Fine-Scale Meiotic Recombination Rate Variation and Potential Genetic Causes in Crows

by

LyAndra Antoinette Lujan

A dissertation accepted and approved in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

in Biology

Dissertation Committee:

William Cresko, Chair

Nadia Singh, Advisor

Peter Ralph, Core Member

Matthew Barber, Core Member

Nelson Ting, Institutional Representative

University of Oregon

Fall 2025

DISSERTATION ABSTRACT

LyAndra Antoinette Lujan

Doctor of Philosophy in Biology

Title: Fine-Scale Meiotic Recombination Rate Variation and Potential Causes in Crows

Recombination is a vital meiotic process that adds novel genetic variation to species. In addition to adding variation, meiotic recombination displays variation as well. This variation is in the rate of recombination. There is small-scale variation across the genome, but there is also large-scale recombination rate variation between different species. We see this recombination rate variation even between closely related species. Hooded and carrion crows are two genetically similar species with constant gene flow between them. In this dissertation, I characterize the recombination rate variation between hooded and carrion crows, and I identify potential genes contributing to this variation. In chapter 1, I create fine-scale recombination rate maps of hooded and carrion crows. I analyze the fine-scale recombination rate variation across the hooded and carrion genomes independently. Then, I compare the differences between the two species' recombination rates, analyzing the large-scale recombination rate variation between these two species. In chapter 2, I investigate meiotic recombination genes found within the crow genome. I use computational methods to identify genes with a pattern of genetic variation consistent with a deviation from neutrality. This work characterizes recombination rate variation in two closely related bird species, it starts to uncover the genetic contributions for this variation, and it builds a foundation for studying fine-scale meiotic recombination rate variation and its causes in natural populations.

DEDICATION

This dissertation is dedicated to my mom, Eloisa Lujan, and my grandmothers, Isabel Lujan and Elsa Najera. Moms are supposed to tell you that you can do anything, but mine assumes I can and expects me to do it well. Her unwavering belief in me has motivated everything I've accomplished. Isabel had the foresight to open a savings account for me that always magically had enough money for at least one semester of textbooks. She is kind and supportive despite not being able to remember a single thing about my research. Elsa is my model for how much you can accomplish if you refuse to acknowledge human limitations.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Meiotic recombination is the process of DNA exchange between homologous chromosomes. This DNA exchange is critical for fitness from both the individual and evolutionary perspectives. For an individual's fitness, recombination is a key component for proper chromosome segregation during meiosis. Improper chromosome segregation can lead to aneuploidy, resulting in pregnancy loss or birth defects (Hassold & Hunt, 2001; Koehler et al., 1996; Pezza, 2015; Webster & Schuh, 2017). From an evolutionary perspective, meiotic recombination plays a significant role in increasing population-level variation. Recombining the existing genetic variation results in increased genomic variation through the creation of novel haplotypes. These novel haplotypes can increase a population's fitness through an increase in the ability to adapt to changing environments (Burt, 2000; Muller, 1932). Recombination also aids in selection through creating combinations of beneficial alleles or removing deleterious alleles from haplotypes that would otherwise be beneficial (Hill & Robertson, 1966; Muller, 1932; Smith & Haigh, 1974).

While recombination is important for population variation, meiotic recombination also experiences variation. Variation in meiotic recombination is everywhere! Recombination rate variation has been studied across a wide range of different mammal species (Segura et al., 2013). This variation has also been found when studying species of the same family, such as closely related species of *Drosophila* (Brand et al., 2018), and flycatchers (Kawakami et al., 2017). Recombination rate differences are even found between populations. The saltwater and

freshwater threespine stickleback (*Gasterosteus aculeatus*) ecotypes shared a general pattern of recombination rate variation across their genomes (Shanfelter et al., 2019). While the pattern of recombination rate peaks and valleys was similar, the recombination rate itself was higher in the saltwater population compared to the freshwater population (Shanfelter et al., 2019). In populations of great tits, not only is there recombination rate variation between populations, but the differences in male and female recombination rates also vary between populations (Van Oers et al., 2014).

Recombination rate also exhibits fine-scale variation across the genome, where loci within close proximity to each other can have different recombination rates. The location where crossover events occur can vary significantly as well. Fine-scale variation along the genome is found in southern blue gum (*Eucalyptus globulus*) (Gion et al., 2016), black cottonwood (*Populus trichocarpa*) (Abeyratne et al., 2023), the wood white butterfly (*Leptidea sinapis*) (Torres et al., 2023), cattle (*Bos taurus*) (Yang et al., 2022), flycatchers (*Ficedula albicollis* and *F. hypoleuca*) (Kawakami et al., 2017), fruit flies (*Drosophila melanogaster*) (Chan et al., 2012; Winbush & Singh, 2022) (*D. pseudoobscura*) (Samuk et al., 2020), great tits (*Parus major*) (Van Oers et al., 2014), Eurasian blackcap (*Sylvia atricapilla*) (Bascón-Cardozo et al., 2022), and threespine stickleback (*Gasterosteus aculeatus*) (Shanfelter et al., 2019). As stated above, although the recombination rate was different between each stickleback population, the pattern of fine-scale recombination rate variation between the threespine stickleback populations remained similar (Shanfelter et al., 2019).

Obtaining these fine-scale recombination rate maps is accomplished through computational methods. Until fairly recently, fine-scale recombination rate maps were often

obtained with population-level genetic data through linkage disequilibrium-based approaches (LDhat, LDhelmet, Pyrho) (Auton & McVean, 2007; Chan et al., 2012; Spence & Song, 2019). However, with the increased accessibility of artificial intelligence and machine learning platforms, machine learning has been employed to help improve the accuracy of fine-scale recombination rate mapping with less data (Adrion et al., 2020). Using statistical methods, we can compare these fine-scale recombination rate maps between species.

What is responsible for this variation in recombination rate within and between genomes? It is clear that many factors play a role. Stress (Parsons, 1988), environment (Dreissig et al., 2019), temperature (Altindag et al., 2020; Kohl & Singh, 2018; Lloyd et al., 2018; Modliszewski et al., 2018; Saini et al., 2017; Stern, 1926), starvation (Mostoufi & Singh, 2022; Parsons, 1988), and infections (Chiriac et al., 2006; Mostoufi & Singh, 2022; Singh, 2019; Singh et al., 2015; Zilio et al., 2018) have all been found to impact recombination rate in some way. Genetic variation has also been found to impact recombination rate (Chowdhury et al., 2009a; Ritz et al., 2017). Most notable is the *mei217/218* gene in *Drosophila* (Brand et al., 2018, 2019). *Mei217/218* encodes part of a fly-specific mini chromosome complex that aids in crossing over events during meiotic recombination (Brand et al., 2019). Brand et al. found that replacing the *mei217/218* gene in *D. melanogaster* with the *D. pseudoobscura* version altered crossover events (2018, 2019), suggesting that it is possible for the genes responsible for meiotic recombination to impact recombination rate.

In many organisms, recombination hot spots have been used to study genetic contributions to recombination rate variation. This is due to the hotspot mediating gene *PRDM9*. *PRDM9* is responsible for hotspot locations in most vertebrates (Shanfelter et al., 2019). The

evolutionary history of *PRDM9* has been of particular interest because it is not found in every organism with hotspots (Singhal et al., 2015), and it does not always appear to influence hotspot location variation in every organism it is found in (Shanfelter et al., 2019).

Although most research on meiotic recombination has been on *Drosophila* and mammals, occasionally researchers look at recombination rate variation in natural populations of birds. Singhal et al. used an LD-based approach to infer fine-scale recombination rate in the zebra finch and long-tailed finch (2015). They found a correlation between GC content and recombination hot spots (Singhal et al., 2015). Later, Kawakami et al. created recombination rate maps of collared and pied flycatchers, also using an LD-based approach, and found recombination rate correlated with CpG islands (2017). However, although birds have hotspots, they have not been found to have *PRDM9,* making their recombination rate variation and any possible genetic contributions to it an interesting topic of study. Like flycatchers and great tits, the crow family offers an exceptional opportunity to study recombination rate variation in birds. The Wolf lab has studied the genetic diversity of crows across Europe and Asia, creating a hooded crow reference genome and sequences of multiple populations of hooded and carrion crows in the process. Although this is an excellent system to study recombination rate variation and any genetic contributions to it, very little is known about meiotic recombination in crows.

In chapter 1, I create fine-scale recombination rate maps for hooded and carrion crows. I calculate summary statistics in windows across the genomes. I use a wavelet analysis to compare the correlation of these summary statistics with recombination rate. I then characterize the differences between the two species' recombination rates using wavelet analysis and ANOVA.

These analyses show statistically significant differences in fine-scale recombination rate between the hooded and carrion crow species.

In chapter 2, I investigate potential genetic causes for the recombination rate differences between hooded and carrion crows. I look for deviations from neutrality in genes that are responsible for some aspects of meiotic recombination. Comparing hooded and carrion crows to collared crows and rooks, I ran McDonald Kreitman tests and CodeML to identify genes with divergent sites that are consistent with deviations from neutrality. I found seven candidate genes for further analysis to uncover genetic contributions to recombination rate diversity.

The work described in this dissertation builds a foundation for studying fine-scale recombination rate variation and potential genetic contributions to that variation in Corvidae. I characterize fine-scale recombination rate variation in hooded and carrion crows. I compare this variation to genomic features and compare similarities and differences between the two species. Then, I evaluate potential genetic contributions to the recombination rate variation between the crow species. More generally, the framework of this dissertation could be applied to study these questions in other natural populations. My doctoral work contributes to the study of recombination rate diversity, the genetic contributions of recombination, and ornithology.

References

Abeyratne, C. R., Macaya-Sanz, D., Zhou, R., Barry, K. W., Daum, C., Haiby, K., Lipzen, A., Stanton, B., Yoshinaga, Y., Zane, M., Tuskan, G. A., & DiFazio, S. P. (2023). High-resolution mapping reveals hotspots and sex-biased recombination in Populus trichocarpa. G3: Genes, Genomes, Genetics, 13(1). https://doi.org/10.1093/g3journal/jkac269

Adrion, J. R., Galloway, J. G., & Kern, A. D. (2020). Predicting the Landscape of Recombination Using Deep Learning. Molecular Biology and Evolution, 37(6), 1790–1808. https://doi.org/10.1093/molbev/msaa038

Altindag, U. H., Taylor, H. N., Shoben, C., Pownall, K. A., & Stevison, L. S. (2020). Putative condition-dependent viability selection in wild type stocks of Drosophila pseudoobscura. https://doi.org/10.1101/2020.04.10.036129

Auton, A., & McVean, G. (2007). Recombination rate estimation in the presence of hotspots. Genome Research, 17(8), 1219–1227. https://doi.org/10.1101/gr.6386707

Bascón-Cardozo, K., Bours, A., Ishigohoka, J., Odenthal-Hesse, L., & Liedvogel, M. (2022). Historical recombination maps diverge between Eurasian blackcap populations with distinct migratory strategies. In Authorea. https://doi.org/10.22541/au.166790167.72861799/v1

Brand, C. L., Cattani, M. V., Kingan, S. B., Landeen, E. L., & Presgraves, D. C. (2018). Molecular Evolution at a Meiosis Gene Mediates Species Differences in the Rate and Patterning of Recombination. Current Biology, 28(8), 1289-1295.e4. https://doi.org/10.1016/j.cub.2018.02.056

Brand, C. L., Wright, L., & Presgraves, D. C. (2019). Positive selection and functional divergence at meiosis genes that mediate crossing over across the Drosophila phylogeny. G3: Genes, Genomes, Genetics, 9(10), 3201–3211. https://doi.org/10.1534/g3.119.400280

Burt, A. (2000). Perspective: Sex, recombination, and the efficacy of selection - Was Weismann right? In Evolution (Vol. 54, Issue 2, pp. 337–351). Society for the Study of Evolution. https://doi.org/10.1111/j.0014-3820.2000.tb00038.x

Chan, A. H., Jenkins, P. A., & Song, Y. S. (2012). Genome-Wide Fine-Scale Recombination Rate Variation in Drosophila melanogaster. PLoS Genetics, 8(12). https://doi.org/10.1371/journal.pgen.1003090

Chiriac, G. I., Andronic, L. I., Bujoreanu, V. V., & Marii, L. I. (2006). Features of crossing-over in virus-infected tomato. Central European Journal of Biology, 1(3), 386–398. https://doi.org/10.2478/s11535-006-0022-6

Chowdhury, R., Bois, P. R. J., Feingold, E., Sherman, S. L., & Cheung, V. G. (2009). Genetic analysis of variation in human meiotic recombination. PLoS Genetics, 5(9). https://doi.org/10.1371/journal.pgen.1000648

Dreissig, S., Mascher, M., Heckmann, S., & Purugganan, M. (2019). Variation in Recombination Rate Is Shaped by Domestication and Environmental Conditions in Barley. Molecular Biology and Evolution, 36(9), 2029–2039. https://doi.org/10.1093/molbev/msz141

Gion, J. M., Hudson, C. J., Lesur, I., Vaillancourt, R. E., Potts, B. M., & Freeman, J. S. (2016). Genome-wide variation in recombination rate in Eucalyptus. BMC Genomics, 17(1), 1–12. https://doi.org/10.1186/s12864-016-2884-y

Hassold, T., & Hunt, P. (2001). To err (meiotically) is human: the genesis of human aneuploidy. Nature Reviews Genetics, 2(4), 280–291. https://doi.org/10.1038/35066065

Hill, W. G., & Robertson, A. (1966). The effect of linkage on limits to artificial selection. Genetical Research, 8(3), 269–294. https://doi.org/10.1017/S0016672300010156

Kawakami, T., Mugal, C. F., Suh, A., Nater, A., Burri, R., Smeds, L., & Ellegren, H. (2017). Whole-genome patterns of linkage disequilibrium across flycatcher populations clarify the causes and consequences of fine-scale recombination rate variation in birds. Molecular Ecology, 26(16), 4158–4172. https://doi.org/10.1111/mec.14197

Koehler, K. E., Hawley, R. S., Sherman, S., & Hassold, T. (1996). Recombination and nondisjunction in humans and flies. In Human Molecular Genetics (Vol. 5). Oxford University Press. http://hmg.oxfordjournals.org/

Kohl, K. P., & Singh, N. D. (2018). Experimental evolution across different thermal regimes yields genetic divergence in recombination fraction but no divergence in temperature associated plastic recombination. Evolution, 72(4), 989–999. https://doi.org/10.1111/evo.13454

Lloyd, A., Morgan, C., Franklin, F. C. H., & Bomblies, K. (2018). Plasticity of meiotic recombination rates in response to temperature in Arabidopsis. Genetics, 208(4), 1409–1420. https://doi.org/10.1534/genetics.117.300588

Modliszewski, J. L., Wang, H., Albright, A. R., Lewis, S. M., Bennett, A. R., Huang, J., Ma, H., Wang, Y., & Copenhaver, G. P. (2018). Elevated temperature increases meiotic crossover frequency via the interfering (Type I) pathway in Arabidopsis thaliana. PLoS Genetics, 14(5). https://doi.org/10.1371/journal.pgen.1007384

Mostoufi, S. L., & Singh, N. D. (2022). Diet-induced changes in titer support a discrete response of Wolbachia-associated plastic recombination in Drosophila melanogaster. G3: Genes, Genomes, Genetics, 12(1). https://doi.org/10.1093/G3JOURNAL/JKAB375

Muller, H. J. (1932). Some Genetic Aspects of Sex. The American Naturalist, 66(703), 118–138. https://doi.org/10.1086/280418

Parsons, P. A. (1988). Evolutionary rates: effects of stress upon recombination. Biological Journal of the Linnean Society, 35(1), 49–68. https://doi.org/10.1111/j.1095-8312.1988.tb00458.x

Pezza, R. J. (2015). Mechanisms of chromosome segregation in meiosis - New views on the old problem of aneuploidy. In FEBS Journal (Vol. 282, Issue 13, pp. 2411–2412). Blackwell Publishing Ltd. https://doi.org/10.1111/febs.13318

Ritz, K. R., Noor, M. A. F., & Singh, N. D. (2017). Variation in Recombination Rate: Adaptive or Not? In Trends in Genetics (Vol. 33, Issue 5, pp. 364–374). Elsevier Ltd. https://doi.org/10.1016/j.tig.2017.03.003

Saini, R., Singh, A. K., Dhanapal, S., Saeed, T. H., Hyde, G. J., & Baskar, R. (2017). Brief temperature stress during reproductive stages alters meiotic recombination and somatic mutation rates in the progeny of Arabidopsis. BMC Plant Biology, 17(1). https://doi.org/10.1186/s12870-017-1051-1

Samuk, K., Manzano-Winkler, B., Ritz, K. R., & Noor, M. A. F. (2020). Natural Selection Shapes Variation in Genome-wide Recombination Rate in Drosophila pseudoobscura. Current Biology, 30(8), 1517-1528.e6. https://doi.org/10.1016/j.cub.2020.03.053

Segura, J., Ferretti, L., Ramos-Onsins, S., Capilla, L., Farré, M., Reis, F., Oliver-Bonet, M., Fernández-Bellón, H., Garcia, F., Garcia-Caldés, M., Robinson, T. J., & Ruiz-Herrera, A. (2013). Evolution of recombination in eutherian mammals: Insights into mechanisms that affect recombination rates and crossover interference. Proceedings of the Royal Society B: Biological Sciences, 280(1771). https://doi.org/10.1098/rspb.2013.1945

Shanfelter, A. F., Archambeault, S. L., & White, M. A. (2019). Divergent Fine-Scale Recombination Landscapes between a Freshwater and Marine Population of Threespine Stickleback Fish. Genome Biology and Evolution, 11(6), 1573–1585. https://doi.org/10.1093/gbe/evz090

Singh, N. D. (2019). Wolbachia infection associated with increased recombination in Drosophila. G3: Genes, Genomes, Genetics, 9(1), 229–237. https://doi.org/10.1534/g3.118.200827

Singh, N. D., Criscoe, D. R., Skolfield, S., Kohl, K. P., Keebaugh, E. S., & Schlenke, T. A. (2015). Fruit flies diversify their offspring in response to parasite infection. Science, 349(6249), 747–750. https://doi.org/10.1126/science.aab1768

Singhal, S., Leffler, E. M., Sannareddy, K., Turner, I., Venn, O., Hooper, D. M., Strand, A. I., Li, Q., Raney, B., Balakrishnan, C. N., Griffith, S. C., McVean, G., & Przeworski, M. (2015). Stable recombination hotspots in birds. Science, 350(6263), 928–932. https://doi.org/10.1126/science.aad0843

Smith, J. M., & Haigh, J. (1974). The hitch-hiking effect of a favourable gene. Genetical Research, 23(1), 23–35. https://doi.org/10.1017/S0016672300014634

Spence, J. P., & Song, Y. S. (2019). Inference and analysis of population-specific fine-scale recombination maps across 26 diverse human populations. Science Advances, 5(10). https://doi.org/10.1126/sciadv.aaw9206

Stern, C. (1926). An Effect of Temperature and Age on Crossing-Over in the First Chromosome of Drosophila Melanogaster. Proceedings of the National Academy of Sciences of the United States of America, 12(8), 530–532. https://doi.org/10.1073/pnas.12.8.530

Torres, A. P., Höök, L., Näsvall, K., Shipilina, D., Wiklund, C., Vila, R., Pruisscher, P., & Backström, N. (2023). The fine-scale recombination rate variation and associations with genomic features in a butterfly. Genome Research, 33(5), 810–823. https://doi.org/10.1101/gr.277414.122

Van Oers, K., Santure, A. W., De Cauwer, I., Van Bers, N. E. M., Crooijmans, R. P. M. A., Sheldon, B. C., Visser, M. E., Slate, J., & Groenen, M. A. M. (2014). Replicated high-density genetic maps of two great tit populations reveal fine-scale genomic departures from sex-equal recombination rates. Heredity, 112(3), 307–316. https://doi.org/10.1038/hdy.2013.107

Webster, A., & Schuh, M. (2017). Mechanisms of Aneuploidy in Human Eggs. In Trends in Cell Biology (Vol. 27, Issue 1, pp. 55–68). Elsevier Ltd. https://doi.org/10.1016/j.tcb.2016.09.002

Winbush, A., & Singh, N. D. (2022). Variation in fine-scale recombination rate in temperatureevolved Drosophila melanogaster populations in response to selection. G3: Genes, Genomes, Genetics, 12(10). https://doi.org/10.1093/g3journal/jkac208

Yang, L., Gao, Y., Li, M., Park, K. E., Liu, S., Kang, X., Liu, M., Oswalt, A., Fang, L., Telugu, B. P., Sattler, C. G., Li, C. jun, Cole, J. B., Seroussi, E., Xu, L., Yang, L., Zhou, Y., Li, L., Zhang, H., … Liu, G. E. (2022). Genome-wide recombination map construction from single sperm sequencing in cattle. BMC Genomics, 23(1). https://doi.org/10.1186/s12864-022-08415-w

Zilio, G., Moesch, L., Bovet, N., Sarr, A., & Koella, J. C. (2018). The effect of parasite infection on the recombination rate of the mosquito Aedes aegypti. PLoS ONE, 13(10). https://doi.org/10.1371/journal.pone.0203481

CHAPTER 2

FINE-SCALE RECOMBINATION RATE VARIATION IN HOODED AND CARRION CROWS

Introduction

Meiotic Recombination, the exchange of genetic information between homologous chromosomes, is crucial for proper chromosome segregation during meiosis and for increasing population variation. At least one crossover event per chromosome is necessary because without a crossover event, meiosis can result in chromosome nondisjunction, resulting in a gamete with an incorrect number of chromosomes. This, in turn, could lead to genetic disorders or loss of pregnancy (Hassold & Hunt, 2001; Koehler et al., 1996; Pezza, 2015; Webster & Schuh, 2017). However, recombination is also an important process in increasing population variation. The exchange of genetic information between chromosomes during meiosis creates novel haplotypes, increasing genetic variation. This increase in a population's genetic variation provides more genetic and phenotypic possibilities when the population is adapting to a novel environment. Genetic variation increases the population's ability to adapt to changing fitness landscapes.

It has been widely accepted that only one crossover event per chromosome is necessary for proper segregation (Hassold & Hunt, 2001). However, we often see variation in the number

and chromosomal location of crossover events. Some organisms, like humans (Coop & Przeworski, 2007) and yeast (Kohli & Bähler, 1994; Munz, 1994), are known to have multiple crossover events per chromosome. We can also see this through the differences in large-scale recombination rates across mammals (Dumont & Payseur, 2008), and between the cottonwood species *Populus tremula*, *P. tremuloides*, and *P. trichocarpa* (J. Wang et al., 2016).

Variation in the location of the crossover events is seen in fine-scale recombination rate maps. We see this variation in the fine-scale recombination rate maps in many organisms, including southern blue gum (*Eucalyptus globulus*) (Gion et al., 2016), black cottonwood (*Populus trichocarpa*) (Abeyratne et al., 2023), the wood white butterfly (*Leptidea sinapis*) (Torres et al., 2023), and cattle (*Bos taurus*) (Yang et al., 2022). When we compare the fine-scale recombination rate maps between closely related species, we find variation as well, for example, the variation in the recombination rate maps of the flycatcher species *Ficedula albicollis* and *F. hypoleuca* (Kawakami et al., 2017). This variation is not limited to species recombination rate maps; we also find recombination rate variation between populations or ecotypes of the same species. In the fruit fly *Drosophila sp.*, there is recombination rate variation between populations of *melanogaster* (Chan et al., 2012; Winbush & Singh, 2022), as well as between populations of *pseudoobscura* (Samuk et al., 2020). The freshwater and saltwater ecotypes of threespine stickleback (*Gasterosteus aculeatus*) are known to have variation in fine-scale recombination rate maps as well (Shanfelter et al., 2019).

Recombination rate maps are vital to understanding this fine-scale recombination rate variation. These recombination rate maps were created using linkage disequilibrium-based approaches, like LDhat (Auton & McVean, 2007), or LDhelmet (Chan et al., 2012), and

population-level whole-genome data. However, with the increased accessibility of artificial intelligence, machine learning approaches have been developed for fine-scale recombination rate mapping. The software ReLERNN uses machine learning to produce more accurate recombination rate maps with a smaller sample size compared to traditional LD-based approaches (Adrion et al., 2020).

Birds offer an amazing opportunity to study meiotic recombination rate variation. We have evidence of fine-scale recombination rate differences between closely related species of flycatchers (Kawakami et al., 2017), and finches (Singhal et al., 2015). Population-level variation has been seen in populations of the great tit (Van Oers et al., 2014), as well as the Eurasian blackcap (*Sylvia atricapilla*) (Bascón-Cardozo et al., 2022, 2024). Like many other vertebrates, birds also have recombination hot spots; however, unlike many vertebrates, birds do not have the *PRDM9* gene that has been associated with vertebrate hot spots (Singhal et al., 2015). These hotspots appear to be conserved across bird species (Singhal et al., 2015). Despite these conserved hotspots, we still find variation in recombination rate within birds. This recombination rate variation, as well as conserved hotspots, makes birds an excellent system to study fine-scale recombination rate variation between species.

Due to the diligence of ornithologists, there is a wide selection of publicly available population-level whole-genome data available within birds. This study utilizes publicly available data generated to study the genetic variation between hooded and carrion crows (Poelstra et al., 2014; Vijay et al., 2016). Up until a decade ago, hooded and carrion crows were thought to be one species (Parkin et al., 2003). Hooded and carrion crows have distinct habitat boundaries with a narrow hybrid zone where they meet. Hooded and carrion crows provide a remarkable

opportunity to study fine-scale recombination rate variation between closely related species from a class known for having conserved hotspots.

This study utilizes publicly available population-level whole-genome data and machine learning approaches to create fine-scale recombination rate maps of hooded and carrion crows. We compare those recombination rate maps between the two species, as well as the population genetics summary statistics that have been shown to correlate with recombination rate. We show that species-level fine-scale recombination rate variation is evident in these extremely genetically similar species with constant and consistent gene flow.

Methods

We created a dataset using 30 *Corvus c. carrion* samples and 30 *Corvus c. cornix* samples from Poelstra et al., 2014 (Table 1). All samples were aligned to the *Corvus c. cornix* reference genome (Poelstra et al., 2014). Bwa mem (Li, 2013) was used to align samples to the reference genome. Reads were merged, validated, and haplotypes were called with GATK (Van der Auwera & O'Connor, 2020). VCF tools (Danecek et al., 2011a) were used to filter out quality scores under 15, and then GATK was used to recalibrate and recall haplotypes. Quality score filtering, recalibration, and haplotype calling were repeated until convergence. Then all individuals were combined into one variant call format (VCF) file using GATK. Single nucleotide polymorphisms (SNPs) with missing data were filtered out of our final VCF file. The species were separated into their own VCF files.

Table 1: Sample sizes

Species	Population	N
Carrion *C. cornix*	Poland	15
	Sweden	15
Hooded *C. corone*	Germany	15
	Spain	15

The recombination rate mapping software, ReLERNN (Adrion et al., 2020), was used to create fine-scale recombination rate maps for each species. We simulated the training data using the hooded crow reference genome (Poelstra et al., 2014) with a mutation rate of 3.18e-9 and a generation time of 5.79 years (Vijay et al., 2016). Neural networks were trained on separate GPUs for the hooded and carrion crows using simulated data. These neural networks were then used to predict recombination rates for the hooded and carrion crows independently. Ninety-five percent confidence intervals were calculated. Each chromosome was run separately.

The ReLERNN neural network determines the best window size to use for predicting the recombination rates. The seven chromosomes that were smaller than 10,000,000 bp long also had window sizes that resulted in 10 or fewer recombination rate estimates across the entire chromosome. We filtered out these chromosomes from further analysis. The rest of the chromosomes were assigned recombination rates for 1000 bp windows.

We used VCFTools (Danecek et al., 2011a) for pi and Fst with 1000 bp windows on SNPs with no missing data. The R package Pegas (Paradis, 2010) for Watterson's theta averaged over 1000 bp windows. The fractional GC content was calculated on 1000 bp windows using the R package seqinr (Charif & Lobry, 2007).

Wavelet was used to compare correlations between recombination rate and each summary statistic between the two crow species. The wavelet analysis was conducted in R based on a script from Spencer et al. (2006). For each species, we ran a linear model based on wavelet decompositions comparing the recombination rate as the dependent variable to pi, Watterson's theta, Fst, and GC content as independent variables. The same linear model was performed on smoothed wavelet decompositions.

ANOVA was used to evaluate species differences between recombination rates. Where recombination rate and/or summary statistics were not normally distributed, data were aligned rank transformed with the R package ARTools (Elkin et al., 2021) before undergoing the ANOVA analysis. Tukey's HSD was used as a post hoc statistical significance test.

Results

We created fine-scale recombination rate maps for hooded and carrion crows using the neural network-based approach, ReLERNN (Adrion et al., 2020). ReLERNN uses simulated data to train a neural network to predict fine-scale recombination rate maps. The deep learning approach allows ReLERNN to predict recombination rate with more accuracy than a strictly linkage disequilibrium-based approach, like LDhelmet and LDhat, on smaller sample sizes (n=20) (Adrion et al., 2020). The datasets for the hooded and carrion crow recombination rate maps were comprised of 30 individuals each. The hooded and carrion crow recombination rate maps each consist of 109,503 recombination rate estimates across 28 chromosomes. We filtered out chromosomes with 10 or fewer individual recombination rate estimates; this corresponds to chromosomes smaller than 10,000,000 bp. The resulting maps are 109,471 recombination rate estimations across 21 chromosomes (Figure 1). The ReLERNN neural network determined and assigned window sizes between 5 and 487 kb for each chromosome. To standardize window sizes across chromosomes and analysis, we assigned the ReLERNN estimated recombination rate to all 1kb windows within the larger ReLERNN-assigned windows.

We looked at Watterson's estimator, pi, number of SNPs, GC content, and Fst calculated over 1kb windows in the hooded and carrion crows (Figure 1). Pi and the number of SNPs have a significant overlap between the hooded and carrion crows in all but 3 chromosomes. Chromosomes 17, 18, and 19 have higher pi and SNP numbers in the carrion crows compared to the hooded crows. Chromosomes 17, 18, and 19 display a decrease in Watterson's estimator for both species when compared to the rest of the genome.

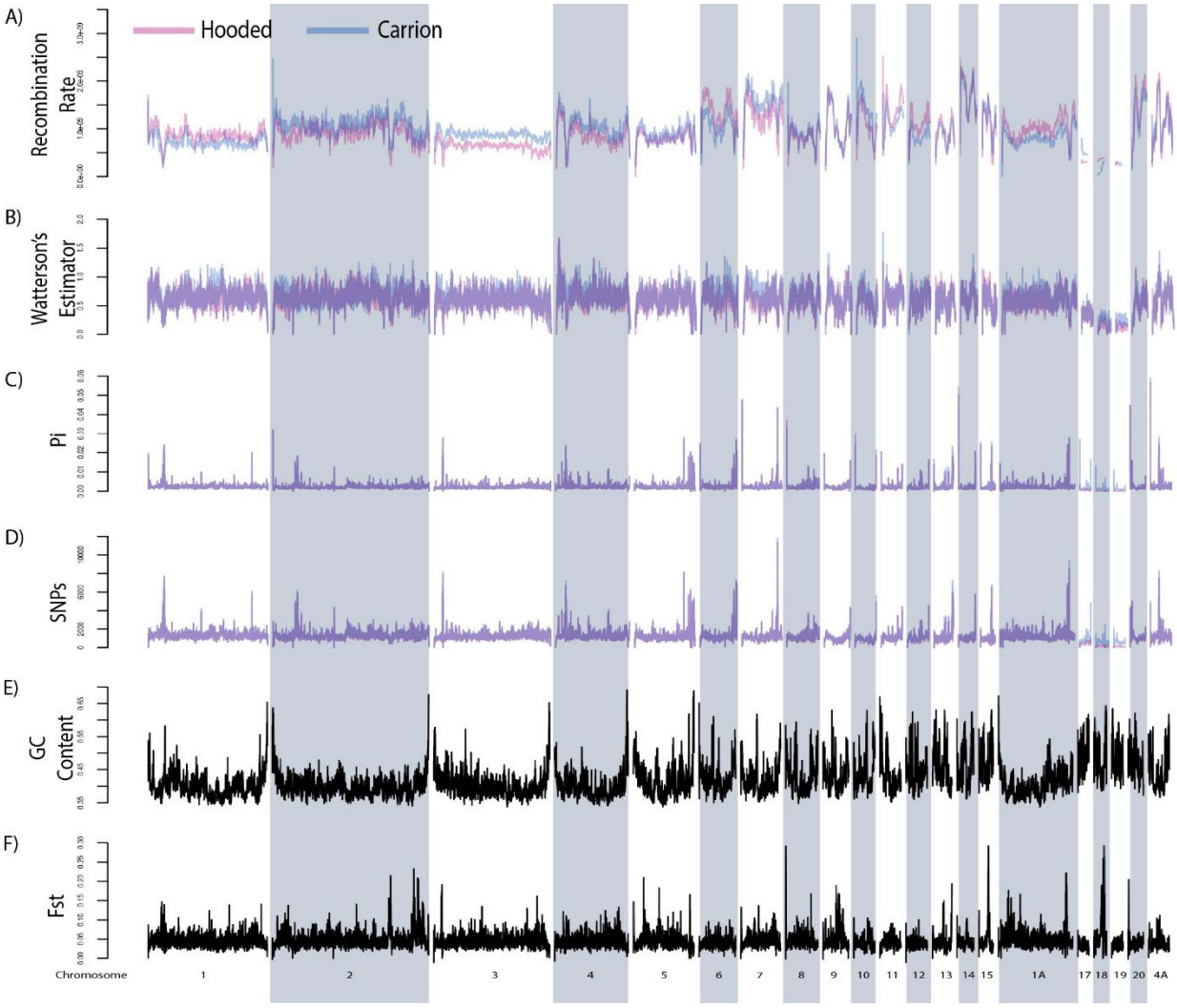

Figure 1. Hooded and Carrion crow genomic features across 21 chromosomes. Hooded crows in pink, carrion crows in blue, and purple is the overlap of both. A) Fine-scale recombination rate maps averaged over 100,000 base pair windows. B) Watterson's estimator averaged over 100,000 base pair windows. C) Pi averaged over 100,000 base pair windows. D) Total SNPs counted within 100,000 base pair windows. E) Percentage of GC content of the reference genome within 1000 base pair windows, averaged over 100,000 base pair windows. F) Fst calculated between the hooded and carrion species averaged over 100,000 base pair windows

We wavelet transformed our data to calculate the correlation between the recombination rate and each of these summary statistics. Wavelet uses short wavelike functions to provide multi-resolution data for further comparison analysis. This allowed us to run linear models on our data at window sizes from 2 kb to 131072 kb by powers of 2 (Figure 2). Instead of choosing a single arbitrary window size, we transformed our data into wavelets to compare hooded and carrion crows across many different scales at once. This allows for a more thorough investigation of recombination rate correlation with genomic features.

Hooded

	2 kb	4 kb	8 kb	16 kb	32 kb	64 kb	128 kb	256 kb	512 kb	1024 kb	2048 kb	4096 kb	8192 kb	16384 kb	32768 kb	65536 kb	131072 kb
Position	1.25	3.7	3.87	0.56	0.07	0.06	0.3	1.77	2.54	5.13	1.26	1	0.61	0.25	0.06	0.78	0.94
Chrom	Inf	0.47	0.16	1.36	0.07	0.28	0.11	0.1	1.37	0.03	5.45	0.44	0.57	1.56	0.21	0.06	0.77
fst	0.37	0.26	0.19	0.29	0.27	0.39	0.29	0.12	0.95	0.01	0.42	1.07	0.44	0.09	1.29	0.4	1.06
SNPs	1.21	1.06	0.37	0.93	1.46	1.29	0.17	0.54	1.78	1.44	0.46	2.66	2.26	1.54	0.51	0.6	1.09
pi	22.17	0.47	0.91	0.36	2.55	1.09	0.06	0.41	2	1.38	0.17	2.3	1.65	1.67	0.58	0.32	1.06
GC	Inf	0.51	2.63	9.33	36.16	36.44	26.51	20.51	22.78	16.95	4.75	0.68	0.53	0.22	1.54	1.22	1.27
Wat	Inf	39.25	118.57	Inf	Inf	Inf	264.66	165	116.43	82.3	64.63	34.81	8.07	3.15	2.44	0.03	1.23
Adj r^2	0.67	0	0	0.03	0.09	0.13	0.17	0.22	0.34	0.44	0.63	0.69	0.61	0.55	0.58	0.34	1

Carrion

	2 kb	4 kb	8 kb	16 kb	32 kb	64 kb	128 kb	256 kb	512 kb	1024 kb	2048 kb	4096 kb	8192 kb	16384 kb	32768 kb	65536 kb	131072 kb
Position	Inf	1.28	2.57	0.77	0.91	0.79	0.29	0.57	1.26	1.5	0.06	1.14	0.3	0.43	1.23	0.36	0.25
Chrom	Inf	11.16	1.56	1.86	5.25	1.09	1.27	0.84	1.16	4.26	1.74	0.88	0.8	2.63	2.02	0.58	0.01
fst	78.71	0.03	0.47	2.08	1.59	1.24	1.73	0.26	1.57	0.42	0.15	0.18	0.45	0.35	1.74	0.07	0.04
SNPs	6.54	0.09	0.23	0.37	0.38	0.72	0.83	0.79	0.46	0.22	0.68	0.22	0.13	0.01	0.39	1.38	0.88
pi	9.71	0.14	0.53	1.57	0.15	0.19	0.18	0.38	0.07	0.27	0.47	0.54	0.17	0.03	0.42	1.52	0.95
GC	Inf	0.51	0.47	13.31	17.17	40.53	29.45	15.22	9.4	9.74	1.08	2.96	0.45	0.56	0.09	0.69	0.16
Wat	Inf	102.02	Inf	Inf	Inf	Inf	252	176.5	116.24	57.88	42.86	22.27	14.07	4.77	2.2	1.71	1.11
Adj r^2	0.63	0	0.02	0.05	0.1	0.14	0.17	0.23	0.31	0.34	0.49	0.67	0.62	0.6	0.54	0.31	0.96

Figure 2. Recombination rate LM. Linear models comparing recombination rate to position, chromosome, Fst, SNP count, pi, GC content, and Watterson's estimator. Red indicates a positive correlation, and Blue indicates a negative. Intensity denotes strength.

Carrion crows had a significant positive correlation between recombination rate and Watterson's theta in 4 kb windows and the window sizes between 128 and 32768 kb, although significance decreased in the larger windows. Similarly, hooded crows showed a significant positive correlation between recombination rate and Watterson's theta in the 4kb, 8 kb, and 128 to 32768 kb windows, with the same decrease in significance in the larger windows.

While pi had the same negative correlation at 2 kb windows with a strong significance, the hooded crows also had a slightly significant positive correlation at 32 kb and 4096 kb windows. Position in the genome was negatively correlated with recombination in the 8kb windows for carrion crows. Hooded crows shared this correlation as well as a negative correlation in 4 kb windows and additionally had two more positive significant correlations in 512 kb and 1024 kb windows. Together, Watterson's theta and pi indicate that recombination rate is a good predictor of genetic diversity between both species.

The correlation between recombination rate and GC content is also remarkably similar between these two crow species. Carrion crows have a significant negative correlation in window sizes from 16 kb to 1024 kb, with a less significant correlation at 4096 kb windows. Hooded crows have the same significant negative correlation in the 16 kb to 1024 kb window sizes, with less significant correlations in the windows at both ends of that range, the 8kb windows and 1048 kb windows.

Chromosome number and SNP count had combinations of positive and negative correlations at different window sizes in one species, while having a single positive correlation in the other species at a window size that does not correlate with recombination rate in the other species. Fst has two positive correlations with recombination rate, one with strong significance for 2 kb windows and a slightly weaker significance in 16 kb windows. In hooded crows, Fst is an outlier in that it has no correlation to recombination rate at any window size.

The statistically significant correlations change drastically when we smooth our data to reduce noise (Figure 3). In carrion crows, recombination rate and chromosome were positively correlated in windows between 2 kb and 128 kb. GC content and Watterson's theta also had a

positive correlation with recombination rate in window sizes between 2 kb and 8192 kb or 65536 kb, respectively. Position has a negative correlation in 2 kb to 256 kb windows, while Fst has a negative correlation that starts at 256 kb window size and stops at the 8192 kb window size. SNP count is positively correlated from 2 kb windows to 16 kb windows but then swaps to a negative correlation at 128 kb windows, on to 32768 kb windows. This is the opposite of what we see for pi, which is negatively correlated from 2 kb to 16 kb, then positively correlated from 128 kb windows up to 65536 kb windows.

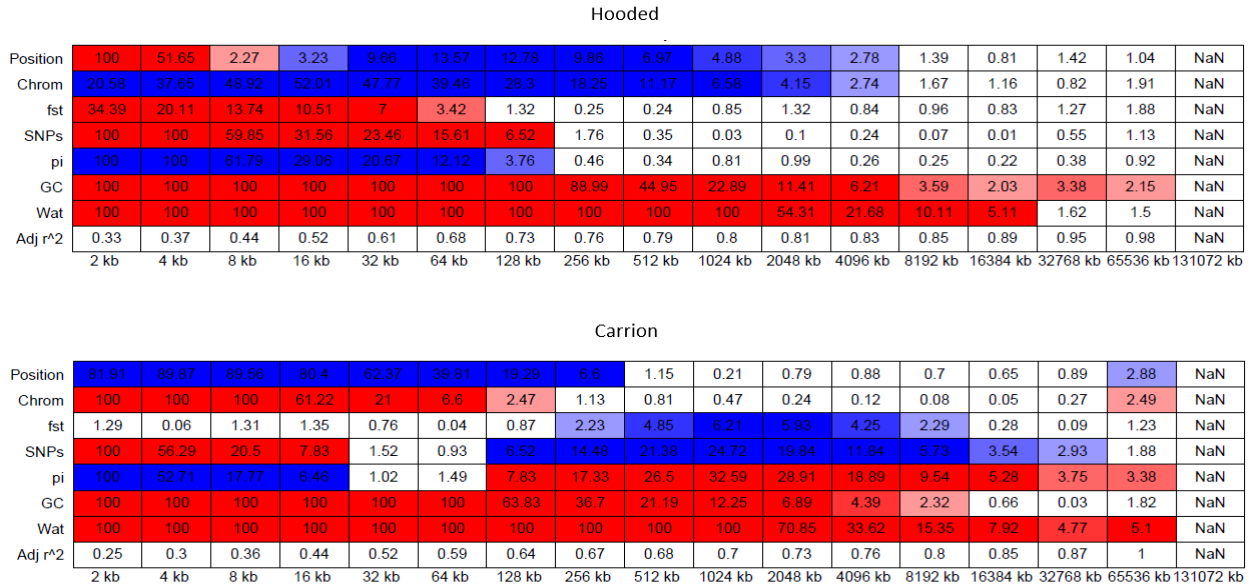

Figure 3. Recombination rate smoothed LM. Linear models comparing recombination rate to position, chromosome, Fst, SNP count, pi, GC content, and Watterson's estimator on smoothed data. Red indicates a positive correlation, and Blue indicates a negative. Intensity denotes strength.

The smoothed hooded data show that Fst is now positively correlated with recombination rate. Fst, SNP count, GC content, and Watterson's theta are all positively correlated with recombination rate starting at 2kb windows and ending at 64 kb, 128 kb, 65536 kb, and 16384 kb windows, respectively. Pi and chromosome are negatively correlated with recombination rate starting at the 2 kb windows through to the 128 kb and 4096 kb windows, respectively. Position starts positively correlated at 2 kb to 8 kb windows, then switches to negatively correlated in 16

kb windows until 4096 kb windows. These results indicate that recombination rate is an unreliable predictor of Fst, chromosome, and position when compared to measures of genetic diversity.

The pairwise correlation of recombination rate, Watterson's estimator, GC content, pi, SNP count, Fst, chromosome number, and position across all window sizes shows that all but chromosome and position are correlated throughout a wide range of window sizes (Figure 4). However, although there is a significant correlation across many different window sizes, the power analysis indicates that the smallest window size, 2 kb, is the strongest. The 2 kb window has a significant correlation between every pair for both hooded and carrion crows. Chromosome and position are opposite. The power analysis indicates that comparisons with chromosome only have power at the larger windows, but the pairwise comparisons indicate that there are no significant correlations for any possible pair with chromosome. Position is only correlated at the smallest scale, but the power analysis indicates the smallest window size is only reliable for hooded crows.

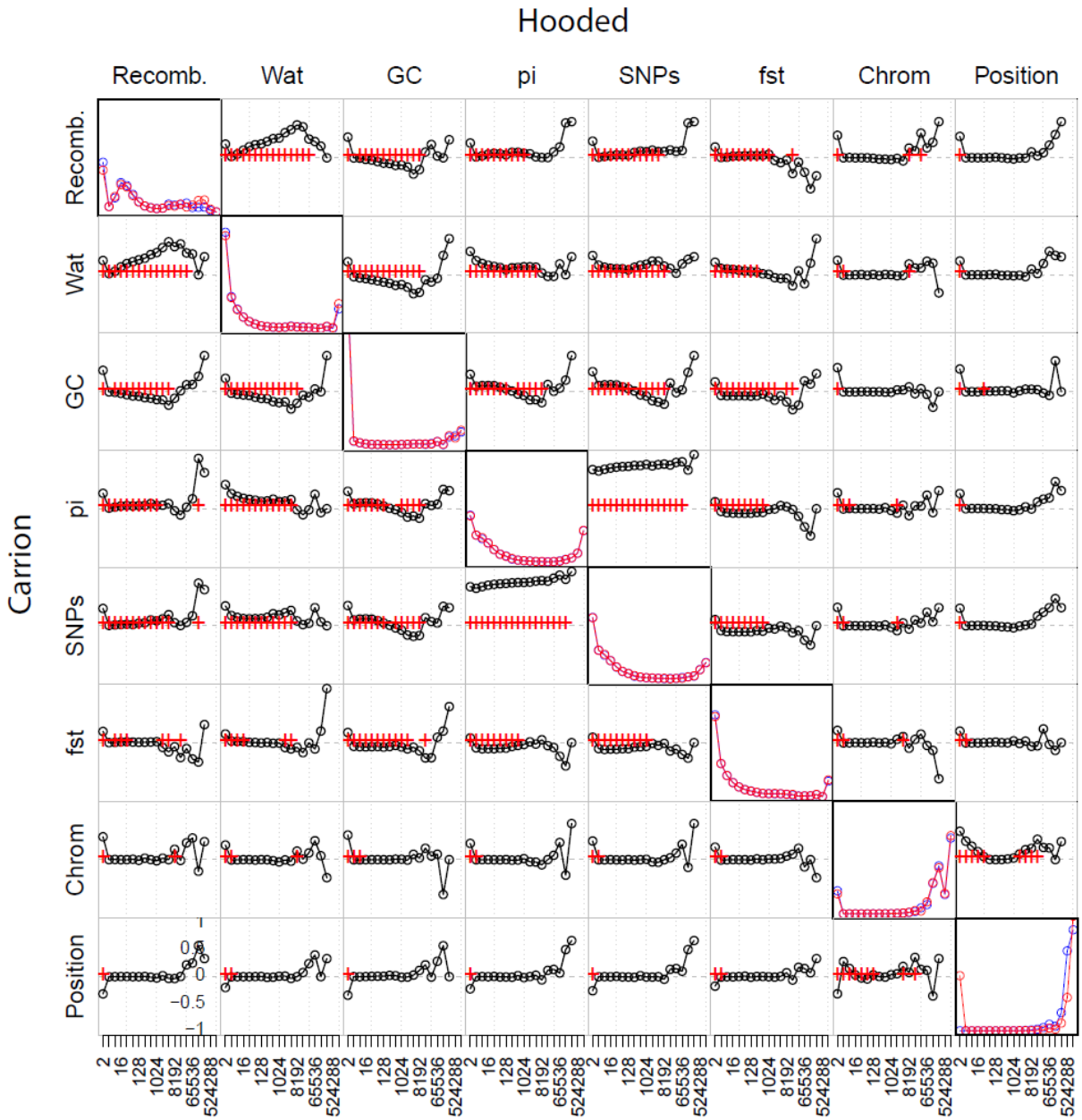

Figure 4. Power Analysis and Pairwise Correlation. The power analysis is the diagonal plots. Hooded crows in red. Carrion crows in blue. Upper right plots represent the hooded crow pairwise comparisons. The lower left represents the carrion crow pairwise comparisons. Red crosses indicate statistically significant correlations.

We used an ANOVA analysis to determine if recombination rate differences were due to the species. In the event that recombination rate was not normally distributed, we align rank transformed the data before conducting the ANOVA. Tukey's HSD was done as a post-hoc statistical test (Table 2). Chromosomes 9, 14, and 4A had no statistically significant p-values, indicating that differences in recombination rate between hooded and carrion crows along these three chromosomes are not attributed to species differences. However, every other chromosome suggests that at some level differences in recombination rate can be attributed to species differences.

Table 2. Tukey's HSD p-values

Chrom	64KB	128KB	512KB
1	<0.001	<0.001	<0.001
2	<0.001	<0.001	<0.001
3	<0.001	<0.001	<0.001
4	<0.001	<0.001	<0.001
5	<0.001	<0.001	<0.001
6	<0.001	<0.001	<0.001
7	<0.001	<0.001	<0.001
8	0.001	0.006	0.097
9	0.947	0.904	0.973
10	<0.001	<0.001	0.014
11	<0.001	<0.001	0.010
12	<0.001	<0.001	<0.001
13	0.009	0.036	0.231
14	0.377	0.514	0.811
15	0.035	0.103	0.333
1A	<0.001	<0.001	<0.001
17	<0.001	<0.001	<0.001
18	<0.001	<0.001	0.006
19	<0.001	<0.001	0.017
20	<0.001	0.001	0.075
4A	0.486	0.606	0.725

P-values for Tukey's HSD after ANOVA for each chromosome. ANOVA and Tukey's HSD were calculated across 4 different window sizes: 64 kb, 128 kb, and 512 kb. Orange denotes statistically significant p-values, intensity of color corresponds to intensity of significance.

Discussion

Fine-scale differences between hooded and carrion recombination rates.

The recombination rate maps for hooded and carrion crows share the same general structure, with one species having slightly higher recombination rates than the other for a chromosome. Which species has the higher recombination rate is not consistent throughout the genome. Additionally, for 3 of the chromosomes, there is an initial peak in recombination rate at the beginning of a chromosome in one species but not the other. For chromosomes 2 and 10, this occurs in the

carrion crows. In chromosome 11, we see the peak in hooded crows, although the carrion crows mimic the same pattern, but to a lesser extent. The smaller chromosomes, 17, 18, and 19 stand out for having a consistently low recombination rate compared to every other chromosome. We also see less variation in the recombination rate in the hooded crows along these three chromosomes. Even though the overall pattern of fine-scale recombination rate variation appears rather similar between the hooded and carrion species, there is still a marked difference in recombination rate between the two species.

The statistics back up these observations. There are statistically significant differences in the recombination rate maps between hooded and carrion crows. An ANOVA comparing recombination rates by species run on the full genome recombination rate map indicated that species had a statistically significant impact on recombination rate when run on 64 kb, 128 kb, and 512 kb windows. Due to the similarity in the structure of the recombination rate maps, we can infer that the differences we see are truly recombination rate differences and not an artifact of any differences in the quality of the maps.

Despite their genetic similarity, the recombination rate differences observed between hooded and carrion crows are consistent with what we expect when comparing recombination rate variation in bird species. Hooded and carrion crows are found to have only 0.28% of their genome that consist of differences between the two species (Poelstra et al., 2014). Much of this genetic difference is found on chromosome 18 around genes that encode coloration (Poelstra et al., 2014). Recombination rate variation is consistently found when comparing recombination rate maps of different species. However, what makes hooded and carrion crows interesting is

how genetically similar they are; they maintain phenotypic diversity in feather color yet maintain consistent gene flow. We can see they also maintain differences in fine-scale recombination rate.

Associations With Recombination Rate

The wavelet analysis suggests that the genetic features we looked at are all correlated with the recombination rate. This is not surprising considering these features have previously been found to be associated with recombination rate within different species. However, what is surprising is how different the correlation within the hooded and carrion crows can be. In the smoothed data LM, position, Fst, pi, and SNP count had differences in correlation to recombination rate. For position and Fst, those differences were positive correlations in one species and negative in the other. For pi and SNP count, the correlation started positive or negative and swapped at a larger window size in one species but remained positively or negatively correlated in the other.

The general trend was that the hooded and carrion crows had remarkably similar genetic features. Chromosomes 17, 18, and 19 tended to be different, where carrion crows had higher scores as well as more variation in scores. While the recombination rate maps are quite different for chromosomes 17 and 18, they are not uncharacteristically different when looking at the recombination rate maps across the genome.

The correlation of Fst and recombination rate is a noticeable deviation. Fst was calculated as the fixation index between the hooded and carrion crow species. Therefore, the Fst values reported are the same for both species. In the carrion wavelet model, we see Fst has a positive correlation with recombination rate at 2kb and 16 kb window sizes. The hooded wavelet model shows no correlation. However, the smoothed model shows a negative correlation between

recombination rate and Fst between the 256 kb windows and the 8192 kb windows. When we smooth out the data, we lose that positive correlation among closer sites, but we find a negative correlation across larger windows. In the hooded crows, the smoothed model shows a positive correlation within window sizes 2 kb to 64 kb. When we smooth the data, we find a positive correlation when looking at those much closer sites. This suggests that more research into the relationship between recombination rate and genetic fixation in crows is necessary.

There are some similarities when comparing the hooded and carrion crows. GC content shows a negative correlation in the wavelet model and a positive correlation in the smoothed model for both species. Watterson's estimator shows a positive correlation in both models for both species over most window sizes. This is especially interesting considering how similar the genetic features are between these two closely related species. Despite these similarities the hooded and carrion crow recombination rates still manage to display statistically significant differences.

Hooded and carrion crow recombination rate maps have a similar structure but differences in recombination rates. These differences can be attributed to species differences. Despite the genetic similarities and consistent gene flow between the two species, we still see variation in fine-scale recombination rate. This suggests the variation is likely due to what little genetic variation they have and environmental factors. Understanding where this variation in crow meiotic recombination rate comes from would help further our understanding of meiotic recombination.

Bridge

This chapter investigates the fine-scale recombination rate variation in the two crow species, hooded and carrion. Despite genetic similarity and gene flow between these two crow species, we see that there are statistically significant differences in fine-scale recombination rate. The causes of this variation remain unknown. The next chapter describes my work initiating the investigation of potential genetic determinants of recombination rate variation in crows.

References

Abeyratne, C. R., Macaya-Sanz, D., Zhou, R., Barry, K. W., Daum, C., Haiby, K., Lipzen, A., Stanton, B., Yoshinaga, Y., Zane, M., Tuskan, G. A., & DiFazio, S. P. (2023). High-resolution mapping reveals hotspots and sex-biased recombination in Populus trichocarpa. G3: Genes, Genomes, Genetics, 13(1). https://doi.org/10.1093/g3journal/jkac269

Adrion, J. R., Galloway, J. G., & Kern, A. D. (2020). Predicting the Landscape of Recombination Using Deep Learning. Molecular Biology and Evolution, 37(6), 1790–1808. https://doi.org/10.1093/molbev/msaa038

Auton, A., & McVean, G. (2007). Recombination rate estimation in the presence of hotspots. Genome Research, 17(8), 1219–1227. https://doi.org/10.1101/gr.6386707

Bascón-Cardozo, K., Bours, A., Ishigohoka, J., Odenthal-Hesse, L., & Liedvogel, M. (2022). Historical recombination maps diverge between Eurasian blackcap populations with distinct migratory strategies. In Authorea. https://doi.org/10.22541/au.166790167.72861799/v1

Bascón-Cardozo, K., Bours, A., Manthey, G., Durieux, G., Dutheil, J. Y., Pruisscher, P., Odenthal-Hesse, L., & Liedvogel, M. (2024). Fine-Scale Map Reveals Highly Variable Recombination Rates Associated with Genomic Features in the Eurasian Blackcap. Genome Biology and Evolution, 16(1). https://doi.org/10.1093/gbe/evad233

Chan, A. H., Jenkins, P. A., & Song, Y. S. (2012). Genome-Wide Fine-Scale Recombination Rate Variation in Drosophila melanogaster. PLoS Genetics, 8(12). https://doi.org/10.1371/journal.pgen.1003090

Charif, D., & Lobry, J. R. (2007). Seqin{R} 1.0-2: a contributed package to the {R} project for statistical computing devoted to biological sequences retrieval and analysis. In U. Bastolla, M. Porto, H. E. Roman, & M. Vendruscolo (Eds.), Structural approaches to sequence evolution : molecules, networks, populations (p. 367). Springer.

Coop, G., & Przeworski, M. (2007). An evolutionary view of human recombination. In Nature Reviews Genetics (Vol. 8, Issue 1, pp. 23–34). https://doi.org/10.1038/nrg1947

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. Bioinformatics, 27(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dumont, B. L., & Payseur, B. A. (2008). Evolution of the genomic rate of recombination in mammals. Evolution, 62(2), 276–294. https://doi.org/10.1111/j.1558-5646.2007.00278.x

Elkin, L. A., Kay, M., Higgins, J. J., & Wobbrock, J. O. (2021). An Aligned Rank Transform Procedure for Multifactor Contrast Tests. The 34th Annual ACM Symposium on User Interface Software and Technology, 754–768. https://doi.org/10.1145/3472749.3474784

Gion, J. M., Hudson, C. J., Lesur, I., Vaillancourt, R. E., Potts, B. M., & Freeman, J. S. (2016). Genome-wide variation in recombination rate in Eucalyptus. BMC Genomics, 17(1), 1–12. https://doi.org/10.1186/s12864-016-2884-y

Hassold, T., & Hunt, P. (2001). To err (meiotically) is human: the genesis of human aneuploidy. Nature Reviews Genetics, 2(4), 280–291. https://doi.org/10.1038/35066065

Kawakami, T., Mugal, C. F., Suh, A., Nater, A., Burri, R., Smeds, L., & Ellegren, H. (2017). Whole-genome patterns of linkage disequilibrium across flycatcher populations clarify the causes and consequences of fine-scale recombination rate variation in birds. Molecular Ecology, 26(16), 4158–4172. https://doi.org/10.1111/mec.14197

Koehler, K. E., Hawley, R. S., Sherman, S., & Hassold, T. (1996). Recombination and nondisjunction in humans and flies. In Human Molecular Genetics (Vol. 5). Oxford University Press. http://hmg.oxfordjournals.org/

Kohli, J., & Bähler, J. (1994). Homologous recombination in fission yeast: Absence of crossover interference and synaptonemal complex. Experientia, 50(3), 295–306. https://doi.org/10.1007/BF01924013

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. http://arxiv.org/abs/1303.3997

Munz, P. (1994). An analysis of interference in the fission yeast Schizosaccharomyces pombe. Genetics, 137(3), 701–707. https://doi.org/10.1093/genetics/137.3.701

Paradis, E. (2010). pegas: an R package for population genetics with an integrated–modular approach. Bioinformatics, 26(3), 419–420. https://doi.org/10.1093/bioinformatics/btp696

Parkin, D. T., Collinson, J. M., Helbig, A. J., Knox, A., & Sangster, G. (2003). The taxonomic status of Carrion and Hooded Crows. British Birds, 6(96), 274–290. https://www.researchgate.net/publication/287844978

Pezza, R. J. (2015). Mechanisms of chromosome segregation in meiosis - New views on the old problem of aneuploidy. In FEBS Journal (Vol. 282, Issue 13, pp. 2411–2412). Blackwell Publishing Ltd. https://doi.org/10.1111/febs.13318

Poelstra, J. W., Vijay, N., Bossu, C. M., Lantz, H., Ryll, B., Müller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M. G., & Wolf, J. B. W. (2014). The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science, 344(6190), 1410–1414. https://doi.org/10.1126/science.1253226

Samuk, K., Manzano-Winkler, B., Ritz, K. R., & Noor, M. A. F. (2020). Natural Selection Shapes Variation in Genome-wide Recombination Rate in Drosophila pseudoobscura. Current Biology, 30(8), 1517-1528.e6. https://doi.org/10.1016/j.cub.2020.03.053

Shanfelter, A. F., Archambeault, S. L., & White, M. A. (2019). Divergent Fine-Scale Recombination Landscapes between a Freshwater and Marine Population of Threespine Stickleback Fish. Genome Biology and Evolution, 11(6), 1573–1585. https://doi.org/10.1093/gbe/evz090

Singhal, S., Leffler, E. M., Sannareddy, K., Turner, I., Venn, O., Hooper, D. M., Strand, A. I., Li, Q., Raney, B., Balakrishnan, C. N., Griffith, S. C., McVean, G., & Przeworski, M. (2015). Stable recombination hotspots in birds. Science, 350(6263), 928–932. https://doi.org/10.1126/science.aad0843

Spencer, C. C. A., Deloukas, P., Hunt, S., Mullikin, J., Myers, S., Silverman, B., Donnelly, P., Bentley, D., & McVean, G. (2006). The influence of recombination on human genetic diversity. PLoS Genetics, 2(9), 1375–1385. https://doi.org/10.1371/journal.pgen.0020148

Torres, A. P., Höök, L., Näsvall, K., Shipilina, D., Wiklund, C., Vila, R., Pruisscher, P., & Backström, N. (2023). The fine-scale recombination rate variation and associations with genomic features in a butterfly. Genome Research, 33(5), 810–823. https://doi.org/10.1101/gr.277414.122

Van der Auwera, Geraldine., & O'Connor, Brian. (2020). Genomics in the Cloud. O'Reilly Media, Inc.

Van Oers, K., Santure, A. W., De Cauwer, I., Van Bers, N. E. M., Crooijmans, R. P. M. A., Sheldon, B. C., Visser, M. E., Slate, J., & Groenen, M. A. M. (2014). Replicated high-density genetic maps of two great tit populations reveal fine-scale genomic departures from sex-equal recombination rates. Heredity, 112(3), 307–316. https://doi.org/10.1038/hdy.2013.107

Vijay, N., Bossu, C. M., Poelstra, J. W., Weissensteiner, M. H., Suh, A., Kryukov, A. P., & Wolf, J. B. W. (2016). Evolution of heterogeneous genome differentiation across multiple contact zones in a crow species complex. Nature Communications, 7. https://doi.org/10.1038/ncomms13195

Wang, J., Street, N. R., Scofield, D. G., & Ingvarsson, P. K. (2016). Natural selection and recombination rate variation shape nucleotide polymorphism across the genomes of three related populus species. Genetics, 202(3), 1185–1200. https://doi.org/10.1534/genetics.115.183152

Webster, A., & Schuh, M. (2017). Mechanisms of Aneuploidy in Human Eggs. In Trends in Cell Biology (Vol. 27, Issue 1, pp. 55–68). Elsevier Ltd. https://doi.org/10.1016/j.tcb.2016.09.002

Winbush, A., & Singh, N. D. (2022). Variation in fine-scale recombination rate in temperatureevolved Drosophila melanogaster populations in response to selection. G3: Genes, Genomes, Genetics, 12(10). https://doi.org/10.1093/g3journal/jkac208

Yang, L., Gao, Y., Li, M., Park, K. E., Liu, S., Kang, X., Liu, M., Oswalt, A., Fang, L., Telugu, B. P., Sattler, C. G., Li, C. jun, Cole, J. B., Seroussi, E., Xu, L., Yang, L., Zhou, Y., Li, L., Zhang, H., … Liu, G. E. (2022). Genome-wide recombination map construction from single sperm sequencing in cattle. BMC Genomics, 23(1). https://doi.org/10.1186/s12864-022-08415-w

CHAPTER 3

GENETIC VARIATION IN MEIOTIC RECOMBINATION GENES IN CROWS

Introduction

Meiotic recombination, the exchange of DNA between homologous chromosomes, is critical for fitness. From an individual fitness perspective, meiotic recombination is vital for proper chromosome segregation during meiosis. Improper chromosome segregation can lead to aneuploidy, resulting in pregnancy loss or birth defects (Hassold & Hunt, 2001; Koehler et al., 1996; Pezza, 2015; Webster & Schuh, 2017). From an evolutionary perspective, meiotic recombination is important for population fitness. Meiotic recombination plays a significant role in increasing population-level variation. Recombining the existing genetic variation in a population creates novel haplotypes, resulting in increased genomic variation. This variation can aid in a population's ability to adapt to changing environments (Burt, 2000; Muller, 1932). Recombination also aids in selection through creating combinations of beneficial alleles or removing deleterious alleles from haplotypes that would otherwise be beneficial (Hill & Robertson, 1966; Muller, 1932; Smith & Haigh, 1974).

It is generally understood that only one crossover event per chromosome is necessary for proper segregation (Hassold & Hunt, 2001); however, in some organisms, we see multiple crossover events per chromosome (Coop & Przeworski, 2007; Kohli & Bähler, 1994; Munz,

1994). We see this in the differences of recombination rate averages between cottonwood species *Populus tremula*, *P. tremuloides*, and *P. trichocarpa* (J. Wang et al., 2016), and within mammals (Dumont & Payseur, 2008).

The location where crossover events occur can vary significantly as well. Fine-scale variation along the genome is found in the southern blue gum (*Eucalyptus globulus*) (Gion et al., 2016), black cottonwood (*Populus trichocarpa*) (Abeyratne et al., 2023), the wood white butterfly (*Leptidea sinapis*) (Torres et al., 2023), and cattle (*Bos taurus*) (L. Yang et al., 2022). Variation in crossover location is evident in the differences within fine-scale recombination rate maps between the flycatcher species *Ficedula albicollis* and *F. hypoleuca* (Kawakami et al., 2017). Similarly, we see crossover location differences in numerous studies that compare fine-scale recombination rate variation that exists between different populations of the same species of fruit flies *Drosophila melanogaster* (Chan et al., 2012; Winbush & Singh, 2022) and *D. pseudoobscura* (Samuk et al., 2020), great tits (*Parus major*) (Van Oers et al., 2014), Eurasian blackcap (*Sylvia atricapilla*) (Bascón-Cardozo et al., 2022), and threespine stickleback (*Gasterosteus aculeatus*) (Shanfelter et al., 2019).

The causes and consequences of this recombination rate variation have important implications for evolution. A correlation between recombination rate and fitness has been found in *D. melanogaster* (Duranton & Pool, 2022) and passerines (Gossmann et al., 2014). It has been shown that GC content and recombination rate are correlated in humans (Meunier & Duret, 2004), other mammals (Duret & Galtier, 2009), *D. melanogaster* (Chan et al., 2012), and birds (Singhal et al., 2015). Transposable elements and recombination rate have been found to be

correlated in *D. melanogaster* (Rizzon et al., 2002), mammals (Jensen-Seaman et al., 2004), and rice (Tian et al., 2009). That recombination rate is tied to many characteristics of the genome underscores the importance of understanding recombination rate variation within and between species.

However, we do not know what is causing a substantial portion of the variation in recombination rate. Environmental stressors such as temperature, starvation, and microbial infections have been found to cause differences in recombination rates. The effects of temperature have been studied in *D. melanogaster* (Kohl & Singh, 2018; Parsons, 1988; Stern, 1926), *D. pseudoobscura* (Altindag et al., 2022), *Caenorhabditis elegans* (Rose & Baillie, 1979), and *Arabidopsis thaliana* (Lloyd et al., 2018; Modliszewski et al., 2018; Saini et al., 2017). In *D. melanogaster,* the effects of Wolbachia infection on recombination rate (Singh, 2019), the effect of nutrition on recombination rate (Parsons, 1988), and the combined effects of both (Mostoufi & Singh, 2022) have been studied. Additionally, the recombination effects of viral infection in tomatoes (*Lycopersicon esculentum*) (Chiriac et al., 2006), and parasite infection in *D. melanogaster* (Jackson et al., 2015) and the mosquito *Aedes aegypti* (Zilio et al., 2018) have been studied. This type of variation, which is environment-dependent, is referred to as recombination plasticity and can be dependent on when and how long the organisms were experiencing the stressors (Jackson et al., 2015; Rybnikov et al., 2024). The plastic recombination response to these changes accounts for some of the rate variation, but what is responsible for the variation across organisms when they are experiencing the same environment?

Although genetic variation only accounts for a small amount of recombination rate variation, it has been found to impact recombination rate in many different species. In mammals, key genes have been identified to contribute to recombination rate variation. *RNF212, SYCP2, PRDM7, MEI1,* and *MSH4* have been shown to impact recombination rate differences in pigs (Brekke et al., 2022). *PRDM9*, *HFM1, MLH3, MSH4, MSH5, RNF212,* and *RNF212B* are associated with recombination rate differences in cattle (Kadri et al., 2016; Sandor et al., 2012). *REC8* and *RNF212B* have been associated with recombination rate differences in red deer (Johnston et al., 2018). *RNF212* is associated with recombination rate differences in Soay sheep (Johnston et al., 2016). *Spo11* has been shown to impact recombination in mice XY chromosome pseudo-autosomal region (Giannattasio et al., 2023). The *RNF212* gene has been associated with recombination rate variation in humans (Chowdhury et al., 2009b; Kong et al., 2008). *REC8* has also been associated with recombination rate variation in cattle (Sandor et al., 2012). *PRDM9* is linked to meiotic recombination hotspot locations in mammals (Alleva et al., 2021; Baudat et al., 2013; Sandor et al., 2012). In other systems, much less is known. In fruit flies, differences in the *D. melanogaster* and *D. pseudoobscura mei217-218* gene have been found to impact recombination events between markers on the second chromosome (Brand et al., 2019).

Recombination rate differences between species have been studied across the tree of life (Haenel et al., 2018; Kawakami et al., 2017; Singhal et al., 2015; Zhou et al., 2024). In general, meiotic recombination genes tend to be highly conserved across species (Arter & Keeney, 2024; Grishaeva & Bogdanov, 2017). Many aspects of meiotic recombination have a narrow margin of error, making the accumulation of variation in protein structure unlikely. This limited tolerance for error means we often see the proteins maintaining their role within meiotic recombination

throughout the tree of life (Arter & Keeney, 2024; Grishaeva & Bogdanov, 2017). When comparing recombination-associated gene polymorphisms between two *D. melanogaster* populations and *D. simulans,* six genes showed a significant number of nonsynonymous differences: *klp3A, ku80, mre11, mtrm, ord,* and *rad50* (Anderson et al., 2009). For instance, the recombination-associated genes *IHO1, SHOC1, SYCP2,* and *TEX11* were shown to have increased evolutionary rates signifying increased divergence within mammals (Dapper & Payseur, 2019). This divergence could be associated with the differences in recombination rates among mammals. It is tempting to speculate that these nonsynonymous divergent sites between species could be impacting the differences in the species' recombination rate. In addition, much of the genetic variation underlying variation in recombination rates is found in genes outside of the meiotic recombination pathway (Hunter et al., 2016).

However, recent evidence points to the *mei 217-218* meiotic recombination gene underlying recombination rate differences between *Drosophila mauritiana* and *D. melanogaster* species (Brand et al., 2018). Thus, genetic variation within recombination genes may hold the key to the evolution of meiotic recombination variation. Interestingly, this gene shows signals of recurrent positive selection. In an attempt to identify genes potentially underlying variation in recombination rates, we utilize publicly available whole-genome data to probe the evolutionary history of genes responsible for meiotic recombination in wild populations of Corvids. Our hope was to identify genes evolving adaptively in these lineages, which could in turn be candidates for genes underlying variation in recombination rate in this species.

Corvids allow a unique opportunity to study genes associated with meiotic recombination across two closely related species. Hooded and carrion crows have only been considered two separate species for the past twenty years (Parkin et al., 2003). Hybrid crows can be found where the hooded and carrion crow habitat zones overlap (Vijay et al., 2016). This has led to interest in these two species' evolutionary histories (Poelstra et al., 2014). This research has provided many publicly available genome sequences that we have utilized to study the genetic variation in the recombination genes within corvids.

In addition to being an ideal system in terms of data availability and evolutionary relationships, there are some examples of recombination rate differences between closely related species of birds (Kawakami et al., 2017; Singhal et al., 2015). Fine-scale recombination rate differences have been found between the flycatcher species *Ficedula albicollis* and *F. hypoleuca* (Kawakami et al., 2017), and between zebra finch and long-tailed finches (Singhal et al., 2015), indicating recombination rate differences that we have observed in fish (Shanfelter et al., 2019) and mammals (Baudat et al., 2013) are also present in birds. Similarly to other vertebrates, recombination rate hotspots have also been found in birds; however, unlike many vertebrates, the hotspot-associated gene, *PRDM9*, is not part of the bird genome (Singhal et al., 2015).

This study utilizes publicly available whole-genome data to analyze the evolutionary histories of genes responsible for meiotic recombination in *Corvidae*. We use McDonald Kreitman tests (McDonald, J. H., and Kreitman, 1991) and PAML's CodeML (Z. Yang, 2007) to identify meiotic recombination genes undergoing recent selection between carrion and hooded corvids. We find evidence of selection in *BRCC3, CNTD1, MEIOB, HFM1, RAD21l1, SYCP1,*

and *TEX11*. These genes could be contributing to the recombination rate differences between these crow species. Before further research into how the genetic variation of the recombination proteins can be conducted, we must first identify the genes with notable variation.

Methods

This study focused on 30 genes responsible for key aspects of meiotic recombination. We started with the list of genes from Dapper & Payseur, 2019 and Szasz-Green et al., 2025, and used the NCBI Genome Data Viewer to find the genes in the *C. c. cornix* reference genome assembly ASM73873v6 (GCF_000738735.6). We downloaded coding sequences of the 24 genes in the *C. c. cornix* reference genome from NCBI. For genes with multiple transcripts, we selected the transcripts with the longest aligned length. If two transcripts had the longest aligned length, we selected the one that spanned the smallest area.

Table 3. Sample sizes including outgroups

Species	Population	N
C. cornix	Poland	15
	Sweden	15
C. corone	Germany	15
	Spain	15
C. frugilegus	Ireland	1
C. pectoralis	China	1

We created a dataset using 30 *Corvus c. corone* samples and 30 *Corvus c. cornix* samples, one *Corvus frugilegus* sample, and one *Corvus c. pectoralis* from (Poelstra et al., 2014; Vijay et al., 2016) (Table 3). All samples were aligned to the *Corvus c. cornix* reference genome (Poelstra et al., 2014). Bwa mem (Li, 2013) was used to align samples to the reference genome. Reads were merged, validated, and haplotypes were called with GATK (Van der Auwera & O'Connor, 2020). VCF tools (Danecek et al., 2011b) were used to filter out quality scores under 15, and then GATK was used to

recalibrate and recall haplotypes. Quality score filtering, recalibration, and haplotype calling were repeated until convergence. Then all individuals were combined into one variant call format (VCF) file using GATK. SNPs with missing data were filtered out of our final VCF file.

For species comparison analysis, we selected 1 sample each from the hooded and carrion groups. The *C. c. cornix* reference coding sequence obtained from NCBI was used as our hooded sequence. We used the *C. c. carrion* sample SAMN02743832 as our representative carrion sample. We created a species tree with IQtree (Nguyen et al., 2015) using these 4 samples. The 4 species were aligned for the species tree using the vcf2phylip Python script from GitHub (Ortiz, 2019).

We ran McDonald Kreitman tests using the Degenotate python script from GitHub (Mirchandani et al., 2024). We obtained the annotation file for the reference genome from NCBI. We used 30 hooded samples and 30 carrion samples for the McDonald Kreitman tests comparing each species to the rook (*C. frugilegus*) sample independently. The McDonald Kreitman test was conducted on the entire chromosome for every chromosome that contained a recombination gene.

We did a phylogenetic maximum-likelihood analysis using the CodeML program in PAML (Z. Yang, 2007) with one sample from each of the four species. We used the *C. cornix* reference genome to represent hooded crows, the *C. corone* sample SAMN02743832 to represent carrion crows, and the *C. frugilegus* and *C. pectoralis* samples from the dataset to represent the rook and collared crows, respectively. For each sample, we created a FASTA file of each gene coding sequence using GATK. We MUSCLE (Edgar, 2004) aligned our coding sequences via

MEGA-CC (Tamura et al., 2021). Each alignment was examined and edited using the MEGA GUI.

We ran CodeML (Z. Yang, 2007) using the PAML command line module from the developer's website. We conducted comparisons between models M0 to M1a, M1a to M2a, M7 to M8, M0 to the branch model, and M0 to the branch site model. The branch site model was run with hooded as the foreground node and with both hooded and carrion as foreground nodes. We conducted a likelihood test and calculated p-values for each gene in R (R Core Team, 2023) using methods from Álvarez-Carretero et al. (2023).

Table 4. Meiotic recombination genes and their function

Gene	Function
MRE11	DNA repair
MLH1	Mismatch repair and Crossover resolution
NBN	DNA repair
RAD21	Sister chromatid binding
MSH5	Regulates crossovers at DSBs.
MEI4	DSB promoter complex through spo11 activation
RNF212	Stabilizes MSH4 and MSH5 to DSBs
MLH3	Mismatch repair and crossover resolution
RAD51b	Homologous chromosome pairing
RAD51	Homologous chromosome pairing
MSH4	Regulates crossovers at DSBs
HFM1	Crossover formation and MLH1 localization
REC14	DSB promoter complex through spo11 activation
IHO1	DSB promoter complex through spo11 activation
RAD50	DSB processor complex- holds DNA ends together
MEIOB	Strand invasion
HORMAD2	Synapsis surveillance
DMC1	Homologous chromosome pairing
RAD51ap1	Homologous chromosome pairing
EME1	Resolves Holliday junctions
SPATA22	Strand inversion
RAD51d	Homologous chromosome pairing
RAD51c	Homologous chromosome pairing
RAD21l1	Sister chromatid binding
SYCP2	Homologous chromosome binding
SPO11	DSB generator
TEX11	Crossover designator protein recruitment
BRCC3	DNA repair
CNTD1	Recruits MLH1 and MLH3
SYCP1	Homologous chromosome binding

Results

Corvidae recombination genes

Using the literature, we searched for genes known to encode proteins responsible for some function within the process of

meiotic recombination. Care was used to ensure we gathered genes from every step of the process, starting with double-stranded break formation to resolution and DNA repair. The literature provided a list of 41 genes responsible for, or associated with, a process within meiotic recombination. Genes responsible for meiotic recombination tend to be highly conserved within and across species (Arter & Keeney, 2024). However, not every meiotic recombination gene is found in every organism. Using the NCBI genome viewer, we found 30 of the 41genes within the C. c. cornix reference genome (Table 4). The NCBI genome viewer was used to collect gene locations and transcript IDs (Table 5).

Table 5. Genetic locations and transcripts used for all analysis for each meiotic recombination gene

Gene	Location	Transcript
MRE11	1: 38,062,321 - 38,082,670	XM_010400630.3
MLH1	2: 31,276,485 - 31,294,680	XM_039549204.1
NBN	2: 128,650,412 - 128,673,607	XM_039548284.1
RAD21	2: 140,086,756 - 140,112,015	XM_039549057.1
MSH5	3: 59,597,230 - 59,600,353	XM_019290328.3
MEI4	3: 82,615,720 - 82,710,389	XM_039568607.1
RNF212	4: 66,980,948 - 66,999,795	XM_019286955.3
MLH3	5: 23,183,774 - 23,191,578	XM_010393002.4
RAD51B	5: 33,448,410 - 33,872,332	XM_010390711.3
RAD51	5: 54,577,456 - 54,583,903	XM_010405350.4
MSH4	6: 12,603 - 37,297	XM_019291271.2
HFM1	8: 16,394,871 - 16,440,184	XM_010409089.4
REC14	10: 18,996,785 - 19,013,145	XM_039557816.1
IHO1	12: 11,596,824 - 11,622,143	XM_010390143.4
RAD50	13: 11,498,122 - 11,517,110	XM_010413071.4
MEIOB	14: 7,759,143 - 7,771,224	XM_020584049.2
HORMAD2	15: 12,132,229 - 12,156,338	XM_039561316.1
DMC1	1A: 50,303,830 - 50,316,808	XM_039570704.1
RAD51AP1	1A: 59,491,945 - 59,500,419	XM_039570147.1
EME1	18: 5,643,704 - 5,667,092	XM_010407574.4
SPATA22	19: 7,810,698 - 7,816,300	XM_019287634.3
RAD51D	19: 5,351,789 - 5,357,889	XM_020584764.2
RAD51C	19: 8,236,105 - 8,252,240	XM_039562955.1
RAD21L1	20: 7,633,306 - 7,646,080	XM_010396934.4
SYCP2	20: 10,996,682 - 11,021,591	XM_019284397.3
SPO11	20: 12,093,745 - 12,318,497	XM_019284446.2
TEX11	4A: 16,139,559 - 16,166,662	XM_019288180.2
BRCC3	4A: 16,461,747 - 16,467,508	XM_039572077.1
CNTD1	27: 1,270,330 - 1,274,548	XM_010412793.4
sycp1	Unplaced Scaffold NW_024108678.1: 112,446 – 134,248	XM_039572397.1

Population-level data show deviation from neutrality in the MEIOB gene in hooded crows

The McDonald Kreitman test uses polymorphism and divergence data to test for departures from neutrality Under neutrality, we expect the ratio synonymous changes to nonsynonymous changes to be the same for divergent sites and polymorphic sites. Because the hooded and carrion crows are too genetically similar to compare and very few loci across the genome exhibited divergent sites, hooded and carrion crows were compared independently to a more distant crow species, the rook.

When looking at the recombination genes, the McDonald Kreitman test did not show departures from neutrality for carrion crows for any genes (Table 6). The hooded crows had a gene with a significant p-value: *MEIOB* has a p-value of 0.029. This indicates a departure from neutrality in this gene. Given that the pN/pS ratio is 0/4, and the dN/dS ratio is 3/0, this could be consistent with an excess of fixed non-synonymous sites, which could be driven by positive selection.

Table 6. McDonald Kreitman

Gene	Hooded p-value	Carrion p-value
BRCC3	1	1
CNTD1	1	1
DMC1	1	1
EME1	1	1
HFM1	1	0.72
HORMAD2	1	1
IHO1	0.30	0.55
MEI4	1	0.5
MEIOB	**0.03**	0.1
MLH1	0.58	1
MLH3	0.67	0.26
MRE11	1	0.52
MSH4	1	1
MSH5	0.61	0.35
NBN	0.16	0.07
RAD21	1	1
RAD21L1	1	1
RAD50	1	0.2
RAD51	1	1
RAD51AP1	1	1
RAD51B	0.55	0.52
RAD51C	1	1
RAD51D	1	1
REC14	1	1
RNF212	1	1
SPATA22	1	1
SPO11	1	1
SYCP1	1	1
SYCP2	0.46	0.46
TEX11	1	1

p-values for McDonald Kreitman test for hooded vs. rook and carrion vs. rook.

CodeML

The CodeML program in the PAML package detects deviations from neutrality or negative selection using a maximum-likelihood phylogenetic approach to analyze codon sequence alignments. CodeML offers a complementary approach to the McDonald Kreitman, and we therefore leveraged this tool to determine if any genes were evolving in a way that was not compatible with a neutral model. CodeML fits different selection models and their corresponding neutral models. We compared the fit of each selection model to the corresponding neutral model to determine if there was evidence of selection (Table 7). The M0, or one-ratio, model applies one dn/ds ratio to every branch of the phylogeny. This is a model of neutrality and is used as the null model to compare branch or branch-site models. M1a is a nearly neutral model that allows for a dn/ds ratio below or equal to one at every codon site; it models neutrality and negative selection.

Comparing the nearly neutral M1a model to the one-ratio neutral M0 model does not assess for selection; it indicates which neutral model fits best. The genes *BRCC3* (p-value = 5e-11) and *TEX11*(p-value = 1.5e-10) fit the nearly neutral M1a model better than the neutral model M0 best, suggesting that dn/ds ratios for the genes can vary at different branches of the tree, but further analysis is necessary to determine if the gene is under selection.

Table 7. Likelihood fit for CodeML models.

Gene	Model	M0 vs. M1a p-value	M1a vs. M2a p-value	M7 vs. M8 p-value	M0 vs. Branch p-value	M0 vs. Branch site Hooded p-value	M0 vs. Branch Site Carrion p-value	M0 vs. Branch Site Hooded and Carrion p-value
BRCC3	Branch, Branch Site Hooded	≤0.001	1	0.26	<0.01	≤0.001	1	1
CNTD1	Branch	0.06	1	1	≤0.001	1	0.06	1
DMC1	Null	1	1	1	1	1	1	1
EME1	Null	1	1	1	1	1	1	1
HFM1	Branch	1	1	1	≤0.001	1	1	1
HORMAD2	Null	1	1	1	1	1	1	1
IHO1	Null	1	1	1	1	1	1	1
MEI4	Null	1	1	1	1	1	1	1
MEIOB	M8	1	1	≤0.001	1	1	1	1
MLH1	Null	1	1	1	1	1	1	1
MLH3	Null	1	1	1	1	1	1	1
MRE11	Null	1	1	1	1	1	1	1
MSH4	Null	1	1	1	1	1	1	1
MSH5	Null	1	1	1	1	1	1	1
NBN	Null	1	1	1	1	1	1	1
RAD21	Null	1	1	1	1	1	1	1
RAD21L1	B. S. Hooded and Carrion	0.18	1	1	1	1	0.18	<0.01
RAD50	Null	1	1	1	1	1	1	1
RAD51	Null	0.2	1	1	1	0.14	0.2	0.14
RAD51AP1	Null	1	1	1	1	1	1	1
RAD51B	Null	1	1	1	1	1	1	1
RAD51C	Null	1	1	1	1	1	1	1
RAD51D	Null	1	1	1	1	1	1	1
REC14	Null	1	1	1	1	1	1	1
RNF212	Null	1	1	1	1	1	1	1
SPATA22	Null	1	1	1	1	1	1	1
SPO11	Null	0.13	1	1	1	1	0.13	1
SYCP1	M2a	1	<0.01	1	1	1	1	1
SYCP2	Null	1	1	1	1	1	1	1
TEX11	M8	≤0.001	1	≤0.001	1	1	1	1

First column lists the genes, second column is the model or models with the best likelihood fit and the subsequent columns are the p-values for each model.

Site Models

Site models apply dn/ds ratios to codon sites along the sequence alignment. The nearly neutral model M1a fit dn/ds ratios equal to or below one to every codon in the sequence for every species. The corresponding selection model, M2a, allows for some codon sites to have a dn/ds greater than 1. M7 models neutrality with beta-distributed dn/ds ratios for codon sites. The selection model M8 builds upon the M7 beta model by including a class of dn/ds ratios greater than 1.

We compared the M2a selection model to the M1a null model. The gene *SYCP1* (p-value = 0.00797) had a maximum likelihood fit for the M2a model. The genes *MEIOB* (p-value = 0.000062) and *TEX11* (p-value = 0.00001) fit M8 with significant p-values. This indicates some codons within *SYCP1*, *MEIOB*, and *TEX11* are deviating from neutrality or negative selection.

Branch Model

CodeML's Branch model assigns different dn/ds ratios to the different species' branches on the gene tree. The branch model is compared to the M0 neutral model, which only assigns one dn/ds ratio to all branches. Two of our meiotic recombination genes, *BRCC3* and *CNTD1*, appear to deviate from neutrality in at least one crow species. We compared the Branch model fit to the M0 null model's fit for all 28 genes, in the case of three of our genes, *BRCC3* (p-value = 0.003), *HFM1* (p-value = 1.13e-10) and *CNTD1* (p-value= 0.00027), the branch model fit better than the null model suggesting at least one branch of the tree has a different dn/ds ratio than the others.

The Branch model for *HFM1* assigned a dn/ds ratio of 14.72 to carrion crows and 0.31 to all other species.

Branch-site Models

Branch-site models combine the dn/ds ratio schemes of branch models and site models. Each codon can be assigned a different dn/ds ratio, and they can differ for each species. Running branch-site models requires designating focal species. We ran the branch-site model 3 times, once with the hooded as our focal species, once with the carrion, and once with both the hooded and carrion as focal species. The Branch-site model is suggestive that *BRCC3* may be under selection in hooded crows. We ran the Branch-site selection model with the hooded species in the foreground and compared it to the M0 null model, and *BRCC3* fits the branch site model best with a p-value of 1.3e-11. When we compare the same branch site model but with the carrion species in the foreground to the M0 null model, none of the genes fit the M0 model best. Using that same branch site model with both the hooded and carrion species in the foreground together compared to the M0 null model, *RAD21l1* (p-value=0.005) fits the branch site selection model best. This suggests that at least one codon site in *BRCC3* is under selection in hooded crows, and at least one codon site in *RAD21l1* is under selection in both hooded and carrion crows.

Discussion

Genetic Variation in Crossover Genes

During meiosis, double-strand breaks form along the DNA strand. Those breaks can be repaired, forming a non-crossover site, or they can cross over and become recombinant sites. At least one crossover site per chromosome is necessary for proper meiosis. Conservation of the proteins responsible for processing and repairing DNA crossovers is important. Mutations that jeopardize proper crossover formation would be quickly removed from the population. However, what we see is that proteins responsible for determining sites of crossover formation, either directly or by recruiting proteins that are directly responsible for crossover sites, have been found to have divergent sites consistent with patterns of natural selection.

RAD21L1, HFM1, and CNTD1 facilitate crossovers through the activation of other proteins. RAD21L1 holds sister chromatids together to allow for crossover formation (Lee & Hirano, 2011). PAML's CodeML indicated that the hooded and carrion crow *RAD21L1* gene had markers consistent with positive selection. CNTD1 is considered a master regulator facilitating crossovers (Gray et al., 2020). CNTD1 also activates MLH1, along with MLH3, and removes recombination factors leading to non-crossover sites (Holloway et al., 2014). CodeML fits a branch model best, suggesting one or more of the branches of the phylogenetic tree have a dn/ds ratio above one, consistent with genes that have been selected in one or more species of the tree. HFM1 is part of the complex that forms the majority of crossovers (Guiraldelli et al., 2013). It is required for the localization of MSH4, which is responsible for stabilizing Holliday junctions and

directing them toward crossing over. The CodeML Branch model had a most likely fit for the *HFM1* gene, indicating that at least one branch of the phylogenetic tree had a dn/ds ratio greater than 1. These results suggest that variation in the proteins that are responsible for aiding those proteins responsible for determining where and if a crossover event occurs, could be beneficial between different crow species.

MEIOB is a dosage-sensitive regulator of crossover formation. It has been observed that when MEIOB is downregulated, there is a reduction in crossover formation (Guo et al., 2021). Despite its role in crossover regulation, MEIOB works with SPATA22 after the strand invasion phase to support the double stranded breaks (Guo et al., 2021); however, how it regulates crossover formation is unclear. The McDonald Kreitman test comparing hooded crows to the outgroup rook found that patterns of nonsynonymous and synonymous polymorphisms and divergent sites within *MEIOB* are similar to patterns in genes undergoing positive selective pressures. CodeML fits the site model M8, suggesting that there is a site within the gene that exhibits patterns consistent with positive selection.

TEX11 has also been found to be directly linked to the recombination rate in some organisms. TEX11 promotes the formation of crossovers, interacts with SYCP1, and is a precursor for the localization of MLH1 (F. Yang et al., 2008). CodeML's M8 site model had the best fit for *TEX11,* indicating that there are markers suggesting at least one codon has markers consistent with a gene undergoing natural selection.

Protein facilitating DNA repair

We find multiple proteins facilitating DNA repair with genetic variation consistent with positive selection. BRCC3 facilitates DNA repair by modulating chromatin structure, diverting from homologous recombination to non-homologous end joining (Ng et al., 2016). Knockdowns of *BRCC3* have been shown to result in inefficient DNA repair (Wang et al., 2024). CodeML had a maximum likelihood fit of a model with a dn/ds ratio higher than one in at least one codon site on the hooded crow branch of the phylogenetic tree. This is consistent with the pattern found in divergent sites undergoing natural selection in a species. Variation in the protein responsible for "marking" a double-strand break for repair before strand invasion could impact recombination rate through the reduction of DSB sites undergoing strand invasion, therefore deciding their fate before the crossover or non-crossover determining phase.

Holliday junction

The resolution in the Holliday junction is an important process, whether it ends in a crossover or a non-crossover event. While the genetic variation in the MLH1-MLH3 complex might be low due to the protein's important function, many proteins that interact with the complex appear to have some level of variation that could be positively selected for. SYCP1 is part of the transverse filaments in the synaptonemal complex, it recruits other proteins and is required for the function of MLH1 and MLH3. The CodeML site model M2a suggests that there could be a codon with markers suggesting it has a positively selected divergent site. RAD21L1,

TEX11, and CNTD1 have also been found to interact with MLH1 either through activation or through aiding in MLH1 localization.

Recombination is a vital meiotic step; it is important that the genes that encode proteins which work together in the recombination process function. However, there is no known source of the variation in recombination rate found between the hooded and carrion crow species. In this study we show that the genes responsible for crossing over, DNA repair, and Holliday junctions have genetic variation consistent with the pattern found in genes that are deviating from neutrality. It is possible that part of this recombination rate variation is caused by the genetic differences in the genes responsible for meiotic recombination.

In this chapter, I explore potential genetic contributions to recombination rate variation between hooded and carrion crows. I evaluate the evolutionary history of the genes that encode for meiotic recombination proteins. I created a list of 7 genes with the potential to contribute to this variation. The next step for this research is to further investigate the differences of each of these genes and how those differences might contribute to meiotic recombination rate variation between hooded and carrion crows.

References

Abeyratne, C. R., Macaya-Sanz, D., Zhou, R., Barry, K. W., Daum, C., Haiby, K., Lipzen, A., Stanton, B., Yoshinaga, Y., Zane, M., Tuskan, G. A., & DiFazio, S. P. (2023). High-resolution mapping reveals hotspots and sex-biased recombination in Populus trichocarpa. G3: Genes, Genomes, Genetics, 13(1). https://doi.org/10.1093/g3journal/jkac269

Alleva, B., Brick, K., Pratto, F., Huang, M., & Camerini-Otero, R. D. (2021). Cataloging Human *PRDM9* Allelic Variation Using Long-Read Sequencing Reveals *PRDM9* Population Specificity and Two Distinct Groupings of Related Alleles. Frontiers in Cell and Developmental Biology, 9. https://doi.org/10.3389/fcell.2021.675286

Altindag, U. H., Taylor, H. N., Shoben, C., Pownall, K. A., & Stevison, L. S. (2022). Putative Condition-Dependent Viability Selection in Wild-Type Stocks of Drosophila pseudoobscura. Cytogenetic and Genome Research, 162(1–2), 76–93. https://doi.org/10.1159/000522585

Álvarez-Carretero, S., Kapli, P., & Yang, Z. (2023). Beginner's Guide on the Use of PAML to Detect Positive Selection. In Molecular Biology and Evolution (Vol. 40, Issue 4). Oxford University Press. https://doi.org/10.1093/molbev/msad041

Anderson, J. A., Gilliland, W. D., & Langley, C. H. (2009). Molecular population genetics and evolution of Drosophila meiosis genes. Genetics, 181(1), 177–185. https://doi.org/10.1534/genetics.108.093807

Arter, M., & Keeney, S. (2024). Divergence and conservation of the meiotic recombination machinery. In Nature Reviews Genetics (Vol. 25, Issue 5, pp. 309–325). Nature Research. https://doi.org/10.1038/s41576-023-00669-8

Bascón-Cardozo, K., Bours, A., Ishigohoka, J., Odenthal-Hesse, L., & Liedvogel, M. (2022). Historical recombination maps diverge between Eurasian blackcap populations with distinct migratory strategies. In Authorea. https://doi.org/10.22541/au.166790167.72861799/v1

Baudat, F., Imai, Y., & De Massy, B. (2013). Meiotic recombination in mammals: Localization and regulation. In Nature Reviews Genetics (Vol. 14, Issue 11, pp. 794–806). https://doi.org/10.1038/nrg3573

Brand, C. L., Cattani, M. V., Kingan, S. B., Landeen, E. L., & Presgraves, D. C. (2018). Molecular Evolution at a Meiosis Gene Mediates Species Differences in the Rate and Patterning of Recombination. Current Biology, 28(8), 1289-1295.e4. https://doi.org/10.1016/j.cub.2018.02.056

Brand, C. L., Wright, L., & Presgraves, D. C. (2019). Positive selection and functional divergence at meiosis genes that mediate crossing over across the Drosophila phylogeny.

G3: Genes, Genomes, Genetics, 9(10), 3201–3211. https://doi.org/10.1534/g3.119.400280

Brekke, C., Berg, P., Gjuvsland, A. B., & Johnston, S. E. (2022). Recombination rates in pigs differ between breeds, sexes and individuals, and are associated with the RNF212, SYCP2, PRDM7, MEI1 and MSH4 loci. Genetics Selection Evolution, 54(1). https://doi.org/10.1186/s12711-022-00723-9

Burt, A. (2000). Perspective: Sex, recombination, and the efficacy of selection - Was Weismann right? In Evolution (Vol. 54, Issue 2, pp. 337–351). Society for the Study of Evolution. https://doi.org/10.1111/j.0014-3820.2000.tb00038.x

Chan, A. H., Jenkins, P. A., & Song, Y. S. (2012). Genome-Wide Fine-Scale Recombination Rate Variation in Drosophila melanogaster. PLoS Genetics, 8(12). https://doi.org/10.1371/journal.pgen.1003090

Chiriac, G. I., Andronic, L. I., Bujoreanu, V. V., & Marii, L. I. (2006). Features of crossing-over in virus-infected tomato. Central European Journal of Biology, 1(3), 386–398. https://doi.org/10.2478/s11535-006-0022-6

Chowdhury, R., Bois, P. R. J., Feingold, E., Sherman, S. L., & Cheung, V. G. (2009). Genetic Analysis of Variation in Human Meiotic Recombination. PLoS Genetics, 5(9), e1000648. https://doi.org/10.1371/journal.pgen.1000648

Coop, G., & Przeworski, M. (2007). An evolutionary view of human recombination. In Nature Reviews Genetics (Vol. 8, Issue 1, pp. 23–34). https://doi.org/10.1038/nrg1947

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. Bioinformatics, 27(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dapper, A. L., & Payseur, B. A. (2019). Molecular evolution of the meiotic recombination pathway in mammals. Evolution, 73(12), 2368–2389. https://doi.org/10.1111/evo.13850

Dumont, B. L., & Payseur, B. A. (2008). Evolution of the genomic rate of recombination in mammals. Evolution, 62(2), 276–294. https://doi.org/10.1111/j.1558-5646.2007.00278.x

Duranton, M., & Pool, J. E. (2022). Interactions between Natural Selection and Recombination Shape the Genomic Landscape of Introgression. Molecular Biology and Evolution, 39(7). https://doi.org/10.1093/molbev/msac122

Duret, L., & Galtier, N. (2009). Biased gene conversion and the evolution of mammalian genomic landscapes. In Annual Review of Genomics and Human Genetics (Vol. 10, pp. 285–311). https://doi.org/10.1146/annurev-genom-082908-150001

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32(5), 1792–1797. https://doi.org/10.1093/nar/gkh340

Giannattasio, T., Testa, E., Faieta, M., Lampitto, M., Nardozi, D., di Cecca, S., Russo, A., & Barchi, M. (2023). The proper interplay between the expression of Spo11 splice isoforms and the structure of the pseudoautosomal region promotes XY chromosomes recombination. Cellular and Molecular Life Sciences, 80(10). https://doi.org/10.1007/s00018-023-04912-7

Gion, J. M., Hudson, C. J., Lesur, I., Vaillancourt, R. E., Potts, B. M., & Freeman, J. S. (2016). Genome-wide variation in recombination rate in Eucalyptus. BMC Genomics, 17(1), 1–12. https://doi.org/10.1186/s12864-016-2884-y

Gossmann, T. I., Santure, A. W., Sheldon, B. C., Slate, J., & Zeng, K. (2014). Highly variable recombinational landscape modulates efficacy of natural selection in birds. Genome Biology and Evolution, 6(8), 2061–2075. https://doi.org/10.1093/gbe/evu157

Gray, S., Santiago, E. R., Chappie, J. S., & Cohen, P. E. (2020). Cyclin N-Terminal Domain-Containing-1 Coordinates Meiotic Crossover Formation with Cell-Cycle Progression in a Cyclin-Independent Manner. Cell Reports, 32(1). https://doi.org/10.1016/j.celrep.2020.107858

Grishaeva, T. M., & Bogdanov, Y. F. (2017). Evolutionary conservation of recombination proteins and variability of meiosis-specific proteins of chromosomes. Russian Journal of Genetics, 53(5), 542–550. https://doi.org/10.1134/S1022795417040081

Guiraldelli, M. F., Eyster, C., Wilkerson, J. L., Dresser, M. E., & Pezza, R. J. (2013). Mouse HFM1/Mer3 Is Required for Crossover Formation and Complete Synapsis of Homologous Chromosomes during Meiosis. PLoS Genetics, 9(3). https://doi.org/10.1371/journal.pgen.1003383

Guo, R., Xu, Y., Leu, N. A., Zhang, L., Fuchs, S. Y., Ye, L., & Wang, P. J. (2021). The ssDNA-binding protein MEIOB acts as a dosage-sensitive regulator of meiotic recombination. Nucleic Acids Research, 48(21), 12219–12233. https://doi.org/10.1093/nar/gkaa1016

Haenel, Q., Laurentino, T. G., Roesti, M., & Berner, D. (2018). Meta-analysis of chromosome-scale crossover rate variation in eukaryotes and its significance to evolutionary genomics. Molecular Ecology, 27(11), 2477–2497. https://doi.org/10.1111/mec.14699

Hassold, T., & Hunt, P. (2001). To err (meiotically) is human: the genesis of human aneuploidy. Nature Reviews Genetics, 2(4), 280–291. https://doi.org/10.1038/35066065

Hill, W. G., & Robertson, A. (1966). The effect of linkage on limits to artificial selection. Genetical Research, 8(3), 269–294. https://doi.org/10.1017/S0016672300010156

Holloway, J. K., Sun, X., Yokoo, R., Villeneuve, A. M., & Cohen, P. E. (2014). Mammalian CNTD1 is critical for meiotic crossover maturation and deselection of excess precrossover sites. Journal of Cell Biology, 205(5), 633–641. https://doi.org/10.1083/jcb.201401122

Hunter, C. M., Huang, W., Mackay, T. F. C., & Singh, N. D. (2016). The Genetic Architecture of Natural Variation in Recombination Rate in Drosophila melanogaster. PLoS Genetics, 12(4), 1–31. https://doi.org/10.1371/journal.pgen.1005951

Jackson, S., Nielsen, D. M., & Singh, N. D. (2015). Increased exposure to acute thermal stress is associated with a non-linear increase in recombination frequency and an independent linear decrease in fitness in Drosophila. BMC Evolutionary Biology, 15(1). https://doi.org/10.1186/s12862-015-0452-8

Jensen-Seaman, M. I., Furey, T. S., Payseur, B. A., Lu, Y., Roskin, K. M., Chen, C. F., Thomas, M. A., Haussler, D., & Jacob, H. J. (2004). Comparative recombination rates in the rat, mouse, and human genomes. In Genome Research (Vol. 14, Issue 4, pp. 528–538). https://doi.org/10.1101/gr.1970304

Johnston, S. E., Bérénos, C., Slate, J., & Pemberton, J. M. (2016). Conserved genetic architecture underlying individual recombination rate variation in a wild population of soay sheep (Ovis aries). Genetics, 203(1), 583–598. https://doi.org/10.1534/genetics.115.185553

Johnston, S. E., Huisman, J., & Pemberton, J. M. (2018). A genomic region containing REC8 and RNF212B is associated with individual recombination rate variation in a wild population of red deer (Cervus elaphus). G3: Genes, Genomes, Genetics, 8(7), 2265–2276. https://doi.org/10.1534/g3.118.200063

Kadri, N. K., Harland, C., Faux, P., Cambisano, N., Karim, L., Coppieters, W., Fritz, S., Mullaart, E., Baurain, D., Boichard, D., Spelman, R., Charlier, C., Georges, M., & Druet, T. (2016). Coding and noncoding variants in HFM1, MLH3, MSH4, MSH5, RNF212, and RNF212B affect recombination rate in cattle. Genome Research, 26(10), 1323–1332. https://doi.org/10.1101/gr.204214.116

Kawakami, T., Mugal, C. F., Suh, A., Nater, A., Burri, R., Smeds, L., & Ellegren, H. (2017). Whole-genome patterns of linkage disequilibrium across flycatcher populations clarify the causes and consequences of fine-scale recombination rate variation in birds. Molecular Ecology, 26(16), 4158–4172. https://doi.org/10.1111/mec.14197

Koehler, K. E., Hawley, R. S., Sherman, S., & Hassold, T. (1996). Recombination and nondisjunction in humans and flies. In Human Molecular Genetics (Vol. 5). Oxford University Press. http://hmg.oxfordjournals.org/

Kohl, K. P., & Singh, N. D. (2018). Experimental evolution across different thermal regimes yields genetic divergence in recombination fraction but no divergence in temperature

associated plastic recombination. Evolution, 72(4), 989–999. https://doi.org/10.1111/evo.13454

Kohli, J., & Bähler, J. (1994). Homologous recombination in fission yeast: Absence of crossover interference and synaptonemal complex. Experientia, 50(3), 295–306. https://doi.org/10.1007/BF01924013

Kong, A., Thorleifsson, G., Stefansson, H., Masson, G., Helgason, A., Gudbjartsson, D. F., Jonsdottir, G. M., Gudjonsson, S. A., Sverrisson, S., Thorlacius, T., Jonasdottir, A., Hardarson, G. A., Palsson, S. T., Frigge, M. L., Gulcher, J. R., Thorsteinsdottir, U., & Stefansson, K. (2008). Sequence Variants in the RNF212 Gene Associate with Genome-Wide Recombination Rate. Science, 319(5868), 1398–1401. https://doi.org/10.1126/science.1152422

Lee, J., & Hirano, T. (2011). RAD21L, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. Journal of Cell Biology, 192(2), 263–276. https://doi.org/10.1083/jcb.201008005

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. http://arxiv.org/abs/1303.3997

Lloyd, A., Morgan, C., Franklin, F. C. H., & Bomblies, K. (2018). Plasticity of meiotic recombination rates in response to temperature in arabidopsis. Genetics, 208(4), 1409–1420. https://doi.org/10.1534/genetics.117.300588

McDonald, J. H., and Kreitman, M. (1991). Adaptive protein evolution at the Adh locus in Drosophila. Nature, 351, 652–654.

Meunier, J., & Duret, L. (2004). Recombination drives the evolution of GC-content in the human genome. Molecular Biology and Evolution, 21(6), 984–990. https://doi.org/10.1093/molbev/msh070

Mirchandani, C. D., Shultz, A. J., Thomas, G. W. C., Smith, S. J., Baylis, M., Arnold, B., Corbett-Detig, R., Enbody, E., & Sackton, T. B. (2024). A Fast, Reproducible, High-Throughput Variant Calling Workflow for Population Genomics. Molecular Biology and Evolution, 41(1). https://doi.org/10.1093/molbev/msad270

Modliszewski, J. L., Wang, H., Albright, A. R., Lewis, S. M., Bennett, A. R., Huang, J., Ma, H., Wang, Y., & Copenhaver, G. P. (2018). Elevated temperature increases meiotic crossover frequency via the interfering (Type I) pathway in Arabidopsis thaliana. PLoS Genetics, 14(5). https://doi.org/10.1371/journal.pgen.1007384

Mostoufi, S. L., & Singh, N. D. (2022). Diet-induced changes in titer support a discrete response of Wolbachia-associated plastic recombination in Drosophila melanogaster. G3: Genes, Genomes, Genetics, 12(1). https://doi.org/10.1093/G3JOURNAL/JKAB375

Muller, H. J. (1932). Some Genetic Aspects of Sex. The American Naturalist, 66(703), 118–138. https://doi.org/10.1086/280418

Munz, P. (1994). An analysis of interference in the fission yeast Schizosaccharomyces pombe. Genetics, 137(3), 701–707. https://doi.org/10.1093/genetics/137.3.701

Ng, H. M., Wei, L., Lan, L., & Huen, M. S. Y. (2016). The Lys63-deubiquitylating enzyme BRCC36 limits DNA break processing and repair. Journal of Biological Chemistry, 291(31), 16197–16207. https://doi.org/10.1074/jbc.M116.731927

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution, 32(1), 268–274. https://doi.org/10.1093/molbev/msu300

Ortiz, E. M. (2019). vcf2phylip v2.0: convert a VCF matrix into several matrix formats for phylogenetic analysis (v2.0). Zenodo.

Parkin, D. T., Collinson, J. M., Helbig, A. J., Knox, A., & Sangster, G. (2003). The taxonomic status of Carrion and Hooded Crows. British Birds, 6(96), 274–290. https://www.researchgate.net/publication/287844978

Parsons, P. A. (1988). Evolutionary rates: effects of stress upon recombination. Biological Journal of the Linnean Society, 35(1), 49–68. https://doi.org/10.1111/j.1095-8312.1988.tb00458.x

Pezza, R. J. (2015). Mechanisms of chromosome segregation in meiosis - New views on the old problem of aneuploidy. In FEBS Journal (Vol. 282, Issue 13, pp. 2411–2412). Blackwell Publishing Ltd. https://doi.org/10.1111/febs.13318

Poelstra, J. W., Vijay, N., Bossu, C. M., Lantz, H., Ryll, B., Müller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M. G., & Wolf, J. B. W. (2014). The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science, 344(6190), 1410–1414. https://doi.org/10.1126/science.1253226

R Core Team. (2023). R: A Language and Environment for Statistical Computing. https://www.R-project.org/.

Rizzon, C., Marais, G., Gouy, M., & Biémont, C. (2002). Recombination rate and the distribution of transposable elements in the Drosophila melanogaster genome. Genome Research, 12(3), 400–407. https://doi.org/10.1101/gr.210802

Rose, A. M., & Baillie, D. L. (1979). THE EFFECT OF TEMPERATURE AND PARENTAL AGE ON RECOMBINATION AND NONDISJUNCTION IN CAENORHABDITIS ELEGANS. Genetics, 92(2), 409–418. https://doi.org/10.1093/genetics/92.2.409

Rybnikov, S. R., Hübner, S., & Korol, A. B. (2024). A Numerical Model Supports the Evolutionary Advantage of Recombination Plasticity in Shifting Environments. American Naturalist, 203(3), E78–E91. https://doi.org/10.1086/728405

Saini, R., Singh, A. K., Dhanapal, S., Saeed, T. H., Hyde, G. J., & Baskar, R. (2017). Brief temperature stress during reproductive stages alters meiotic recombination and somatic mutation rates in the progeny of Arabidopsis. BMC Plant Biology, 17(1). https://doi.org/10.1186/s12870-017-1051-1

Samuk, K., Manzano-Winkler, B., Ritz, K. R., & Noor, M. A. F. (2020). Natural Selection Shapes Variation in Genome-wide Recombination Rate in Drosophila pseudoobscura. Current Biology, 30(8), 1517-1528.e6. https://doi.org/10.1016/j.cub.2020.03.053

Sandor, C., Li, W., Coppieters, W., Druet, T., Charlier, C., & Georges, M. (2012). Genetic variants in *REC8*, *RNF212*, and *PRDM9* influence male recombination in cattle. PLoS Genetics, 8(7). https://doi.org/10.1371/journal.pgen.1002854

Shanfelter, A. F., Archambeault, S. L., & White, M. A. (2019). Divergent Fine-Scale Recombination Landscapes between a Freshwater and Marine Population of Threespine Stickleback Fish. Genome Biology and Evolution, 11(6), 1573–1585. https://doi.org/10.1093/gbe/evz090

Singh, N. D. (2019). Wolbachia infection associated with increased recombination in drosophila. G3: Genes, Genomes, Genetics, 9(1), 229–237. https://doi.org/10.1534/g3.118.200827

Singhal, S., Leffler, E. M., Sannareddy, K., Turner, I., Venn, O., Hooper, D. M., Strand, A. I., Li, Q., Raney, B., Balakrishnan, C. N., Griffith, S. C., McVean, G., & Przeworski, M. (2015). Stable recombination hotspots in birds. Science, 350(6263), 928–932. https://doi.org/10.1126/science.aad0843

Smith, J. M., & Haigh, J. (1974). The hitch-hiking effect of a favourable gene. Genetical Research, 23(1), 23–35. https://doi.org/10.1017/S0016672300014634

Stern, C. (1926). An Effect of Temperature and Age on Crossing-Over in the First Chromosome of Drosophila Melanogaster. Proceedings of the National Academy of Sciences of the United States of America, 12(8), 530–532. https://doi.org/10.1073/pnas.12.8.530

Szasz-Green, T., Shores, K., Vanga, V., Zacharias, L., Lawton, A. K., & Dapper, A. L. (2025). Comparative Phylogenetics Reveal Clade-specific Drivers of Recombination Rate Evolution Across Vertebrates. Molecular Biology and Evolution, 42(5). https://doi.org/10.1093/molbev/msaf100

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tian, Z., Rizzon, C., Du, J., Zhu, L., Bennetzen, J. L., Jackson, S. A., Gaut, B. S., & Ma, J. (2009). Do genetic recombination and gene density shape the pattern of DNA elimination in rice long terminal repeat retrotransposons? Genome Research, 19(12), 2221–2230. https://doi.org/10.1101/gr.083899.108

Torres, A. P., Höök, L., Näsvall, K., Shipilina, D., Wiklund, C., Vila, R., Pruisscher, P., & Backström, N. (2023). The fine-scale recombination rate variation and associations with genomic features in a butterfly. Genome Research, 33(5), 810–823. https://doi.org/10.1101/gr.277414.122

Van der Auwera, Geraldine., & O'Connor, Brian. (2020). Genomics in the Cloud. O'Reilly Media, Inc.

Van Oers, K., Santure, A. W., De Cauwer, I., Van Bers, N. E. M., Crooijmans, R. P. M. A., Sheldon, B. C., Visser, M. E., Slate, J., & Groenen, M. A. M. (2014). Replicated high-density genetic maps of two great tit populations reveal fine-scale genomic departures from sex-equal recombination rates. Heredity, 112(3), 307–316. https://doi.org/10.1038/hdy.2013.107

Vijay, N., Bossu, C. M., Poelstra, J. W., Weissensteiner, M. H., Suh, A., Kryukov, A. P., & Wolf, J. B. W. (2016). Evolution of heterogeneous genome differentiation across multiple contact zones in a crow species complex. Nature Communications, 7. https://doi.org/10.1038/ncomms13195

Wang, J., Street, N. R., Scofield, D. G., & Ingvarsson, P. K. (2016). Natural selection and recombination rate variation shape nucleotide polymorphism across the genomes of three related populus species. Genetics, 202(3), 1185–1200. https://doi.org/10.1534/genetics.115.183152

Wang, Z., Wang, C., Zhai, Y., Bai, Y., Wang, H., & Rong, X. (2024). Loss of Brcc3 in Zebrafish Embryos Increases Their Susceptibility to DNA Damage Stress. International Journal of Molecular Sciences, 25(25). https://doi.org/10.3390/ijms252212108

Webster, A., & Schuh, M. (2017). Mechanisms of Aneuploidy in Human Eggs. In Trends in Cell Biology (Vol. 27, Issue 1, pp. 55–68). Elsevier Ltd. https://doi.org/10.1016/j.tcb.2016.09.002

Winbush, A., & Singh, N. D. (2022). Variation in fine-scale recombination rate in temperatureevolved Drosophila melanogaster populations in response to selection. G3: Genes, Genomes, Genetics, 12(10). https://doi.org/10.1093/g3journal/jkac208

Yang, F., Gell, K., Van Der Heijden, G. W., Eckardt, S., Leu, N. A., Page, D. C., Benavente, R., Her, C., Höög, C., McLaughlin, K. J., & Wang, P. J. (2008). Meiotic failure in male mice lacking an X-linked factor. Genes and Development, 22(5), 682–691. https://doi.org/10.1101/gad.1613608

Yang, L., Gao, Y., Li, M., Park, K. E., Liu, S., Kang, X., Liu, M., Oswalt, A., Fang, L., Telugu, B. P., Sattler, C. G., Li, C. jun, Cole, J. B., Seroussi, E., Xu, L., Yang, L., Zhou, Y., Li, L., Zhang, H., … Liu, G. E. (2022). Genome-wide recombination map construction from single sperm sequencing in cattle. BMC Genomics, 23(1). https://doi.org/10.1186/s12864-022-08415-w

Yang, Z. (2007). PAML 4: Phylogenetic Analysis by Maximum Likelihood. Molecular Biology and Evolution, 24(8), 1586–1591. https://doi.org/10.1093/molbev/msm088

Zhou, W., Zhang, N., Huang, K., Lin, H., Tu, J., Zheng, C., Que, P., Chiang, C.-Y., Martinez, J., Naerhulan, H., Székely, T., Zhang, Z., & Liu, Y. (2024). Divergent Selection in Low Recombination Regions Shapes the Genomic Islands in Two Incipient Shorebird Species. Molecular Biology and Evolution, 41(2). https://doi.org/10.1093/molbev/msae006

Zilio, G., Moesch, L., Bovet, N., Sarr, A., & Koella, J. C. (2018). The effect of parasite infection on the recombination rate of the mosquito Aedes aegypti. PLoS ONE, 13(10). https://doi.org/10.1371/journal.pone.0203481

CHAPTER 4

CONCLUSION

This dissertation describes my work investigating recombination rate variation in crows. Recombination rate variation is everywhere. We see variation on large scales, within and between species and populations. We also find fine-scale variation along the genome. While we know that environmental and genetic factors can play a part in the large-scale and fine-scale variation, the nuances of their contributions are still being uncovered.

Before my research, we knew that some genes responsible for recombination were linked to variation in flies. We also knew that some variation was often associated with the hotspot mediating gene *PRDM9.* Birds do not lack hotspots, but they do lack *PRDM9*. Hotspots in birds appear to be conserved across species, however they still exhibit species level variation in recombination rate. My goal was to characterize recombination rate variation and identify genes that could be contributing to that variation in crows, a system that does not have *PRDM9*.

In my first chapter, I investigate the fine-scale recombination rate variation between hooded and carrion crows. Before my research, we did not have information on the fine-scale recombination rate in crows. I was able to map recombination rate across the genome in two crow species and confirm that hooded and carrion crows have statistically significant fine-scale recombination rate variation. My results also demonstrated that the recombination rate was correlated with a plethora of summary statistics in both hooded and carrion crows. Findings from

this chapter contribute to a growing body of fine-scale recombination rate maps and an understanding of genome characteristics associated with recombination rate.

In my second chapter, I explored patterns of selection in the genes that encode proteins responsible for meiotic recombination in crows. My findings demonstrate that some meiotic recombination genes have characteristics consistent with a deviation from neutrality that we associate with positive selection in crows. These results suggest that there is genetic variation in these crow species that could be contributing to the recombination rate variation that was found in Chapter 1. Future work can map the genetic sites that have deviated from neutrality to the protein structure. Estimating how these polymorphisms could impact protein structure and function could aid in our understanding of whether and how these genes are contributing to recombination rate variation.

Prior to my dissertation, recombination rate variation in crows had not been studied. Therefore, there was no consideration into what could be responsible for recombination rate variation in crows. Now we know that there is recombination rate variation between hooded and carrion crows. We also have a list of candidate genes that could be aiding in this variation. These findings will allow us to further analyze genetic components of recombination rate variation and develop a framework for investigating the genetic contributions to the variation in natural populations.

APPENDIX A

SUPPLEMENTAL DATA FOR DATASET

Supplemental Table 1. Samples included in VCF dataset

Sample	Species	BioProject	Population
SAMEA5089891	*Corvus pectoralis*	PRJEB9057	China Guangxi
SAMN02843223	*Corvus frugilegus*	PRJNA192205	Ireland LoughMoneyFarm Belfast
SAMN02743847	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743846	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743845	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743844	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743843	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743842	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743841	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743840	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743839	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743838	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743837	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743836	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743835	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743834	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743833	*Corvus corone*	PRJNA192205	Spain Villaseca de la Sorriba
SAMN02743832	*Corvus corone*	PRJNA192205	Germany Radolfzell
SAMN02743831	*Corvus corone*	PRJNA192205	Germany Radolfzell
SAMN02743830	*Corvus corone*	PRJNA192205	Germany Radolfzell
SAMN02743829	*Corvus corone*	PRJNA192205	Germany Radolfzell
SAMN02743828	*Corvus corone*	PRJNA192205	Germany Radolfzell
SAMN02743827	*Corvus corone*	PRJNA192205	Germany Radolfzell
SAMN02743826	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743825	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743824	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743823	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743822	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743821	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743820	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743819	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02743818	*Corvus corone*	PRJNA192205	Germany Konstanz
SAMN02439834	*Corvus cornix*	PRJNA192205	Sweden Uppsala

Continued on next page.

Supplemental Table 1 Continued

Sample	Species	BioProject	Population
SAMN02439833	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439832	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439831	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439830	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439829	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439828	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439827	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439826	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439825	*Corvus cornix*	PRJNA192205	Sweden Uppsala
SAMN02439824	*Corvus cornix*	PRJNA192205	Sweden Rimbo
SAMN02439823	*Corvus cornix*	PRJNA192205	Sweden Rimbo
SAMN02439822	*Corvus cornix*	PRJNA192205	Sweden Rimbo
SAMN02439821	*Corvus cornix*	PRJNA192205	Sweden Rimbo
SAMN02439820	*Corvus cornix*	PRJNA192205	Sweden Rimbo
SAMN02439819	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439818	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439817	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439816	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439815	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439814	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439813	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439812	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439811	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439810	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439809	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439808	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439807	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439806	*Corvus cornix*	PRJNA192205	Poland Warsaw
SAMN02439805	*Corvus cornix*	PRJNA192205	Poland Warsaw