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Evolutionary ecology of avian neo-sex chromosomes

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Feb 1st 2021 - Oct 9th 2026

Studia Chromosomatum Sexualium Neoformatorum

Evolutionary ecology of avian neo-sex chromosomes

SIMON JACOBSEN ELLERSTRAND

DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY

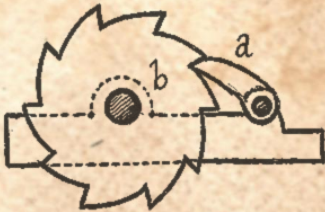


fig. 1



fig. 2



Alauda razae

We discovered a species of lark in flocks. These birds are so tame that we could have knocked many over with sticks.

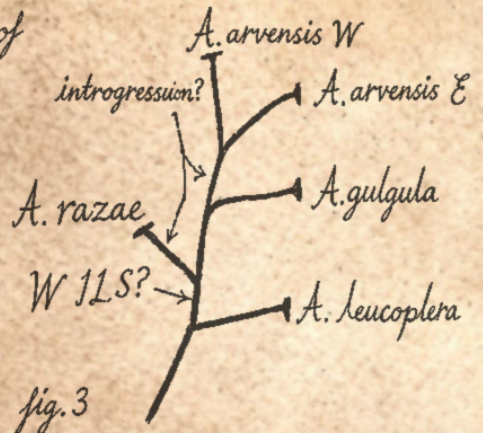
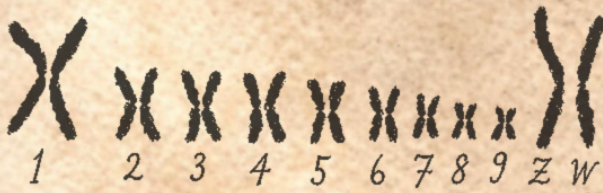


fig. 3

Karcyotype: *Alauda arvensis* (2n=76)



The karyotypes of larks are characterized by unusually large sex chromosomes and variable numbers of macroautosomes.



Evolutionary ecology of avian neo-sex chromosomes

Evolutionary ecology of avian neo-sex chromosomes

Simon Jacobsen Ellerstrand



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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Science at Lund University to be publicly defended on the 9th of September at 09.00 in the Blue Hall, Department of Biology, Ecology Building, Kontaktvägen 10, Lund 223 62

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Abstract:

Females and males are alternative reproductive phenotypes that can differ through striking suites of correlated phenotypic traits, such as behaviour, morphology and life-history traits. These sex-specific adaptations are sometimes facilitated through supergenes — sex chromosomes — which link sexually antagonistic alleles and the sex-determining locus into sex-specific haplotypes. However, recombination cessation reduces the ability of selection to maintain sex-limited Y/W haplotypes, often leading to progressive Y/W degeneration and fitness consequences for the heterogametic sex (male XY or female ZW). Despite their acknowledged importance in adaptation, life-history variation and speciation, sex chromosomes remain understudied in many areas of evolutionary ecology. Recent advances in DNA sequencing technology and sequencing initiatives have accumulated evidence that sex chromosome systems across the tree of life vary extensively in age, turnover, evolutionary transitions, autosomal fusions and stratum formation. Thus, now is an ideal time for biologists to investigate the role of sex chromosomes in evolutionary ecology. In this thesis, I studied sex chromosomes in larks (Alaudidae). Several autosomal translocations with subsequent recombination cessation in larks have resulted in greatly expanded neo-sex chromosomes. This system, with multiple evolutionary strata formed at different times, provides a powerful framework for studying different stages of sex chromosome evolution and for performing comparative analyses. I developed a bioinformatic pipeline that extracts Y/W sequences from short-read data, which enabled me to address questions regarding sex chromosome evolution and its role in evolutionary ecology. I studied Z–W gametolog evolution to characterise the longevity and functional role of W chromosomes under extreme demographic scenarios, and dissected the faster-Z effect using a gametolog-level approach. I studied sex chromosomes during speciation, characterising the evolutionary history of a large recombination-cessation polymorphism, and described factors influencing introgression across the Z chromosome. I studied the predictability of sex chromosome evolution and found that several gene properties predict gametolog co-evolution. I characterised remarkable variation in recombination cessation among lark species, with repeated formation of evolutionary strata, linking this variation to sexual dimorphism, pace-of-life strategy, and IUCN Red List status. Taken together, this thesis provides the first chromosome-level genome assembly for larks and lays a foundation for using larks as a model system to study sex chromosomes in evolutionary ecology.

Key words: sex chromosome evolution, recombination cessation, evolutionary strata, bioinformatic pipeline, Y/W degeneration, faster-X/Z effect, population genomics, mutational load, conservation genomics, speciation, large-X/Z effect, local adaptation, genome evolution, repeated evolution, sexual dimorphism, life-history variation

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Simon Jacobsen Ellerstrand



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MADE IN SWEDEN 

*“We are going to fight. We are going to be hurt. And in the end
we will stand.”*

—Roland Deschain

The Drawing of the Three by
Stephen King

“I have not failed. I've just found 10,000 ways that won't work.”

—Thomas Edison

Inventor

*“Jag ser mig själv ingen rolighet mer hava att förvänta förr än
den eviga roligheten.”*

—Gustav II Adolf

King of Sweden

“The sooner we're rid of it, the sooner to rest.”

—Samwise Gamgee

The Return of the King by
J.R.R Tolkien

“I need a vacation.”

—T-800

Arnold Schwarzenegger in
Terminator 2: Judgment Day



Christen Jacobsen



Jacob Borup
(Bagger)

Y

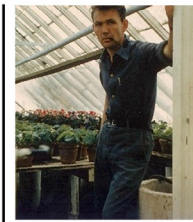


Anna Jørgensen

Aksel Jacobsen

Y

X



Elif Jacobsen

Y



Lissie Severinsen

X

Y



Leif Jacobsen



Henrik Jacobsen

Y

X



Thomas Ellerstrand

Anne-Marie Norberg

X



Christina Ellerstrand

X



Linnea Olofsson

Johannes Jacobsen

X

Y

Rufus Jacobsen

Herbert Jacobsen



Simon and Rufus
building sand castles



Simon Jacobsen
Ellerstrand



Aksel, Elif and Henrik chasing butterflies.

This thesis is dedicated to the carriers of my Y lineage and the co-inherited name of Jacobsen. We are sensitive men of deep thought and hot temperament, who find love and joy in the small things. But lest I forget the women who chose a life alongside us, for without their love, patience, and guarding X chromosomes, we would be nothing.

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Still, I know that I have sometimes appeared to be all over the place, and have not always been easy to supervise, especially during the first year. However, I have really appreciated your letting me pursue tangents with various side projects and trusting in me to balance them with my main projects. I think that this, together with your guidance, has really benefited me in finding my own way, developing independence, and making this research feel like my own. At the same time, you are a present supervisor. I have really appreciated your open door policy, seeing each other every other day, and always making time for me (although I know that I have overused it at times). If I have one complaint, it is that you are too nice sometimes, letting me get away with breaking my own deadlines!

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Thank you for introducing me to Skraeckoedlan, and for putting up with my ignorance of all pop-cultural references, which I am like 8 years (?) too young to get. A lot must really have happened during the years that separate us in age. Most of all, thank you for setting a good example of how to keep a (relatively) healthy work-life balance. It keeps me anchored to the world outside the department.

The original three: After three years as a civil engineer student, I decided to take a break and give biology a try. Only for a year. Maybe two. Now I am here, finishing my doctoral studies, and never have I looked back (well, maybe glanced at my previous classmates' engineering salaries). Natural sciences definitely hold a special place in my heart, and it is no wonder I stayed in the field of biology. However, my

new classmates that first year were what really decided it for me, making me feel as if I had found where I belonged.

I want to thank you all for some of the greatest years of my life, both my first and later course mates. Most of you have moved on to new endeavours over the years, but three of you went for the full marathon. Thank you, **Johan Kjellberg Jensen**, **Hanna Eriksson Thosteman**, and **Erica Winslott**. Having you and your friendship around all this time has given me a sense of stability in a field where turnover is high, and where new friends leave as soon as they arrive.

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Last, but not least: I know that for most of you reading this, this is probably the first page you opened in search for your name and in the hope of finding a personal acknowledgement. I am sorry to disappoint you, but I have decided not to name any more names than the ones above. So dry your tears and hear me out!

Throughout my long time at the department, there have been so many new faces who have come and gone that my memory has sometimes blurred who was there when, who said such, who taught me that, and who did what. And that is why I am less worried about making someone disappointed than making myself guilty of recency bias. I don't want to forget anyone who might feel they deserved acknowledgement.

But that is not to say that, for most of you, your presence and friendship have not meant the world to me. During my time as a doctoral student, I have in periods suffered from severe depression due to loneliness, feelings of inadequacy, and hopelessness. These symptoms have not all stemmed from the doctoral studies themselves, although doctoral studies are certainly not a context, nor academia an environment, that mitigates them.

I therefore want each and every one of you (if you feel as though you should be included here, you probably are, and perhaps even if you don't think you should) to know that I acknowledge the smallest of gestures, whether a passing nod in the corridor, a flash of a smile, an invitation to take a seat at lunch. I acknowledge science-related discussions and help with analyses. I acknowledge the time some of you have taken to listen to my deepest personal problems and insecurities, which I seldom share with anyone. I acknowledge everything in between, such as ridiculous discussions escalating at lunch, nerding out over cool insects, plants, and other natural phenomena, for listening to my rants and tangents about random things, and for sharing a beer at the end of every week at the Friday pub.

Thank you for making the department a space and part of my life where I rarely felt unwelcome, and often felt as a second home. Thank you for keeping me in the light!

Abstract

Females and males are alternative reproductive phenotypes that can differ through striking suites of correlated phenotypic traits, such as behaviour, morphology and life-history traits. These sex-specific adaptations are sometimes facilitated through supergenes — sex chromosomes — which link sexually antagonistic alleles and the sex-determining locus into sex-specific haplotypes. However, recombination cessation reduces the ability of selection to maintain sex-limited Y/W haplotypes, often leading to progressive Y/W degeneration and fitness consequences for the heterogametic sex (male XY or female ZW). Despite their acknowledged importance in adaptation, life-history variation and speciation, sex chromosomes remain understudied in many areas of evolutionary ecology. Recent advances in DNA sequencing technology and sequencing initiatives have accumulated evidence that sex chromosome systems across the tree of life vary extensively in age, turnover, evolutionary transitions, autosomal fusions and stratum formation. Thus, now is an ideal time for biologists to investigate the role of sex chromosomes in evolutionary ecology. In this thesis, I studied sex chromosomes in larks (Alaudidae). Several autosomal translocations with subsequent recombination cessation in larks have resulted in greatly expanded neo-sex chromosomes. This system, with multiple evolutionary strata formed at different times, provides a powerful framework for studying different stages of sex chromosome evolution and for performing comparative analyses. I developed a bioinformatic pipeline that extracts Y/W sequences from short-read data, which enabled me to address questions regarding sex chromosome evolution and its role in evolutionary ecology. I studied Z–W gametolog evolution to characterise the longevity and functional role of W chromosomes under extreme demographic scenarios, and dissected the faster-Z effect using a gametolog-level approach. I studied sex chromosomes during speciation, characterising the evolutionary history of a large recombination-cessation polymorphism, and described factors influencing introgression across the Z chromosome. I studied the predictability of sex chromosome evolution and found that several gene properties predict gametolog co-evolution. I characterised remarkable variation in recombination cessation among lark species, with repeated formation of evolutionary strata, linking this variation to sexual dimorphism, pace-of-life strategy, and IUCN Red List status. Taken together, this thesis provides the first chromosome-level genome assembly for larks and lays a foundation for using larks as a model system to study sex chromosomes in evolutionary ecology.

Popular Science Summary

The study of evolution and ecology took off in the mid-19th century, during a time of European exploration and colonial expansion. While the dark history of these fields is in part rooted in exploitation, they also developed out of, and contributed, to the birth of modern science. This was a time when a naturalist could step off a boat onto a foreign shore and start describing new species (to science, anyway) by the hundreds. These fields came about at a time when invasive species had already been — and continued to be — spread globally, when the Industrial Revolution increased the release of pollutants, and when agricultural intensification drastically reshaped the landscape. At the same time, modern scientific practice emphasised systematic documentation of the diversity of life, and how it interacted with the environment that we had begun to erode.

Today, it might seem as if that era of exploration and discovery is over. Many species have been described, and it is often said that few truly unexplored regions remain on Earth, such as the Mariana Trench. But the era of exploration never stopped; the only thing that shifted was the frontiers. One of these frontiers is the diversity of genomes. Genomes can be found all around us: in the grass of a park, in your pet, in your food, and in microorganisms in the air. The millions of cells in your own body carry your genome. What separates you from that new frontier of exploration is not an expedition by boat over the course of months, but technology that allows you to extract DNA and sequence it.

In this thesis, I set out to discover what lies beyond this frontier, specifically with an emphasis on the evolution of sex chromosomes in birds and their connection to species ecology. Just as the early naturalists required specific instruments such as insect nets, microscopes, and other equipment to perform science, modern biologists have expanded their toolbox to include DNA extraction techniques, population genomic models, and computer software to study the genome. Therefore, I started my journey by developing a “bioinformatic pipeline”, a computational method that allowed me to study new aspects of sex chromosomes.

When I started this thesis, the Skylark was the bird with the largest sex chromosomes yet known to science. The expansion of lark sex chromosomes has occurred in several steps, through a process in which large segments containing many genes move from regular chromosomes to the sex chromosomes at different points in time. This movement to sex chromosomes suggests that those genes are

important blueprints for biological differences between females and males, or that they might eventually evolve such functions.

I described interesting and complex dynamics in the evolution of the Y and X chromosomes (called Z and W in birds) and how they interact and evolve in response to one another. I also found some indications that genes newly transferred to the sex chromosomes might benefit female birds relative to males in several respects, especially in threatened species with small populations. This result is intriguing, as it supports a role for sex chromosome evolution in species conservation management.

I also studied different aspects of how sex chromosomes become distributed among newly forming species, how they are later exchanged between species through hybrids, and how sex chromosome may facilitate adaptation to geographically different environments. The Skylark's geographical distribution spans most of northern Eurasia, from Europe in the west to Japan and beyond in the east. The genomes of western and eastern Skylarks carry different versions of the sex chromosomes. This distribution between west and east is likewise reflected in closely related species depending on where they occur in Eurasia, suggesting that sex chromosomes play a role in species adaptation to the environment.

One of the ultimate goals of science is to understand a system well enough to — with some accuracy — predict how it will behave in the future. That is how science has enabled us to forecast the development of things such as weather, and the spread of disease. Throughout my journey, I found several properties of genes and chromosomes that appear to be associated with their evolutionary fate. This means that such knowledge might eventually allow us to forecast which chromosomal segments might become sex chromosomes, and the fate evolution has in store for those genes.

Finally, I explored, discovered, and described an amazing diversity of previously undocumented sex chromosomes in almost 50 species. This revealed more diversity in sex chromosomes than had previously been documented among birds. Furthermore, I managed to link this variation to species ecology. Lark species with larger sex chromosomes often have males with larger beaks than females, suggesting adaptation to different feeding niches between the sexes. Larger sex chromosomes are also associated with species characteristics often seen among threatened species, further supporting a role for sex chromosome evolution in species conservation management. These observations raise new questions about which underlying processes might be causing these associations.

Taken together, I have studied processes and found results that are sometimes in line with, and sometimes contradict, current theory. I have discovered and described biodiversity previously unknown to science. I have pushed the frontiers of research in sex chromosome evolution and ecology slightly further, and uncovered many new avenues for future modern explorers to travel.

Populärvetenskaplig Sammanfattning

Evolution och ekologi som forskningsfält tog fart under mitten av 1800-talet, i en tid präglad av europeiskt upptäcktsresande och kolonial expansion. Trots sitt mörka ursprung med rötter i exploatering växte de också fram ur och bidrog till den moderna vetenskapen. Det var en tid då naturforskare kunde kliva av en båt på en främmande strand och börja beskriva hundratals nya arter (åtminstone nya för vetenskapen). Dessa forskningsfält uppstod i en tid då invasiva arter redan hade spridits och fortsatte att spridas världen över, då den industriella revolutionen skapade stora utsläpp av föroreningar, och då ett intensifierat jordbruk drastiskt omformade landskapet. Samtidigt betonade den vetenskapliga praktiken en systematisk dokumentation av livets mångfald och hur organismer samspelade med den miljö som vi redan hade börjat förändra och utarma.

Kanske tror många att denna tid av utforskning och upptäckter idag är över. Många arter är beskrivna, och det sägs ofta att få verkligt outforskade områden återstår på jorden, såsom Marianergraven. Men tiden för utforskande tog aldrig slut; det enda som skiftade var forskningsfronten. En av dessa nya forskningsfronter är genomens mångfald. Organismers genom finns överallt omkring oss: i gräset i en park, i ditt husdjur, i din mat och i mikroorganismerna i luften. De miljontals celler som bygger upp din egen kropp bär på ditt eget genom. Det som skiljer dig från denna nya upptäcktsfront är inte längre en flera månader lång båtresa, utan teknik som gör det möjligt att extrahera och sekvensera DNA.

I denna avhandling gav jag mig i kast med att utforska vad som döljer sig i genomet, med särskilt fokus på könskromosomernas evolution hos fåglar och kopplingen till deras ekologi. Precis som de tidiga naturforskarna behövde särskilda verktyg såsom insektsnät, mikroskop och annan utrustning för att bedriva vetenskap, har moderna biologer utökat sin repertoar med labbtekniker för DNA, populationsgenetiska modeller och programvara för att studera genomet. Därför började jag min upptäcktsresa med att utveckla en metod och datorprogram som gjorde det möjligt för mig att undersöka nya aspekter av könskromosomer.

När jag påbörjade denna avhandling var sånglärkan den fågel som hade de största könskromosomerna som då var kända för vetenskapen. Lärkornas könskromosomer har förstörats i flera steg genom en process där stora kromosomsegment och gener har flyttats från vanliga kromosomer till könskromosomerna vid olika tidpunkter. Att dessa segment har förflyttats till könskromosomerna antyder att generna i dem

är viktiga för biologiska skillnader mellan honor och hanar, eller att de med tiden kan komma att få sådana egenskaper.

Jag beskrev intressanta och komplexa dynamiker i evolutionen av Y- och X-kromosomerna (som hos fåglar kallas W- respektive Z-kromosomer) och hur deras evolution samspelar med varandra. Jag fann också indikationer på att gener som nyligen förflyttats till könskromosomerna kan gynna honfåglar i förhållande till hanar på flera sätt, särskilt bland hotade arter med små populationer. Detta resultat är fascinerande eftersom det antyder att könskromosomernas evolution kan få betydelse inom bevarandebiologi.

Jag studerade även olika aspekter av hur könskromosomer fördelas mellan nybildade arter, hur de senare kan överföras mellan arter genom hybridisering, samt huruvida könskromosomer kan underlätta anpassning till geografiskt skilda miljöer. Sånglärkans utbredning sträcker sig över större delen av norra Eurasien, från Europa i väst till Japan och vidare i öst. Genomen hos västliga och östliga sånglärkor bär på olika varianter av könskromosomerna. Detta geografiska samband mellan väst och öst återfinns även hos närbesläktade arter beroende på var de förekommer i Eurasien, vilket antyder att könskromosomer spelar en roll i arters anpassning till sin miljö.

Ett av vetenskapens yttersta mål är att förstå ett system till den grad att vi med viss träffsäkerhet kan förutsäga dess framtida beteende. Det är på så vis vetenskapen har möjliggjort vår förmåga att förutse utvecklingen av sådant som väder och sjukdomsspridning. Under min upptäcktsresa fann jag flera egenskaper hos gener och kromosomer som verkar vara kopplade till deras evolutionära bana. Detta innebär att sådan kunskap kan komma att göra det möjligt för oss att förutse vilka kromosomsegment som i framtiden kan komma att utvecklas till könskromosomer och hur dessa gener kan komma att formas av evolution över tid.

Slutligen utforskade, upptäckte och beskrev jag en imponerande mångfald av sedan tidigare odokumenterade könskromosomer hos nästan 50 arter, vilket avslöjade en större variation av könskromosomer än vad som tidigare har dokumenterats hos fåglar. Dessutom lyckades jag finna kopplingar mellan denna variation och arternas ekologi. Lärkararter med större könskromosomer har ofta hanar med större näbbar än honor, vilket tyder på anpassningar till olika födonischer mellan könen. Större könskromosomer är även kopplade till egenskaper som ofta återfinns hos hotade arter, vilket ytterligare antyder att könskromosomer kan ha betydelse för bevarandebiologi. Dessa observationer väcker nya frågor om vilka underliggande processer som kan tänkas ligga bakom dessa samband.

Sammantaget har jag studerat processer och funnit resultat som ibland överensstämmer med, och ibland motsäger, rådande teoretiska ramverk. Jag har upptäckt och beskrivit biologisk mångfald som tidigare var okänd för vetenskapen. Jag har flyttat forskningsfronten inom könskromosomernas evolution och ekologi en liten bit framåt och öppnat många nya vägar för framtidens forskare att utforska.

List of Papers

- I. **Ellerstrand, S.J.**, Churcher, A., Kutschera, V.E., Hansson, B., 2026. PhaseWY: A pipeline for haplotype phasing, sex chromosome identification and extraction of sex-limited sequences. *bioRxiv* preprint. <https://doi.org/10.64898/2026.06.17.732863>
- II. **Ellerstrand, S.J.**, Hansson, B., 2026. Selective regimes and evolutionary dynamics of Z and W gametologs across an expanded avian neo-sex chromosome. *Genome Biology and Evolution*, 18(8), evag204. <https://doi.org/10.1093/gbe/evag204>
- III. **Ellerstrand, S.J.**, Brooke, M. de L., Kutschera, V.E., Hansson, B., 2026. An enlarged neo-W chromosome maintains female functional genetic variation in the critically endangered Raso lark. Submitted to *Molecular Ecology*.
- IV. **Ellerstrand, S.J.**, Alström, P., Hansson, B., 2026. Dynamics of enlarged neo-sex chromosomes during speciation of *Alauda* larks. Manuscript.
- V. Brown, T.J., **Ellerstrand, S.J.**, Sigeman, H., Melo, M., Lundberg, M., Hansson, B., 2026. Unique sex chromosome translocations and evolutionary strata in two Sylvioidea songbird families. *BMC Genomics*, 27, 401. <https://doi.org/10.1186/s12864-026-12861-1>
- VI. **Ellerstrand, S.J.**, Proux-Wéra, E., Gallard, S., Fu, Y., Alström, P., Hansson, B., 2026. Sex chromosome evolution predicts sexual dimorphism and life-history outcomes across larks. Manuscript.

Author Contributions

- I. **SJE** and BH conceived the study. **SJE** developed the code of the pipeline and performed the analyses with input from BH. AC and VEK converted the code into a Snakemake pipeline with input from **SJE**. **SJE** and BH wrote the paper with input from AC and VEK.
- II. **SJE** and BH conceived the study. **SJE** performed the analyses with input from BH. **SJE** and BH wrote the paper.
- III. **SJE** and BH conceived the study. MdeLB performed field work. **SJE** performed the analyses with input from BH. VEK performed GERP score estimation with input from **SJE**. **SJE** and BH wrote the paper with input from MdeLB and VEK.
- IV. **SJE**, PA and BH conceived the study. PA performed sample collection. **SJE** performed the analyses with input from PA and BH. **SJE** and BH wrote the paper with input from PA.
- V. TJB, HS and BH conceived the study. MM performed field work. TJB, **SJE** and ML performed analyses with input from BH. TJB and BH wrote the paper with input from **SJE**, HS, MM and ML.
- VI. **SJE**, PA and BH conceived the study. **SJE**, BH and PA performed sample collection. **SJE** performed the analyses with input from BH. EPW assembled the genomes. SG collected and analysed trait data. YF generated data on evolutionary strata. **SJE** and BH wrote the paper with input from EPW, SG, YF and PA.

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Additional papers

The following papers were published during my doctoral studies but are not included in this thesis:

- Jayaprasad, S., Peona, V., **Ellerstrand, S.J.**, Rossini, R., Bunikis, I., Pettersson, O.V., Olsen, R.-A., Rubin, C.-J., Einarsdottir, E., Bonath, F., Bradford, T.M., Cooper, S.J.B., Hansson, B., Suh, A., Kawakami, T., Schielzeth, H., Palacios-Gimenez, O.M., 2024. Orthopteran Neo-Sex Chromosomes Reveal Dynamics of Recombination Suppression and Evolution of Supergenes. *Molecular Ecology* 33, e17567. <https://doi.org/10.1111/mec.17567>
- Sigeman, H., **Ellerstrand, S.J.**, Hansson, B., 2026. Evolutionary dynamics of enlarged neo-sex chromosomes and novel pseudoautosomal regions in Sylvioidea songbirds. *Communications Biology* 9, 898. <https://doi.org/10.1038/s42003-026-10603-3>

Introduction

Sex chromosome evolution

The evolution of diverse sex chromosome systems

Most uninitiated sex chromosome enthusiasts are at the very least aware of the human XY system. Women carry two copies of the X chromosome, while men carry a maternally inherited X, and a paternally inherited Y chromosome. It is the system found among humans and all eutherian mammals (Cortez *et al.*, 2014). Consequently, the XY sex chromosome system is perhaps the most studied sex determination system. However, other sex determining systems exist (**Box 1; Figure 1A**; Bachtrog *et al.*, 2014), such as the ZW chromosome system in which the female is the heterogametic sex (carrying two different chromosomes, Z and W), and the male is the homogametic sex (carrying two Z chromosomes; **Figure 1B**). In UV systems, sex is expressed in the haploid phase. Females carry the U chromosome and males carry the V chromosome (Bachtrog *et al.*, 2014). Furthermore, there are extraordinary examples of sex chromosome systems consisting of several chromosomes, such as the five pairs of XY chromosomes in the platypus (*Ornithorhynchus anatinus*; Veyrunes *et al.*, 2008), and systems in which two non-homologous chromosomes can independently serve the role of the Z chromosome, such as in the Adélie penguin (*Pygoscelis adeliae*; Gunski *et al.*, 2017).

Sex chromosomes typically originate from homologous autosomes that acquire a sex-determining mutation, after which recombination suppression may expand around the sex-determining locus (Ohno, 1967; **Figure 2**). Thus, chromosomes with sex-determining loci are referred to as sex chromosomes, which contain a non-recombining region that is sex-linked. Consequently, the size of sex chromosomes and their sex-linked region can vary widely among organisms. The sex-linked region can consist of only a single nucleotide polymorphism (SNP), as observed in the Tiger puffer fish (*Takifugu rubripes*; Kamiya *et al.*, 2012). On the other hand, the White campion (*Silene latifolia*) has a ~320 Mb homologous sex-linked region encompassing approximately 2,500-3,000 genes, with the Y chromosome being as large as ~550 Mb due to repeat accumulation (Moraga *et al.*, 2025).

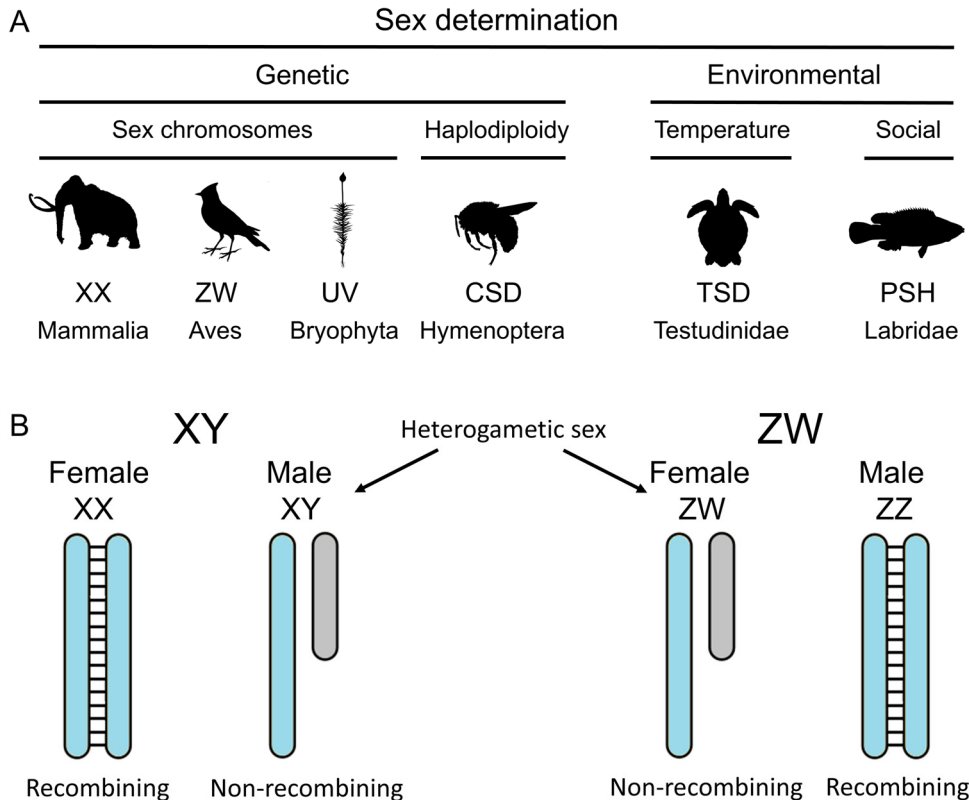


Figure 1. Diversity of sex determination systems. (A) Sex determination systems can be broadly divided into genetic and environmental. Genetic sex determination includes sex chromosome systems with male heterogamety (XY, e.g. mammals and beetles) and female heterogamety (ZW, e.g. birds, butterflies), and systems where sex is only expressed in the haploid phase (UV, e.g. mosses and brown algae). Genetic sex determination can also be based on haplodiploidy, such as complementary sex determination (CSD, e.g. bees, ants, and wasps). Environmental sex determination can occur through temperature during incubation (TSD, e.g. turtles and crocodiles), or social cues, such as protogynous sequential hermaphroditism (PSH), where an individual is born female and later transitions to male in response to changes in the social hierarchy (e.g. wrasses). **(B)** The XY and ZW systems are named according to which sex carries two similar sex chromosomes (the homogametic sex, female XX or male ZZ), and which carries differentiated sex chromosomes (the heterogametic sex, male XY or female ZW). In the homogametic sex, recombination occurs freely between the similar sex chromosome pair, while in the heterogametic sex, recombination is suppressed or has ceased completely across the sex-linked region. Note that these examples do not represent a complete overview of sex determination systems.

Expansion of the non-recombining region is generally thought to be driven by selection favouring tighter linkage between the sex-determining locus and sexually antagonistic alleles, thereby creating sex-specific haplotypes (Bull, 1983; Fisher, 1931; Rice, 1987; **Figure 2**). Large expansions can occur through structural rearrangements such as inversions, which may encompass many additional genes beyond the one driving fixation of the rearrangement (Bergero & Charlesworth, 2009). Autosomal translocations and fusions can further recruit genes to the sex

chromosome, creating so-called neo-sex chromosomes (Pala *et al.*, 2012). These expansions can in turn generate evolutionary strata, each characterised by distinct levels of divergence between sex-linked haplotypes (Lahn & Page, 1999; Xu *et al.*, 2019). Evolutionary strata occur across diverse taxa, including mammals, birds, fish, plants, fungi, mosses and algae (Bellott *et al.*, 2014; Bergero *et al.*, 2007; Cortez *et al.*, 2014; McDaniel *et al.*, 2013; Peichel *et al.*, 2020; Votintseva & Filatov, 2009; Xu *et al.*, 2019; Zhou *et al.*, 2014a). In many old sex chromosome systems, the sex-limited region has ceased recombining across most of its length, leaving a small pseudoautosomal region (PAR) that ensures proper segregation during meiosis (Graves *et al.*, 1998; Otto *et al.*, 2011; Zhou *et al.*, 2014a).

Box 1: The evolution of sex and sex determination.

Meiosis is a commonly used definition of sex as a biological process. Meiosis leads to the exchange of genetic material when two haploid cells (gametes) fuse to form a diploid cell through fertilisation. Prior to meiosis, the genetic material is replicated, after which homologous chromosome pairs exchange DNA through recombination. By this definition, asexual reproduction only involves mitotic cell division, also known as apomixis, or clonal reproduction (Beukeboom & Perrin, 2014). This definition further restricts sex to eukaryotes, where it likely has its single origin (Dacks & Roger, 1999; Speijer *et al.*, 2015). The common ancestor of eukaryotes was likely facultatively sexual, since sexual reproduction is widespread among eukaryotes, including basal lineages (Dacks & Roger, 1999). But there are exceptions to facultative sex in eukaryotes. If we look at the multicellular animals (Metazoa) and vascular plants (Tracheophyta) which make up a relatively small proportion of eukaryotic diversity, we find that obligate sexuality is often the rule and that facultative sexuality and obligate asexuality exist but are less common (Speijer *et al.*, 2015).

There are several benefits of having sex. Meiosis acts as a DNA repair mechanism, in which the homologous chromosome is used as a template (Bernstein *et al.*, 1988). Recombination may create new combinations of alleles, generating genetic variation for selection to act upon, and dissociating deleterious alleles from beneficial ones, enabling efficient purging (Burt, 2000; Weismann, 1889). However, sex can also break apart favourable allele combinations, resulting in reduced offspring fitness. In infinite populations, sex is only favoured under very narrow conditions (Beukeboom & Perrin, 2014). In infinite populations all possible combinations of alleles already exist at any given moment, and the most optimal combination will outcompete other combinations and rise in frequency in response to environmental change (Fisher, 1930; Muller, 1932). However, for most species, a stable environment is seldom the reality, and neither is a large and stable population size. Thus, sex can increase the adaptive potential of a population in a constantly changing environment (Bell, 1982), and beneficial mutations can be spread across several lineages and subsequently combined by recombination (Muller, 1932).

The process by which either female or male development is triggered — known as sex determination — is diverse and can be broadly categorised into genetic- and environmental sex determination, although systems can sometimes be a combination of both (**Figure 1A**). Environmental sex determination can be favoured when specific environmental conditions are beneficial for a particular sex. However, such systems can be problematic when the environment is unpredictable or so stable that only a single sex is produced. For example, in a warming climate, species in which sex is determined through temperature may be vulnerable if an excess of one sex is produced (Bachtrog *et al.*, 2014). Genetic sex determination provides a mechanism for preventing extreme sex ratios. When sex is determined by sex chromosomes — and inherited according to Mendelian segregation — offspring sex ratios are more likely to remain balanced, even in smaller populations (Beukeboom & Perrin, 2014; Fisher, 1930). While genetic sex determination can be especially favourable under some conditions, such systems can be vulnerable to other processes, such as degeneration of the sex-limited Y/W chromosome and reduced fitness in the heterogametic sex (**Figure 2**).

Consequences of being sex-limited

Because the sex chromosomes are allocated differently in females and males, they will experience different selection dynamics and effective population sizes than that on autosomes. Under even sex ratios, the effective population size of the X/Z chromosome is expected to be three-quarters that of autosomes, whereas the Y/W chromosomes have an effective population size of one-quarter of autosomes (Ellegren, 2011). Furthermore, the X/Z chromosome spends more evolutionary time in one sex than the other, while the Y/W chromosome is transmitted exclusively through one sex (Klein *et al.*, 2021). Recombination cessation, skewed transmission and reduced effective population size of the sex chromosomes increase their vulnerability to genetic drift, background selection and selective sweeps (Hill & Robertson, 1966; Smith & Haigh, 1974; Charlesworth *et al.*, 1993; Ellegren & Galtier, 2016). These effects are strongest in the non-recombining Y/W regions, which accumulate deleterious mutations and transposable elements through Muller's ratchet, the irreversible accumulation of deleterious mutations in non-recombining genomic regions (Muller, 1964; Bachtrog, 2008, 2013).

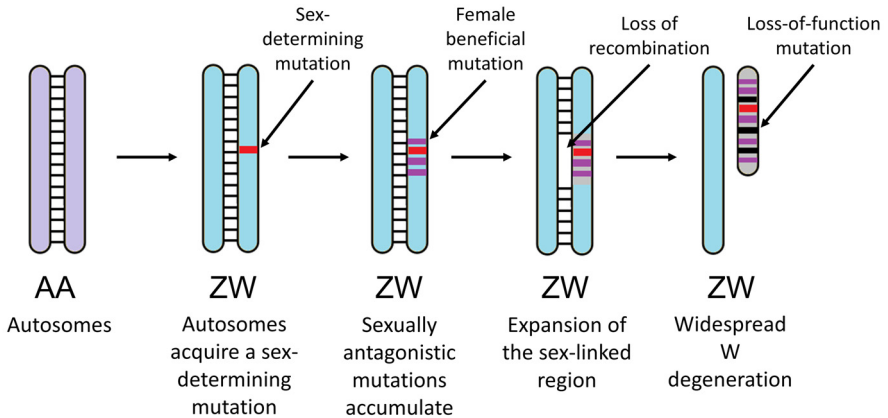


Figure 2. Sex chromosome evolution. In the classic model of sex chromosome evolution, a pair of autosomes gain a sex-determining mutation. The sex-linked region subsequently expands as sexually antagonistic alleles are linked to the sex-determining locus to create sex-specific haplotypes. Inefficient selection may ultimately lead to widespread degeneration across the sex-limited Y/W chromosome.

The reduced efficacy of selection often leads to gradual degeneration of Y/W-linked genes (Bachtrog, 2008; **Figure 2**), contributing to the gene-poor and repeat-rich mammalian Y chromosomes, where only a handful of genes persist through strong purifying selection (Bellott *et al.*, 2014, 2017). While extensive theory exists on the rate of Y/W degeneration, empirical observations are often made from old and already degenerated systems such as the mammalian Y and avian W. Consequently, empirical observations of degeneration in young and intermediate-aged sex chromosome systems remain relatively limited (Bachtrog, 2008; Huang *et al.*, 2022; Sigeman *et al.*, 2024; Xu *et al.*, 2024).

Due to reduced effective population size and inefficient selection, Y/W chromosomes generally harbour low levels of genetic variation and limited functional and adaptive potential (Bachtrog, 2013; Ellegren & Galtier, 2016). Only very rarely do Y/W-linked genes outlast their X/Z gametologs, as exemplified by the gene *PRSSLY* — a male fertility gene — which has survived in some mammalian lineages (Hughes *et al.*, 2022). Nevertheless, despite these constraints, some Y/W-linked genes can continue to play functional and adaptive roles over long evolutionary time scales (Zhou & Bachtrog, 2012b). For example, genes with sex-specific functions, such as the male-determining gene *SRY* in mammals (Sinclair *et al.*, 1990), and various male fertility genes (Lahn & Page, 1997). Furthermore, functional polymorphisms can sometimes be maintained on the Y/W chromosome, potentially through balancing selection acting on phenotypic traits (Meisel, 2022), as observed in male colour morphs in guppies (*Poecilia parae*; Sandkam *et al.*, 2021) and female – and potentially egg – colour morphs in cuckoos (Fossøy *et al.*, 2016; Merondun *et al.*, 2024). While documented examples of adaptive Y/W evolution remain relatively rare, these cases suggest that Y/W-linked genes can sometimes retain functional and adaptive roles over long evolutionary timescales.

XY versus ZW-systems

Due to the differences in the heterogametic sex between XY and ZW systems, organellar genomes — such as mitochondria and plastids — can be co-inherited with the sex-limited chromosome. In many species, these genomes are predominantly maternally inherited, and therefore co-segregate with the W chromosome in ZW systems. This shared inheritance can generate strong associations between the W chromosome and organellar genomes, despite their being separate genomic elements (Berlin & Ellegren, 2001; Berlin *et al.*, 2007; Greiner *et al.*, 2015).

While XY and ZW sex chromosome systems share many aspects of selection and evolution, they may differ in the sex-specific forces that shape their evolutionary dynamics. In particular, male-biased mutation rates and male-biased variance in reproductive success (i.e. sexual selection) can generate systematic differences in evolutionary pressures acting on sex chromosomes across systems (Naurin *et al.*, 2010). These effects are expected to be most pronounced in animals, whereas plant life histories may often experience weaker sex-specific selective regimes (Beukeboom and Perrin, 2014). In addition, plants may expose a larger fraction of their genome to haploid purifying selection during the gamete stage, potentially limiting degeneration and the evolution of dosage compensation (Charlesworth and Charlesworth, 1992; Joseph and Kirkpatrick, 2004).

Everything else being equal; under male-biased mutation and sexual selection, sex chromosomes are expected to experience different evolutionary pressures relative to autosomes. In XY systems, this will lead to a lower mutation rate and more

efficient purging of deleterious alleles on the X relative to the Y, while the Y experiences a higher mutation rate and reduced efficacy of purifying selection. Conversely, in ZW systems this will lead to a higher mutation rate and relaxed purifying selection on the Z relative to the W. These processes may result in a faster rate of Y relative to W degeneration. For these reasons, selection for dosage compensation on the Z chromosome may be weaker when W gametologs remain functional for longer. Indeed, dosage compensation does not appear as prominent in bird ZW systems compared to mammalian and *Drosophila* XY (Naurin *et al.*, 2010). Furthermore, stronger sexual selection in males and male-biased mutation rates may affect evolutionary rates of X, Y, Z, and W chromosomes in different ways, adding additional layers of complexity discussed in the following sections.

Rates of evolution can vary

A phenomenon termed faster-X/Z evolution (Charlesworth *et al.*, 1987; Vicoso & Charlesworth, 2009) refers to the observation that X/Z-chromosomes often evolve more rapidly than autosomes, documented in several systems, including mammals (XY), birds (ZW) and Lepidoptera (ZW; Baines and Harr, 2007; Mank *et al.*, 2010b; Sackton *et al.*, 2014; Torgerson and Singh, 2003). However, this is not a general pattern among species, as there are likewise observations of similar evolutionary rates between X/Z and autosomes (Counterman *et al.*, 2004; Vicoso *et al.*, 2008; Mongue *et al.*, 2022), or even relatively slower evolution (slower-X/Z; Baird *et al.*, 2025; Rousselle *et al.*, 2016). Many factors can influence these patterns in complex ways. Therefore, faster X/Z theory requires a thorough decomposition (**Figure 3**).

- **Neutral evolution:** Starting from a model of strictly neutral evolution without sex-specific influences, Y/W degeneration or dosage compensation, we do not expect different evolutionary rates between chromosomes. Although variation in effective population size and recombination rate can influence the speed of fixation of neutrally segregating polymorphisms, they do not alter the probability of fixation (Kimura, 1968). Thus, the substitution rate is expected to remain the same across chromosomes when observed over longer evolutionary time spans.
- **Male-biased mutation rate:** If we introduce male-biased mutation rate into our neutral model, we change the influx of new mutations on chromosomes relative to the evolutionary time they spend in males. This leads to relative differences in substitution rates: $Y > \text{autosomes} > X$, and $Z > \text{autosomes} > W$ (Ellegren, 2007).
- **Efficacy of selection (via effective population size and recombination rate):** If we introduce selection into the neutral model, differences in effective population size and recombination rate among chromosomes affect the efficacy of selection accordingly (Charlesworth *et al.*, 1993; Hill

& Robertson, 1966; Smith & Haigh, 1974). Relaxed purifying selection results in drift elevating the rate of non-synonymous substitution among chromosomes: $Y/W > X/Z > \text{autosomes}$ (Vicoso & Charlesworth, 2009).

- **Male-biased sexual selection:** If we introduce male-biased sexual selection, the increased variance in male reproductive success will further reduce the efficacy of selection on chromosomes relative to the evolutionary time they spend in males (i.e. drift). This leads to relative differences in non-synonymous substitution rates: $Y > \text{autosomes} > X$, and $Z > \text{autosomes} > W$ (Vicoso & Charlesworth, 2009).
- **Reduced male-biased recombination rate:** If we introduce reduced male-biased recombination rate, linked selection will reduce the efficacy of selection further on chromosomes relative to the evolutionary time they spend in males (Hill & Robertson, 1966; Smith & Haigh, 1974; Charlesworth *et al.*, 1993). This leads to differences in non-synonymous substitution rates: $Y > \text{autosomes} > X$, and $Z > \text{autosomes} > W$ (Vicoso & Charlesworth, 2009).
- **Hemizygous exposure:** If we introduce degeneration of Y/W-linked gametologs into the model, we allow hemizygous exposure to selection of recessive alleles segregating on the X/Z when occurring in the heterogametic sex. If *de novo* recessive X/Z alleles are beneficial, hemizygous exposure is expected to increase the non-synonymous substitution rate of the X/Z chromosome relative to autosomes. However, if adaptive evolution instead proceeds from standing variation at higher allele frequencies, recessive alleles are more likely to already occur in homozygous form, reducing the relative impact of hemizygous exposure on X/Z-linked evolutionary rates.

If recessive X/Z alleles are moderately to strongly deleterious, hemizygous exposure to purifying selection is expected to reduce the non-synonymous substitution rates of the X/Z chromosome relative to autosomes. However, for weakly deleterious mutations, rates may still be elevated if Y/W degeneration in the absence of dosage compensation reduces expression of X/Z-linked genes, thereby weakening purifying selection against mildly deleterious alleles (Charlesworth *et al.*, 1987).

- **Dosage compensation:** If we incorporate dosage compensation, increased expression of hemizygously expressed X/Z genes strengthens selection acting on recessive alleles by increasing their phenotypic effects in the heterogametic sex. If recessive alleles are beneficial, it is expected to increase the rate of the X/Z chromosome. If recessive alleles are deleterious, it is expected to reduce the rate of the X/Z chromosome (Charlesworth *et al.*, 1987).

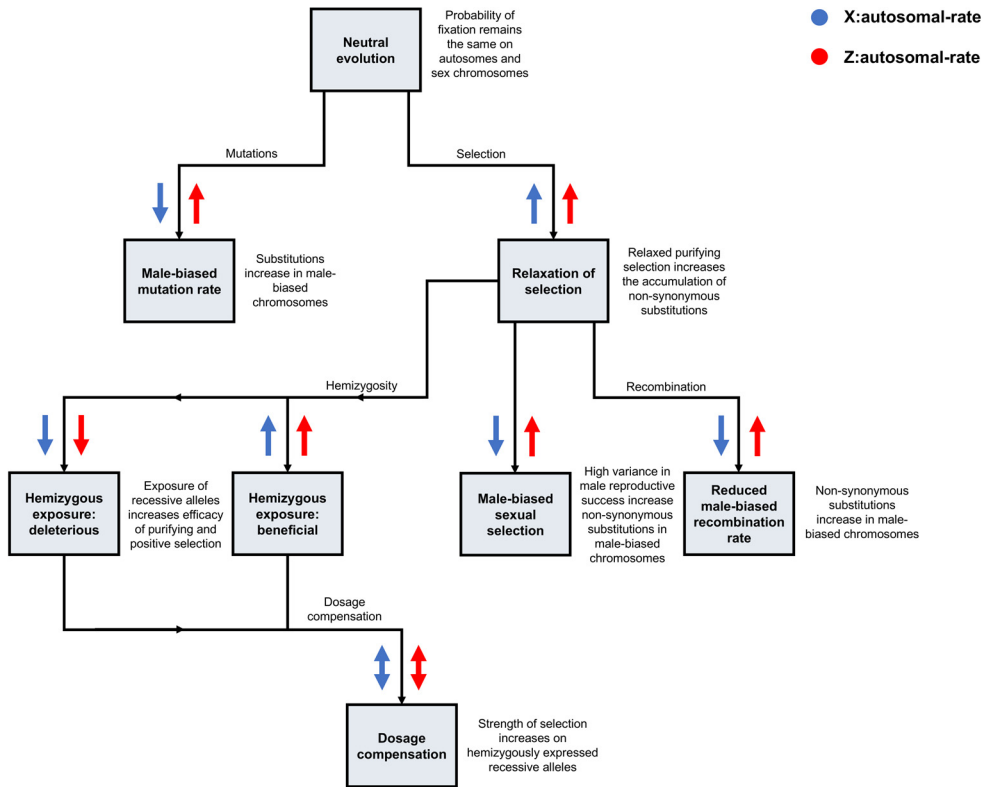


Figure 3. The faster-X/Z effect. Under a model of strictly neutral evolution, the substitution rate is expected to remain constant across chromosomes. Incorporating additional processes into the model is expected to affect the substitution rate of sex chromosomes relative to autosomes in specific ways, e.g. non-neutral processes affect the rate of non-synonymous substitutions. The relative impact of these processes on evolutionary rates is often unknown and system dependent, and with sex-specific effects depending on the sex chromosome system (XY or ZW). Upward-pointing arrows indicate a contribution to a faster-X/Z effect, whereas downward-pointing arrows indicate a contribution to a slower-X/Z effect.

Of course, several of these processes may act simultaneously, but it is often not known to what extent each of them contributes to evolutionary rates, and therefore what relative evolutionary rates to expect. However, these processes are often broadly discussed in terms of adaptive faster-X/Z evolution (efficient selection due to hemizygous exposure of beneficial alleles) and drift-driven faster-X/Z evolution (relaxed purifying selection due to male-biased sexual selection increasing drift), or slower-X/Z evolution (efficient selection due to hemizygous exposure of deleterious alleles; Meisel & Connallon, 2013). Subsequently, XY and ZW are often expected to — and found to — follow specific patterns (Mank *et al.*, 2010b; 2010a). In several studied XY systems with global dosage compensation, selection-driven adaptive faster-X or slower-X evolution is often inferred to be the net pattern (Torgerson & Singh, 2003; Baines & Harr, 2007; Mank *et al.*, 2010b; Zhou & Bachtrog, 2012b):

- **Male-biased mutation rate:** X experiences slower evolutionary rates due to male-biased allocation. Y experiences faster degeneration
- **Male-biased sexual selection:** X experiences less drift-driven evolutionary rates due to male-biased allocation.
- **Efficacy of selection:** X experiences higher drift-driven evolutionary rates due to smaller effective population size.
- **Hemizygous exposure:** X evolutionary rates are more affected by hemizygous exposure due to faster Y degeneration.
- **Dosage compensation:** Dosage compensation leads X to be more affected by hemizygous exposure due to faster Y degeneration.

In ZW systems like birds and Lepidoptera that are lacking global dosage compensation, drift-driven faster-Z evolution is often inferred to be the main process (Mank *et al.*, 2010a; Wright *et al.*, 2015; Xu *et al.*, 2019; Hayes *et al.*, 2020; Chase *et al.*, 2025):

- **Male-biased mutation rate:** Z experiences higher evolutionary rates due to male-biased allocation. W experiences slower degeneration
- **Male-biased sexual selection:** Z experiences higher drift-driven evolutionary rates due to male-biased allocation.
- **Efficacy of selection:** Z experiences higher drift-driven evolutionary rates due to smaller effective population size.
- **Hemizygous exposure:** Z evolutionary rates are less affected by hemizygous exposure due to slower W degeneration.
- **Dosage compensation:** Lack of dosage compensation leads Z to be less affected by hemizygous exposure due to slower W degeneration. However, mildly deleterious alleles are more likely to increase drift-driven evolutionary rates.

Moreover, these outcomes may be modulated by sex specific recombination rates, which vary widely between systems (Sardell & Kirkpatrick, 2020), and by other factors, such as differences in the allocation or enrichment of gene-specific properties across chromosomes, chromosome specific recombination rates, skewed sex ratios, and the demographic history of the population (Ellegren, 2011; Bachtrog, 2013; Soni & Eyre-Walker, 2022). However, many studies stop at the observation of a faster or slower-X/Z effect and seldom attempt to dissect the causes. While the simple observation of different rates of evolution is interesting, it is insufficient for drawing conclusions of the contribution of underlying processes. However, by studying how individual X/Z genes co-evolve with their Y/W gametologs, coupled with gene-specific properties, it might be possible to disentangle how these

processes shape differences in evolutionary rates, e.g. the functional state of Y/W and hemizygous exposure to selection on X/Z.

Evolution and gene properties

Genes can have various properties that may determine their evolutionary fate, the fate of the gametolog pair, and the fate of the chromosome on which they reside. It follows that the average evolutionary rate of a chromosome is highly dependent on the allocation of various gene properties. Typical gene properties include, but are not limited to:

- **Gene length:** Longer genes constitute a larger mutational target and can therefore be more difficult to maintain by selection than shorter genes. Thus, purifying selection may be relaxed and lead to Y/W degeneration (Ellegren, 2011). However, longer genes can sometime carry important functions, thereby being maintained by stronger purifying selection (Lipman *et al.*, 2002; Soni & Eyre-Walker, 2022).
- **Dosage sensitivity:** Dosage-sensitive genes require balanced expression for proper function. This can be achieved by retaining two functional gene copies, or through dosage compensatory mechanism that modify expression imbalance between sex chromosomes and autosomes. These therefore expected to be conserved under strong purifying selection on both X/Z and Y/W chromosomes (Bellott *et al.*, 2014, 2017).
- **Gene essentiality:** Genes that are essential for organismal function tend to be dosage sensitive and broadly expressed across tissues. These are likewise expected to be conserved under strong purifying selection on both X/Z and Y/W chromosomes (Bellott *et al.*, 2014, 2017; Wright *et al.*, 2014; Peichel *et al.*, 2020).
- **Sex-specific function:** Genes with sex-specific functions and expression experience sex-specific selective regimes. The efficiency and outcomes of selection may depend on their allocation on the X/Z or Y/W chromosomes (Ellegren, 2011; Meisel, 2011; Bachtrog, 2013).
- **Sexual antagonism:** Sexually antagonistic genes experience opposing fitness effects in males and females, which can be partially resolved by sex chromosome linkage. This may affect their selective regimes and outcome of selection depending on dominance and sex chromosome allocation (Ellegren, 2011; Bachtrog, 2013; Klein *et al.*, 2021).

How these gene properties shape faster-X/Z evolution may be predictable in some cases, but is not well understood. Thus, empirical evidence remains limited on how

specific gene properties, and their allocation to the sex chromosomes versus autosomes shape degeneration and evolutionary rates.

Repeated evolution

Once Y/W degeneration has proceeded, the putative long-term fitness cost in the heterogametic sex of carrying single copies of many genes can be circumvented through sex chromosome turnover, in which a new chromosome gains the sex determining function. The previous X/Z can then be fixed as an autosome, and the degenerated Y/W can be relinquished. Thus, degeneration can start over with a fresh and non-degenerated Y/W (Blaser *et al.*, 2013; Bachtrog *et al.*, 2014; Beukeboom & Perrin, 2014). Such events appear to be recurrent within some fish (Kikuchi & Hamaguchi, 2013; Phillips, 2013), amphibians (Jeffries *et al.*, 2018; Uno & Matsubara, 2024) and reptiles (Kratochvíl *et al.*, 2021). In contrast, sex chromosome turnovers are not observed in birds and eutherian mammals, where sex chromosomes are old and highly differentiated. In these systems, degeneration has instead been accompanied by the evolution of dosage compensation mechanisms of varying complexity that modify expression imbalance between sex chromosomes and autosomes (Naurin *et al.*, 2010; Bachtrog *et al.*, 2014; Beukeboom & Perrin, 2014; Papanicolaou *et al.*, 2025). However, once sex chromosomes become highly differentiated, they may become constrained in their capacity for subsequent turnover, because replacement would have to accommodate gene loss, complex dosage compensatory mechanisms, and sex-specific specialization on the existing sex chromosomes.

Interestingly, sex chromosome turnover and neo-sex chromosome translocations within vertebrates appear to repeatedly involve a non-random set of homologous chromosomal regions (Uno & Matsubara, 2024). However, it is ultimately the genes contained within these regions that are the drivers of such events. Thus, some genes or gene enriched regions might be more predisposed to sex determination or sex-specific functions, and therefore drive repeated events of sex chromosome formation (Rice, 1987; van Doorn & Kirkpatrick, 2007). In addition, once recombination ceases, some genes may be more or less essential for individual fitness regardless of sex, and consequently more or less likely to degenerate on the sex-limited Y/W chromosome (Bachtrog, 2008; Huang *et al.*, 2022). While observations of Y/W-degeneration and repeated recruitment of chromosomes in evolutionary stratum-formation suggest that the nature of sex chromosome evolution is predictable to some degree, observations are often not abundant enough to analyse statistically. Thus, empirical evidence remains limited on which processes and gene properties govern the degeneration and selective regimes across gametolog pairs, and whether the repeated recruitment of genomic regions in stratum-formation is non-random.

Sex chromosomes in evolutionary ecology

Sex chromosomes are supergenes

The study of structural genomic rearrangement in non-model organisms has greatly benefited from recent advances in long-read genomic sequencing techniques and declining sequencing costs (**Box 2**). Structural genomic rearrangements — such as inversions — can suppress recombination across extensive chromosomal regions and thereby create large, non-recombining haplotypes that maintain substantial genetic variation in wild populations (Kirkpatrick, 2010; Wellenreuther *et al.*, 2019). When these haplotypes encompass multiple linked loci that contribute to correlated phenotypes, they form so-called supergenes. Supergenes can underlie striking co-segregation of behavioural and morphological traits, such as alternative mating strategies in the Ruff (*Calidris pugnax*; Küpper *et al.*, 2016; Lamichhaney *et al.*, 2016) and ecomorphs in the Rough periwinkle (*Littorina saxatilis*; Faria *et al.*, 2019; Morales *et al.*, 2019). Such structural polymorphisms are often maintained over long evolutionary timescales through processes such as heterozygous advantage, negative frequency-dependent selection, life-history trade-offs, and environmental heterogeneity (Mérot *et al.*, 2020; Nosil *et al.*, 2023).

Moreover, even if recombination has ceased between haplotypes, recombination within each haplotype in homozygous individuals can facilitate the purging of deleterious mutations, thereby contributing to their long-term persistence (Muller, 1964; Bachtrog, 2013). Nevertheless, in some cases one haplotype shows substantial fitness costs when homozygous due to the accumulation of recessive deleterious mutations. A prominent example is the 2M inversion in the White-throated sparrow (*Zonotrichia albicollis*), where 2M/2M homozygotes are extremely rare and typically in poor condition, whereas heterozygotes and the alternative homozygotes do not exhibit these costs (Tuttle *et al.*, 2016; see also the Ruff, Küpper *et al.*, 2016).

Females and males are alternative reproductive phenotypes that can differ through striking suites of correlated phenotypic traits. Females and males can differ in behaviour (e.g. parental care, foraging, dispersal), morphology (e.g. reproductive organs, pigmentation, body shape and size), and other life-history traits (e.g. survival and longevity; Beukeboom & Perrin, 2014). An extreme example of sexual dimorphism occurs in deep-sea anglerfishes (Ceratioidei), where females are free-swimming predators, whereas dwarfed males may adopt a parasitic strategy by permanently attaching to and fusing with the female's body (Pietsch, 2005). An example linking sex chromosomes to sexual dimorphism comes from seed beetles (*Callosobruchus maculatus*), in which genetic variation on the Y chromosome can alone produce a 30 % male-biased sexual size dimorphism, illustrating the substantial effects sex-linked variation can have on quantitative traits (Kaufmann *et al.*, 2021).

Box 2: Sex chromosomes in the age of genomics.

The age of genomics is upon us, and genomic data is being generated and published at a rate faster than ever (**Figure 4**). While the first human genome was a multi-collaborative effort, 13 years in the making and with a total cost of 2.7 billion dollars, a human genome can now be sequenced for only a thousand dollars (National Human Genome Research Institute, 2021). In 2025, the world record for the fastest sequencing a human genome was set at 3 hours and 59 minutes by researchers at Broad Clinical Labs (Guinness World Records, 2025). The sequencing techniques that were once restricted to humans and a few model species can now be applied to most non-model species. Wet lab extraction, next generation sequencing, and bioinformatic analyses do not require expert knowledge or equipment, but can be outsourced. This new frontier in human exploration has given rise to multiple sequence initiatives under the umbrella of the Earth Biogenome Project, with the aim to “sequence, catalogue and characterize the genomes of all of Earth’s eukaryotic biodiversity over a period of ten years” (Earth Biogenome Project, 2026). We now live in a time with the technological infrastructure to sequence genomes rapidly and affordably, and public databases containing most of Earth’s sequenced biodiversity. Our potential for answering questions in genomics, evolution, ecology, and medicine is larger than ever. In fact, we have trouble keeping up with analysing these data at the same rate at which they are being generated (Stephens et al., 2015).

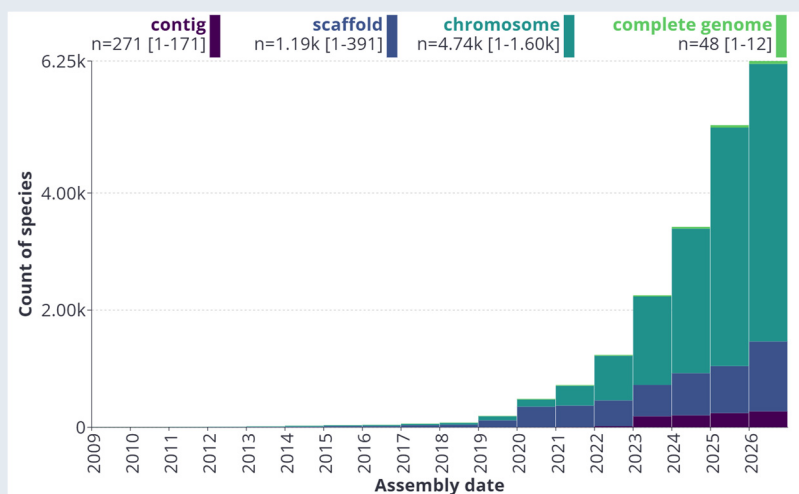


Figure 4. Species sequencing progress. Cumulative number of assemblies for eukaryotic species generated by Earth Biogenome Project over time. Accessed 7th of May 2026 (<https://goat.genomehubs.org/projects/EBP>).

Over the past decades, the extensive sequencing of non-model organisms and the development of long-read sequencing techniques have greatly improved our knowledge of structural variants. As such, evidence has accumulated that sex chromosome systems across the tree of life can vary extensively in age, turnover, evolutionary transitions, autosomal fusions, and stratum formations, even within systems otherwise considered relatively stable, such as mammals and birds (Bachtrog et al., 2014; Cortez et al., 2014; Jeffries et al., 2018; Sigeman et al., 2019; Xu et al., 2019). As the field of genomics continues to grow, bioinformatic methods and pipelines are being developed that lower the bar for researchers who want to pursue research in genomics, or simply implement genomics as a tool to answer questions in other fields. Yet, several limitations to studying sex-limited Y/W chromosomes remain due to the biased accumulation of repetitive content, and sequence homology with X/Z chromosomes (Tomaszkiewicz et al., 2017). To overcome these limitations, several approaches have been developed that identify sex chromosomes using short-read sequencing of males and females to detect sex-specific genomic signatures (Palmer et al., 2019). Such methodological advancements are welcome, as they may further research into sex chromosome evolution, their role in evolutionary ecology, and their use as tools for inferring sex-specific aspects of population ecology.

Consequently, sexual antagonism has long been proposed as an important driver of sex chromosome evolution. Tight linkage between sexually antagonistic alleles and the sex-determining locus can mitigate intralocus sexual conflict by creating sex-specific haplotypes that facilitate sex-specific adaptation (Bull, 1983; Fairbairn & Roff, 2006; Fisher, 1931; Rice, 1987). Therefore, sex chromosomes can be viewed as a special class of supergenes in which recombination suppression links sex determination and sex-specific function – the Y chromosome in XY males and the W chromosome in ZW females – providing a direct link between sex chromosome evolution and evolutionary ecology (Wellenreuther *et al.*, 2019; Meisel, 2022). In addition to Mendelian segregation, negative frequency-dependent selection may render the persistence of sex-chromosomes unusually stable, because the rarer sex often gains a reproductive advantage (Beukeboom & Perrin, 2014; Fisher, 1930; Hamilton, 1967).

However, the cessation of recombination in the sex-limited Y/W haplotypes severely reduces the capacity to purge deleterious mutations, eventually leading to the degeneration of Y/W chromosomes observed in eutherian mammals and birds (Bellott *et al.*, 2014, 2017). Consequently, sexual dimorphism may arise not only through adaptive evolution, but can also reflect Y/W degeneration-associated effects, including reduced gene expression and maladaptation in the heterogametic sex corresponding their X/Z gametologs are not being dosage compensated (Bachtrog, 2013; Bellott *et al.*, 2014, 2017; Bruhn-Olszewska *et al.*, 2025). The role of sex chromosomes as adaptive supergenes and the progressive Y/W degeneration over time raises the question of whether variation in the extent of recombination suppression across sex chromosomes can predict patterns of sexual dimorphism and life-history evolution.

Fitness consequences in the heterogametic sex

Fitness in the heterogametic sex (XY males or ZW females) may be particularly affected by sex chromosomes, because they can be effectively heterozygous or hemizygous across large portions of the genome depending on the stage of Y/W degeneration (Bachtrog, 2013). The costs associated with old Y/W chromosomes can stem from several processes. Inefficient selection on Y/W chromosomes can increase mutational load by providing a refugium for transposable elements to accumulate (the toxic Y/W hypothesis; Brown *et al.*, 2020; Peona *et al.*, 2021), and degeneration of Y/W gametologs can disrupt dosage balance (the dosage hypothesis; Chandler, 2017; Ohno, 1967; Zhu *et al.*, 2025).

Moreover, loss of Y/W gametologs can expose recessive deleterious mutations segregating on the shared X/Z sex chromosome (the unguarded X/Z hypothesis; Marais *et al.*, 2018; Trivers, 1985). Classical examples in humans include male-biased X-linked recessive disorders ranging from colour blindness to haemophilia. Taken together, such effects may cumulatively contribute to reduced lifespan in the

heterogametic sex, as observed across animal taxa with different sex chromosome systems (Xirocostas *et al.*, 2020). Reduced heterogametic fitness can have impacts seen on the population level, such as skewed sex ratios. For example, male-biased sex ratios are common among threatened bird species (Donald, 2007). The Raso lark (*Alauda razae*) experienced strongly male-biased adult sex ratios of 1.4–3.0 males per female during draught years (Donald *et al.*, 2007, 2005, 2003), explained by higher female mortality and limited population growth (Dierickx *et al.*, 2019). However, the unguarded-X/Z effect may be modulated by demography, because small effective population sizes can increase inbreeding, reduce functional genetic variation, and increase the frequency of deleterious alleles. Such an effect has been suggested in *Drosophila melanogaster*, in which XX females were observed to experience a disproportionate reduction in lifespan compared with XY males, consistent with a scenario in which homogametic individuals cease to benefit from masking of X-linked deleterious alleles when their two inherited X chromosomes are identical by descent (Sultanova *et al.*, 2018).

By contrast, young sex chromosomes may still retain functional Y/W copies of most genes (Mrnjavac & Vicoso, 2025; Zhou & Bachtrog, 2012b). Analogous to typical supergenes, heterozygous individuals could potentially benefit from increased functional X–Y/Z–W heterozygosity or from Y/W-mediated masking of recessive deleterious mutations on the X/Z chromosome — effectively a reversed unguarded-X/Z effect, here termed the guarded-X/Z effect. For example, a large inversion polymorphism in the Zebra finch (*Taeniopygia guttata*) provides a fitness advantage in heterozygous males through enhanced sperm quality and siring success (Knief *et al.*, 2017). Furthermore, deleterious to lethal effects of inversion polymorphisms can be expressed in individuals homozygous for the alternative haplotype, but absent in heterozygous individuals, presumably through the masking of recessive deleterious alleles (the Ruff and White-throated sparrow; Küpper *et al.*, 2016; Tuttle *et al.*, 2016). Consistent with these ideas, neo-sex chromosomes in the Raso lark have been hypothesised to buffer the loss of functional genetic variation and the accumulation of X/Z-linked deleterious alleles in ZW females during a severe population bottleneck (Brooke, *et al.*, 2010). Thus, a central unresolved question in evolutionary biology is whether, and for how long, neo-Y/W chromosomes can enhance or maintain fitness in the heterogametic sex beyond sex-specific adaptation.

A central role in speciation theory

The influence of sex chromosomes on heterogametic fitness also extends to central speciation theory, as reflected in the two “rules of speciation” (Orr & Coyne, 1989). Haldane’s rule states that reduced hybrid fitness is predicted to be strongest in the heterogametic sex (Haldane, 1922; Cowell, 2023), and the large-X/Z effect describes the disproportionate contribution of X/Z chromosomes to reduced hybrid

fitness relative to autosomes (Orr & Coyne, 1989; Presgraves, 2018). Several theories focusing on postzygotic incompatibility mechanisms have been proposed to explain these patterns. These include recessive Dobzhansky–Muller incompatibilities exposed on the hemizygous X/Z chromosome (Dobzhansky, 1937; Muller, 1942), selection-driven faster-X/Z evolution generating incompatibilities in hybrids (Charlesworth *et al.*, 1987), and the introduction of harmful transposable elements accumulated on Y/W chromosomes into a naïve genetic background (Peona *et al.*, 2021). Additionally, lower recombination on sex chromosomes relative to autosomes may reduce the efficacy of selection in decoupling linked incompatibilities, thereby increasing selection against larger blocks of introgressed ancestry (Butlin, 2005; Fraïsse and Sachdeva, 2021). Consequently, these processes predict that sex chromosomes may act as stronger barriers to gene flow than autosomes (Presgraves, 2018; Fraïsse & Sachdeva, 2021).

However, if sex chromosomes act as supergenes that facilitate local adaptation, secondary contact could also promote introgression when sex-linked haplotypes provide an immediate adaptive advantage in the local environment. For example, in guppies (*Poecilia reticulata*), male colour patterns are correlated with female mate choice and are often linked to the Y-chromosome. However, this association is shaped by predation regime, because colourful males are more vulnerable in high-predation environments (Gordon *et al.*, 2012). In brood-parasitic cuckoos (*Cuculus canorus*), host-specific egg phenotypes and host preference are both associated with maternally inherited W chromosomes and mitochondria (Fossøy *et al.*, 2016). In both scenarios, introgression of the Y/W chromosomes could facilitate rapid adaptation to the local environment, potentially outweighing the deleterious effects of linked incompatibilities. However, evidence for Y/W introgression remains limited (but see Dixon *et al.*, 2019; Jensen *et al.*, 2024; Macholán *et al.*, 2008; Shogren *et al.*, 2024). As such, Y/W chromosomes are expected to introgress rarely, whereas X/Z chromosomes are predicted to show reduced signatures of introgression.

Moreover, the impact of these processes should depend on the age and degree of Y/W degeneration, as well as degree of divergence, which is in turn caused by mutation rate and the efficacy of purifying selection, which may differ among different organisms. Older and more differentiated sex chromosomes might be less prone to introgression, because they are expected to show greater hemizygous exposure of recessive X/Z-linked incompatibilities in the heterogametic sex, as well as a greater accumulation of harmful transposable elements on the Y/W chromosome (Bachtrog, 2013; Lima, 2014; Peona *et al.*, 2021). On the other hand, older X/Z chromosomes may become unusually conserved over long evolutionary timescales due to optimization of gene function and repeated exposure to efficient purifying hemizygous selection in the heterogametic sex (a slower-X/Z effect; Bachtrog *et al.*, 2009; Charlesworth *et al.*, 1987; Orr, 2005; Rousselle *et al.*, 2016).

In such a scenario, older X/Z regions may be less likely to accumulate new recessive incompatibilities compared to other genomic regions and thus show less resistance to gene flow. This suggests that sex chromosome introgression is likely a trade-off between various costs and benefits, a balance which is modulated by population genomic processes acting over evolutionary time. Thus, the probability of Y/W introgression and proportion of introgression among X/Z chromosomes should reflect the degenerative state of the Y/W chromosome, divergence between species, and the efficacy of selection approximated by metrics such as regional linkage decay and phylogenetic conservation of genes.

Population ecology leaves its signatures in the genome

On top of inherently molecular-biological processes such as mutations and recombination, genetic variation across the genome can be shaped by various ecological and demographic processes. Such signatures can be used to infer selection, demographic history, population structure, introgression, etc. Due to the sex-biased allocation and transmission of sex chromosomes, sex-specific processes can leave genomic signatures that differ from those in autosomes. For example, sexual selection, defined as variation in reproductive success (Jones, 2016) is often stronger in males than in females. In many species, females invest more resources in egg production and parental care than males, which limits the number of offspring they can produce. Consequently, females can become a limited reproductive resource for males and may therefore be more selective in their choice of mate. However, the number of offspring a male can produce is often limited by access to mating opportunities, since the production of sperm is comparatively inexpensive. As such, males can sometimes gain substantial reproductive benefits if they take risks in increasing mating opportunities, and may be less selective in mate choice than females (Lipshutz, 2018).

Therefore, if sex chromosome sequences can be identified and extracted from male and female genomes (**Box 2**), they can be used to study processes beyond sex chromosome evolution itself and potentially serve as a tool for inferring sex-specific aspects of population ecology with applications in conservation management. As previously mentioned, sex-specific processes can result in different evolutionary rates among sex chromosomes (**Figure 3**), and thus reflect long-term differences in sexual selection and mating strategies. Likewise, there may be signatures that reflect contemporary processes, such as sex differences in dispersal, skewed sex ratios, and variation in reproductive success (Ellegren and Galtier, 2016).

Under mutation–drift equilibrium and neutral evolution, the relative difference in genetic diversity between autosomes and sex chromosomes is expected to reflect the relative effective population sizes of the respective chromosomes (Kimura, 1983). Therefore, under even sex ratios, X/Z chromosomes should show three-

quarters the diversity of autosomes, whereas Y/W chromosomes are expected to show one-quarter the diversity of autosomes (Ellegren, 2011). Thus, deviations from these patterns can reflect differences in the number of females and males contributing to reproduction, which may arise from skewed sex ratios through sex-biased births or survival, mating systems deviating from monogamy, sex-biased extra-pair matings, or differences in sexual selection (Ellegren & Galtier, 2016). For example, many species experience male-biased sexual selection, which often results in $X:A > 0.75$ and $Z:A < 0.75$, as the X and Z chromosomes respectively spend less and more time in males compared to autosomes (Frankham, 2012).

Historical demography also leaves genomic signatures in the distribution of genetic diversity and linkage disequilibrium across the genome. Such signatures can be used to infer demographic trajectories through time using, for example, coalescent- and linkage disequilibrium-based methods (Nadachowska-Brzyska *et al.*, 2022). Consequently, contrasting demographic trajectories inferred from autosomes and sex chromosomes may provide insight into whether sex-specific population ecological processes are recent phenomena or have persisted over longer timescales. For example, such studies suggest that in ancestral humans, fewer men than women contributed to reproduction, with a substantial reduction in reproductive skew following the Out-of-Africa dispersal 50-100 KYA (Amster *et al.*, 2020), and contrasting patterns of the paternally inherited Y chromosome and maternally inherited mitochondrial DNA suggest a major male-biased bottleneck ca. 10 KYA (Karmin *et al.*, 2015). Thus, analyses of sex-biased demography can provide insights into whether biased sex ratios are recent phenomena, have persisted over longer timescales, or whether specific periods of environmental change or human interference have perturbed the relative reproductive contributions of females and males (Clemente *et al.*, 2018).

Genetic differentiation between geographic locations can inform us about whether individuals in these locations represent distinct populations or are connected through dispersal with gene flow (Alexander *et al.*, 2009; Nadachowska-Brzyska *et al.*, 2022). Likewise, sex-biased dispersal may be inferred by contrasting genetic structure between autosomes and sex chromosomes. For example, sex-biased dispersal may arise from various reasons such as limited local mating opportunities, resource competition, and inbreeding avoidance (Greenwood, 1980; Pusey, 1987). In a scenario of male-biased dispersal, female-biased genomic elements such as X/W chromosomes and mitochondria are expected to exhibit stronger population structure and isolation by distance than autosomes, whereas male-biased elements such as Y/Z chromosomes are expected to show greater admixture between populations. Just as dispersal may be sex-biased, so too may the costs and risks associated with mating across species boundaries (Lipshutz, 2018). Therefore, sex-biased dispersal and hybridization can be confounding factors in interpreting patterns of the previously discussed large-X/Z effect (Lipshutz, 2018; Presgraves, 2018; Fraïsse & Sachdeva, 2021).

Thesis aims

In this thesis, my overarching goal is to contribute to the research field of sex chromosome evolution, and its role in evolutionary ecology. I address this goal through aims which can be broadly placed in four themes (**Table 1**): Method development (**Paper I-II**); Gametolog evolution (**Paper II-III**); Sex chromosomes in speciation (**Paper IV**); and Predictability of sex chromosome evolution (**Paper II, IV-VI**).

Method development

I develop a method for identifying and extracting Y/W sequences from short-read sequencing data using a novel approach based on haplotype phasing and clustering (**Paper II**). I implement this method as a user-friendly and reproducible Snakemake workflow, and benchmark its performance under different biological scenarios, study designs, and parameter settings (**Paper I**).

Gametolog evolution

I investigate whether the W chromosome and its genes are destined to degenerate or whether they continue to play a role in evolution and female function. I assess the rate of W degeneration over time (**Paper II**). I also investigate how Z—W gametologs co-evolve and how degeneration and selection shape their evolutionary dynamics and rate of evolution (**Paper II**). Furthermore, I test whether W-linked genes are capable of adaptive evolution (**Paper II-III**). Finally, I investigate whether functional W gametologs can enhance female functional heterozygosity and mask deleterious variation on the Z chromosome (**Paper III**).

Sex chromosomes in speciation

I investigate the role of sex chromosomes in speciation (**Paper IV**). I set up and test hypotheses of what processes underlie a W chromosome polymorphism observed in the *Alauda* lark species complex. I also test whether the Z chromosome acts as a barrier to gene flow compared to autosomes and pseudoautosomes. I then investigate whether introgressed tracts are involved in local adaptation.

Predictability of sex chromosome evolution

I investigate whether sex chromosome evolution is predictable. I analyse how gene-specific properties predict degeneration and selective regimes of Z- and W-linked gametologs (**Paper II**) and the genomic landscape predicts introgression across the Z chromosome (**Paper IV**). I also investigate whether certain genes and genomic regions are predisposed to sex-linkage and sex-specific function. I identify and characterise neo-sex chromosomes in previously unstudied passerine species, describing repeated recruitment of genomic regions in stratum-formation (**Paper V-VI**). Finally, I investigate if the extent of sex-linkage across different chromosomal fusions in larks correlates with ecological and evolutionary traits, including sexual dimorphism, life-history traits, and IUCN Red List status (**Paper VI**).

Table 1. Thesis aims. In the papers of this thesis, I address aims which can be broadly placed under four themes.

Theme	Aim	Question	Paper
Method development	1a	Can Y/W sequences be extracted from short-read data using haplotype phasing and clustering?	I-II
	1b	Which factors influence sex chromosome identification and Y/W sequence extraction?	I
	1c	Can this method be implemented in a user-friendly and reproducible workflow?	I
Gametolog evolution	2a	How long can W-linked genes remain functional?	II
	2b	How do W-linked genes co-evolve with Z gametologs?	II
	2c	Are W-linked genes capable of adaptive evolution?	II
	2d	Can functional W-linked genes buffer genetic variation in females?	III
Sex chromosomes in speciation	3a	What processes underlie polymorphic W haplotypes during speciation?	IV
	3b	Is the Z chromosome a barrier to introgression?	IV
	3c	Are introgressed Z-tracts involved in local adaptation?	IV
Predictability of sex chromosome evolution	4a	Can the fate of sex-linked genes and regions be predicted?	II, IV
	4b	Are some genes and genomic regions predisposed to sex-linkage?	V-VI
	4c	Does the amount of sex-linkage correlate with ecological and evolutionary traits?	VI

Methods overview

Study system - sex chromosomes in larks and related passerines

Biological systems with dynamic sex chromosome evolution and several well-defined evolutionary strata offer rare opportunities to investigate evolutionary processes at different stages of sex chromosome evolution following recombination cessation (Bachtrog, 2013). The passerine superfamily Sylvioidea (sensu Alström *et al.*, 2006; Fregin *et al.*, 2012) presents one such system (**Figure 5**).

Avian sex chromosomes

The evolution of the ancestral avian sex chromosomes began ~135 million years ago (MYA), when the putative male-determining gene *DMRT1* (Smith *et al.*, 2009) was encompassed by the first avian evolutionary stratum (stratum S0) in the ancestor of all extant birds (Neornithes; Cortez *et al.*, 2014; Wright *et al.*, 2012; Zhou *et al.*, 2014). Additional evolutionary strata have subsequently formed independently in several bird lineages. A second stratum (S1) formed ~92 MYA in Neognathae after their divergence from Palaeognathae (e.g., ostriches and emus; Jarvis *et al.*, 2014; Stiller *et al.*, 2024; Zhou *et al.*, 2014). A third stratum (S2) formed ~78 MYA in Neoaves after their divergence from Galloanseres (e.g., chicken and ducks; Jarvis *et al.*, 2014; Stiller *et al.*, 2024; Zhou *et al.*, 2014). The fourth and most recent stratum (S3) in Passerides and Corvides formed ~40 MYA after their divergence from the Rifleman (*Acanthisitta chloris*; Oliveros *et al.*, 2019; Stiller *et al.*, 2024; Xu *et al.*, 2019).

While avian genomes, and their sex chromosomes in particular, in general appear highly conserved in gene order and karyotypes across species (Nanda *et al.*, 2008; Ellegren, 2010; Degrandi *et al.*, 2020), structural rearrangements involving the avian sex chromosomes have recently been described in several species based on both cytogenetic and genome sequencing studies. These all involve autosomal translocations and fusions forming neo-sex chromosomes with subsequent recombination cessation (Pala *et al.*, 2012; Gunski *et al.*, 2017; Gan *et al.*, 2019; Kretschmer *et al.*, 2020; Burley *et al.*, 2022; Huang *et al.*, 2022; Shogren *et al.*, 2024; Souza *et al.*, 2024; Xu *et al.*, 2024).

Sylvioidea

The superfamily Sylvioidea comprises ~10% of all extant bird species, and shows evidence of multiple sex chromosome translocations (Leroy *et al.*, 2021; Pala *et al.*, 2012; Sigeman *et al.*, 2022, 2021, 2020, 2019; **Figure 5**). The ancestor of Sylvioidea experienced a translocation of chromosome 4A to the ancestral sex chromosomes (Pala *et al.*, 2012; Sigeman *et al.*, 2019). Recombination subsequently ceased independently on at least three occasions: in the common ancestor of Alaudidae and Panuridae (Sigeman *et al.*, 2021), partly in the common ancestor of remaining Sylvioidea, and partly in the common ancestor of Sylvioidea excluding Macrosphenidae, which retains only partial recombination cessation across 4A (Sigeman *et al.*, 2019, 2021, 2022c). Additional translocations and stratum formations subsequently formed across Sylvioidea; chromosome 3 in Alaudidae and Panuridae (Sigeman *et al.*, 2021), chromosome 4 in Cisticolidae (Sigeman *et al.*, 2024, 2020), chromosome 5 in Alaudidae (Sigeman *et al.*, 2019), and chromosome 8 in Macrosphenidae (Sigeman, Zhang, *et al.*, 2022a; **Figure 5**).

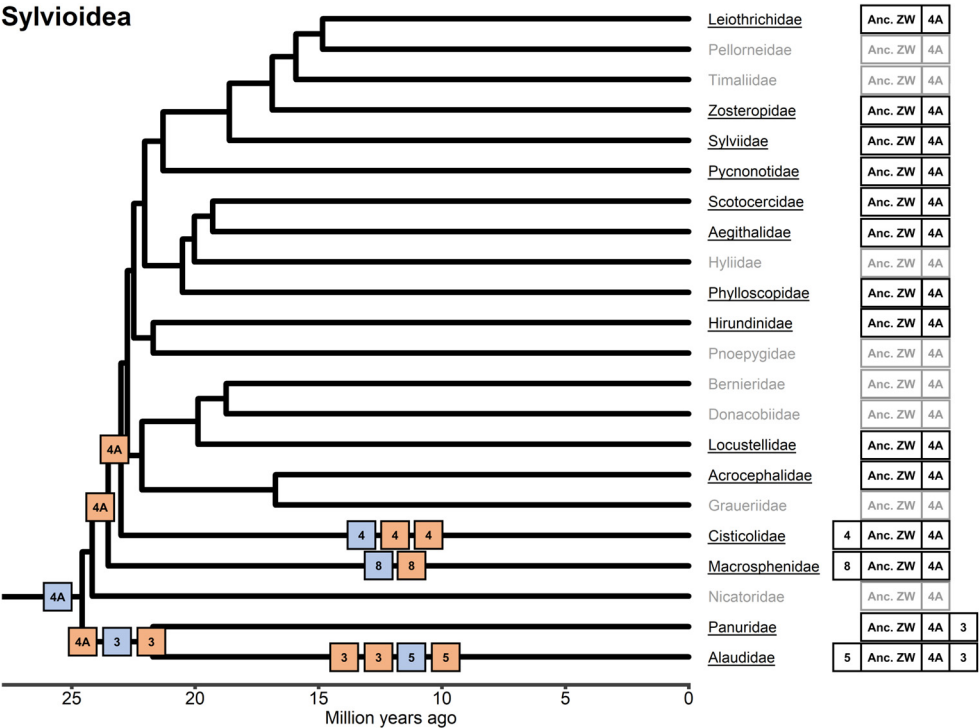


Figure 5. Neo-sex chromosomes in Sylvioidea. Phylogenetic relationships and divergence times of 22 families in Sylvioidea based on Oliveros *et al.*, (2019), with translocation events (blue) and stratum formation events (orange). At least one species observation has been made within highlighted families. Hypothetical sex chromosome structure for respective family is shown on the left.

Alaudidae - larks

The Eurasian Skylark (*Alauda arvensis*; Skylark hereafter) and the Raso lark possess the largest known avian neo-sex chromosomes, formed through three chromosomal translocations, and comprising four to five additional evolutionary strata (Brooke *et al.*, 2010; Dierickx *et al.*, 2020; Sigeman *et al.*, 2021, 2019). In the basal Sylvioidea, lineage Panuridae (with a single extant species, the bearded reedling — *Panurus biarmicus*) and Alaudidae (larks), the translocated 4A region ceased recombining ~22 MYA (stratum 4A; Alström *et al.*, 2023; Oliveros *et al.*, 2019; Sigeman *et al.*, 2021, 2019; Stiller *et al.*, 2024). In the same lineage, a translocation of chromosome 3 occurred, inferred from an 8.0 Mb region with recombination cessation (stratum 3-a) in both bearded reedling and larks (Sigeman *et al.*, 2019, 2022b). It is possible that stratum 4A and 3-a formed in the same event, in which case stratum 3-a would likewise have formed ~22 MYA. Further recombination cessation occurred after larks diverged from the bearded reedling ~20 MYA (Alström *et al.*, 2023; Sigeman *et al.*, 2019; Stiller *et al.*, 2024). These include a 3.6 Mb stratum (stratum 3-b) observed in all investigated lark species (Sigeman *et al.*, 2019, 2024; Dierickx *et al.*, 2020). A translocation of chromosome 5 and independent stratum-formations are apparent in some lark genera, including a 36.3 Mb region (stratum 5) formed in *Alauda* and *Galerida* (Sigeman *et al.*, 2019; Dierickx *et al.*, 2020; Shakya *et al.*, 2022). Finally, a 64.9 Mb region of chromosome 3 (stratum 3-c) ceased recombining in the genus *Alauda* (Alström *et al.*, 2023; Dierickx *et al.*, 2020; Sigeman *et al.*, 2019). Stratum 3-c is present in the Raso lark and European Skylark populations, but appears absent in East Asian Skylarks and its closest sister species, the Oriental lark (*A. gulgula*; Dierickx *et al.*, 2020). In a scenario of incomplete lineage sorting, this suggests an origin ~6 MYA (Alström *et al.*, 2023), although there are other processes such as introgression or repeated stratum formation that could make its appearance more recent.

A system for sex chromosome evolution

The lark family comprises ~100 species, whereof only five were analysed with genomic data when I began my thesis project (Dierickx *et al.*, 2020; Sigeman *et al.*, 2024). Nor did any chromosome-level assembly exist for any lark or the bearded reedling, limiting inference of the physical structure and true sex chromosome size. However, karyotypes consistently show large sex chromosomes with little heteromorphism and fewer macrochromosomes relative to that of other passerines (Udagawa, 1952; Bulatova *et al.*, 1971; Bulatova, 1973; Roy & Kaul, 1985; Sultana & Bhunya, 1986; Bian *et al.*, 1988; Li *et al.*, 1994; Malinovskaya *et al.*, 2022). In summary, Sylvioidea possesses multiple well-defined evolutionary strata formed at different times, but many species remain uninvestigated. This system could

potentially prove powerful for comparative analyses, and possess many undiscovered neo-sex chromosomes. Taken together, Sylvioidea — and larks in particular — are a promising system for studying different stages of sex chromosome evolution, and their implications for evolutionary ecology.

Sample acquisition and sequencing

For **Paper I-III**, blood samples from Skylarks were collected by Pavel Zehtindjiev in Italy (2007), and by Arne Hegeman in the Netherlands (2009), and DNA was extracted using a phenol–chloroform-based protocol. Blood samples from Raso larks were collected by Michael de L. Brooke on the islet of Raso, Cape Verde (2015 and 2017), and DNA from these samples were extracted from dried blood on tissue paper with using the Qiagen blood and tissue kit (Qiagen, Hilden, Germany). Individuals were sequenced as paired-end 150 bp reads on an Illumina NovaSeq 6000 platform. Library preparation and sequencing were performed by National Genomics Infrastructure (NGI), Uppsala, Sweden (<http://ugc.igp.uu.se/our-services/ngs-technologies/>). Additional sequences from one male and one female of each species were retained from Sigeman *et al.* (2019).

DNA from additional species used in **Paper IV-VI** were obtained from fresh samples or museum collections provided by several collaborators, including Yang Liu, Derek Engelbrecht, and Per Alström. Library preparation and sequencing were performed by NGI, Stockholm/Uppsala, SciLifeLab, and sequenced as paired-end 150 bp reads on an Illumina NovaSeq 6000 platform (Bengt Hansson & Per Alström, personal communication).

To generate chromosome-level assemblies used in **Paper I, IV, VI**, we caught one male Skylark using mist nets at Kämpinge, Sweden in 2021 October 12th, and one male Bearded reedling at Lake Kvismaren, Sweden in 2021, August 9th. Blood was collected from each individual and immediately flash-frozen on dry ice. High-molecular-weight DNA was extracted using the Monarch Cell/Blood DNA Extraction Protocol. Two PacBio HiFi libraries were constructed and sequenced on two 25M SMRT Cells using the Pacific Biosciences Revio platform at NGI Uppsala, SciLifeLab. Hi-C libraries were prepared using the Omni-C protocol and sequenced on one lane of Illumina NovaSeq X Plus 10B with 150-bp paired-end reads at NGI Stockholm, SciLifeLab.

Genomes and annotations

For genomic analyses, I mainly used two different Skylark reference genomes: a scaffold-level assembly (GenBank accession GCA_902810485.1; **Paper II-III**), and the chromosome-level assembly developed in this thesis (**Paper I, IV, VI**). This reference genome was assembled with the *Earth-Biogenome pipeline* (<https://github.com/NBISweden/Earth-Biogenome-Project-pilot>).

Scaffolds were annotated by performing a synteny alignment to the Great tit (*Parus major*; NCBI RefSeq accession GCF_001522545.3) and the Zebra finch (NCBI RefSeq accession GCF_003957565.2; Rhie *et al.*, 2021), using *Satsuma* (<https://github.com/bioinfologics/satsuma2>; Grabherr *et al.*, 2010; **Paper II**) and *MUMmer* (Marçais *et al.*, 2018; **Paper I, VI**).

Repetitive elements were annotated with *RepeatMasker* (Smit *et al.*, 2013), using the repeat database “Aves” as the source of query DNA (**Paper I-IV**). Genes were annotated by performing lift-overs from the Zebra finch assembly using *Liftoff* (Shumate and Salzberg, 2021; **Paper I-III, VI**).

Processing of raw sequence data

For Illumina short reads, raw sequences were trimmed for quality and adapter removal with *Trimmomatic* (Bolger *et al.*, 2014). Read pairs were aligned to the reference genome with *BWA-MEM* (Li, 2013), duplicates removed with *MarkDuplicates* from *Picard* (<http://broadinstitute.github.io/picard>), and quality filtered with *SAMtools* (Li *et al.*, 2009).

Variants were called with *Freebayes* (Garrison and Marth, 2012; **Paper II-III**), or *BCFtools* (Danecek *et al.*, 2021; **Paper I, IV**). Variants were filtered using *VCFtools* (Danecek *et al.*, 2011), *vcflib* (Garrison *et al.*, 2022), *BCFtools*, and *vt* (Tan *et al.*, 2015). Filters were applied to exclude sites of low quality, low and high depth, high missingness, strand bias, and excess heterozygosity. Variants located within repetitive elements were excluded. Complex variants were decomposed and normalized into constituent SNPs and INDELs. Only sites that remained polymorphic or fixed for the alternate allele were retained for downstream analyses.

My approach of alignment to a homogametic reference assembly requires some specific considerations of how to approach filtering. For example, I did not consider filters for allelic balance suitable, since there are likely alignment biases between Z and W sequence to the homologous Z reference. Also, in very divergent sex chromosome regions where female W-reads do not align to the homologous Z reference, female genotypes will be about half the mean female autosomal depth. Therefore, I attempted to retain those sites by adjusting the minimum mean depth

filter. The depth of female individuals is divided by two, then a new and corrected mean is taken for all individuals, which guides setting of the lower mean depth.

Detecting sex chromosomes and extracting W-sequences

To identify sex-linked regions, I used the bioinformatic pipeline *FindZX* (Sigeman *et al.*, 2022a; **Paper II-III, V-VI**). *FindZX* takes a homogametic reference assembly and genomic reads of at least one female–male pair of a species and outputs normalized differences in alignment depth and heterozygosity between the sexes. These can then be manually inspected to classify sex-linked regions. Older and degenerated W regions will have trouble aligning to the Z reference, resulting in lower female alignment depth. In younger W regions, such W reads might still align to the Z reference, but their divergence will give rise to patterns of inflated female heterozygosity. In the species I have been working on, all neo-sex chromosomal regions appear more or less in large coherent strata. Therefore, these are easy to identify in the *FindZX* output, due to similar divergence patterns across their lengths.

To identify and extract W-sequences (**Paper I-IV**), I applied various versions of my bioinformatic pipeline *PhaseWY* developed in **paper I-II**. *PhaseWY* uses whole-genome sequencing data from multiple females and males to identify sex-chromosomal regions and extract the corresponding W sequences. It uses bam-files to identify callable regions based on depth and missingness thresholds, which I base on the outcome of my variant filtering. *PhaseWY* also uses the bam-files to detect sex differences in alignment depth. It applies read-based and statistical haplotype phasing, and identifies sex-linked regions using haplotype clustering. Finally, *PhaseWY* subsets autosomal, Z- and W-linked variants for downstream analyses.

Analyses

To analyse my data, I apply a wide range of methods common within the field of evolutionary ecology. To benchmark the accuracy of *PhaseWY*, I simulated neutral sex chromosome divergence with individual-based forward simulations using *SLiM* (Haller & Messer, 2023) and *msprime* (Kelleher *et al.*, 2016), and simulated paired-end reads with using NGSNGS (Henriksen *et al.*, 2023; **Paper I**).

I worked with protein-coding sequences, which were aligned with *MACSE* (Ranwez *et al.*, 2018), analysed for branch-specific rates of sequence evolution and hypothesis testing for selection using *HyPhy* (Kosakovsky Pond *et al.*, 2020; **Paper II-III**), and estimated mutational impact on open reading frames using *SnPEff* (Cingolani *et al.*, 2012; **Paper II-III**). I estimated the distribution of fitness effects

using *polyDFE* (Tataru & Bataillon, 2018; **Paper III**), and phylogenetic conservation of sites in genes using *GenErode* (Kutschera *et al.*, 2022; **Paper III**).

I inferred demographic history using *MSMC2* (Wang *et al.*, 2020) and *GONE* (Santiago *et al.*, 2020; **Paper III**), and phylogenetic relationships with *IQ-TREE* (Wong *et al.*, 2026; **Paper I, IV**). I inferred population structure with PCAs using *Plink* (Purcell *et al.*, 2007), and *ADMIXTURE* (Alexander *et al.*, 2009; **Paper III-IV**), and inferred introgression using *Dsuite* (Malinsky *et al.*, 2021; **Paper IV**).

I performed genome scans for nucleotide diversity, Tajima's D, and introgression using *popgenWindows.py* and *ABBABABAwindows.py* (https://github.com/simonhmartin/genomics_general; **Paper I, III-IV**), linkage disequilibrium using *PopLDdecay* (Zhang *et al.*, 2019; **Paper III-IV**), and runs of homozygosity using *BCFtools* (**Paper III**). I classified phylogenetic topologies across the genome using *Saguaro* (Zamani *et al.*, 2013). I investigated signs of sexually antagonistic polymorphisms using F_{ST} and absolute divergence with *popgenWindows.py*, and a genome-wide association study using *GEMMA* (Zhou and Stephens, 2012; **Paper I**).

I performed ancestral reconstruction of traits with *phytools* (Revell, 2024) *evomap* (Smaers *et al.* 2016; **Paper II**). I analysed enrichment of gene functions with GO and KEGG terms using *clusterProfiler* (Yu *et al.*, 2012; **Paper I-IV, VI**). I also implemented forward simulations of stratum formation along a species tree.

In most papers, I applied statistical models to analyse my data. I analysed datasets with binomial and Gaussian response variables using generalized linear models with *glmmTMB* (Brooks *et al.*, 2017; **Paper II, III**). For datasets requiring modelling of spatial autocorrelation, I used *glmmTMB* for generalized linear mixed models, and *nlme* (<https://cran.r-project.org/web/packages/nlme/index.html>) for generalized least squares models (**Paper IV**). I analysed datasets with categorical response variables using Bayesian generalized linear multivariate models with *brms* (Bürkner, 2017; **Paper III**). I analysed dataset with proportional response variables using a beta-inflated distribution implemented in *gamlss* (Rigby and Stasinopoulos, 2005; **Paper I, IV**). Datasets requiring phylogenetic correction were analysed using Bayesian linear mixed-effects models *MCMCglmm* (Hadfield, 2010; **Paper VI**).

For several papers, I included publicly available resources in my statistical models. I used the predicted probability of haploinsufficiency index (the necessity of two functional gene copies for normal function) from humans as a proxy for gene essentiality (Collins *et al.* 2022; **Paper II-III**). I utilised published species data on sexual selection and sexual dichromatism (Dale *et al.*, 2015; **Paper VI**), sex - specific size measurements (Tobias *et al.*, 2022; **Paper VI**), life history (Bird *et al.*, 2020; Tobias *et al.*, 2022; **Paper II, VI**), and IUCN Red List status (IUCN, 2025; **Paper VI**). For analyses of comparative genomics, I accessed several published genomes and gene annotations at the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>; **Paper II-III, V-VI**).

Results and Discussion

Identifying and extracting sex-limited sequences

To analyse W-linked sequences in **paper II-III** I began developing *PhaseWY*, a method for the identification and extraction of sex-limited sequences (**Aim 1a**). The method was later improved, benchmarked (**Aim 1b**), and converted into a Snakemake workflow which is presented in **paper I** (**Aim 1c**). Throughout my thesis *PhaseWY* has formed the basis for analysis in **papers II-IV**.

PhaseWY

PhaseWY uses short-read sequencing data of several females and males and classifies callable genomic regions as sex-linked or autosomal. Highly degenerated regions are classified based on sex differences in sequencing depth, where heterogametic genotypes are recoded as haploid for the shared sex chromosome. Non-degenerated regions are classified using a new implementation of phasing and haplotype clustering, approaches similar to those applied by Almeida *et al.*, (2021). Since the number of samples from each sex is known, the expected proportion of sex chromosome haplotypes in the dataset is also known, which can be used to infer sex-linkage. Within these regions, the X/Z and Y/W haplotypes are extracted separately, and genotypes are recoded as haploid in heterogametic individuals (**Figure 6; Paper I, Figure 1; Aim 1a**).

A distinguishing feature of *PhaseWY* is the systematic and explicit classification, extraction, subsetting and ploidy-correction of sex-linked genotypes (**Figure 7**). The output is summarised in VCF and BED format, which can be directly used in downstream analyses with commonly used software packages in population genomics and phylogenomics. Importantly, there are tasks that the pipeline is not designed to perform, which make it best used as a complement to other methods and workflows. The main inputs of the pipeline are sequence alignments to a homogametic reference in BAM format, as well as a filtered VCF file. Furthermore, the sex-determination system (XY or ZW) and the sex of each sequenced individual are required.

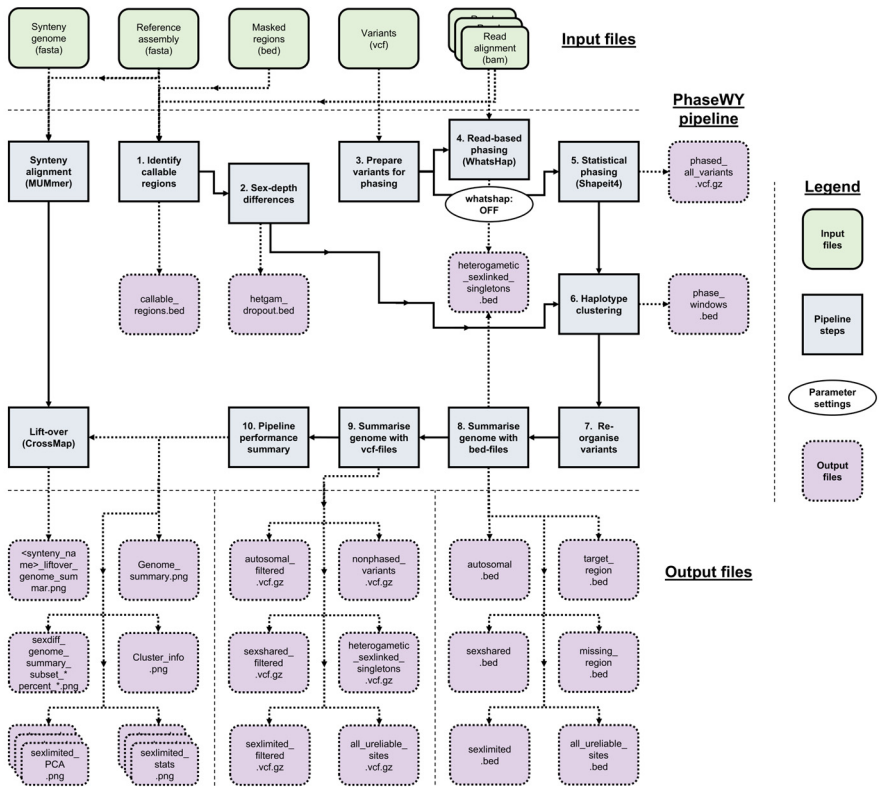


Figure 6. PhaseWY flowchart. An overview of the PhaseWY workflow. Figure from **paper I**.

PhaseWY is published on GitHub, and is my methodological contribution to the scientific community. *PhaseWY* provides a novel method for sex-limited sequence extraction, and lowers the threshold for sex chromosome analyses in evolutionary and ecological research in non-model organisms (**Aim 1c**).

Benchmarking

In **paper I**, I generated simulated data to benchmark the performance of *PhaseWY* in relation biological factors (time since divergence of the sex chromosomes and effective population size), study design (number of sequenced females and males used to infer haplotypes) and parameter settings for the identification of sex-linkage through haplotype clustering (e.g. window size, minor allele frequency filter, and haplotype clustering model) to guide future users in applying the pipeline (**Aim 1b**).

I find that the identification of sex-linkage through sex chromosome divergence — measured as sex-differences in heterozygosity — is possible relatively early

following recombination cessation, estimated at 1,000–10,000 generations. However, this signal takes longer to be evident in large populations, probably because it is obscured by high levels of background genetic variation, as well as slower divergence of neutrally evolving alleles in large populations. In contrast, sex differences in alignment depth were generally not detectable until after 1,000,000–10,000,000 generations. Thus, sex-depth differences are primarily effective for identifying relatively older, highly degenerated sex chromosome systems, while heterozygosity is powerful for identifying very young sex chromosomes.

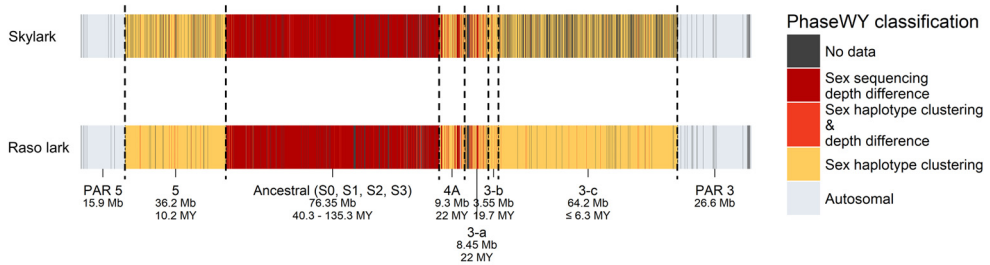


Figure 7. PhaseWY classification of sex-linkage. The sex chromosomes of the Skylark and Raso lark. Strata are annotated by name, size, and age. The Skylark is a widespread and genetically diverse species, while the Raso lark is severely bottlenecked and retains little genetic diversity. Despite both datasets being aligned to the Skylark reference assembly, it is evident in the two youngest strata — 5 and 3-c — that high levels of background genetic diversity interferes with classification in low-divergence regions. Figure adapted from **paper I**.

For *PhaseWY* specifically, I found that biological factors, study design, and parameter settings interact in complex ways to affect sensitivity (the proportion of variants classified as true positives), and precision (the proportion of true positives out of all both true and false positives). My simulations suggest that evolutionary time generally improves the sensitivity of sex-linked variant detection but reduces the precision of X-linked variants. Increasing population size reduces sensitivity overall. Larger window sizes are generally recommended when running *PhaseWY*, as they improve performance across most scenarios. However, large window sizes can reduce performance in large populations (likely due to inefficient statistical phasing when linkage disequilibrium decays quickly rather than because of high background genetic diversity) and can reduce precision in older sex chromosome systems (likely due to increased noise from patchy alignments in degenerated regions). I further found that enabling read-based phasing can improve sensitivity, particularly in older systems and in large populations with rapid linkage decay. In addition, read-based phasing improves the construction of longer, more coherent haplotypes, thereby allowing the use of larger window sizes (**Paper I, Figure 3**).

Furthermore, I use empirical data from the Skylark and Raso lark to showcase the information produced by the pipeline (**Paper I, Figure 2**), and to illustrate how the output can be applied for downstream analyses (**Paper I, Figure 6**). Consistent with

the simulations, I found that high background nucleotide diversity interferes with reliable classification in regions of low sex chromosome divergence, which was evident when comparing the outcomes in the two youngest strata (5 and 3-c), between the highly genetically diverse Skylark (0.016) and the bottlenecked Raso lark (0.0013; **Figure 7; Paper I, Figure 5**).

W gametologs play dynamic roles in evolution

In **paper II**, I estimate rate of W degeneration over evolutionary time and found that W gametologs can remain structurally intact for millions of generations (**Aim 2a**). In **paper II**, I further investigate how the degeneration and selective regime of W gametologs co-evolve with Z gametologs, and found that in neo-sex chromosome strata, haploinsufficient genes are more likely to follow similar evolutionary trajectories under similar selective regimes, although W gametologs evolve under weaker efficacy of selection (**Aim 2b**). I also found that the functional status of the W gametolog affect the rate of Z gametolog evolution, unravelling insight into complex processes shaping faster-Z evolution. In **paper II-III**, I investigated the capability of the W chromosome to undergo adaptive evolution on a per-gene level, and by modelling the type of mutations that contribute to standing variation across coding regions of the W chromosome (**Aim 2c**). I found that individual W genes can evolve under positive selection, as well as intensified selection relative to their Z gametolog, but that beneficial mutations do not contribute to standing variation. In **paper III**, I then investigate whether functional W-linked genes can buffer functional genetic variation in females. I found that females experience elevated Z–W functional heterozygosity in young to intermediate aged strata, and that the relative contrasts to males were larger in the bottlenecked Raso lark due to overall lower heterozygosity in males. However, only rarely does females benefit from W-mediated masking of deleterious Z alleles (**Aim 2d**).

W degeneration proceeds over millions of generations

In **paper II**, I characterise the rate of W degeneration over evolutionary time by using the age span of the nine avian strata identified in the Skylark and Raso lark. To estimate the ages of strata, I performed a comprehensive compilation of information from published resources. On the ancestral Z chromosome, strata S0–S3 span approximately 135–40 MY (S0: 135 MYA, S1: 92 MYA, S2: 78 MYA, and S3: 40 MYA), whereas the five strata on the Skylark and Raso lark neo-sex chromosome range from approximately 22 to ≤ 6 MY (4A: 22 MYA, 3-a: 22 MYA, 3-b: 20 MYA, 5: 10 MYA, and 3-c: ≤ 6 MYA). Since evolution unfolds on the timescale of generations rather than calendar years, I modelled ancestral generation times as a continuous branch trait on a dated avian phylogeny (**Figure 8B**). I used a

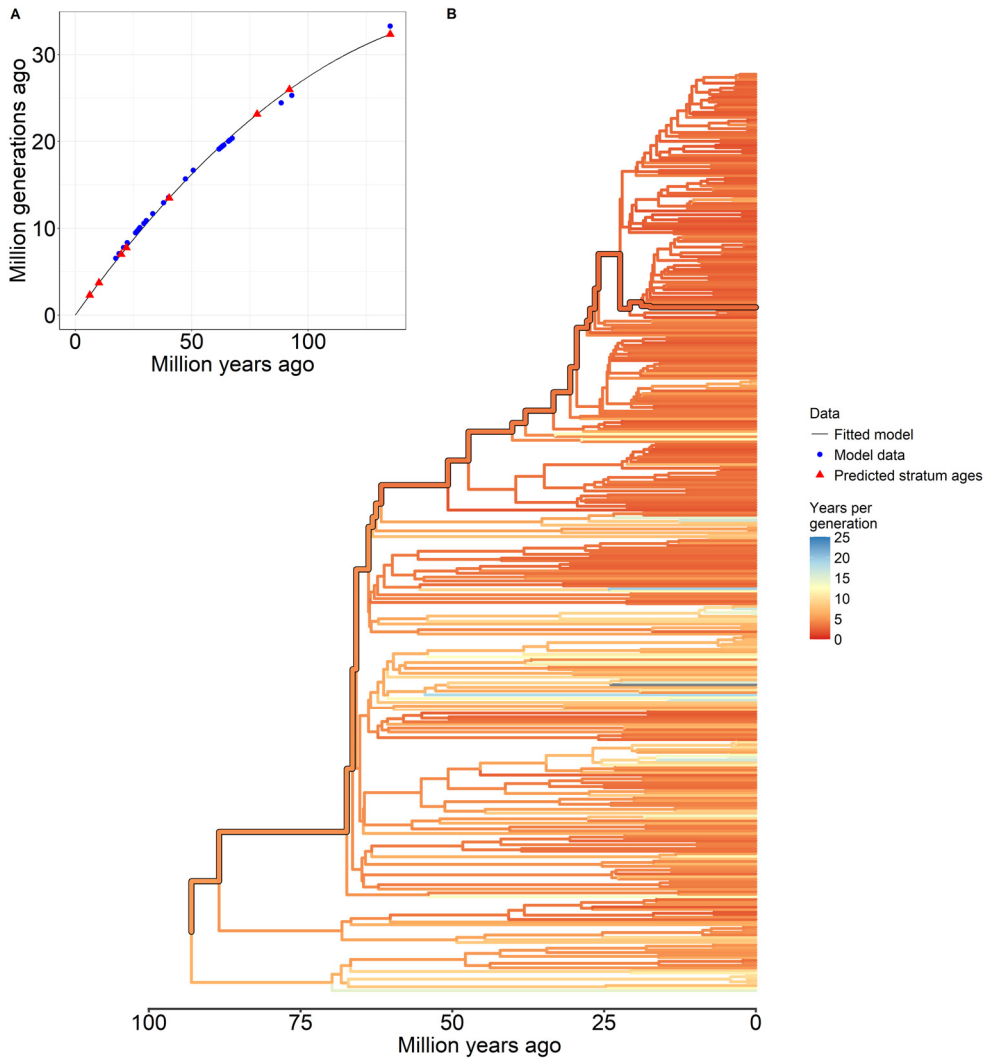


Figure 8. Evolutionary time in years and generations among birds. (A) Relationship between phylogenetic node age measured in million generations and million years respectively. Blue circles represent dated nodes traced from the Skylark to the root of the dated phylogeny. The black line shows the fitted polynomial function based on these nodes. Red triangles indicate predicted ages of strata, derived from the fitted model. **(B)** Estimated ancestral years per generation in birds, modelled as a branch trait and visualised on the dated phylogeny from (Stiller *et al.*, 2024). Branch colours represent estimated years per generation, with warm colours indicating shorter, and cold colours longer, generation lengths. Highlighted branches visualise the evolutionary path from the root to the Skylark. Figure adapted from **Paper II**.

polynomial model to estimate the gradual reduction in generation time from 5.3 years/generation in the ancestor of all extant birds, down to 2.7 years/generation in the Skylark (**Figure 8A**). I subsequently used this model to transform stratum ages

of 6-135 million years into 2.3 to 32.3 million generations (MG). Individual W genes were classified as non-functional if they had lost at least one exon (inferred from sex-differences in alignment depth), or if they carried a loss-of-function mutation. Genes in the PAR were included as an estimate of age 0. As expected, younger strata retained more functional W genes than older strata. However, many W genes appear structurally intact on strata of intermediate age. From these data, I estimated that a typical W gametolog reaches a 95% probability of losing structural function after approximately 14.7 MG, corresponding to 43.5 MY in passerines/larks (**Paper II, Figure 2; Aim 2a**).

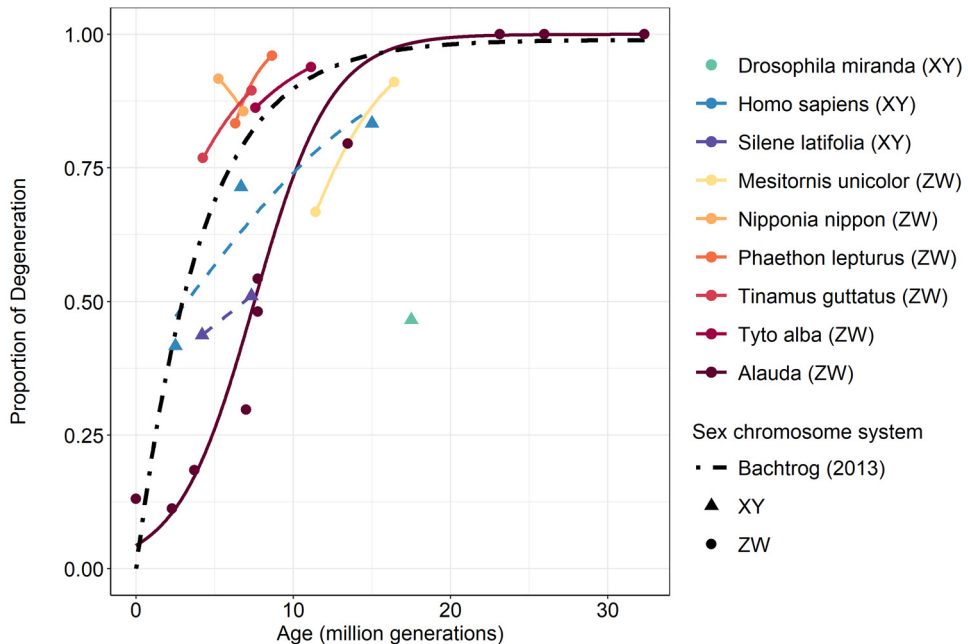


Figure 9. Rate of Y/W degeneration. Predicted probability of non-functionality in larks (*Alauda*) as a function of stratum age (in million generations, MG), with other predictors held at their median values. Points and triangles represent the observed proportion of non-functional genes on each stratum (and PAR 3 and 5 combined at age zero). In addition, empirical values from Krasovec *et al.*, (2018) are added, with model fit using a quasibinomial GLM with a logit link in ggplot2. The black dash-dotted line represents a conceptual degeneration curve adapted from (Bachtrog, 2013). Figure adapted from **Paper II**.

The shape of degeneration is often described as initially rapid, followed by a gradual deceleration over time (Bachtrog, 2013; **Figure 9**). This is attributed to the fact that immediately following stratum formation, the average efficiency of selection per gene is reduced due to the sudden addition of many linked genes, subsequently creating an increasing target for mutations to occur, and selection to maintain. Consequently, many genes with low fitness consequences are allowed to degenerate at the beginning of recombination cessation, and subsequently the rate slows down as individual genes irrevocably lose function and lessen the burden of selection on

the remaining genes. The rate of degeneration then levels out at a stage with a few important genes remaining under strong purifying selection. In contrast, my estimated shape of degeneration appeared sigmoidal (**Figure 9; Paper II, Figure 2B**). This suggests that W genes initially remain structurally intact for some time before the rate of structural degeneration increases. However, I found strong support for the importance of mutational target size, as longer W genes showed a much higher probability of loss-of-function. While I did not have information on gene functions to include in my model, I did use haploinsufficiency (the necessity of two functional copies for normal function) as a proxy for gene essentiality. Consistent with the theory of gene importance delaying degeneration, I found that high haploinsufficiency mitigates the effects of gene length and stratum age.

I believe that one limitation of my approach is the inference of W gene functionality based on structural integrity. Because my classification focuses on coding changes, it likely underestimates the earliest stages of decay, such as regulatory erosion, as documented in *Drosophila miranda* (Zhou and Bachtrog, 2012b). This might further explain the sigmoidal shape of the degeneration curve; the regulatory functions of genes are lost first, while coding degeneration is a delayed symptom of interrupted selection following functional loss.

W genes influence the evolution of Z gametologs

In **paper II**, I characterise selection regimes and evolutionary dynamics of Z-W gametologs. I found that Z-W gametologs can co-evolve according to certain patterns, and that haploinsufficiency of gametologs — used as a proxy for gene essentiality — has a major influence on these patterns. Furthermore, I found that biased allocation of haploinsufficiency between autosomes and Z chromosomes can be a major confounding factor in the interpretation of observed faster or slower-Z effects, which may not be evident until contributing effects are dissected on a per gene level.

I found that haploinsufficient gametologs are more likely to follow similar evolutionary trajectories, are under stronger purifying selection, and are less likely to lose their W gametolog. However, W gametologs consistently evolved under relatively more relaxed purifying selection, and this relaxation was more pronounced once the W copy had lost its function.

At a glance, when I compare the rate of evolution on autosomal (PAR) genes against all Z genes, I only found a trend suggesting that the rate of non-synonymous evolution is faster on the Z chromosome ($P = 0.069$). Under a basic model of evolutionary rates, with reduced efficacy of selection, and potentially a male-biased mutation rate and sexual selection, I would expect higher rates of Z evolution (**Figure 3**; although I control for mutation rate by contrasting non-synonymous to

synonymous substitutions). Still, it is surprising that I only found a weak signal of elevated rate of Z evolution.

However, by analysing differences in rates between genes with a functional W, or with a degenerated W (i.e. hemizygous exposure), it is possible to further disentangle whether the effect is due to relaxed purifying selection through drift, or efficient positive selection acting on beneficial hemizygously expressed alleles. I found that overall, Z genes with a degenerated W gametolog showed elevated rates of non-synonymous divergence relative to both PAR genes and Z genes with a functional W gametolog (**Figure 10A; Paper II, Figure 3D**). The fact that the rate is higher for hemizygous genes is consistent with a positive selection-driven faster-Z effect, as opposed to a slower-Z effect. Thus, I found that the functional state of the W gene influences the selective regime of the Z gametologs on a per-gene basis (**Aim 2b**). However, chromosome-wide averages may themselves be strongly confounded by differential allocation of highly constrained genes. At this stage of interpretation, these results do not rule out that drift also contributes to the faster-Z effect.

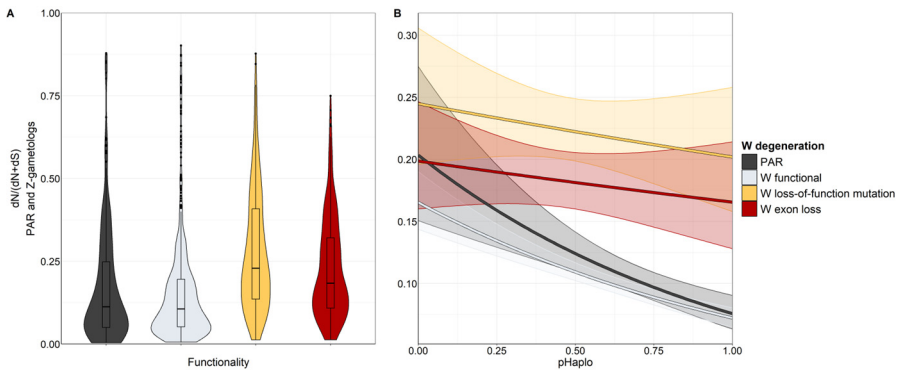


Figure 10. Signatures of selection on Z gametologs. (A) Distribution of $dN/(dN + dS)$ for PAR genes and Z gametologs grouped by W functional–degeneration category. **(B)** Estimated interaction between W degeneration and haploinsufficiency (pHaplo), with other predictors set to their median values ($\pm 95\%$ CI). Purifying selection strengthens (lower $dN/(dN + dS)$) with increasing haploinsufficiency (high pHaplo), but this relationship is weaker for Z genes whose W copy is degenerated. Figure adapted from **Paper II**.

I found that the rate of evolution in Z genes with a degenerate W is not affected by haploinsufficiency, while PAR genes and Z genes with a functional W experience much stronger purifying selection as a function of haploinsufficiency ($P \leq 1.7 \times 10^{-6}$; **Figure 10B; Paper II, Figure 3E**). Less essential genes (low haploinsufficiency) are less likely to evolve under strong purifying selection, and are therefore more influenced by drift (Bellott *et al.*, 2014, 2017; Collins *et al.*, 2022). Therefore, under a drift-driven faster-Z effect, I would expect differences in rates between non-haploinsufficient PAR and Z genes. Yet, among genes with low haploinsufficiency I found no difference when comparing PAR to all Z genes ($pHaplo \leq 0.5$), nor PAR

to Z genes with a degenerated W (pHaplo = 0.0; **Figure 10B; Paper II, Figure 3E**). Furthermore, Z genes with a degenerated W experience higher rates of non-synonymous evolution than Z genes with a functional W across the whole gradient of haploinsufficiency (pHaplo = 0.0-1.0, $P \leq 0.0056$; **Figure 10B; Paper II, Figure 3E**), suggesting that hemizygous exposure contributes to the faster-Z effect also in non-essential genes. By comparing the evolutionary rate with the incorporation of polymorphisms (Direction of Selection), I again found that Z genes with W loss-of-function mutation and W exon loss were both significantly higher than Z genes with a functional W ($P \leq 0.033$), but no difference between PAR genes and any Z gene category. Finally, I found that PAR genes are enriched for haploinsufficient genes relative to each Z category. An enrichment of haploinsufficient genes on PAR could have biased results toward the observation of a drift-driven faster-Z effect when comparing the dataset as a whole, but could now be controlled for by incorporating a proxy for gene essentiality. Taken together, the observed pattern of higher rate of non-synonymous evolution, across the full gradient of haploinsufficiency, in hemizygous genes compared to Z genes with a functional W — but not PAR genes — is primarily consistent with a selection-driven faster-Z effect, with little to no influence of drift. The absence of a drift-driven faster-Z effect suggest little male-biased sexual selection, consistent with social monogamy in the Skylark and Raso lark (Hutchinson & Griffith, 2008; Brooke *et al.*, 2010a).

In addition, it is important to note that I had to exclude about three quarters of the genes on ancestral Z (407 of 533) due to absence, or too few detectable substitutions over ~23 MY (since the divergence from the Great tit). This is interesting, since 98.5% of these genes were hemizygous, and the extreme conservation may reflect long-term purifying selection against recessive deleterious mutations, potentially driven by hemizygous selection (Charlesworth *et al.*, 1987; Rousselle *et al.*, 2016). While these genes could not be included in the analyses due to insufficient data, they were more abundant than the genes I had to exclude from PAR for the same reasons (19 of 153; $P = < 2.2 \times 10^{-16}$). Therefore, these genes could reflect slower-Z evolution in a relatively large gene set.

By dissecting the evolutionary rates of subsets of genes, I go from inferring only a trend of faster-Z evolution to finding strong evidence for some genes experiencing an adaptive faster-Z effect driven by hemizygous exposure to selection, without strong evidence supporting a contribution of drift. Furthermore, for ancestral Z genes I found indicative evidence of a slower-Z effect. From my results it is apparent that the inferred processes can vary depending on how the dataset is analysed, and whether genes of various properties are allocated differently between autosomes and sex chromosomes. In this case, incorporating haploinsufficiency into my analysis gave us much deeper insights into the processes governing evolutionary processes operating on sets of sex chromosomal genes, rather than only looking at patterns of large groups of genes.

Finally, it is possible to speculate that these effects could be temporal, which I have not investigated with this dataset (but may be possible). In young strata with little hemizygosity, the faster-Z effect may be weak or absent. As W genes begin to degenerate, the faster-Z effect may increase due to hemizygous exposure (Mrnjavac *et al.*, 2023; Höök *et al.*, 2024). Later, once hemizygous Z genes are optimized, they may primarily be maintained under purifying hemizygous selection causing a slower effect, as indicated by the ancestral Z genes in my dataset (Orr, 2005; Bachtrog *et al.*, 2009; Rousselle *et al.*, 2016).

W genes can be adaptive

In **paper II**, I estimate the functional role of W gametologs, and their potential to undergo adaptive evolution. I performed branch-length tests across 824 gametolog pairs, applying methods for positive selection and comparative intensification/relaxation of selection between branches, and found multiple W genes under selection (**Aim 2c**). I found that eight W genes have evolved under an episode of positive selection following recombination cessation (*BRWD3*, *CKAP5*, *FAM161B*, LOC100223640/*FNDC3A*, *OGFRL1*, *SMOC1*, *SPDYA*, *STEEP*), but I could not identify any enrichment for GO or KEGG terms. I also found six genes in which the W gametolog has experienced intensified selection relative to its Z gametolog (*ALKBH1*, *NEK9*, *PRSS35*, *SPDYA*, *WDR43*, *WDR44*), which were enriched for the human KEGG terms “Progesterone-mediated oocyte maturation” and “Oocyte meiosis”. Notably, *SPDYA*, which showed both significant positive selection on the W branch and relaxed Z/intensified W selection, was associated with several human GO terms related to female reproduction, including “oogenesis” and “female gamete generation” (**Figure 11; Paper II, Figure 5**).

In **paper III**, I further infer the direction of selection (DoS), as well as the distribution of fitness effects (DFE) in autosomes, the Z, and the W chromosome, in the Skylark and Raso lark, respectively. Both DoS and DFE operate on more contemporary timescales, as they are based on segregating polymorphisms as well as substitutions.

DoS is a metric often used to infer recurrent positive selection. I found that DoS values were in general distributed close to zero, consistent with many genes evolving under neutral evolution or strong purifying selection (few substitutions and few segregating polymorphisms). However, I found that the DoS distribution was skewed towards negative values across all genomic regions in the Raso lark (mean DoS for autosomes/Z/W: -0.12/-0.14/-0.035) and for the Skylark W (-0.064; autosomes/Z: 0.0062/0.012; **Paper III, Figure 3F**). This pattern suggests an excess of weakly deleterious mutations segregating in a fraction of genes in the Raso lark, which purifying selection has not efficiently removed. Alternatively, it could mean that polymorphisms are scarce, reflecting low effective population sizes. Moreover,

positive DoS values were present, suggesting that positive selection may have acted on a subset of genes (**Aim 2c**).

The DFE estimates how the fitness impacts of new mutations are distributed using patterns of standing variation. For the DFE, the best-fitting model for the Skylark autosomes and Z chromosome included beneficial mutations. On the Skylark W chromosome, as well as all genomic regions in the Raso lark, the best-fitting models did not include a detectable contribution of beneficial mutations (**Paper III, Figure 3E**). This is not surprising in the Raso lark, which shows low genome-wide variation due to recent and severe population bottlenecks (**Paper III, Figure 2 & 3A**). However, the Skylark is a species with large panmictic populations, where beneficial mutations are expected to contribute to genetic variation on autosomes and the Z chromosome. This species therefore provides a useful system for assessing potential adaptive evolution on the W chromosome. However, the lack of detectable contribution of beneficial mutations mainly suggests reduced standing variation on the W chromosome overall, rather than indicating that *de novo* mutations cannot undergo adaptive evolution. This is corroborated by the observation that nucleotide diversity on the Skylark W was much lower than on autosomes and Z, relative to the same comparison in the Raso lark (**Paper III, Figure 3B-C**). Further, the DFE suggested that purifying selection is still acting on the W chromosome relative to the Raso lark (**Paper III, Figure 3E**). This suggests that the low nucleotide diversity on the Skylark W is influenced by purifying selection, rather than just drift alone (**Aim 2c**; Charlesworth *et al.*, 1993).

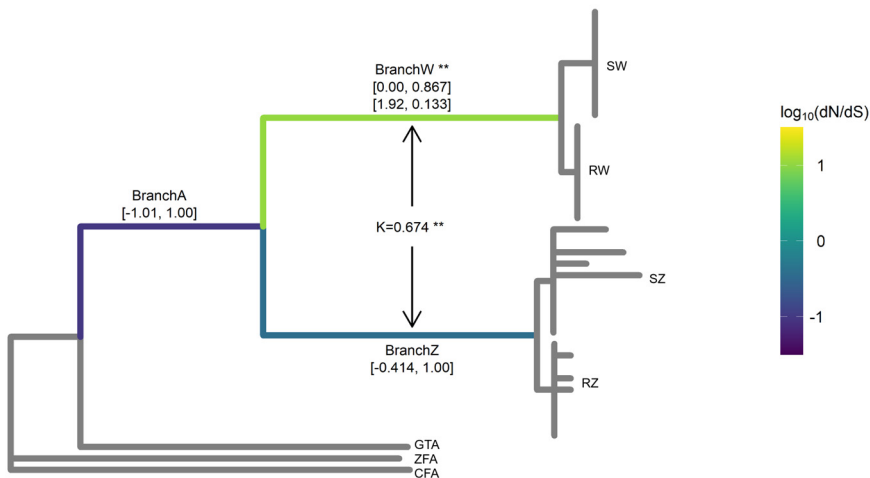


Figure 11. Positive and intensified selection on SPDYA-W. Maximum-likelihood estimates of branch lengths and branch-specific $\log_{10}(\text{dN}/\text{dS})$ for SPDYA gametologs. The W branch shows significant positive selection ($\log_{10}(\text{dN}/\text{dS}) > 0$; $\log_{10}$ of aBSREL-estimated rate distributions in square brackets), as well as significantly intensified selection relative to the Z branch ($\log_{10}(K) > 0$). Branch labels: SW = Skylark W, RW = Raso lark W, SZ = Skylark Z, RZ = Raso lark Z, GTA = Great tit autosomal, ZFA = Zebra finch autosomal, CFA = Collared flycatcher autosomal. Tips correspond to Z and W sequences of individual females. Figure adapted from **paper II**.

W genes may buffer genetic variation during bottlenecks

In **paper III**, I investigate whether functional W-linked genes can buffer functional genetic variation in females. I classified genotypes at both the per-gene and per-individual level in male Z-linked and female Z–W gametologous genotypes. These were classified as functional homozygous (FF), loss-of-function homozygous (LL), masking heterozygous (FL; one functional and one loss-of-function allele), and functional heterozygous (F^1F^2 ; two functional alleles differing by at least one amino-acid substitution). I then evaluated how the proportion of these genotypes varied over time in females and males, in Skylarks and Raso larks respectively, two species with extreme differences in recent demographic histories (**Figure 12; Paper III, Figure 2**).

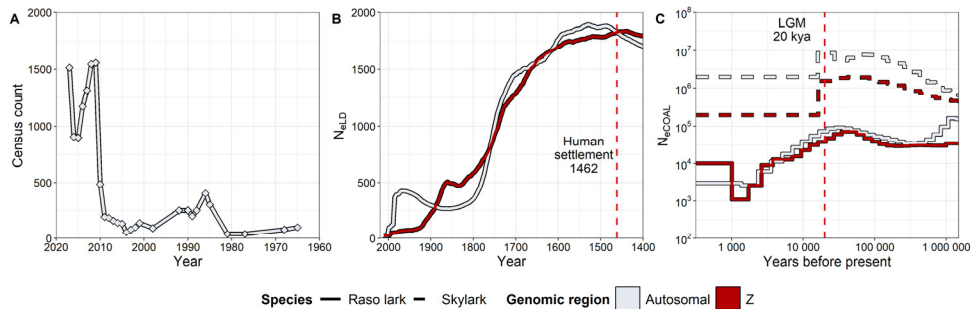


Figure 12. Recent demography of the Raso lark. (A) Census counts of Raso lark individuals between 1965 and 2017 (Dierickx *et al.*, 2020). (B) Effective population size trajectories for Raso lark inferred from autosomes and the Z chromosome with GONE. The red dashed line marks the onset of human settlement of Cape Verde in 1462. (C) Effective population size trajectories for Raso lark and Skylark inferred from autosomes and the Z chromosome with MSMC2. The red dashed line indicates the last glacial maximum (LGM; ~20 Kya). Figure adapted from **paper III**.

I found that as the sex chromosome system becomes older and Y/W genes diverge and degenerate, females in both species experience rapid decline in functional homozygosity (FF), mirrored by a rapid increase in masking heterozygosity (FL). Interestingly, females also show a substantial elevation in functional heterozygosity (F^1F^2), reflecting diverging Z–W gametologs that remain functionally intact. This effect peaks in young strata, but declines in intermediate strata as W gametologs accumulate loss-of-function mutations (**Figure 13A-B; Paper III, Figure 4**). While I did not quantify actual fitness effects, such genotypes have the potential to generate benefits through heterozygote advantage (Knief *et al.*, 2017; **Aim 2d**).

While females showed no obvious differences between species, the contrasting demographic histories of the species were clearly reflected in the males. Raso lark males exhibited very high levels of functional homozygosity (FF) and very little functional heterozygosity (F^1F^2) across strata, consistent with strongly reduced genome-wide diversity following their recent bottleneck. In contrast, Skylark males displayed intermediate levels of both functional homozygosity (FF) and functional

heterozygosity (F^1F^2 ; **Figure 13A-B; Paper III, Figure 4**). Consequently, female-male contrasts differed widely between species, and were mainly driven by male differences in functional homozygosity (FF) and heterozygosity (F^1F^2).

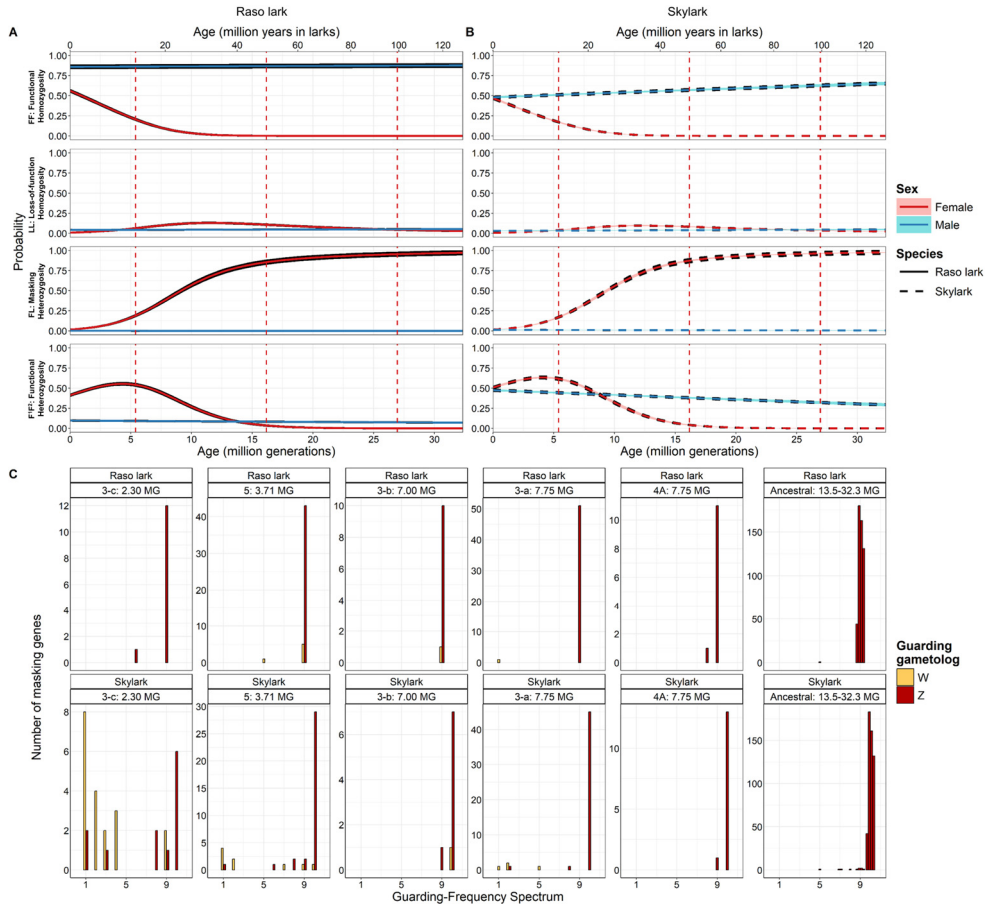


Figure 13. Genotype probabilities and gametolog guarding. Predicted genotype probabilities as a function of age in (A) Raso larks, and (B) Skylarks across sex chromosome stratum ages. Females are marked in red and males in blue with $\pm 95\%$ credible intervals. Genotypes were categorised as: functional homozygosity (FF), loss-of-function homozygosity (LL), masking heterozygosity (FL), and functional heterozygosity (F^1F^2). Vertical red lines indicate the three ages used to calculate probability contrasts, corresponding to sex chromosome strata defined as young (5.4 MG), intermediate (16 MG), and old (27 MG). (C) The frequency of masking loss-of-function genotypes within genes of each stratum and species (Raso lark $n=9$, Skylark $n=10$). Was guarding gametolog shown in yellow, and Z as guarding gametolog shown in red. Figure adapted from **paper III**.

In females with masking heterozygosity (FL), W loss-of-function gametologs were often guarded by functional Z gametologs (F^ZL^W). Rarely, and mainly in young strata in the Skylark, Z loss-of-function gametologs were guarded by functional W gametologs (F^WL^Z), often as low-frequency L^Z alleles. (**Figure 13C; Paper III**,

Figure 5A). Accordingly, the opportunity for W-mediated masking appears to be greatly reduced in Raso lark females, where it is largely confined to the few genes with fixed L^Z alleles. However, in two genes shared by the Skylark and the Raso lark, and in four additional genes in the Raso lark, functional W gametologs masked fixed L^Z alleles. The Raso lark genes were enriched for the chicken and human KEGG term “Folate biosynthesis”, a pathway essential for ovarian function, embryogenesis, and hatchling survival (Zhao *et al.*, 2025). This enrichment suggests that W-mediated guarding is not random, but may be preferentially retained at genes with critical female functions (**Aim 2d**).

Sex chromosomes introgress during speciation

In **paper IV**, I investigated the role of sex chromosomes in introgression and speciation. I verified a ~64 Mb recombination-suppression polymorphism, and found evidence for it predating speciation, consistent with incomplete lineage sorting (**Aim 3a**). I also characterised the length of introgressed tracts along the Z chromosomes between two species. Contrary to theory, older and degenerated strata appear to introgress more and in longer tracts compared to autosomes and younger strata (**Aim 3b**). Finally, I investigated whether introgressed tracts may be involved in local adaptation. I found weak support for reduced divergence between western and eastern skylarks within introgressed tracts, and that these tracts were enriched for functional gene categories, suggesting a potential role for introgression in local adaptation between western and eastern Eurasian (**Aim 3c**).

W polymorphisms can be maintained over evolutionary time

In **paper IV**, I confirm previous observations in Dierickx *et al.*, (2020), that the ~64 Mb stratum 3-c is present in the Raso lark and western Skylark populations, but is absent in the Oriental lark and eastern Skylark populations. Stratum 3-c thus appears to be associated by geography rather than phylogeny (**Figure 14A-C**; **Paper IV**, **Figure 1**). I defined four alternative hypotheses that could exclusively explain this pattern, and the predicted topologies resulting from phylogenetic tree-building under each scenario (**Figure 15A-D**):

- A. Parallel recombination cessation:** Stratum 3-c has ceased recombination separately in the Raso lark and western Skylarks. I predict that Z and W in shared strata will each follow the species tree and diverge basally before speciation, while stratum Z-3-c and W-3-c will cluster together by species.
- B. Re-establishment of recombination:** Stratum 3-c has ceased recombination completely in a common ancestor, with subsequent parallel re-establishment of recombination in eastern skylarks and the Oriental lark.

I predict that Z and W in shared strata will each follow the species tree and diverge basally before speciation, as will stratum Z-3-c and W-3-c.

- C. Incomplete lineage sorting:** Stratum 3-c arose from an ancestral polymorphism with incomplete lineage sorting. I predict that Z in shared strata and Z-3-c will follow the species tree, W in shared strata and W-3-c will cluster by geography, and W-3-c will diverge from Z before speciation.
- D. W introgression:** Stratum 3-c has ceased recombination recently in either the Raso lark or western Skylarks, with subsequent introgression into the opposite lineage. I predict that Z and W in shared strata will diverge basally before speciation, with Z following the species tree, but W clustering by geography. Stratum Z-3-c will follow the species tree and W-3-c will cluster together with Z-3-c of either western Skylark or Raso lark.

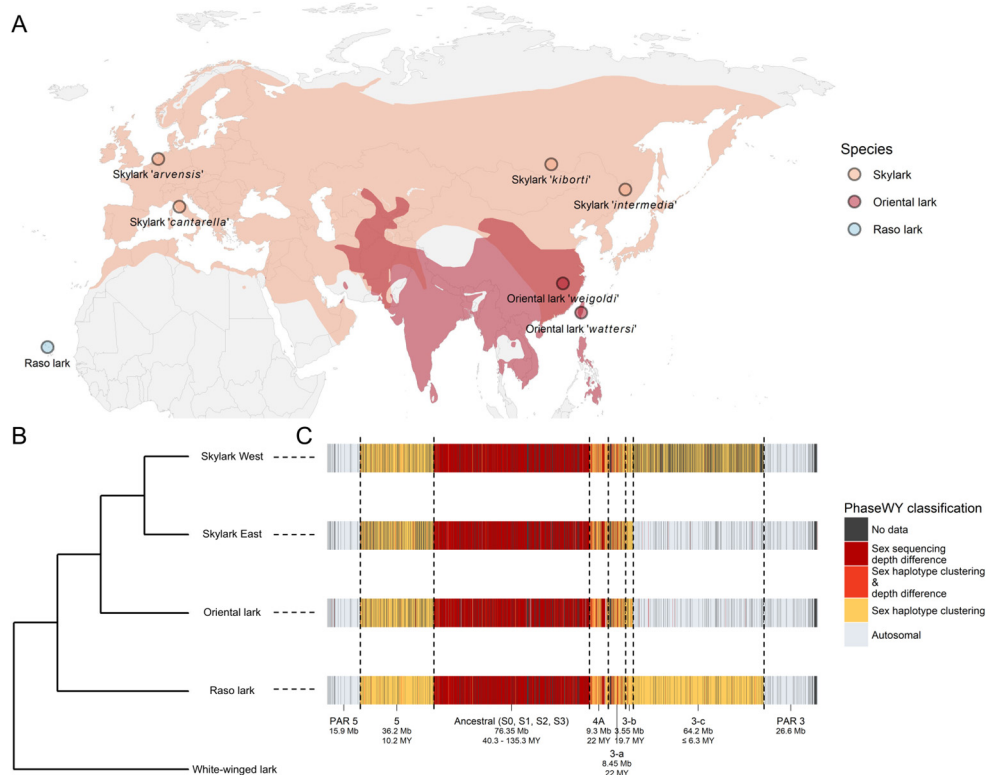


Figure 14. Geographic and phylogenetic relationships of *Alauda* larks. (A) The Geographic distribution of the Raso lark, Skylark and Oriental lark, with respective subspecies. (B) Cladogram of phylogenetic relationships of species within the genus *Alauda* based on Alström *et al.*, (2023). (C) The classification of recombining regions (light blue), or recombination cessation (sex depth differences as red; haplotype clustering as yellow) of the lark sex chromosomes in respective taxa based PhaseWY. Strata are annotated by name, size, and age. Note that the stratum 3-c is absent from eastern populations of Skylark and the Oriental lark. Distribution maps from BirdLife International & Handbook of the Birds of the World (2025). Figure adapted from **paper IV**.

The resulting topologies are consistent with a scenario of an ancestral polymorphism with incomplete lineage sorting (**Aim 3a**): the W lineages and the mitochondrion all cluster by geography rather than species (Raso lark and western Skylarks vs. Oriental lark and eastern Skylarks; **Figure 15E**; **Paper IV**, **Figure 2**). In the tree of stratum 3-c, W lineages all diverge basally before speciation (**Figure 15C**), as opposed to clustering with their respective Z-gametologs in respective species (parallel events of recombination cessation; **Figure 15A**), or within either western Skylarks (**Figure 15D**) or Raso lark Z gametologs (introgression). Furthermore, the tree of the shared W and the mitochondrion do not follow the species tree (re-establishment of recombination; **Figure 15B**). Dating the phylogenies suggests that the W polymorphism arose 5.0 MYA (95% CI: 4.6, 5.5 MYA).

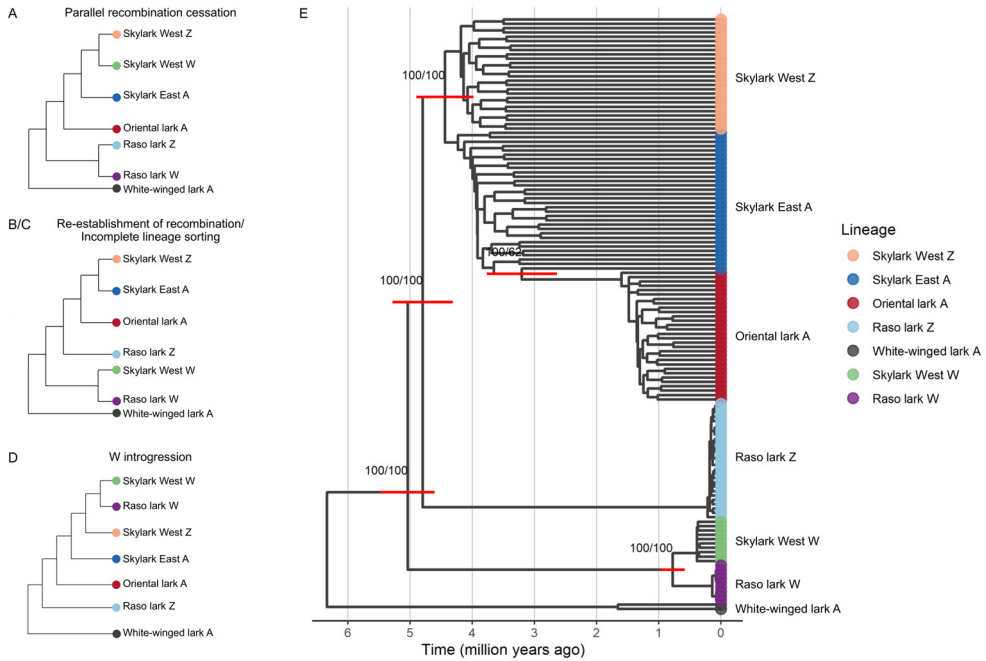


Figure 15. Dated phylogeny of species in stratum 3-c. Schematic trees visualise predicted topologies of stratum 3-c for different hypotheses: **(A)** parallel recombination cessation in western Skylarks and Raso larks, **(B)** re-establishment of recombination in eastern Skylarks and Oriental larks, **(C)** incomplete lineages sorting (this topology looks the same as for re-establishment of recombination in stratum 3-c, and topologies of the shared W or mitochondrion are needed to distinguish the two), and **(D)** introgression from the western Skylark to the Raso lark (introgression from the Raso lark to western Skylarks would instead show W lineages clustering with Raso lark Z). **(E)** Resulting topology of stratum 3-c agreeing with hypotheses B and C. Note that the Oriental lark clusters within eastern Skylarks instead of outside Skylarks, consistent with the Eurasian skylark and the Oriental lark forming a ring species, where the eastern skylark group occurs in between the two (Mayr, 1963; P Alström & Z Liu, unpublished). Genomic regions are abbreviated as A=autosomal, Z=Z chromosome, W=W chromosome. Figure adapted from **paper IV**.

While incomplete lineage sorting is the only exclusive process that can explain these topologies, a combination of processes could result in the same pattern. These

include re-establishment of recombination followed by introgression, although re-establishment of recombination lacks empirical evidence (but see Lenormand and Roze, 2024 for theory). Alternatively, incomplete lineage with subsequent introgression would not necessarily alter the topologies.

I investigated a scenario of recent introgression by testing whether divergence times of the W chromosome and the mitochondrion differed relative to those of the autosomes and Z chromosome. Across divergence dates compared, I found that autosomal and Z lineages generally agreed across trees (Raso lark–western Skylarks: 4.5–4.8 MYA; Oriental lark–eastern Skylarks: 2.7–3.8 MYA; Skylark west–east: 3.9–4.4 MYA), but these were generally older than the same species splits in the W chromosome and mitochondrion. All divergence dates of the W chromosome occurred relatively recently (Raso lark and western Skylarks: 0.78–0.83 MYA; Skylark west–east: 1.3 MYA), with the mitochondrion diverging after autosomal and Z lineage splits, but before W lineages (Raso lark and western Skylarks: 1.9 MYA; Skylark west–east: 3.0 MYA). However, the divergence of the W polymorphism is much older than the corresponding splits on the shared W and mitochondrion (stratum 3-c: 5.0 MYA, shared W: 1.3 MYA, mitochondrion: 3.0 MYA). This discrepancy could be explained by substitution rates differing between genomic elements, and potentially over evolutionary time. I tested this and found that W lineages and the mitochondrion increased in substitution rate towards the present day relative to autosomes and Z lineages, which would result in older rather than recent divergence dates, as an increase in substitution accumulation would increase branch lengths closer to the present. However, substitution rates may differ between genomic elements, which is not explicitly accounted for in my approach. For example, many animal (including birds) have male biased mutation rates, resulting in slower evolution on the female biased W chromosome and mitochondrion (Ellegren, 2007). Therefore, stronger evidence could be obtained using models that explicitly account for variation in substitution rates among branches.

Support for an ancient polymorphism with incomplete lineage sorting is interesting, as W polymorphisms must survive despite strong drift and complete linkage across the W chromosome (Hill & Robertson, 1966; Smith & Haigh, 1974; Charlesworth *et al.*, 1993; Ellegren & Galtier, 2016). However, this could have been facilitated by potentially large historical effective population sizes of *Alauda*, consistent with the observation of high genetic diversity and recent demographic trends in western Skylarks (Dierickx *et al.*, 2020; **Paper III**). It is tempting to ponder whether the underlying driver of these processes is caused by local adaptation to western and eastern Eurasia respectively. Although western and eastern Skylarks are well separated geographically, analyses of admixture suggest that most of their genomes are similar enough to be classified as belonging to the same genomic cluster (**Paper IV, Figure 3**), suggesting that W haplotypes are not necessarily structured due to a historical absence of gene flow.

W degeneration facilitates Z introgression

In **paper IV**, I tested whether the Z chromosome acts as a barrier to introgression, and whether this barrier grows stronger in older strata with high W loss-of-function. I classified phylogenetic topologies across the genome and identified genome-wide introgression tracts consistent with introgression between the Raso lark and ancestral Skylarks, covering 2.6 % of the genome and 4.3 % of the Z chromosome (**Figure 16A, Paper IV, Figure 4A**). I then tested whether introgression tracts lengths varied with the proportion of stratum-degeneration, while accounting for heterogeneity in evolutionary rate, time since introgression, and linkage disequilibrium reflecting the ancestral recombination landscape. I found that the length of introgressed tracts increases with the proportion of W-loss-of-function, a pattern also reflected in the proportion of genomic regions and strata covered by introgressed tracts (**Figure 16E, Paper IV, Figure 4G**).

These results contrast with theory, which predicts that hemizygosity exposes incompatibilities on the Z chromosome (Butlin, 2005; Fraïsse & Sachdeva, 2021). Therefore, introgression in degenerated strata would be expected to show shorter tracts lengths, thereby decoupling such incompatibilities. However, in degenerated strata, Z genes may eventually become unusually conserved and functionally optimised through repeated exposure to efficient purifying hemizygous selection (Charlesworth *et al.*, 1987; Orr, 2005; Bachtrog *et al.*, 2009; Rousselle *et al.*, 2016). This is further supported by my analyses of faster-Z in **paper II**, where I had to exclude about three quarters of the genes on the ancestral Z chromosome from analyses because they contained no, or too few detectable substitutions. These results suggest that W loss-of-function can under some circumstances facilitate introgression across the Z chromosome (**Aim 3b**).

Introgression may facilitate local adaptation

In **paper IV**, I tested whether introgressed tracts may be related to local adaptation between western and eastern Eurasia. In such a scenario, I expected introgressed regions to diverge at a slower rate within the west (between the Raso lark and western Skylarks), compared to between west and east (western and eastern Skylarks), and that this contrast should be larger in introgressed tracts compared to non-introgressed regions. I therefore calculated a normalized divergence metric:

$$LA = \log_{10} \left(\frac{RND_{SLW-SLE}}{RND_{RL-SLW}} \right)$$

in which RND is the relative node depth (divergence controlled for mutation-rate variation) between western and eastern skylarks (SLW and SLE), and between the Raso lark (RL) and western Skylarks. Positive values of LA indicate a greater divergence between western and eastern Skylarks than expected from the average

divergence observed within western Eurasia, whereas negative values indicate lower divergence than expected. I found that divergence between western and eastern Skylarks was 80 % of that observed between the Raso lark and western Skylarks in non-introgressed regions, with a trending increase to 97 % in introgressed tracts (**Figure 16D, Paper IV, Figure 4F**).

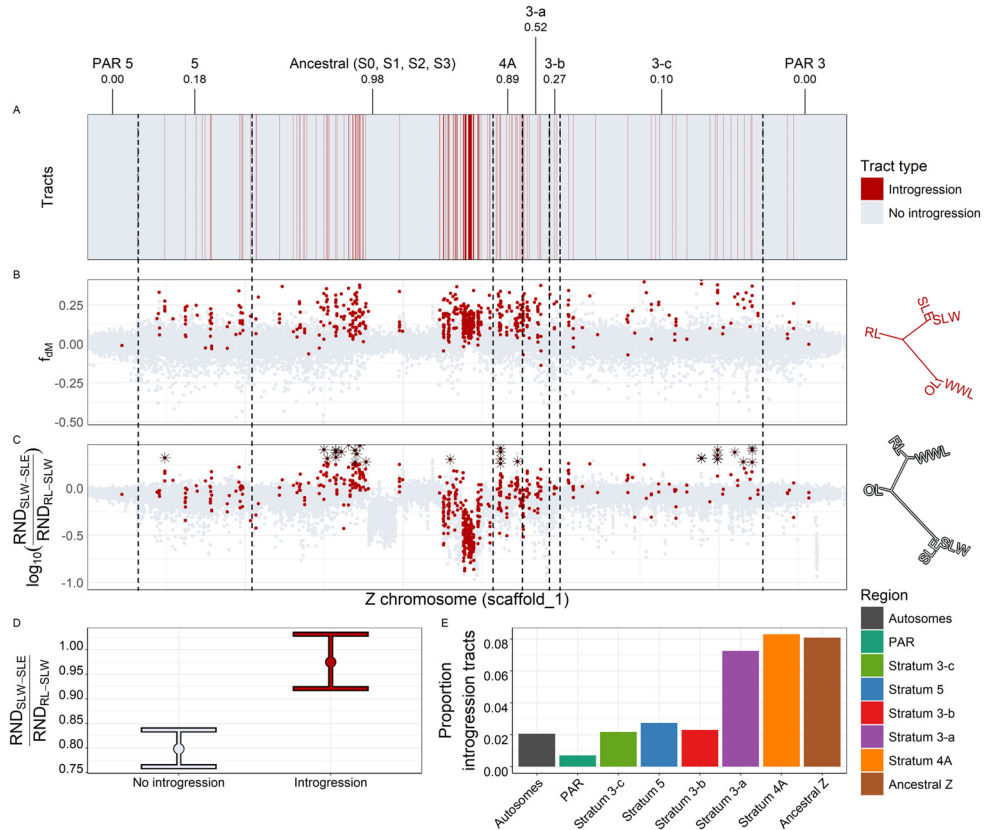


Figure 16. Admixture and introgression across genomic region. Population genomic metrics visualised across the Z chromosome: **(A)** Tracts of introgression, **(B)** the fdM (proportion of introgression), with higher values indicating more introgression between the Raso lark and western skylark, and **(C)** our local adaptation metric (LA), with high values indicating reduced divergence between the Raso lark and western skylarks compared to between western and eastern skylarks. Stars visualise the upper 5% of the residual distribution of LA. Strata are annotated by name and the proportion of W loss-of-function gametologs. Topologies in the margin represent weighted mean topologies, weighted by the proportion of the genome covered by respective tract type (Introgression and non-introgression). **(D)** Predicted relative node depths in non-introgressed and introgressed regions ($\pm$ 95% CI). **(E)** The proportion of genomic regions covered by introgression tracts. Species names are abbreviated as OL=oriental lark, SLE=skylark east, SLW=skylark west, RL=Raso lark, WWL=white-winged lark. Figure adapted from **paper IV**.

While the effect was only a trend, I analysed enrichment for gene functions in introgressed tracts and found enrichment of several chicken and human GO terms.

I also found enrichment of gene functions after restricting the analysis to introgressed genes overlapping introgressed regions within the upper 5% of the residual distribution of LA (**Figure 16C, Paper IV, Figure 4C**). Thus, these results suggest that introgression on the Z chromosome is not random. Taken together, introgressed tracts between the Raso lark and ancestral skylarks are enriched for gene functions, and show reduced conservation following Skylark expansion into eastern Eurasia. These results raise the possibility that introgressed tracts may be involved in local adaptation between western and eastern Eurasia (**Aim 3c**).

Sex chromosome evolution is predictable

In **paper II**, I utilised several gene properties in my statistical analysis of W degeneration and evolutionary dynamics of ZW gametologs. I found that some of these gene properties have a high explanatory power in the fate of sex-linked genes (**Aim 4a**). In **paper IV**, I investigated how the genomic landscape predicts introgression across the Z chromosome, and find that patterns both expected and contrasting with theory (**Aim 4a**). In **paper V-VI**, I characterised sex-linked regions in several species that has previously not been investigated. I found that many of these regions represent repeated events of autosomal translocations, as well as recruitment in stratum-formation (**Aim 4b**). These findings gave me a powerful dataset that in **paper VI** allowed me to statistically investigate whether these genomic regions are predisposed to sex-linkage (**Aim 4b**), and whether the amount of sex-linkage correlated with ecological and evolutionary traits (**Aim 4c**).

Gene properties predict the fate of sex-linked genes

In **paper II**, I specifically focus on the broad-scale impact of various gene properties that I hypothesised could influence selective regimes governing ZW gametologs. These included (i) stratum age, measured as the time since recombination cessation in generations, (ii) species, capturing short-term effects of differences in genetic drift between the Skylark and the Raso lark, (iii) haploinsufficiency, representing the potential negative impact of having only a single functional gene copy, (iv) gene length, (v) W-gametolog functionality, and (vi) the intensity of selection on Z-gametologs.

I found that the probability of W degeneration was primarily predicted by stratum age, as well as gene length, which interacted (**Figure 9, 17A-B; Paper II, Figure 2**). This is not surprising, as reduced effective population size and absence of recombination are expected to severely reduce the efficacy of selection on W chromosomes (Bachtrog, 2013). However, inefficient selection by itself is not sufficient to cause degeneration, which also requires standing loss-of-function

variation or the accumulation of new mutations (Muller, 1964; Bachtrog, 2013). Thus, evolutionary time reflects the duration over which a gene has been able to accumulate deleterious mutations, as well as the time during which such mutations have survived exposure to relaxed purifying selection. In a similar way, long protein-coding sequences present a larger mutational target, and are therefore more difficult to maintain by selection (Ellegren, 2011). However, I also found that haploinsufficiency mitigates the negative effects of both age and gene length (**Figure 17A-B; Paper II, Figure 2D-E**). This suggests that haploinsufficiency reflects the strength of selection acting to prevent W-loss, such that essential genes are more likely to be retained despite being long, as well as to survive over longer evolutionary timespans as W-linked (Bellott *et al.*, 2014, 2017). I found that the severely bottlenecked Raso lark displayed a slightly higher proportion of genes lost (53.2%) than the widely distributed Skylark (51.9%), suggesting that drift may generally result in gene-loss. However, this pattern was measured across the full genome and could not be associated specifically with autosomes, Z-linked or W-linked genes, or any particular stratum. This suggests that most W degeneration pre-dated the species split, and that recent demographic history affects only a small fraction of genes that remain functional.

The effect of gene properties on signatures of Z-selection was extensively discussed above (see “W genes influence the evolution of Z gametologs”), but I briefly summarise it here. I found that haploinsufficiency influenced the strength of selection and interacted with the functional state of the W gametolog. I specifically found that genes with a functional W copy experienced stronger purifying selection as a function of increasing haploinsufficiency, a relationship that was indistinguishable from the relationship between PAR genes and haploinsufficiency. However, the strength of selection on Z gametologs that had lost their W copy did not show any such association with haploinsufficiency (**Figure 10B; Paper II, Figure 3E**). Similarly to W degeneration, I found that long Z genes were more likely to experience relaxed purifying selection, an effect mitigated by high haploinsufficiency (**Paper II, Figure 3F**).

To conclude, the fate of Z and W gametologs can be predicted by gene properties. Evolutionary time since recombination cessation increases the probability of relaxation and degeneration of W gametologs. Haploinsufficient genes are more likely to retain their W gametolog, and Z and W gametologs of such genes are more likely to follow parallel evolutionary trajectories. Longer genes provide a larger target for mutations to accumulate, and thus are more difficult for selection to maintain. Long W genes are therefore more likely to degenerate, although in young strata they may persist if they carry important functions that promote their maintenance. By contrast, short and non-haploinsufficient gametolog pairs are more likely to follow independent evolutionary trajectories from one another (**Aim 4a**).

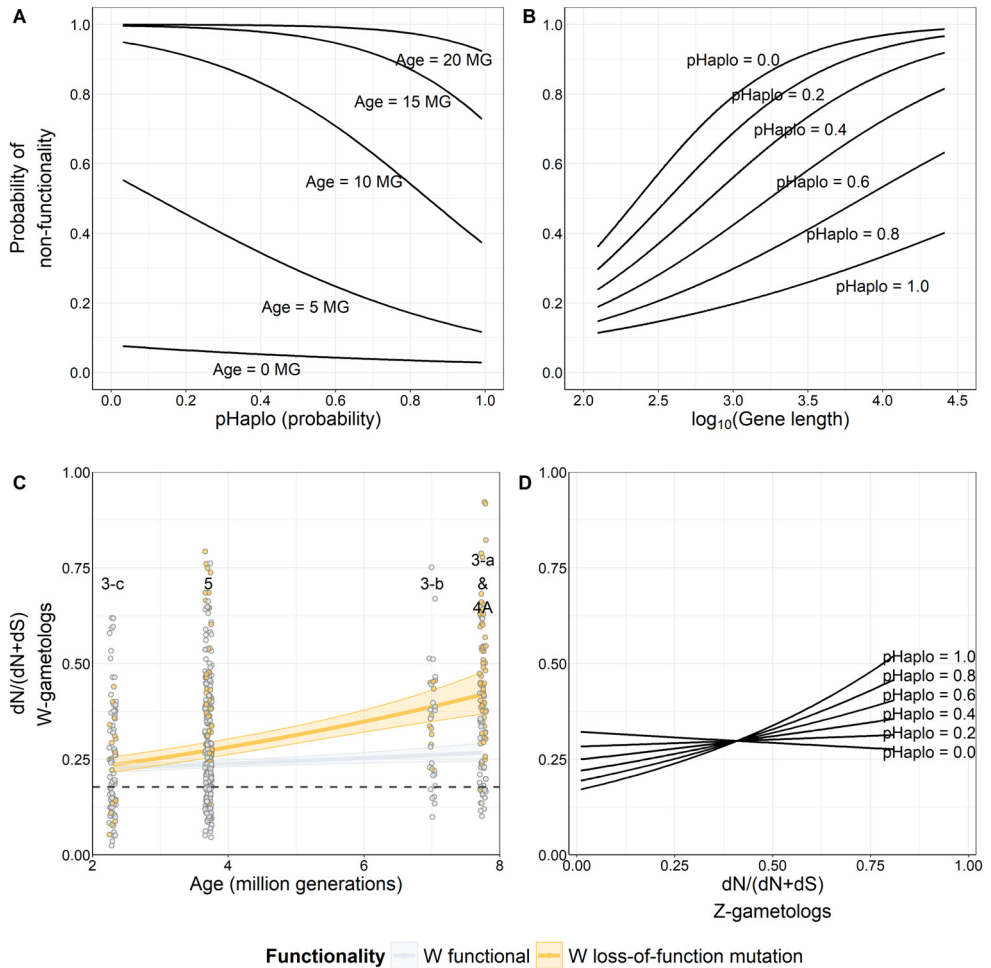


Figure 17. Predictors of W selection and degeneration. (A) Predicted probability of W-gametolog non-functionality as a function of haploinsufficiency (pHaplo) across strata of different ages (MG), with other predictors held at their median values. (B) Predicted probability of W-gene non-functionality as a function of gene length across various values of pHaplo with strata age set at 7.5 MG. (C) Interaction between stratum age and W functional status (functional or loss-of-function) on W-selection signature ($dN/(dN + dS)$; $\pm 95\%$ CI). Individual data points are transparent and jittered horizontally for visibility. The dashed line indicates the median Z-selection strength (D) Relationship between $dN/(dN + dS)$ of W and Z gametologs at different pHaplo. Figure adapted from **paper II**.

The genomic landscape predicts introgression of sex-linked regions

In **paper IV**, I investigated how the genomic landscape predicts introgression tract lengths across the Z chromosome. I specifically investigated the effects of (i) the proportion of W loss-of-function in respective strata to test for the influence of hemizygous exposure of incompatibilities to selection, (ii) the mean absolute

divergence (d_{XY}) between the white-winged lark and respective studied taxa to test for heterogeneity in evolutionary rate, (iii) linkage disequilibrium (LD) in respective species to test for the ancestral recombination landscape, and RND between the Raso lark and western Skylarks to test for the time since introgression.

In contrast to expectations by large-X/Z theory, hemizygosity appears to facilitate long introgression tracts (discussed above under “W degeneration facilitates Z introgression”). However, in accordance with theory, high LD regions appear to act as barriers to gene flow, reducing tract lengths. This could be because such regions often form tightly co-evolved haplotypes, vulnerable to incompatibilities, or less capable of decoupling them through recombination (Butlin, 2005; Fraïsse & Sachdeva, 2021). I found that LD interacted with the mean d_{XY} , such that tract lengths became smaller in rapidly evolving regions with low LD (**Paper IV, Figure 4D**). This could reflect that rapidly evolving regions accumulate incompatibilities, which are efficiently decoupled in highly recombining regions. High mean d_{XY} has less effect on tract lengths in high LD regions, contradicting the expectation that low-recombining regions are less capable of decoupling incompatibilities. One interpretation is that rapidly evolving, tightly linked haplotypes are adaptive, allowing introgression to confer benefits that outweigh the potential costs of exposing incompatibilities (Bull, 1983; Rice, 1987; Wellenreuther *et al.*, 2019; Meisel, 2022). Furthermore, I expected a negative association of RND and introgressed tract lengths, as these high RND should reflect older introgressed regions that have broken into shorter segments over time. I find this association as an interaction with mean d_{XY} : putatively recently introgressed tracts show little association to divergence rate, but older introgressed tracts become increasingly smaller in rapidly diverging regions (**Paper IV, Figure 4E**). This pattern might reflect that introgressed tract are most strongly fragmented in rapidly diverging regions, where incompatibilities accumulate fast and need to be decoupled. These results suggest that introgression of genomic material on sex chromosomes can be predicted by several factors describing the genomic landscape (**Aim 4a**).

Repeated stratum-formation on chromosome 4

In **paper V**, we present evidence for a translocation and stratum formation of passerine chromosome 4 (Zebra finch - chromosome 4) in the yellow-throated Nicator (*Nicator vireo*). Translocation and stratum-formation involving regions of chromosome 4 have previously been observed in Cisticolidae (the Zitting cisticola – *Cisticola juncidis*, and the Grey-backed camaroptera - *Camaroptera brevicaudata* Sigeman *et al.*, 2024, 2020; **Figure 18; Paper V, Figure 2**). Thus, this new observation marks a second and independent case of translocation and stratum formation involving chromosome 4. We infer that both events occurred at the opposite end of the 4A fusion known in all Sylvioidea (**Paper V, Figure 3**), and that the fission/fusion point of chromosome 4 is remarkably similar, varying only by 1.6

Mb between events (**Figure 18B; Paper V, Figure 2**). Both the Zitting cisticola and the Grey-backed camaroptera share a single stratum, but have experienced one stratum-forming event each, expanding the sex-linked region to roughly similar extents. The yellow-throated Nicator has likewise experienced two stratum-forming events, of which the first encompasses the full region observed in the Zitting cisticola and the Grey-backed camaroptera.

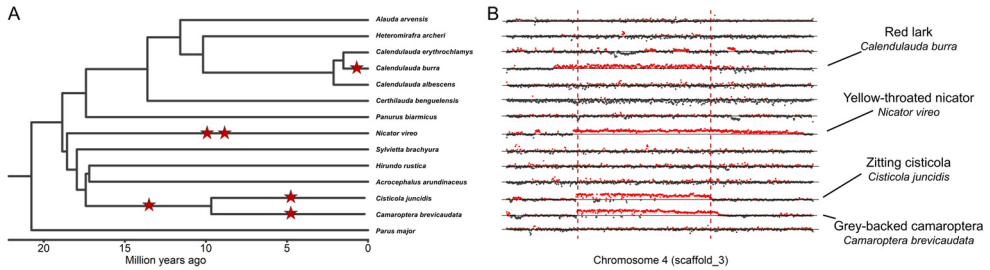


Figure 18. Chromosome 4 has repeatedly formed neo-sex chromosomes in Sylviidae. (A) Phylogenetic relationships and divergence times of species. Red stars are placed on branches corresponding to an event of independent recombination cessation for 4. Tree and divergence times based on Oliveros *et al.*, (2019) and Alström *et al.*, (2023) **(B)** Normalized sex heterozygosity differences in 100 kb windows on Zebra finch chromosome 4 (Skylark scaffold_3). Windows coloured red corresponds to female inflated heterozygosity above the 25 % quantile. Thin black lines correspond to the y-intercept at 0. Dashed red lines mark shared sex-linked regions of the Red lark, Yellow-throated nicator, Zitting cisticola, and Grey-backed camaroptera. Figure adapted from **paper VI**.

In **paper VI**, I present evidence for a third translocation with a stratum-forming event of chromosomes 1 and 4 in the Red lark (*Calendula burra*). The stratum on chromosome 4 encompasses the region shared by the previous three species, resulting in a sex-linked region shared among all four species that constitutes almost half the passerine chromosome 4 (30.6 Mb) (**Figure 18B; Paper VI, Figure 2 & 4**). In the absence of a genome assembly, I was unable to infer the fission/fusion point in the Red lark. However, since the Red lark appears to be sex-linked across all of the translocated chromosomes 3 and 5, it is likely that chromosomes 1 and 4 have fused at either end of the lark sex chromosome (**Paper VI, Figure 2**). While we were only able to describe these events without statistical support, it is remarkable that the same genomic region has translocated and formed evolutionary strata across a 30.6 Mb region in three independent lineages in a ca. 22 MY-old clade (**Figure 18A; Paper VI, Figure 4**).

In a broader context, chromosome 4 has likewise been recruited as a sex chromosome in some amphibian and lizard lineages (Uno & Matsubara, 2024). Considering that neo-sex chromosome translocations appear rare among birds in general (Nanda *et al.*, 2008; Ellegren, 2010; Degrandi *et al.*, 2020), the case of chromosome 4 suggests that this region could be predisposed to sex-linkage formation, potentially being enriched in sex-determining candidates, sexually

antagonistic loci, or sex-biased regulatory networks (Bachtrog *et al.*, 2014; Rice, 1987; van Doorn and Kirkpatrick, 2007; **Aim 4b**).

Repeated stratum-forming events across larks

In **paper VI**, I documented remarkable variation in recombination cessation across the lark family. I investigated female–male pairs in 44 lark species and inferred 45 unique neo-sex chromosome strata (**Paper VI, Figure 3**). The species investigated include cases with no additional stratum formation from the ancestral lark state, as well as cases with ceased recombination across all or most of the neo-sex chromosome 3 and 5 regions. One species — the Red lark — appears to have recruited not only chromosome 4, but also chromosome 1, in stratum formation (**Figure 19B; Paper VI, Figure 2**). A striking observation is that chromosome 5, which fused to the ancestral sex chromosome without stratum formation in the lark ancestor, has experienced 14 independent stratum-forming events that expanded the PAR border from the fusion point (**Figure 19A; Paper VI, Figure 5A-B**). In species carrying the basal stratum 3-b, there have likewise been 12 independent stratum formations expanded the PAR border from stratum 3-b (**Figure 19A; Paper VI, Figure 5A**).

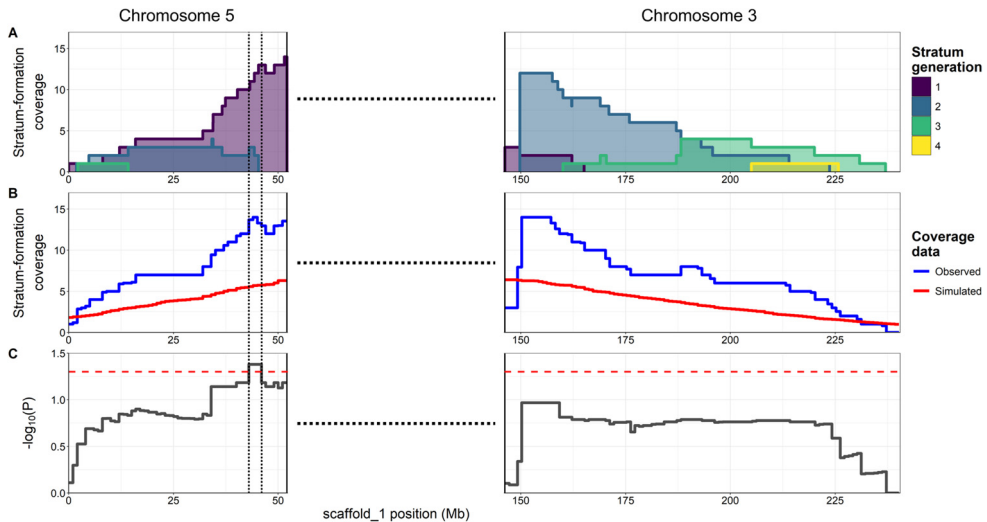


Figure 19. Observed stratum-formations diverges from simulated expectations. (A) Tetris plot showing identified neo-strata at their size and genomic position. **(B)** Stratum-formation coverage along neo-sex chromosomes. Strata are color-coded by their generation (e.g. first PAR border expansion from ancestral lark PAR is purple, and following expansion is blue, etc.). **(C)** Observed and simulated stratum-formation coverage along neo-sex chromosomes. Simulated 95 % confidence interval is barely visible behind simulated mean line. **(D)** FDR corrected P-values representing stratum-formation coverage exceeding expectations of simulated coverage. Dashed red line correspond to P-value threshold (0.05). Figure from **paper VI**.

I attempted to statistically detect stratum-formation hotspots by simulating stratum formation in the lark phylogeny along chromosomes 3 and 5. It is important to note that, in this analysis, I assumed that stratum-formation is common and used a stratum-formation rate inferred from the observed data. Thus, this analysis did not evaluate whether stratum formation is common, but rather whether some regions were more commonly involved than expected under a null hypothesis of stratum expansion. I identified a 3 Mb region encompassing 59 genes on chromosome 5 that appear to be involved in more stratum-forming events than expected by chance (**Figure 19B-C; Paper VI, Figure 5B-C**). This region is located 6.1 Mb from the fusion point and is involved in all but one first-generation stratum-forming event, as well as one second-generation event.

In a broader context, it appears that both chromosomes 3 and 5 have repeatedly been recruited for sex-linkage among vertebrates. Chromosome 3 shares homology with the platypus sex chromosomes (Veyrunes *et al.*, 2008), as well as sex chromosomes in amphibian and lizard lineages (Uno & Matsubara, 2024). Chromosome 5 shares homology with sex chromosomes in turtles and amphibian lineages (Montiel *et al.*, 2016; Uno & Matsubara, 2024). In larks, chromosomes 3 and 5 both appear to represent single translocation events of their respective chromosomes. However, the high number of repeated stratum-forming events involving these regions likewise suggests that they are predisposed to sex-linkage (**Aim 4b**).

The extent of recombination cessation predicts evolutionary outcomes

In **paper VI**, I utilised the great variation in extent of recombination cessation among larks to statistically investigate whether sex-linkage predicts evolutionary outcomes in species traits and ecology. I therefore combined these observations together with published species trait data and performed phylogenetically aware statistical models, using recombination cessation across chromosome 3 and 5 as independent predictors. I found that the extent of sex-linkage on either neo-sex chromosome correlates with different outcomes (**Aim 4c**).

I found that a compound variable (reduced dimensionality with PCA) of various male-to-female body size estimates was explained by the extent of sex-linkage on chromosome 3, with species with more sex-linkage showing a higher ratio of male-to-female size dimorphism (**Paper VI, Figure 6A-C**). This outcome appears to be primarily driven by a male-biased increase in beak size, rather than female underdevelopment (**Figure 20A**), and is consistent with theory suggesting that sex-specific haplotypes may help resolve sexual conflict and enable evolution towards sex-specific fitness optima (Bull, 1983; Fairbairn & Roff, 2006; R. A. Fisher, 1931; Rice, 1987; **Aim 4c**). Enlargement of male beak size suggests adaptation to sex-specific foraging and feeding niches. While empirical observations of sex-specific foraging in larks remain limited, the Raso lark combines extensive recombination

suppression along chromosome 3 with pronounced dimorphism in bill morphology and body size associated with sex-specific feeding behaviour (Donald *et al.*, 2007).

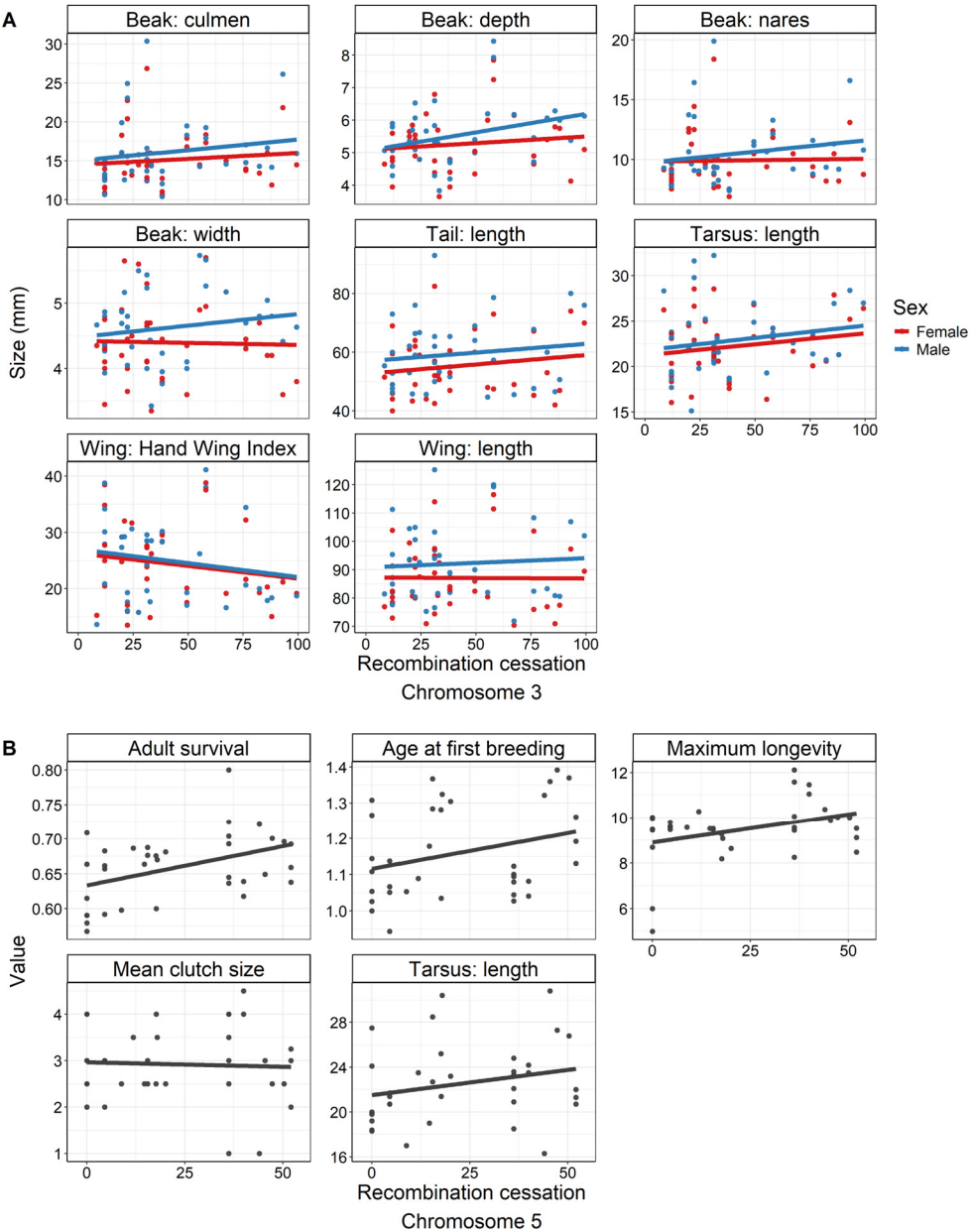


Figure 20. The extent of recombination cessation predicts evolutionary outcomes. (A) Slopes of female and male size traits as a function of the extent of recombination cessation on chromosome 3. **(B)** Slopes of life history traits as a function of the extent of recombination cessation on chromosome 5. Figure adapted from **Paper VI**.

I also found that a compound variable of life-history traits consistent with a pace-of-life gradient was associated with sex-linkage on chromosome 5 (**Paper VI, Figure 6D-F**). Species with more sex-linkage conform to a slower pace-of-life, with traits such as later age at reproduction, higher adult survival rate, larger body size, and smaller clutch size (**Figure 20B**). This result is interesting, as it links sex chromosome evolution specifically to species ecology and life-history strategy (**Aim 4c**). Allocating an increased proportion of the genome to sex chromosomes may impact the efficacy of selection and the accumulation of genetic load, ultimately having consequences for both sexes, and the heterogametic sex in particular. While I did not have sex-specific data for life-history traits such as survival and longevity, it is possible that female-specific consequences of underdevelopment and maladaptation could ultimately provoke trade-offs between survival and reproduction.

Finally, I found that the extent of sex-linkage on chromosome 5 also correlated with elevated IUCN Red List status (**Paper VI, Figure 6G**). This result potentially reflects the association with a slower pace-of-life, as organisms with slow reproduction are often more vulnerable to demographic decline and extinction (Owens & Bennett, 2000; Purvis *et al.*, 2000; **Aim 4c**). However, the credible intervals are wide, reflecting both the small number of threatened species and the uncertainty in the effect size of this association.

Conclusions and future perspectives

Summary of results

In this thesis, I have attempted to address several questions related to the field of sex chromosome evolution, and its role in evolutionary ecology. My findings are summarised below:

Method development

In **paper II**, I began developing *PhaseWY*, a method for the identification and extraction of sex-limited sequences, which formed the basis for the analyses in **papers II-IV**. I thus succeeded in my aim to extract W sequences from short-read data using haplotype phasing and clustering (**Aim 1a**).

In **paper I**, I managed to improve and convert *PhaseWY* into a Snakemake workflow. I generated simulated data to benchmark the performance of *PhaseWY* across several factors, including biological factors, study design, and pipeline parameter settings. This allowed me to fulfil my aim of determining which factors influence sex chromosome identification and Y/W sequence extraction (**Aim 1b**).

PhaseWY was not only converted into a Snakemake workflow, but was also released on GitHub and as a bioRxiv preprint, with extensive documentation, benchmarking analyses, and proof-of-concept applications to empirical datasets. I thereby achieved my goal of developing a reproducible method, implemented in a — hopefully — user-friendly workflow (**Aim 1c**).

Gametolog evolution

In **paper II**, I investigated how long W-linked genes could remain functional. My results suggest that the protein-coding structure of individual W genes can persist for tens of millions of generations, thus potentially contributing to female-specific functions long after recombination has ceased (**Aim 2a**).

I further investigated how W-linked genes co-evolve with Z gametologs in relation to faster-Z theory. By relating per-gene evolutionary patterns to gene properties such as haploinsufficiency and the functional status of W gametologs, I described how

W-linked genes co-evolve with their Z gametologs (**Aim 2b**). I found strong evidence for a selection-driven faster-Z effect in the Skylark and Raso lark. This is unusual, since drift-driven faster-Z is frequently inferred in bird lineages.

I tested whether W-linked genes are capable of adaptive evolution on the per-gene level in **paper II-III**. I found several W genes evolving under positive selection following recombination cessation, with individual genes associated with sex-specific functions (**Aim 2c**). In **paper III**, I further modelled adaptive evolution on the W chromosome as a whole. While I did not detect standing variation contributing to beneficial fitness effects on the W chromosome overall, these results do not exclude the possibility of new mutations undergoing adaptive evolution.

In **paper III**, I investigated whether functional W gametologs can buffer female genetic variation. I found that females experience elevated Z–W functional heterozygosity in young strata, which suggests potential benefits arising from heterozygote advantage. Therefore, females may benefit from buffered functional heterozygosity relative to males during severe bottlenecks, as illustrated in the Raso lark, where male heterozygosity is extremely low. W-mediated masking of deleterious Z alleles appeared rare. However, such genes were enriched for female reproductive functions, suggesting that W-mediated guarding may be preferentially retained at genes critical for female fitness (**Aim 2d**).

Sex chromosomes in speciation

In **paper IV**, I investigated a polymorphic W chromosome, hypothesising introgression as the underlying cause. Instead, I found evidence supporting an ancient W-polymorphism predating speciation, consistent with incomplete lineage sorting (**Aim 3a**).

I further investigated whether the Z chromosome — according to large-X/Z theory — acts as a stronger post-zygotic barrier to gene flow relative to autosomes. Contrary to theory, older and degenerated strata appear to introgress more and in longer tracts compared to autosomes and younger strata, consistent with long-term optimisation of gene functions and efficient hemizygous purifying selection in ancestral Z strata (**Aim 3b**).

I then investigated whether introgressed tracts may be involved in local adaptation. I found some support for reduced divergence in introgressed tracts within western taxa relative to that observed between western and eastern Eurasian taxa. Introgressed tracts were enriched for functional gene categories, indicating that introgression on the Z chromosome is not random. Together, these results suggest that local adaptation may have contributed to introgression across the Z chromosome (**Aim 3c**).

Predictability of sex chromosome evolution

In **paper II**, I investigated whether the fate of genes could be predicted following sex-linkage. I found that time since recombination cessation and longer protein-coding gene length increase the risk of relaxed selection and W degeneration, and that increased haploinsufficiency strengthens purifying selection. I also found that these factors interact in a complex ways, and with the selective regime of gametologs, resulting in high explanatory power for predicting the fate of sex-linked genes (**Aim 4a**). In **paper IV**, I investigated how the genomic landscape predicts introgression across the Z chromosome. I found that tract lengths increase in hemizygous regions, and that high linkage disequilibrium acts as a barrier to gene flow. Rapidly evolving regions are fragmented over time, potentially facilitating decoupling of incompatibilities. However, rapidly evolving, tightly linked regions can introgress in longer tracts, potentially reflecting adaptive haplotypes whose benefits outweigh the costs of incompatibilities. (**Aim 4a**).

In **paper V-VI**, I characterised sex-linked regions in the yellow-throated Nicator and several new species of larks. I found that chromosome 4 has translocated to the sex chromosomes three times within Sylvioidea, with subsequent recombination cessation of similar extent. In **paper VI**, I documented remarkable variation in recombination cessation, and found that several regions across chromosomes 3 and 5 have been repeatedly recruited in stratum-forming events, suggesting that they may carry genes predisposed to sex-linkage (**Aim 4b**).

In **paper VI**, I found that the extent of recombination cessation across chromosome 3 was associated with sexual dimorphism through increased male beaks size, and across chromosome 5 was associated with a slower pace-of-life through later reproduction, longer lifespan, and elevated IUCN Red List status (**Aim 4c**).

Future perspectives

Extraction of W sequences and other applications

I believe that my pipeline *PhaseWY* worked well for studying my research questions. However, the inference of haplotypes is relatively simple, and I am confident that the code I have published could be further improved, not only to increase accuracy, but also to better report its performance in various regions of the genome. Beyond this, *PhaseWY* is a dedicated phasing pipeline that could be adapted to phase and cluster other homologous haplotypes, such as inversions, with relatively little additional effort. To achieve this, the clustering algorithm would need to incorporate a third genotype beyond the female heterogametic and male homogametic states (an alternative homozygote). It would therefore be appropriate to add additional tools for genotype assignment at the individual level.

Transcriptomics is the next step

While I have added considerable insight into sex chromosome evolution at the level of protein-coding structure, I believe that transcriptomic data could provide important insights into several processes. For example, can it verify the functional annotation of W genes? When does gene function cease at the level of gene regulation relative to protein-coding structure? How does per-gene sex-biased expression relate to faster-Z evolution, and how does it predict the selective regime of genes and gametolog pairs? How do tissue-specific genes evolve in relation to these processes?

The rate of structural rearrangements

In discussing repeated recruitment of genomic regions to sex-linkage, I have assumed that these processes are driven by selection for genes predisposed to sex-linkage. However, it is important to recognize that the rate of various structural rearrangements in birds and other species — such as translocations and stratum-forming events — remains largely unknown. The limited evidence currently available suggests that both processes may be exceedingly rare. However, there is a bias in the detection of the two, because the inference of sex-linkage is relatively easy, while the discovery of translocations can go unnoticed without the use of long-read sequencing techniques. As the scientific community continues to investigate genomic stability among birds, it might turn out that translocations to sex chromosomes are not uncommon, but that subsequent recruitment in stratum formation is. However, if translocations prove rare, how can we determine whether the observed recruitment in stratum formation is a consequence of predisposition to sex-linkage, or an inevitable consequence of physical linkage?

Larks as a study system for sex chromosome evolution

The family-level lark dataset and population-level samples of additional *Alauda* taxa became available to me relatively late in my doctoral studies, and I believe that these datasets offer considerable potential for gaining deeper insight into both the questions addressed in this thesis and in related topics. For example, the repeated stratum-formation events along chromosomes 3 and 5 would provide a powerful model for estimating the rate of W loss-of-function in the very early stages of degeneration. Furthermore, such a model could allow detailed investigation of which genes degenerate and which appear to drive stratum formation following recombination cessation.

Furthermore, my investigation of sex chromosomes in the speciation of the genus *Alauda* is the most underdeveloped part of my thesis. The next step towards strengthening my conclusions about divergence times and better understanding the demographic history of the *Alauda* genus would be to use coalescent-based methods. Moreover, this system offers numerous opportunities for further research. For example, if Skylark populations are genetically connected along their distribution, then the W should follow a west-to-east cline in frequency. Determining where this W transition occurs, how abrupt it is, and whether it coincides with a biogeographical boundary could lend support to the hypothesis that the W polymorphism is involved in local adaptation to western and eastern Eurasia. In fact, sequences from several additional populations are available to investigate these questions, but were never included in my project due to time limitations.

Time limitations further prevented me from investigating how per-gene properties predict the probability of genes participating in Z introgression. Gene essentiality, phylogenetic conservation, and W functional status may be particularly informative predictors. I think such an approach as I utilised in **paper II-III** would give better resolution regarding the influence of hemizygous exposure, and gene conservation.

Just as the genus *Alauda* appears to experience a complex history of speciation and secondary contact, many other clades in the lark family may experience similar histories. Further exploration of other lark groups could therefore reveal opportunities for studying speciation and gene flow, while also providing unique opportunities for natural experiments of repeated introgression of the same genomic regions.

Concluding remark

In this thesis, I have contributed a little piece of the puzzle to our understanding of sex chromosome evolution. I have published a well-documented bioinformatic pipeline that enables the study of Y/W chromosomes from short-read data. I have used the capabilities of this pipeline to address several questions regarding the evolution of W and Z gametologs, how they co-evolve, and their potential role in various aspects of evolutionary ecology. Furthermore, I have produced the first chromosome-level genome assembly for larks, allowing me to verify the physical structure of the largest known avian Z chromosome to date. I have provided context for the evolution of this genome throughout lark evolution, and identified clues about the drivers of repeated events of introgression and stratum formation across its length. All in all, I have laid the foundation for larks as a study system for sex chromosome evolution, and showcased the qualities that make them an especially powerful system for addressing central questions about the role of sex chromosomes in evolutionary ecology and genome evolution.

Supplementary material

All figures and supplementary material for unpublished papers can be accessed at GitHub via the link:

https://github.com/sjellerstrand/Thesis_supplementary_material.git

or via the QR code:



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PAPER VI

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