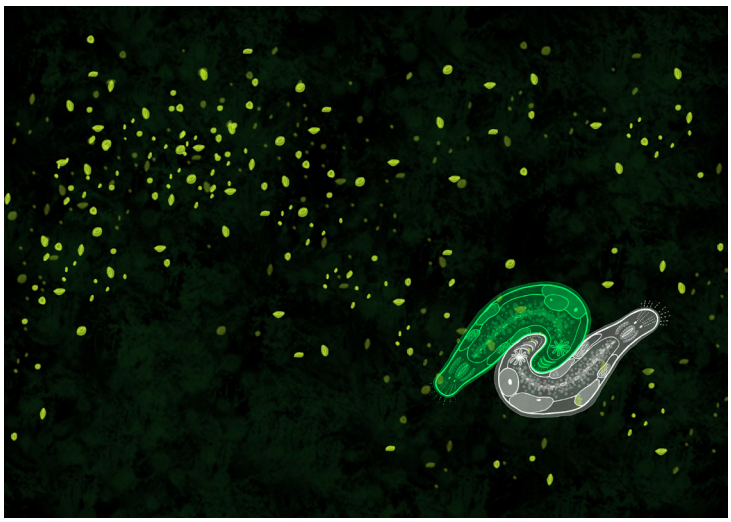
I. Abbott, J. K., Nordén, A. K., and Hansson, B. Sex chromosome evolution: historical insights and future perspectives. *Proc. R. Soc. B 284: 20162806.*

II. Nordén, A. K. and Abbott, J. K. Sex-specific genetic variance for fitness and inheritance of a genetic marker in a simultaneous hermaphrodite. *Manuscript.*

III. Nordén, A. K., Moosmann, M. and Abbott, J. K. Environmental variation in sex-specific fitness in a simultaneous hermaphrodite. *Submitted.*

IV. Nordén, A. K. and Abbott, J. K. Sex-limited selection in a hermaphrodite leads to changes in sex-role related traits. *Manuscript.*

V. Nordén, A. K. and Abbott, J. K. Gene-expression differences in a simultaneous hermaphrodite subjected to sex-limited selection. *Manuscript.*



Printed by Media-Tryck, Lund 2017 NORDIC ECOLABEL, 3041 0903

LUND UNIVERSITY

Faculty of Science
Department of Biology

ISBN 978-91-7753-473-0