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DRIVERS OF INTROGRESSION AND FITNESS IN THE SALTMARSH-NELSON'S
SPARROW HYBRID ZONE

BY

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Baccalaureate Degree (BS), University of New Hampshire, 2013

THESIS

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ABSTRACT

DRIVERS OF INTROGRESSION AND FITNESS IN THE SALTMARSH-NELSON'S
SPARROW HYBRID ZONE

by

Logan M. Maxwell

University of New Hampshire, September, 2018

Hybrid zones can provide an understanding of the genetic basis of biodiversity

maintenance and as well as insight into how interacting species respond to climate change, and

how climate change may alter patterns of introgression. This body of research focuses on

dynamics of hybridization between the Saltmarsh (*Ammospiza caudacutus*) and Nelson's

Sparrow (*A. nelsoni*) across two populations in the center of the hybrid zone to gain a window

into both the evolutionary processes underlying the relationship between these species and the

role of climate change and adaptive introgression on the future persistence of the two sparrows.

In Chapter 1, I determined patterns of introgression between Saltmarsh and Nelson's Sparrows

on a fine-scale across a habitat gradient and on a broad-scale through comparison with known

patterns in the southern range of the zone. I explored the fitness consequences of hybridization to

female Saltmarsh, Nelson's and hybrid sparrows in relation to environmental conditions and tidal

marsh nesting adaptations in Chapter 2. Finally, in Chapter 3, I evaluated the relative fitness of

male Saltmarsh, Nelson's and hybrid individuals in relation to competitive ability and male

condition.

I intensively sampled sparrow adults (n = 218) and chicks (n = 326) and determined the

success of 201 nests over two years at two marshes in the center of the hybrid zone located at

Popham Beach State Park and Wharton Point on Maquoit Bay on the northeastern coast

of the United States, between Brunswick, Maine and Phippsburg, Maine. I used a ddRAD

sequencing approach to identify a panel of135 fixed SNPs, which I used to calculate a hybrid

index and determine the genotypic composition of individuals and the level of admixture of the

populations. In addition, a separate panel of 589 SNPs was used to assign paternity to offspring

and reconstruct mating pairs. I compared genotypic composition and patterns of introgression

across two sites in the center of the hybrid zone with previous work done in the southern portion

of the hybrid zone. I tested for reduced survival of hybrid females in support of Haldane's Rule

and also for assortative mating between the species. I modeled daily nest survival and fledging

success between Saltmarsh, Nelson's and hybrid females in relation to tidal cycles and known

tidal marsh nesting adaptations. Lastly, I compared the number of offspring sired by Saltmarsh,

Nelson's and hybrid males in relation to male condition, as measured by three secondary and one

primary male sexual traits.

I found that population density differences across the hybrid zone influenced patterns of

introgression, such that in the center of the zone there is relatively equal backcrossing in both the

Saltmarsh and Nelson's Sparrow direction compared to asymmetric backcrossing toward the

Saltmarsh Sparrow in the southern hybrid zone (Walsh et al., 2015a). Local site-specific

characteristics of the two study populations influenced the distribution of genotypes and patterns

of introgression across a tidal marsh habitat gradient, such that there were a higher number of

hybrids and more backcrossing towards Nelson's Sparrow at the inland than coastal site. I also

observed twice as many recent-generation hybrid female nestlings than adults in the population,

supporting Haldane's Rule, and a significant correlation between mother and father hybrid index

($r = 0.73$, $P < 0.0001$), indicative of assortative mating.

I found differential fitness among Saltmarsh, Nelson's and hybrid females. Birds with predominantly Saltmarsh Sparrow alleles had higher reproductive success than birds with predominantly Nelson's Sparrows alleles, with hybrids being intermediate between the two. Fledging success models suggested that the number of offspring fledged also increased with two known tidal marsh nesting adaptations: nest height and nesting synchrony with tidal cycles. I found a positive relationship between hybrid index and fitness in daily nest survival in 2016, but not across both breeding seasons (2016 & 2017) combined, likely due to differing levels of nest flooding. The strongest and most consistent predictors of daily nest survival were nesting synchrony with lunar tidal flooding cycles (female behavioral adaptation) and daily maximum tide height. I also found differential male fitness, with Saltmarsh Sparrows siring more offspring than Nelson's Sparrows (ANOVA; $F = 3.81$, $P = 0.04$) and hybrids intermediate in fitness, although more similar to Nelson's Sparrows. Cloacal Protuberance (CP) volume and body mass were significant predictors of interspecific fitness, providing evidence that pre and post copulatory sexual selection may be acting on body size and CP volume (as a proxy for sperm competition) to drive mating patterns within and between the Saltmarsh and Nelson's Sparrows. Saltmarsh-Nelson's Sparrow hybrid zone structure and maintenance appear to be driven by endogenous and exogenous factors at multiple spatial scales. Fitness differences among parental species and hybrids, relative population densities and species distributions, differential adaptation to local environments, and pre-zygotic and post-zygotic reproductive isolating mechanisms all play a role in the dynamics of this hybrid zone.

INTRODUCTION

Natural hybrid zones have long been recognized as "windows into the evolutionary process" (Harrison 1990). Multiple generations of gene flow and recombination between taxa can have largely varying evolutionary outcomes, in some cases stimulating adaptive evolution, and in others disrupting local adaptation (Fitzpatrick, et al. 2015). When hybrid individuals have greater fitness than one or both of the parental taxa, it can lead to hybrid swarms and displacement or extinction of parental species (Rhymer & Simberloff, 1996). Indeed, many species have become extinct due to hybrid swamping, both in animal and plant taxa (Rhymer & Simberloff, 1996, Allendorf et al. 2001). Hybridization can also lead to outbreeding depression, where first-generation hybrids have lower reproductive success and survival than pure species by means of either intrinsic (genetic interactions) or extrinsic (loss of adaptation to local environment) mechanisms (Edmands & Timmerman, 2003). Despite potential negative consequences of hybridization, interspecific gene flow can be beneficial in many situations. Hybridization occurs naturally in many taxa (Mallet 2005), forming hybrid zones where genetically divergent species occur sympatrically and hybridize with no adverse effects (Allendorf et al. 2001, Mallet 2005). Increased gene flow between two genetically distinct species can lead to a corresponding increase in genetic diversity and stimulate an adaptive response (Fitzpatrick et al. 2015). In some cases this can even lead to speciation (Rheindt & Edwards, 2011). Evaluating factors that give rise to natural hybrid zones and identifying what governs their dynamics and structure is important for predicting the evolutionary consequences of hybridization (Culumber et al. 2014).

Hybrid zones have also been deemed important "windows on climate change" (Taylor et al., 2015). Monitoring hybrid zones in relation to anthropogenic climate change holds the power to inform how interacting species respond to climate change, and how climate change may alter patterns of introgression. Hybrid zones provide valuable systems to study changes in species geographical distributions and the role of interspecific gene flow in providing genetic variation that may facilitate the evolution of novel phenotypes to new or changing environments (Taylor et al., 2015). Indeed, adaptive introgression of alleles may be a potential source of evolutionary resilience or rescue in light of climate change, releasing populations from their adaptive constraints (Carlson et al., 2014; Hamilton & Miller, 2015; Taylor et al., 2015). Adaptive introgression has been seen in many hybridizing taxa, including both plants and animals (Lexer et al., 2003; Aiken et al., 2008; Song et al., 2011; Scriber et al., 2014; as reviewed by Taylor et al. 2015). As such, natural hybridization can augment genetic diversity in cases where genetic variation is limited, by extending a species gene-pool and thereby allowing for greater adaptive capacity in response to changing conditions (Lewontin & Birch, 1966; Hamilton & Miller, 2015).

By studying the hybrid zone dynamics between the Saltmarsh (*Ammospiza caudacutus*) and Nelson's Sparrow (*A. nelsoni*), one gains a window into both the evolutionary processes underlying the relationship between these species and the role of climate change and adaptive introgression on the future persistence of the two sparrows. The Saltmarsh and Nelson's sparrows have restricted breeding habitat along the northeastern Atlantic coast of the United States. Nelson's Sparrows breed in marshes from the Canadian Maritimes to Massachusetts and the Saltmarsh Sparrow's range extends from southern Maine to Virginia (Nocera et al., 2007, Greenlaw & Woolfenden 2007). These sister species co-inhabit marshes where their ranges

overlap (Rising & Avise 1993, Hodgman et al. 2002), forming a ~200km hybrid zone currently stretching from South Thomaston, Maine to Newburyport, Massachusetts (Hodgman et al., 2002; Shiver et al., 2005, Walsh et al., 2011, 2015a). Both the Saltmarsh and Nelson's sparrow are endemic to the tidal marsh ecosystem; however, slight differences in habitat affinity, behavior, and morphology exist between Saltmarsh and Nelson's sparrows, which are thought to be due to the differing evolutionary history in tidal marshes (Greenlaw, 1993). Located narrowly along the coastline, tidal marshes are restricted to small shoreland areas with high development pressure, and they are highly susceptible to the impacts of climate-change-associated sea-level rise and alteration in precipitation regimes (IPCC 2014, Tlands 2013). As such, the hybrid zone between the Saltmarsh and Nelson's sparrow represents an ideal system for studying the evolutionary outcomes of hybridization of closely related sister taxa in secondary contact, as well as species response to climate change and the influence of adaptive potential on species persistence.

Anthropogenic climate change and landscape modification threaten the integrity of salt marshes and their importance as breeding grounds for a community of birds uniquely adapted to the ecosystem and influenced by cyclic patterns of tidal inundation (IPCC 2014). The Saltmarsh and Nelson's Sparrows are ground-nesting, and as such, monthly tidal events are the leading cause of nest failure in this system. Consequently, these species are extremely vulnerable to even slight increases in sea level (Greenlaw & Rising, 1994; Shriver et al., 2007; Bayard & Elphick, 2011). During high spring tides, the entire marsh will flood causing nests to be inundated with water for multiple hours (Gjerdrum et al., 2008). Increased tidal flooding due to rising sea levels and more frequent storm events as a result of climate change will reduce, if not eliminate, the sparrows' reproductive ability within the imminent future (Bayard & Elphick, 2011). Due to these effects, compounded with limited habitat, these two tidal-marsh sparrow species are of

high conservation priority in the northeastern U.S. (USDI 2008), and the Saltmarsh Sparrow is also globally at risk of extinction (IUCN 2015).

Previous work in the Saltmarsh and Nelson's Sparrow hybrid zone indicates that high levels of introgression exist throughout the zone; however, species boundaries still remain intact despite gene flow (Walsh et al. 2015a; Walsh et al. 2016b). Asymmetrical introgression towards the Saltmarsh Sparrow, a deficit of recent-generation hybrids with reduced survival of females, and assortative mating exists in the southern end of the zone (Walsh et al. 2015a; Walsh et al., 2016a). There is also evidence that suggests hybrid birds may have differential reproductive success than pure species; however, conclusions from these studies are limited to the southern range of the hybrid zone, where species densities are highly skewed (Saltmarsh to Nelson 5.5:1) and very few intermediate (F1/F2) individuals and pure Nelson's sparrows exist (Walsh et al. 2015a). It is uncertain whether patterns of gene flow are driven by adaptive benefits of increased genetic diversity through admixture or a result of species distributions and the spatial scale of sampling. Working in the center of the hybrid zone where the two species occur in relatively equal proportions holds the power to yield new insight on hybrid fitness and isolate potential drivers of introgression and species boundaries maintenance, without the confounding effects of unequal species proportions and sample sizes.

In this study, I evaluated the relative fitness and adaptive potential of Nelson's, Saltmarsh, and hybrid sparrows though a lens of local adaptation within a changing environment. I explored patterns of hybridization and introgression across multiple spatial scales of the Saltmarsh and Nelson's Sparrow hybrid zone. I aimed to determine potential drivers of hybrid zone structure including relative species densities, environmental (fine-scale microhabitat), genetic (reduced survival of hybrid females), and behavioral (mating patterns) in Chapter 1, as

well as relative fitness between hybrids and pure individuals (females in Chapter 2 and males in

Chapter 3).

CHAPTER 1

INFULENCE OF DEMOGRAPHY AND HABITAT ON BROAD AND FINE-SCALE

VARIATION IN STRUCTURE OF THE SALTMARSH-NELSON'S SPARROW HYBRID[1]

ZONE

Abstract

Exploring variation in dynamics across a hybrid zone allows for better understanding of factors that influence hybrid zone structure. In this study, we investigated patterns of introgression and drivers of gene flow at two spatial scales within the Saltmarsh Sparrow (*Ammospiza caudacutus*) and Nelson's Sparrow (*A. nelsoni*) hybrid zone. By intensively sampling adults (n = 218) and chicks (n = 326) over two years at two locations in the center of the hybrid zone, we determined patterns of introgression on a fine-scale across a habitat gradient and on a broad-scale through comparison with known patterns in the southern range of the zone. Using a ddRAD sequencing approach, a panel of fixed SNPs (135) was used to calculate a hybrid index and determine the genotypic composition of individuals and the level of admixture of the populations. In addition, a separate panel of SNPs (589) was used to assign paternity to offspring and reconstruct mating pairs to test for evidence of assortative mating. We found that patterns of introgression varied at broad and fine spatial scales, in relation to habitat differences, species occurrence ratios, and population densities. We found both the center and southern edge portion of the hybrid zone had high levels of introgression and low numbers of recent-

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[1] Co-authored manuscript prepared for submission to publication

generation hybrids, but patterns of introgression differed. Unequal species densities and asymmetrical introgression towards the Saltmarsh Sparrow characterized the southern end of the hybrid zone, while relatively equal species densities and patterns of bi-directional introgression, with very few pure parental species, occurred in the center of the zone. We also found differences at a fine scale within the center of the hybrid zone. Local site-specific characteristics influenced the distribution of genotypes, extent of hybridization, and patterns of introgression across a tidal marsh habitat gradient, such that there were higher numbers of hybrids and more backcrossing towards Nelson's Sparrow at the inland than coastal site. We observed twice as many recent-generation hybrid female nestlings than adults in the population, providing evidence for reduced survival of hybrid females from nestling to adult stage, supporting Haldane's Rule. The large majority (79%) of mating pairs occurred within species boundaries, with a significant correlation between hybrid index of males and females of each mated pair (r = 0.73, P < 0.0001), indicative of assortative mating. Our study supports a growing body of literature that shows hybrid zones vary structurally across space in relation to endogenous and exogenous factors specific to the locations and populations occupying them.

Introduction

Understanding hybrid zone structure, including patterns of introgression and character variation, can help infer processes that maintain hybrid zones, and provide important insights into the nature of species boundaries (Ross & Harrison, 2002; Harrison & Larson, 2014). Because many hybrid zones vary in structure across geographic space, studying them at different spatial scales and locations may reveal complex patterns. It has been argued that by studying the same taxa in multiple situations and scales, it may be possible to correlate spatial variation in hybrid-zone structure with specific characteristics of locations and the populations occupying

them (Futuyma & Shapiro, 1995). Exploring variation in dynamics across hybrid zones allows for better understanding of hybrid zone structure in general and provides a link to hybrid zone maintenance (Ross & Harrison, 2002; Morgan-Richards & Wallis, 2003).

In a variety of taxa, the extent of hybridization and resulting patterns of introgression have been shown to vary substantially among geographic locations where species hybridize, due to exogenous factors relating to local environmental and ecological conditions. For example, in plants, differences in rates of hybridization and reproductive isolation have been attributed to local conditions such as pollinator choice of flowers (Aldridge & Campbell, 2009) or elevational differences in sites (Aldridge, 2005). In animal systems, reduced introgression occurs in two distinct regions of the field cricket (*Gryllus pennsylvanicus* and *G. firmu*) hybrid zone (Larson et al., 2014). Similarly, in the pupfish (*Cyprinodon atrorus and C. bifasciatus*), complex admixtures of parental and hybrid genotypes occur in intermediate environments, while hybrids are more genetically similar to resident species in parental habitats (Carson et al., 2012). The hybrid zone between Lazuli and Indigo Buntings (*Passerina amoena and P. cyanea*) exemplifies an avian system in which patterns of hybridization are best explained by differential adaptation to environmental variation across the Rocky Mountain and Great Plains ecotone (Carling & Thomassen, 2012).

Patterns of hybridization and asymmetrical gene flow may also be affected by local population size, demographics, and species distributions that vary across a hybrid zone. Specifically, hybridization may be influenced by the relative population size of parental populations. If population sizes are unequal between parental species, Hubbs Principle (Hubbs 1955) suggests that hybridization will be more widespread due to restricted mate choice (Randler

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2002). However, if parental populations are highly skewed, the absolute rate of hybridization may be limited due to the reduced interaction of the two species. This is especially true in promiscuous mating systems that depend on encounter rates, such that members of the rarer species may fail to mate (Baskett & Gomulkiewicz, 2011). When the parents of one species are less common than the other, asymmetrical backcrossing may exist in the direction of the more abundant parent (Ellstrand & Elam, 1993). Spatial distribution of individuals or enhanced immigration of one parental species across hybrid zones can also affect direction and intensity of interspecific gene flow (Vines et al., 2003; Field et al., 2010). In small populations, hybrid fertilizations constitute a larger proportion of the total, and hybrids may backcross differentially to the common parental taxa; in extreme cases this can lead to genetic assimilation (Ellstrand & Elam, 1993; Burgess et al., 2005). For example, in the Golden-winged (*Vermivora chrysoptera*)-Blue-winged Warbler (*V. pinus*) hybrid zone, rates of introgression vary across sites that differ in relative population size and status of the two species (Dabrowski et al., 2005). In locations where Golden-winged Warbler populations were found to be in decline and at minimum, introgression was frequent/prevalent and almost completely unidirectional from Blue-winged into Golden-winged Warblers, while when populations co-exist in more equal proportions, introgression was more bi-directional and affects only 50% of the Golden-winged Warblers (Dabrowski et al., 2005).

Interspecific behavior and assortative mating have also been shown to influence patterns of hybridization and introgression across hybrid zones by means of sexual selection. Some behaviors may promote hybridization and gene flow, while others may inhibit it. Differences in male aggression across the Townsend's (*Setophaga townsendi*) and Hermit Warbler (*S. occidentalis*) hybrid zone suggest a competitive advantage of Townsend's over Hermit warblers

as a driver of asymmetric introgression (Pearson & Rohwer, 2000; Pearson, 2000). Alternatively, mate choice, in the form of assortative mating, may preserve species boundaries and maintain bimodal population structure, as a result of pre or post copulatory behaviors and processes (Culumber et al., 2014). Mate choice may also work in concert with other drivers of hybrid zone structure, such as occurs with Golden (*Manacus vitellinus*) and White-collard Manakins *(M. candei)*, in which there is a balance between trait introgression for yellow and white plumage via sexual selection via female choice. In sympatry, bright yellow plumage is selectively advantageous, while white coloration is selected for in the same plumage trait in a single allopatric population, which has been attributed to plumage looking more or less conspicuous in differing habitats in which they are displayed (Uy & Stein, 2007).

Endogenous factors may also play a part in hybrid zone structure, whereby local genetic makeup of a population may influence patterns of introgression (Teeter et al., 2009), or selection against hybrids contributes to maintaining species boundaries (Steeves et al., 2010). Studies from house mice (*Mus domesticus* and *M. musculus*) have shown large differences in genomic and geographic clines across geographic transects, dependent in part on the genetic structure of local populations, attributed to differing histories of natural selection or genetic drift (Teeter et al., 2009). Selection against hybrids may also differ for the sexes. For example, Haldane's Rule predicts that the heterogametic sex of first generation hybrids should experience greater reductions in fitness (Haldane, 1922; Neubauer et al., 2014). This lower fitness can display itself as lower fertility and/or lower survival, both of which have been observed in avian hybrid zones (Svedin et al., 2008; Neubauer et al., 2014)

In this study, we investigated patterns of introgression and drivers of gene flow at broad and fine spatial scales in the Saltmarsh Sparrow (*Ammospiza caudacutus*) and Nelson's Sparrow (*A. nelsoni*) hybrid zone. These two tidal marsh bird species have restricted breeding habitat along the northeastern Atlantic coast of the United States. Nelson's Sparrows breed in marshes from the Canadian Maritimes to Massachusetts and the Saltmarsh Sparrow's range extends from southern Maine to Virginia (Nocera et al. 2007, Greenlaw & Woolfenden 2007). These sister species co-inhabit marshes where their ranges overlap (Rising & Avise, 1993; Hodgman et al., 2002), forming a ~200km hybrid zone stretching from South Thomaston, Maine to Plum Island, Massachusetts (Hodgman et al., 2002; Shriver et al., 2005; Walsh et al., 2011, 2015a). Slight differences in habitat affinity, behavior, and morphology exist between the Saltmarsh and Nelson's Sparrows, likely due to their differing evolutionary histories in tidal marshes (Greenlaw, 1993). A vicariance event is thought to have split the species into discontinuous distributions, where Nelson's Sparrows evolved as an isolate in more interior non-tidal wetlands, and Saltmarsh Sparrows differentiated in salt marshes along the Atlantic coast (Greenlaw, 1993). Secondary contact was established by recent (Pleistocene) recolonization of the coast by a subspecies of Nelson's sparrow (*A. n. subvirgatus*; Greenlaw 1993, Shriver et al. 2007). As such, Saltmarsh Sparrows are entirely restricted to tidal salt marshes, while Nelson's Sparrows will also breed in brackish, less tidal coastal marshes, and have been known to inhabit hayfields and fens (Greenlaw 1993; Shriver et al. 2005; Nocera et al. 2007).

The Saltmarsh-Nelson's Sparrow hybrid zone is linear, encompassing the narrow strip of coastal marshes along the Atlantic seaboard. However, this habitat is patchy and characterized by larger, more coastal expansive marsh complexes in the south and more isolated fringe marshes in the north (Greenlaw 1993). The hybrid zone habitat is shaped by a complex spatial structing of

marshes with a mix of marsh types, leading to a mosaic model of hybrid zone maintenance along a tidal marsh gradient between coastal and brackish marshes, with selection for traits related to tidal marsh adaptations across the range (Walsh et al. 2015b; Walsh et al. 2016b). Previous work indicates that high levels of introgression exist across the zone; however, levels of admixture vary spatially and species boundaries remain largely intact in the face of high gene flow (Walsh et al. 2015a; Walsh et al. 2016b). Asymmetrical introgression towards the Saltmarsh Sparrow, a deficit of recent-generation hybrids with reduced survival of females, and assortative mating characterize the southern end of the zone (Walsh et al. 2015a; Walsh et al., 2016a); however, species densities are highly skewed on these focal demographic sites (Saltmarsh to Nelson 5.5:1) and very few intermediate (F1) individuals exist in that area (Walsh et al. 2016a). It is unknown, therefore, whether patterns of gene flow are driven by adaptive benefits of increased genetic diversity through admixture, habitat affinities, or differences in demography and species distributions. Comparing patterns of introgression across spatial locations with differing habitats, population densities, and species distributions will yield insight into potential drivers of the structure and maintenance of this hybrid zone.

Objectives

In this study, we explore patterns of hybridization within the Saltmarsh-Nelson's Sparrow hybrid zone in relation to local endogenous and exogenous characteristics. We compare structure across multiple spatial scales, including at a broad scale between the center and southern portion of the hybrid zone, and on a fine scale across a coastal-upriver habitat gradient within the center of the hybrid zone. We aim to 1) determine the extent of hybridization and introgression at two sites in the center of the Saltmarsh and Nelson's Sparrow hybrid zone. We compare these patterns of introgression on a fine scale between the two sites, which span a coastal-upriver habitat gradient,

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and also more broadly with results of prior work from the southern end of the hybrid zone. We also aim to (2) test for evidence of reduced survival of hybrid females via Haldane's Rule, and (3) determine interspecific mating patterns.

Predictions

1) Patterns of introgression will be shaped by relative species densities and local demographic factors such that:

(a) Because the two species occur in relatively similar proportions in the center of the hybrid zone, we expect higher levels of introgression, with equal rates in both directions, and more recent-generation hybrids (F1/F2) than in the southern end of the hybrid zone.

(b) Due to differential habitat affinities, we expect to observe more Nelson's sparrows at the inland site and more Saltmarsh sparrows at the coastal site. Differences in species relative proportions will result in mating asymmetries, with more backcrossing toward Nelson's Sparrows on the inland marsh and more backcrossing toward Saltmarsh Sparrows on the coastal marsh.

2) As predicted by Haldane's Rule, hybrid females will have reduced fitness, resulting in a deficit of first generation hybrid females. This may manifest during either offspring production or juvenile and adult survival, such that:

(a) There will be a male-biased offspring sex ratio and a lower mean hybrid index of hybrid female nestlings compared to males, due to a female's ability to manipulate the sex ratio of hybrid offspring and/or greater inviability of female hybrid eggs; or

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(b) There will be reduced survival of females from nestling to adult stage, such that there will be an even offspring sex ratio and a male-skewed adult sex ratio.

3.) Saltmarsh and Nelson's Sparrows will exhibit assortative mating, such that there will be more matings within species than between species.

Methods

Study Area

Two field sites were selected in the current center of the hybrid zone for their historical importance, including the earliest observations of hybrid individuals. Sites included the marshes at Popham Beach State Park and Wharton Point on Maquoit Bay, located on the northeastern coast of the United States, between Brunswick, Maine and Phippsburg, Maine. We chose these sites with expectations of relatively similar species abundances based on recent abundance estimates (Wiest et al., 2016) and a relatively high number of first generation hybrids based on a peak in interspecific heterozygosity across the hybrid zone (Walsh et al., 2016b). The two sites fall at the two ends of a habitat gradient between coastal and inland tidal marshes and differ slightly in fine-scale habitat (vegetation) characteristics and amount of tidal inundation (Chapter 1; Walsh et al., 2015b). The marshes at Popham Beach State Park are located at the tip of a peninsula, directly on the coast. The area of marsh at Popham is expansive; therefore, we selected to focus on a portion of the marsh consisting of a ~15-hectare plot. The entire marsh at Maquoit Bay is located more inland and is much smaller than Popham, with the selected study area (~5 hectares) about a third the size of the study plot at Popham. Popham marshes are part of an expansive coastal marsh network, while Maquoit is located in a small cove that is surrounded by mostly forest and field. Although both sites experience daily and monthly tidal inundation,

tide heights tend to be dampened in inland marshes relative to coastal (Benvenuti et al., 2018),

suggesting the flooding rates may be lower at Maquoit compared to Popham.

Field Data Collection

To determine the extent of hybridization and patterns of introgression, we monitored and

sampled the population at both sites during the 2016 & 2017 breeding seasons. We followed

standardized protocols established by the Saltmarsh Habitat and Avian Research Program

(SHARP; www.tidalmarshbirds.org). We performed systematic as well as opportunistic netting,

using 2–6, 12-m mist-nets, throughout the breeding season to sample as many resident adults as

possible. To test predictions of Haldane's rule and assortative mating, we sampled as many

offspring as possible. We conducted nest monitoring at both sites during May —August,

encompassing approximately 3 nesting cycles (see Chapter 2 for further methodological details).

From each nest, nestlings were banded with a USGS aluminum leg band and a single site-

specific color band when they were 6 days old. A blood sample (a few drops on a filter card) was

also collected from the medial metatarsal vein of each nestling for genotyping and hybrid

identification. We also collected any deceased, unbanded chicks or eggs that had failed to hatch

to use in genetic analyses. To determine the identity of females associated with each nest, we

conducted targeted mist-netting to capture females off of their nests during incubation or

brooding. Once caught, each female was banded with a USGS aluminum band, a site-specific

color band, and a PIT tag that was attached affixed to a color band for non-invasive detection of

re-nesting attempts. Males were sampled systematically and opportunistically across the extent

of each study site and throughout the breeding season and banded with a USGS aluminum band

and a site-specific color band. We collected standard morphological measurements from all

adults and recorded presence/absence of brood patch for females. Blood samples were drawn

from the cutaneous ulnar vein and stored on blood filter strips at room temperature for genetic analysis.

ddRAD Library Preparation

Samples from adult females, nestlings, and salvaged chicks or eggs from the two field seasons were used to prepare double digest restriction site associated DNA (ddRAD) sequencing libraries. In addition, we also used 30 samples each from allopatric Nelson's Sparrow and allopatric Saltmarsh Sparrow populations from previous sampling of the hybrid zone (Appendix B) for developing a hybrid index. DNA was extracted from blood samples using the either Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) or Zymo Quick DNA kit (Zymo, Irvine, CA) following the manufacturer's protocol. We determined the concentration of resulting DNA samples using Qubit fluorometer Broad Range double-stranded DNA assay kit (Life Technologies, NY, USA). We targeted a DNA concentration of 5–25 ng/ul. Samples below 10ng/ul after initial extraction were vacuum centrifuged to concentrate to within the target range. Samples that were above 25 ng/ul were diluted down to 25 ng/ul. A small number of samples below 5 ng/ul were included and grouped into one index group to ensure the best results. ddRADtags were created using the protocol described in Peterson et al. (2012). DNA was digested with SbfI and MspI, and ligated to P1 and P2 adapters using T4 DNA ligase (30min at 37 ºC and 60min at 20 ºC, held at 10 ºC; Peterson et al., 2012). Samples were pooled into index groups by their unique P1 adapter and cleaned using 1.5x Agencourt AMPure XP beads. Using BluePippin (Sage Science, MA, USA), fragments were size selected between 400–700 bp in length. Low cycle PCR reactions were then preformed to incorporate the Illumina TruSeq primer sequences into the library, as well as a final clean up using AMPure XP beads. Libraries were visualized on a fragment Bioanalyzer to ensure desired fragment size/distribution and index

groups pooled. Resulting libraries were sequenced across three Illumina HiSeq 2500 lanes and one HiSeq 2500 rapid run lane (read length 100 bp) at the Cornell University Institute for Biotechnology (Genomics Facility Research Center).

Bioinformatic Data Processing & SNP Detection

Sequences were initially evaluated for overall quality using FastQC, then trimmed and filtered using FASTX-Toolkit. Specifically, reads were trimmed on the 3' end to 97 bp and eliminated if the Phred quality scores were below 10 or if 95% of the bases had Phred quality scores below 20. Using STACKS (version 1.48), we demultiplexed the remaining sequences. We used the process_radtags command with the following conditions: any reads not meeting Illumina's chastity/purity filter and of low quality were discarded, data were cleaned such that any read with an uncalled base was removed, reads with mismatches in the adapter sequence >1 were removed, and reads were only processed if the sequence had an intact SbfI RAD site and one of the unique barcodes. Subsequently, fastx_trimmer was used to trim all sequences to the length of the shortest sequences. Reads were aligned to the Saltmarsh Sparrow reference genome (Walsh et al., 2018a) using STACKS (version 1.48). Minimum stack depth for a read to be assembled into a catalog was 6. The number of mismatches allowed between sample loci was set at 5. We filtered catalog loci based on the mean log likelihood of the catalog locus in the population, with the minimum log likelihood set at -300. These filtering steps resulted in the recovery of 5,391 SNPs.

We used the program Populations to subset a panel of SNPs for use in calculating a hybrid index. We chose only one SNP per locus and required that a SNP be present in a minimum of 50% of all individuals, with a minimum stack depth of 6, for it to be called.

Subsequently, VCFtools (Danecek et al., 2018) was used to group individuals into 3 populations: 1) all individuals sampled in this study from the center of the hybrid zone, 2) allopatric Nelson's Sparrows, and 3) allopatric Saltmarsh Sparrows. We then calculated the fixation index (F_{st}) for each SNP using VCFtools and subsetted the panel further to include only fixed SNPs ($F_{st} = 1$) between allopatric Nelson's and Saltmarsh Sparrows. This resulted in a panel of 135 fixed SNPs that we used for the development of a hybrid index to classify pure and hybrid individual sparrows.

We also created a separate panel of SNPs to be used in paternity analysis to address questions about assortative mating using only sympatric birds from the Popham and Maquoit study sites (i.e., excluding allopatric samples). For the paternity panel we again chose only one SNP per locus and required that a SNP be present in a minimum of 95% of the individuals with a minimum stack depth of 6. This resulted in a 589-SNP paternity panel.

Patterns of Introgression

Sparrows were assigned to genotypic classes using methods of Milne and Abbot (2008), as in Walsh et al. (2015a). Using this method, which combines our hybrid index and interspecific heterozygosity, we placed each individual into one of five genotypic classes consisting of: pure Nelson's Sparrow, backcrossed Nelson's, F1/F2 (recent generation hybrids), backcrossed Saltmarsh, or pure Saltmarsh Sparrow. Hybrid index was defined as the proportion of alleles inherited from the Saltmarsh Sparrow (0 = pure Nelson's Sparrow and 1 = pure Saltmarsh Sparrow), based on the 30 allopatric Saltmarsh and Nelson's sparrows. Interspecific heterozygosity was defined as the proportion of genotypes that were heterozygous across the species for the parental alleles (0 = all homozygous genotypes, found only in one parental

species, and 1 = all heterozygous genotypes across species). Individuals with intermediate hybrid index (0.25–0.75) and high heterozygosity (>0.3) were considered recent generation hybrids (F1 or F2), and individuals with very low or high hybrid index (0.05–0.24 or 0.75–0.95) and low heterozygosity (<0.3) were considered backcrossed. Pure individuals were defined by a hybrid index of 0–0.05 (Nelson's Sparrow) or 0.95–1 (Saltmarsh Sparrow). The Introgress package in R was used for calculating the hybrid index and interspecific heterozygosity (Gompert & Buerkle, 2010). Analyses did not distinguish between F1 and F2 individuals, which were grouped together into an overall recent-generation hybrid category, used throughout.

We compared the distribution of genotypes for all individuals (adults and nestlings) between sites. Genetic composition of the Popham and Maquoit populations were compared to allopatric parental populations (Saltmarsh and Nelson's) using STRUCTURE, version 2.3.4 (Pritchard et al., 2000) and visualized using CLUMPAK (Kopelman et al., 2015). We determined the genotypic composition of nestlings and adults of each sex. We also compared the distribution of the genotypic classes between Popham Beach and Maquoit Bay using a chi-squared test. We also performed a two-tailed Student's t-test to compare the proportion of backcrossed individuals between the two sites to determine if there was more backcrossing towards Nelson's Sparrow at Maquoit Bay and more backcrossing towards Saltmarsh Sparrow at Popham Beach.

Testing Haldane's Rule

To test Haldane's Rule, we determined: (1) if interspecific mating resulted in male-biased production of offspring due to infertility/reduced viability of females; or (2) if there was observed reduced survival of hybrid females from the nestling to adult stage. The sex of each offspring was identified by PCR amplification of the CDH1 gene (Fridolfsson & Ellegren, 1999;

Griffiths et al.,1996) and visualized using gel electrophoresis. We performed two-tailed
Student's t-tests to compare the hybrid index of male and female offspring across both sites and
the proportion of male offspring produced from interspecific and intraspecific mating events. To
test for reduced survival of females, we compared the proportion of recent generation hybrids
among nestling females, adult female, nestling males, and adult males.

Assessing Mating Patterns

To test for assortative mating, we conducted paternity analyses of nestlings using
genotype data from the SNP paternity panel and reconstructed mating pairs. Candidate fathers
were assigned using the approaches implemented in CERVUS (Marshall et al., 1998) and COLONY
v2.0 (Jones & Wang, 2010). The maximum likelihood approach of CERVUS uses simulated
genotypes from provided data to create a log-likelihood confidence level in true parentage
assignments but does not account for unsampled males in the population. To address this
problem, we used the full likelihood approach in COLONY, which can assign paternity to a
sampled male even if the true father was not among the sampled males. For both methods, we
used a genotyping error rate of 1%, 95% of loci typed, and candidate father sampling of 70%.
We assumed the proportion of sampled mothers to be 95% given the targeted netting
identification of females off of their nests. For each site and year, a list of candidate fathers was
developed. For 2016, all sampled adult males were included, and for 2017, all males that were
sampled in that year, as well as any males from 2016 (adults and offspring as determined from
molecular sexing) were included to account for any hatch years that may have returned to their
natal site, as well as any returning adult males that may have evaded capture in 2017. For each
offspring, we determined the most likely father as assigned by CERVUS (delta trio value ≥95%).
This was then compared to the paternity assignment made in COLONY. For any discrepancies on

confident paternity assignments (>95%) between the two programs, we compared the number of loci mismatches, delta pair confidence, and overall loci typed to identify the best male assignment.

Each mating event was classified into two categories: within species (Nelson's Sparrow/Nelson's Sparrow, and Saltmarsh Sparrow/Saltmarsh Sparrow) and between species (F1/F2 with Nelson's Sparrow or Saltmarsh Sparrow, backcrossed with Nelson's Sparrow or Saltmarsh Sparrow and F1/F2 with backcrossed), and the number of offspring resulting from each group was compared. We also tested for a correlation between the parental hybrid index scores for each offspring using a Pearson product-moment correlation coefficient. Finally, we compared mating patterns between Popham and Maquoit, testing for differences in the proportion of between species and within species mating across the two sites using a two-tailed Student's t-test.

Results

Broad Scale Patterns of Introgression

We banded and genotyped 544 sparrows across both study sites in the 2 years (218 adults, 326 nestlings and eggs). STRUCTURE analysis revealed high admixture at the two study sites. Although few individuals exhibited pure ancestry, most shared a larger proportion of alleles from one parental species than the other (i.e., backcrossed; Figure 1). Using hybrid index to classify individuals into genotypic classes, 33% of adults were backcrossed Nelson's Sparrows (30 females, 42 males), 45% were backcrossed Saltmarsh Sparrows (50 females, 47 males), 12% were recent generation hybrids (8 female, 17 male), 8% were pure Nelson's Sparrows (11 females, 7 males), and 3% were pure Saltmarsh Sparrows (5 females, 1 males; Figure 2; Table

1). Although low levels of recent-generation hybrids, there were many backcrossed individuals. As such, the mean hybrid index was similar between adult males (0.54 ± 0.15) and females (0.57 ± 0.16) and was slightly higher for nestlings (0.65 ± 0.13), although still similar between the sexes (Table 2). Interspecific heterozygosity was comparable between adult male (0.20 ± 0.03), adult female (0.15 ± 0.01), and nestling birds (0.17 ± 0.01; Table 2). The genotypic structure of the population was similar between sampled adults and nestling birds, indicating no reduced survival for any one genotypic class as a whole (Figure 2). The distribution of genotypic classes across nestlings illustrates considerable current interspecific gene flow, such that most offspring are of backcrossed origins, with fewer recent-generation hybrids, and even fewer pure individuals (Figure 3).

Fine Scale Patterns of Introgression

Abundance differed between the two sites along the habitat gradient. Although the marsh at Popham (~15 hectares) is three times larger than the one at Maquoit (~5 hectares), the density of adult breeding birds between the sites was similar with 11.1 birds per hectare at Popham and 10.4 birds per hectare at Maquoit. However, we found a large discrepancy in the density of offspring produced at each site. Popham produced approximately 4 times as many nestlings per marsh area (20.0 birds/ha) than Maquoit (5.2 birds/ha). Sparrows at Maquoit bay (inland) had a larger proportion of Nelson's Sparrow alleles, while sparrows at Popham (coastal) had more Saltmarsh Sparrow alleles (Figure 1). There was also a significant difference in the distribution of genotypes between the two sites (X^2= 12.2, P = 0.002), with significantly more backcrossing towards Nelson's Sparrow at Maquoit than Popham (t = 2.54, P = 0.01). We found a greater number of adult recent-generation hybrids (F1/F2) at Maquoit than at Popham (t = 2.17, P = 0.03). The mean hybrid index and mean interspecific heterozygosity for each site also reflected

these patterns. Overall there were more Saltmarsh Sparrow-like birds at Popham (mean hybrid index = 0.64 ± 0.14) than Maquoit (mean hybrid index = 0.43 ± 0.13; Table 1; Figure 4). The sparrows at Maquoit also showed more mixture between the two species' gene pools, with higher interspecific heterozygosity at Maquoit (mean = 0.21 ± 0.02) than Popham (mean = 0.17 ± 0.15; Table 1, Figure 4).

The distribution of genotypic classes between sites showed the large majority of individuals were of mixed ancestry (Figure 5) at both sites, with backcrossed Nelson's making up a larger portion of the population at Maquoit and backcrossed Saltmarsh individuals a larger portion at Popham. Both backcrossed genotypic classes had relatively equal adult sex ratios at Popham and Maquoit (Figure 5). Maquoit had proportionally more adult F1/F2 individuals (9 total; 4 females, 5 males) than Popham (16 total; 4 females, 12 males), and a higher proportion of them were female at Maquoit and male at Popham. The percentage of pure individuals was low for each site; however, Maquoit possessed a relatively large proportion of pure Nelson's Sparrow females (Figure 5). The adult breeding pool differed in composition from the offspring produced across the two years at Maquoit, while it was similar between the stages at Popham, with most individuals of mixed ancestry. There were no pure nestlings sampled from Maquoit despite higher levels of pure adults, and almost equal numbers of offspring split between recent-generation hybrids and backcrossed sparrows.

Testing Haldane's Rule

We found no difference in mean hybrid index between male and female nestlings (male: 0.66 ± 0.13, female: 0.68 ± 0.12, t = -0.75, P = 0.46) across both study sites and years, suggesting that offspring production and egg viability was not biased in favor of males. We did

find evidence for reduced survival of females to adulthood, however, through the comparison of the percentage of recent-generation hybrids between nestlings and adults of the two sexes. Proportionally, male and female recent-generation hybrid nestlings represented a similar sector of the population, with males and females comprising 8.7% and 7.8% of all nestlings, respectively. For the adult age class, however, recent generation hybrid males outnumbered hybrid females 2:1, with the proportion of recent generation hybrid males (5.2% of all adults) twice that of hybrid females (2.5% of all adults) (Figure 6). Nestling sex ratios were male-skewed (60:40) for backcrossed Nelson's and Saltmarsh Sparrows (Figure 3), and female-biased to the same degree for pure Saltmarsh Sparrows. Sex ratios of first generation hybrid nestlings (51% male) and pure Nelson's Sparrow nestlings (50% male) were relatively equal (Figure 3). Although at both sites there were fewer female than male recent-generation hybrid adults (Figure 6), this was more pronounced at Popham, which had almost four times as many adult hybrid males than females, while the ratio was less skewed at Maquoit (6:4 male to female; Figure 7).

Assessing Mating Patterns

The majority (79%) of all reconstructed mating pairs occurred within species groups (backcrossed Saltmarsh Sparrows and Saltmarsh Sparrow or backcrossed Nelson's Sparrows and Nelson's Sparrows), with 10 times as many matings (217 pairings) within species than between species (21 pairings). The hybrid indices of the parents of each reconstructed mating pair were significantly correlated (r = 0.73, P < 0.001), meaning birds were pairing with others that were more like their own genotype (Figure 8). We also found that assortative mating was stronger at Popham than Maquoit, with significantly more between species pairings at Maquoit (t = 3.30, P = 0.003).

Discussion

Mechanisms of hybrid zone maintenance may depend largely on the context in which hybridization occurs, and their identification requires close inspection of behavior, species interactions—including pre and post mating barriers, habitat, distribution and species ranges (Ross & Harrison, 2002; Harrison & Larson, 2014). In this study we found that patterns of introgression differ across the Saltmarsh – Nelson's Sparrow hybrid zone, at both broad and fine spatial scales. Species relative densities and distributions, habitat gradients, mate choice, and endogenous factors influence hybridization rates and result in variable patterns of introgression across the hybrid zone. Patterns of introgression vary broadly between the southern and central portion of the hybrid zone, related to relative species densities, as well as on a small spatial-scale between coastal-upriver habitat gradient. Despite variation, we also found broad-scale stability and consistency in assortative mating and reduced survival of hybrid females across the hybrid zone, although the strength of assortative mating differed on a fine-scale between coastal and inland sites.

Exogenous Factors: Relative Species Densities and Distribution

We found the relative population size of parental species influenced patterns of asymmetrical gene flow across the Saltmarsh and Nelson's Sparrow hybrid zone at a broad scale. In contrast to the asymmetrical backcrossing towards the Saltmarsh Sparrow previously observed throughout the hybrid zone and an observed majority of backcrossed Saltmarsh Sparrows in the southern range margins of the hybrid zone (Walsh et al. 2015b; Walsh et al., 2016), sparrows in the center of the hybrid zone showed fairly equal backcrossing in each direction, with 33% of individuals classified as backcrossed Nelson's Sparrows and 45% as backcrossed Saltmarsh

Sparrows. Further, the slight bias of backcrossing in the Saltmarsh Sparrow direction could be due to our sampling scheme, as Popham (which had more backcrossing in the Saltmarsh Sparrow direction) was a much larger site with many more birds sampled than Maquoit (which was smaller in size and had more backcrossing in the Nelson's direction but less individuals sampled).

When one parental species is less common than the other, asymmetrical backcrossing may exist in the direction of the more abundant parent (Ellstrand & Elam, 1993). Differences in rates of hybridization and patterns of introgression due to local demographics and population size have been seen in a variety of other taxa, including birds (Vines et al., 2003; Burgess et al., 2005; Dabrowski et al., 2005; Field et al., 2010), playing a key role in hybrid zone structure and maintenance. While high levels of introgression via back-crossing characterized both the southern end (Walsh et al. 2016) and the center of the hybrid zone (this study), the direction and asymmetry of introgression differed. This suggests that species ratios could have been leading to observed asymmetrical introgression towards the Saltmarsh Sparrow in the south of the hybrid zone, and that when species are at more equal proportions (as occurs in the center of the zone), introgression exists readily in both directions. This may be particularly important to consider in light of conservation concerns for the Saltmarsh and Nelson's Sparrows. Both of these species are highly threatened by sea-level rise and coastal development (Greenlaw & Rising, 1994; Bayard & Elphick, 2011; Shriver et al., 2007). With growing threats and increasingly small patches of discontinuous coastal marsh (Tlands, 2013), sparrow populations may become smaller and more disjunct. Neutral processes alone are therefore expected to cause relative species densities to become unequal at more marshes throughout their range, causing differential backcrossing to the common parental taxa and exacerbated threat to the other.

We found broadly consistent results in the extent of hybridization between the southern and central portion of the hybrid zone, with few recent-generation hybrids existing in both locations despite high levels of introgression. There was a consistent number of recent-generation hybrids in south to that observed in the center of the hybrid zone, representing only 12% of the population. The center of the hybrid zone was characterized by relatively equal species densities, while the southern range margins had unequal species ratios (5:1 Saltmarsh to Nelson's; Walsh et al. 2016. Hubbs principle suggests that hybridization will be more widespread with unequal parental species populations (Hubbs, 1955, Randler 2002). This system does not find support for this however, with low frequencies of hybrids in the south despite skewed densities (Walsh et al., 2015b). Conversely, if parental populations are highly skewed, hybridization may be in fact be limited due to the reduced interaction of the two species, especially in promiscuous mating systems where members of the rarer species may fail to mate (Baskett & Gomulkiewicz, 2011). Due to the relatively equal proportions of pure individuals sampled in the center of the hybrid zone, access to interspecific mates was higher and relatively equal in the center than in the south of the hybrid zone; however, we still saw limited recent-generation hybrids at a population level across both study sites. Although relative species densities appear to affect the direction of introgression between the Saltmarsh and Nelson's Sparrow, recent-generation hybrids were relatively uncommon both when species abundances were equal (center) and skewed (south), providing evidence for reproductive barriers between the species.

Exogenous Factors: Habitat

Differing patterns of introgression across sites may be based on habitat as well as resulting relative population sizes of parental species, as has been documented across other

hybrid zones (Vines et al., 2003; Dabrowski et al., 2005). Habitat preference plays a critical role in the fine-scale structure of mosaic hybrid zones (Carson et al., 2012; Culumber et al., 2012). When organisms show a preference for the habitat to which they are adapted, the affinity of and additional immigration of pure types from the periphery helps to sustain the mosaic pattern in the face of hybridization (Vines et al. 2003). Other systems show differential adaptations to environment across hybrid zones such as the hybrid zone between Lazuli and Indigo Buntings (*Passerina amoena and P. cyanea*), in which patterns of hybridization are best explained by differential adaptation to mesic or xeric conditions that vary across the Rocky Mountain and Great Plains (Carling & Thomassen, 2012). Other examples of adaptation include swordtails (*Xiphophorus birchmanni* and *X. malinche*) along a temperature gradient (Culumber et al., 2012), tit tyrant flycatchers (*Anairetes reguloides and A. nigrocristatus*) along an altitudinal gradient (Dubay & Whitt, 2014), and fire-bellied toads (*Bombina bombina and B. variegata*) linked with pond and access to aquatic habitat (Vines et al., 2003).

Local site-specific characteristics within the center of the Saltmarsh and Nelson's Sparrow hybrid zone influenced the distribution of genotypes across the landscape, supporting previous findings supporting a role for exogenous factors shaping patterns of gene flow along a coastal-inland habitat gradient. Genotypic compositions differed between the inland and coastal sites in this study, with significantly more backcrossing towards the Nelson's Sparrow at Maquoit. This is consistent with known differences in habitat affinities and evolutionary histories between the two species, with Saltmarsh Sparrows inhabiting expansive coastal marshes with heavy tidal flow, and Nelson's Sparrows preferring brackish/upriver fringe marshes (Greenlaw 1993; Shriver et al. 2005; Nocera et al. 2007). Additionally, this adds further support to a hypothesized mosaic model of hybrid zone structure in saltmarsh and Nelson's sparrows and

previous niche modeling showing habitat preferences of pure species, such that marshes dominated by Nelson's Sparrow were smaller, more isolated, and drier than those dominated by Saltmarsh Sparrows (Walsh et al., 2015b). Saltmarsh Sparrows alleles have also been found to be more common in coastal sites, with more Nelson's Sparrow alleles in more inland and fringe marshes (Walsh et al., 2015b). Additionally, selection for traits related to tidal marsh adaptations have been found across the range of the Saltmarsh and Nelson's Sparrow hybrid zone (Walsh et al. 2015b; Walsh et al. 2016b). Our results suggest exogenous selection may also play a role in hybrid zone dynamics at a very fine scale across habitat gradients within the center of the hybrid zone.

Maquoit also had a significantly higher proportion of recent-generation hybrid individuals. This pattern is similar to results found in the southern end of the hybrid zone where more recent generation hybrid nestlings were produced at inland sites than coastal (Walsh et al., 2016a). In addition to habitat differences, a higher number of recent-generation hybrids at Maquoit could also be due to site-specific differences in population size and spatial extent, which may influence the frequency and production of recent-generation hybrid individuals. Although there are size differences in population and physical marsh between sites, the bird density is similar. This suggest that physical size of the marsh as well as the population size could affect the rate of hybridization. Individuals at Maquoit may have limited mate choices due to smaller population size and pool of mates to choose from, increasing rates of interspecific interactions and hybridization (Ellstrand & Elam, 1993; Beysard et al., 2012).

Assortative Mating

Behavior and mate choice are important in determining hybrid zone structure and patterns of introgression because the occurrence of hybridization is often due to a breakdown of premating isolation (Taylor et al., 2006; Culumber et al., 2014). Although variation in behavior across hybrid zones can lead to differing patterns of hybridization and introgression (Pearson & Rohwer, 2000; Uy & Stein, 2007), we found that interspecific mate choice behavior was consistent across the Saltmarsh-Nelson's Sparrow hybrid zone at a broad scale. Similar to trends in the southern part of the zone (Walsh et al., 2018b), we observed preference for within-species matings in the center of the hybrid zone, with the large majority of the reconstructed mating events (79%) within species boundaries. Further, we found a significant correlation between the hybrid index of males and females within mate pairs, further supporting the conclusion that individuals prefer genotypically similar (conspecific) mates.

These findings suggest assortative mating and active avoidance of interspecific mating exists between Saltmarsh and Nelson's Sparrows. Due to roughly equal species densities in the center of the zone, the observed patterns of mate choice cannot be explained by limited access to conspecific mates. Rather, some form of pre or post copulatory mechanisms may be acting to limit hybridization and maintain species boundaries in the face of high levels of gene flow in the hybrid zone. This could take shape in the form of male-male competition for access to mates, female choice, or a combination, at either the pre- or post-copulatory stage for either sex (Parker, 1970; Andersson, 1994; Birkhead, 1998). Sperm competition and cryptic female choice may act after mating has occurred; however, intersexual mate choice can also be based on numerous kinds of male secondary sexual traits or sexual signals that influence pre-copulatory decisions (Andersson, 1994). There appears to be limited male dimorphism between Saltmarsh and Nelson's Sparrows that could potentially act as sexual signals; however, they do differ in size,

song, and mating behavior, with Nelson's Sparrows being smaller and more likely to mate guard and exhibit flight displays (Greenlaw, 1993; Shriver et al., 2007, 2010; Hill et al., 2010). The traits that differ between the species are sexual characteristics often involved in competition, fighting (body size), and dominance signaling (song, or mate guarding; Andersson 1994). Differences in competitive ability could be driving patterns of assortative mating between Saltmarsh and Nelson's Sparrows and minimizing the level of hybridization (Greenlaw, 1993; Shriver et al., 2007, 2010; Hill et al., 2010), as well as post-copulatory factors including sperm competition and cryptic female choice.

Although consistent on a broad scale, we did find mating patterns to differ on a fine-scale between sites. Assortative mating was stronger at Popham than Maquoit, with 55% of all parings being between species at Maquoit and only 18% at Popham. Differences in the level of assortative mating could be driven by known difference in genotypic composition and relative densities, or population and marsh size between the two locations. Maquoit is a much smaller marsh, with a smaller population, which could increase the number of interspecific interactions (Ellstrand & Elam, 1993, Beysard et al., 2012).

Endogenous Factors

We found support for Haldane's Rule (Haldane, 1922), the reduced fitness of hybrid females (the heterogametic sex) in the center of the hybrid zone. Low fitness can manifest itself at different stages in the lifecycle, as either lower fertility and/or lower annual survival, both of which have been observed in avian hybrid zones (Svedin et al., 2008; Neubauer et al., 2014). We observed fewer adult hybrid females than nestlings, while we found male hybrids to remain relatively constant from the nestling stage into the breeding population. This pattern was also

seen in the sex ratio between adult and nestling F1/F2s, with similar proportions of each sex in nestlings (47:53), and male bias in adults (68:32). Reduced survival may be acting as a method of post-zygotic isolation between the Saltmarsh and Nelson's Sparrows across the zone. We did not find that interspecific pairs resulted in more male than female offspring; therefore, we found no evidence for reduced vitality or fertility of female hybrids. This suggests selection is not acting on egg production or viability and females do not bias offspring sex ratios, but recent-generation female nestlings may have reduced survival to adulthood. These results mirror what was seen in the southern end of the hybrid zone, where Walsh et al. (2016a) also found low levels of first generation hybrid adult females relative to nestlings, with no evidence for sex biases for any genotypic class, including hybrids, in the nestling stage (Walsh et al., 2016a). Our data suggest that reduced survival of hybrid females may play a role in limiting the extent of hybridization within the Saltmarsh and Nelson's Sparrow hybrid zone, and as such, endogenous selection may play a part in maintaining hybrid zone structure and species boundaries in this system.

Conclusion

Patterns of introgression vary across the Saltmarsh and Nelson's Sparrow hybrid zone. Similar to the southern end of the hybrid zone, we found high levels of introgression in the center of the hybrid zone with few recent-generation hybrids. With more equal species ratios and access to mates in the center than the south of the hybrid zone, introgression occurred on a more equal basis between the species, showing that density differences of species influence patterns of introgression. Genotypic composition and extent of hybridization among sites within the center of hybrid zone differed on a small spatial scale, with more backcrossing towards Nelson's Sparrows and more recent generation hybrids at the inland site than the coastal site. These

differences are likely a result of known differences in habitat affinities between the two species, such that local adaptive differences influence the distribution of genotypic classes on a fine scale across sites. We also found evidence for reduced annual survival of hybrid females via Haldane's Rule and assortative mating within the center of the hybrid zone. Overall, hybrid zone structure and maintenance appear to be driven by endogenous and exogenous factors at multiple spatial scales, including population densities and species distribution, differential adaptation to local environments, and pre-zygotic and/or post-zygotic reproductive isolating mechanisms. Thus, no single factor is driving hybridization patterns in this system but rather multiple drivers act in concert to allow for observed patterns in hybrid zone structure.
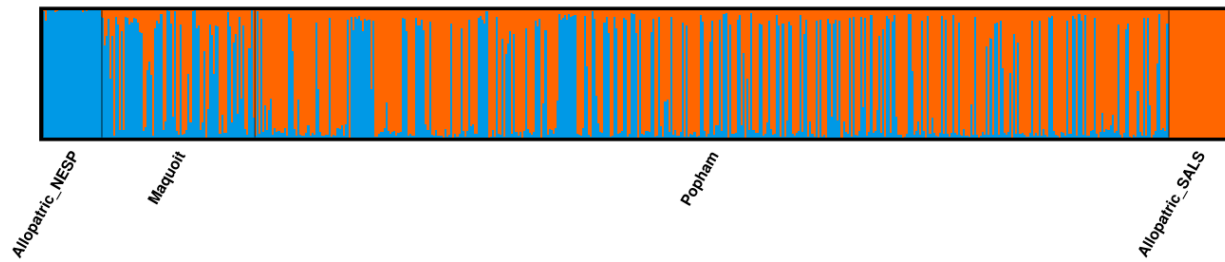
Figure 1.1: Admixture plots from STRUCTURE analysis of Saltmarsh and Nelson's Sparrows from two study locations (Maquoit and Popham) in the center of the hybrid zone compared to allopatric Nelson's Sparrow (Allopatric_NESP) and allopatric Saltmarsh Sparrow (Allopatric_SALS) populations (K = 2). Each vertical bar represents the genetic makeup of an individual, blue representing the Nelson's Sparrow alleles, and orange representing the Saltmarsh Sparrow alleles allopatric Saltmarsh Sparrow population (Allopatric_SALS). Sparrows of pure ancestry have a bar of a solid color, while sparrows of mixed ancestry have bars comprised of both colors.

Table 1.1: Number of birds sampled (adult & nestling) and adult genotypic composition across Popham & Maquoit for the 2016 &2017 breeding seasons. Genotypic classes: pure Saltmarsh Sparrow (SALS), pure Nelson's Sparrow (NESP), first generation hybrids (F1/F2), backcrossed Saltmarsh Sparrow (BC_SALS), and backcrossed Nelson's Sparrow (BC_NESP).

	BC_NESP	BC_SALS	F1/F2	NESP	SALS	Adults	Nestlings	Total Birds
Popham (Coastal Site)	32 % (53)	49% (82)	10% (16)	5% (9)	4% (6)	166	300	466
Maquoit (Inland Site)	37 % (19)	29% (15)	17% (9)	15% (8)	2% (1)	52	26	78
Total Birds	33% (72)	45% (97)	12 % (25)	8% (17)	3% (7)	218	326	544

Table 1.2:Average hybrid index and interspecific heterozygosity for adults and nestlings (male & female) across Popham & Maquoit in the 2016 & 2017 breeding seasons.

	Popham (Coastal Site)	Maquoit (Inland Site)	Male Adults	Female Adults	Male Nestlings	Females Nestlings
Mean Hybrid Index	0.64	0.43	0.54	0.57	0.622	0.64
Mean Interspecific Heterozygosity	0.17	0.21	0.12	0.15	0.17	0.20
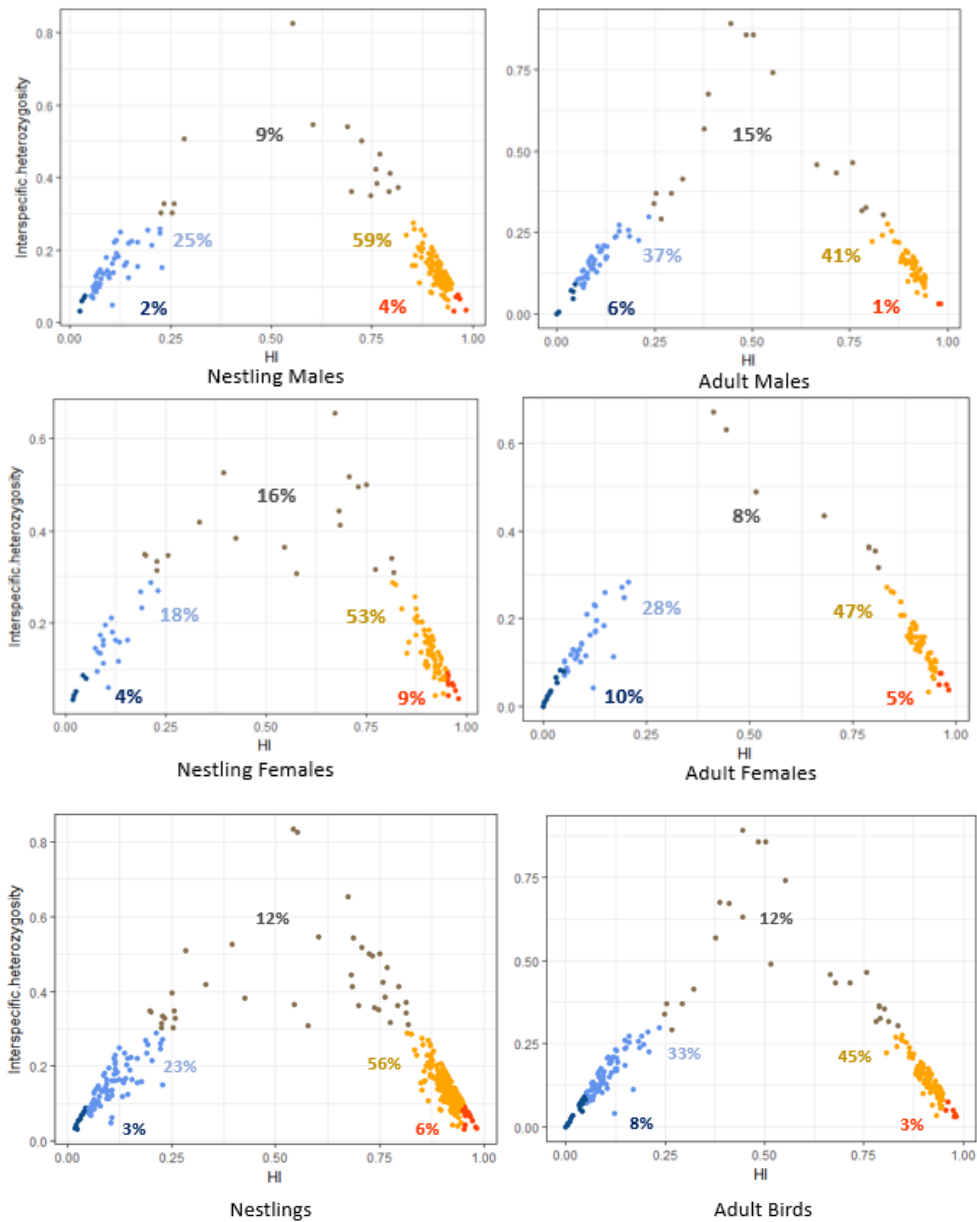
Figure 1.2: Genetic composition by hybrid index (HI) and interspecific heterozygosity of adult and nestling sparrows from two sites in the center of the Saltmarsh-Nelson's hybrid zone. The top panel shows the distribution of genetic composition for all nestling and adult birds, and the lower two panels show the distributions by sex. Colored circles indicate the corresponding genotypic class for the combination of HI and interspecific heterozygosity as follows: dark blue = pure Nelson's Sparrows, light blue = backcrossed Nelson's Sparrows, gray = recent generation hybrids, yellow = backcrossed Saltmarsh Sparrows, and orange = pure Saltmarsh Sparrows.
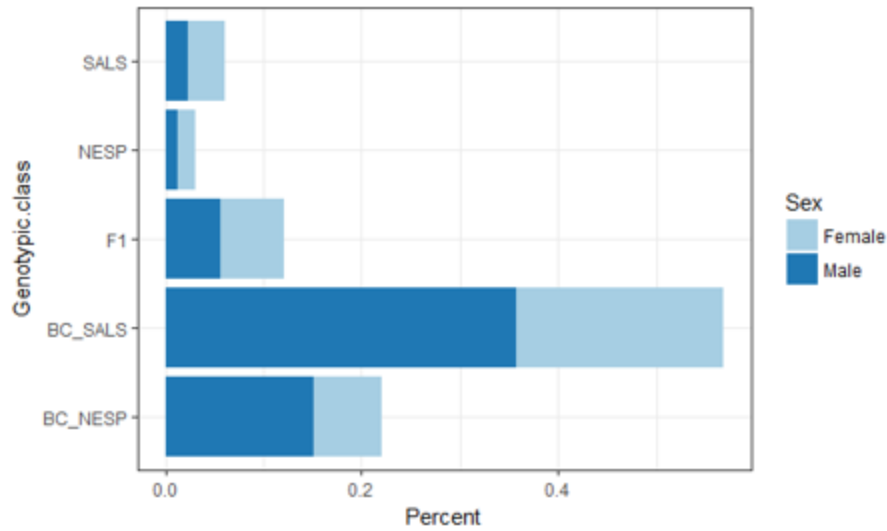
Figure 1.3: Proportional distribution of nestling sparrows of each sex by genotypic class for the study locations in the center of the hybrid zone. Light blue bars represent females and dark blue bars represent males. Genotypic classes: pure Saltmarsh Sparrow (SALS), pure Nelson's Sparrow (NESP), first generation hybrids (F1/F2), backcrossed Saltmarsh Sparrow (BC_SALS), and backcrossed Nelson's Sparrow (BC_NESP).
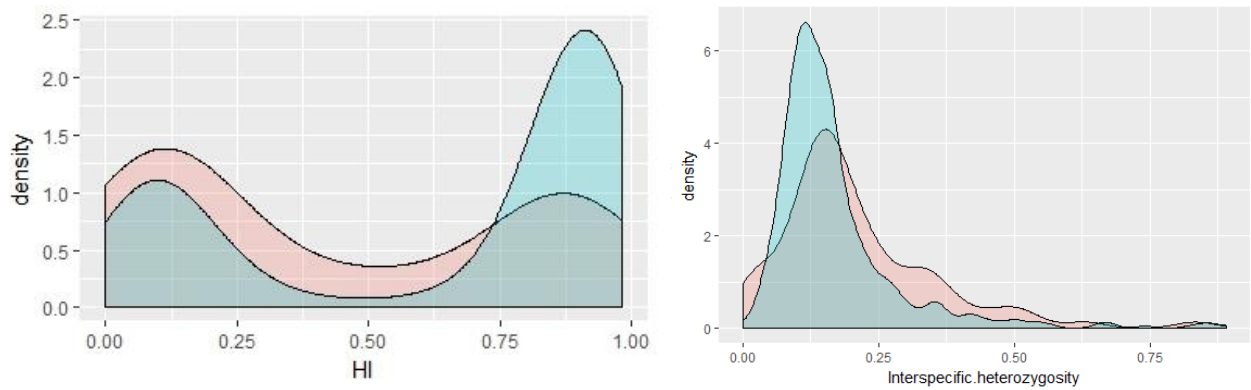
Figure 1.4: Left panel: Relative abundance (density) of sparrows across the range of hybrid index scores (HI; left panel) and interspecific heterozygosities (right panel) between the coastal site (Popham; blue shading) and the inland site (Maquoit; pink shading).

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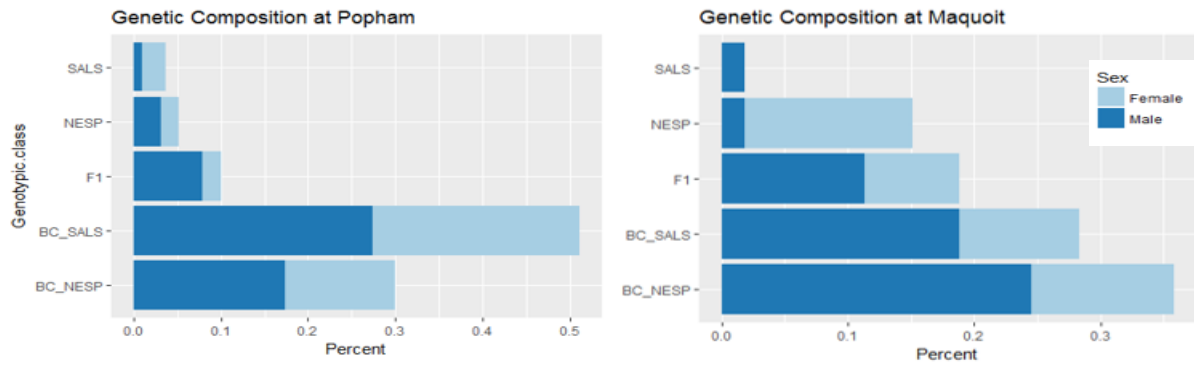
Figure 1.5: Genetic composition of Saltmarsh and Nelson's Sparrow males (dark blue) and females (light blue) across the coastal (Popham) and inland (Maquoit) sites in the center of the hybrid zone (2016 & 2017 seasons). Genotypic classes: pure Saltmarsh Sparrow (SALS), pure Nelson's Sparrow (NESP), first generation hybrids (F1/F2), backcrossed Saltmarsh Sparrow (BC_SALS), and backcrossed Nelson's Sparrow (BC_NESP).
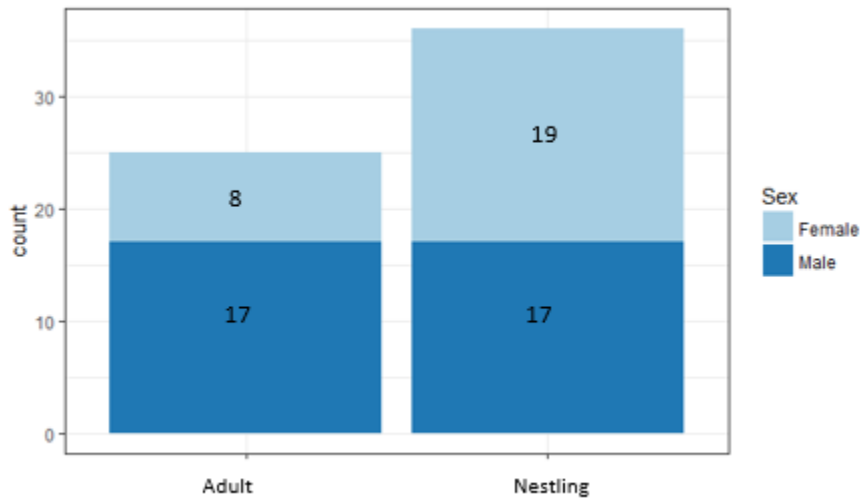
Figure 1.6: Sex ratio of all recent-generation hybrid sparrows across both study sites and years (2016 & 2017) for the two age classes: adult and nestling. The light blue represents females while the dark blue represents males.
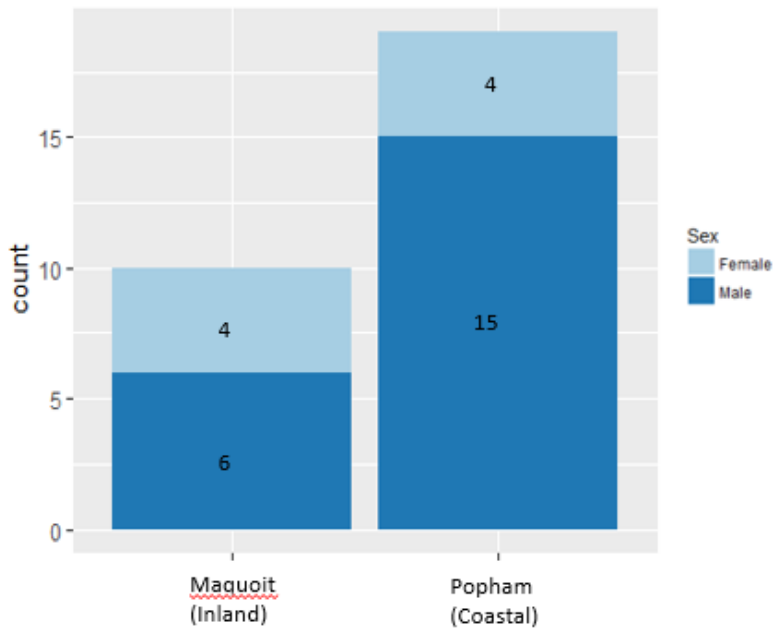
Figure 1.7: Sex ratio of all recent-generation hybrid adults between the coastal (Popham) and inland (Maquoit) study locations (2016 & 2017). Light blue represents the number of recent-generation hybrid adult females and dark blue represents the number of recent-generation hybrid adult males.
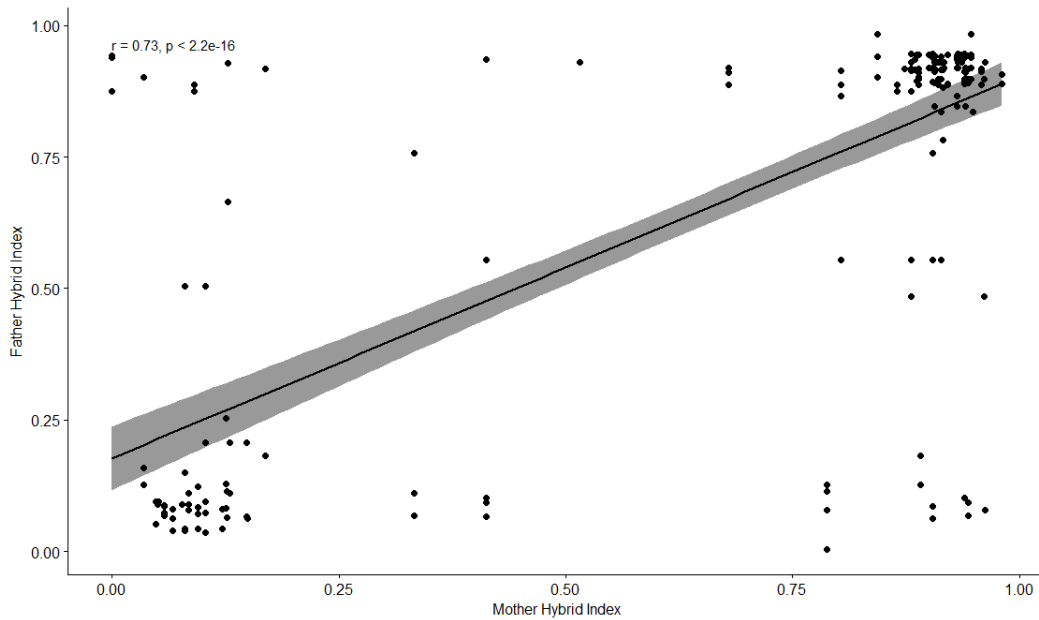
Figure 1.8: Correlation between mother hybrid index (x-axis) and father hybrid index (y-axis) across all reconstructed mating pairs for sparrows in the center of the Saltmarsh-Nelson's Sparrow hybrid zone.

CHAPTER 2

FITNESS CONSEQUENCES OF HYBRIDIZATION FOR NESTING FEMALE

SALTMARSH AND NELSON'S SPARROWS[1]

Abstract

Natural hybridization can augment genetic diversity and may hold a potential source of

evolutionary resilience for species facing the rapid effects of climate change (Carlson et al.,

2014; Hamilton & Miller, 2015; Taylor et al*.,* 2015). When predicting potential outcomes of

hybridization and its role in generating adaptive potential and evolutionary resilience, it is

imperative to look at differential fitness of pure and hybrid individuals because this is indicative

of species responses to shared gene flow. In this study, we address female fitness consequences

of hybridization in two sister species that are endemic to a threatened tidal marsh ecosystem: the

Saltmarsh and Nelson's Sparrows. In the center of the hybrid zone across two years (2016 &

2017), we determined the success of 201 nests of pure and admixed Saltmarsh and Nelson's

Sparrows and determined the hybrid index of adult females (n = 104) using a panel of SNPs from

ddRAD Sequencing. We evaluated five metrics of female fitness, and modeled nesting success in

relation to genotypic, environmental (tidal water level), and nesting characteristics (nest structure

and female behavior). We found differential fitness among Saltmarsh, Nelson's and hybrid

females. Saltmarsh Sparrows had higher fledging and hatching success than Nelson's Sparrows.

Additionally, hybrid index was a predictor of fledging success, such that birds with

predominantly Saltmarsh Sparrow alleles had higher reproductive success than birds with

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[1] Co-authored manuscript prepared for submission to publication

predominantly Nelson's Sparrows alleles. Fledging success models suggested that the number of offspring fledged also increased with two known tidal marsh nesting adaptations: nest height and nesting synchrony with tidal cycles. We found a positive relationship between hybrid index and fitness in daily nest survival in 2016, but not across both breeding seasons (2016 & 2017) combined, likely due to differing level of nest flooding. The strongest and most consistent predictors of daily nest survival were nesting synchrony with lunar tidal flooding cycles (female behavioral adaptation) and daily maximum tide height. Although we observed differential fitness between Saltmarsh and Nelson's Sparrows, flooding rates are so high in many years that they masked any fitness differences between the species or due to hybridization, and all females had poor nesting success, regardless of genetic makeup. Increasing nest flooding rates due to rising sea levels may be limiting any evolutionary outcomes of hybridization due to very low overall nesting success in both the Nelson's and Saltmarsh Sparrows.

Introduction

Assessing the fitness and adaptive potential of organisms in vulnerable natural systems is integral for prioritizing conservation actions. Increasingly, wild populations must respond to the combined effects of climate change and anthropogenic modifications of the landscape, i.e. habitat loss, degradation, and fragmentation. To conserve species in the face of rapid environmental change, understanding and assessing their capacity to cope with or respond to these changes (adaptive capacity) is a current research priority, especially for specialist species and ecosystems most vulnerable to climate change (Nocitra, 2015). The ability to respond to these rapid changes relies on a combination of mechanisms at both short and long-term scales (Aitken, et al., 2008). Plasticity may allow for quick and short-term responses to the environment; however, adaptive or evolutionary responses are needed to allow for genetic and

resulting phenotypic changes to deal with longer-term challenges, such as those faced by climate change (Aitken et al., 2008; Franks et al., 2013; Savolainen et al., 2013). One potential source of evolutionary resilience (Hamilton & Miller, 2015) in light of climate change that has recently gained attention, is the role of hybridization and introgression in releasing populations from their adaptive constraints (Carlson et al., 2014; Taylor et al., 2015). Recombination of genetic variation due to hybridization may allow for rapid evolution in response to changing selective pressure, and in turn increase the rate of demographic recovery or resilience of populations to changing environmental conditions (Hamilton & Miller, 2015). Natural hybridization can augment genetic diversity by extending a species gene-pool, allowing for greater adaptive capacity in response to new environments (Lewontin & Birch, 1966; Hamilton & Miller, 2015). Indeed, adaptive introgression has been seen in a diversity of hybridizing taxa, including both plants and animals (Lexer et al., 2003; Aiken et al., 2008; Scriber et al., 2014; Song et al., 2011; as reviewed by Taylor et al., 2015).

Although multiple generations of gene flow between hybridizing taxa may stimulate adaptive evolution, it can also disrupt local adaptation (Fitzpatrick, et al. 2015). Indeed, hybridization can have largely varying effects on parental populations, ranging from adaptive introgression and speciation (Lewontin & Birch, 1966; Rheindt & Edwards, 2011) to species extinction and outbreeding depression (Allendorf et al., 2001; Edmands & Timmerman, 2003). When trying to understand potential outcomes of hybridization, it is imperative to look at differential fitness between pure and hybrid individuals because this can aid in predicting species responses to shared gene flow (Burke & Arnold, 2001; Lancaster et al. 2007). Due to the complex nature of hybridization and introgression, outcomes are system dependent, and each situation must be taken on a case-by-case basis. In this study, we aimed to identify female fitness

consequences of hybridization between two tidal marsh endemics of conservation concern: the

Saltmarsh Sparrow (*Ammospiza caudacutus*) and the Nelson's Sparrow (*A. nelsoni*).

The Saltmarsh Sparrow's breeding range is restricted to tidal marshes along the

northeastern Atlantic seaboard of the United States and extends from southern Maine to Virginia,

while the Nelson's Sparrow breeds in marshes from the Canadian Maritimes to Massachusetts

(Nocera et al., 2007; Greenlaw & Woolfenden 2007). These sister species co-inhabit marshes

where their ranges overlap (Rising & Avise, 1993; Hodgman et al., 2002) but have differences in

habitat use, behavior, and morphology. Saltmarsh Sparrows are entirely restricted to coastal salt

marshes with heavy tidal flow, while Nelson's Sparrows will also breed in brackish, less tidal

coastal marshes, and have been known to inhabit hayfields and fens (Greenlaw, 1993; Shriver et

al., 2005; Nocera et al., 2007). Historically, the hybrid zone between Saltmarsh and Nelson's

Sparrows was documented in a small range (~50 km) from Scarborough to Popham Beach along

the Maine coast (Greenlaw, 1993). Later results from bird surveys showed a much larger overlap

between Saltmarsh and Nelson's Sparrows than previously documented, with an approximately

200km zone extending from St. Thomaston, Maine as far south as Newburyport, Massachusetts

(Hodgman et al., 2002). Recent genetic studies have indicated that high amounts of introgression

exist throughout the zone and extends north and south the of zone (Walsh et al., 2011), with 52%

of individuals sampled through the hybrid zone consisting of mixed ancestry- mostly

backcrossing in the direction of Saltmarsh Sparrows (Walsh et al., 2015a).

The future of these sparrow species is dramatically influenced by habitat loss and climate

change. Located narrowly along the coastline, tidal marshes are restricted to small shoreland

areas with high development pressure, and they are highly susceptible to the impacts of climate

change associated sea-level rise and alteration in precipitation regimes (IPCC 2014; Tlands, 2013). Because these birds nest only a few centimeters above the marsh surface, monthly tidal events are the leading cause of nest failure in this system, and consequently, these species are extremely vulnerable to even slight increases in sea level (Greenlaw & Rising, 1994; Shriver et al., 2007; Bayard & Elphick, 2011). During these high spring tides, the entire marsh will flood causing nests to be inundated with water for multiple hours (Gjerdrum et al., 2008). Increased tidal flooding due to rising sea levels and more frequent storm events as a result of climate change will reduce, if not eliminate, the sparrows reproductive ability within the imminent future (Bayard & Elphick, 2011). Due to these threats, compounded with limited habitat, these two species are of high conservation priority in the northeastern U.S. (USDI 2008), and the Saltmarsh Sparrow is also globally at risk of extinction (IUCN 2015), with a predicted collapse of the global population within 50 years (Correll et al., 2017). Establishing an understanding of interspecific interactions between Saltmarsh and Nelson's Sparrows and identifying fitness consequences of hybridization will yield important information for the conservation management of these tidal marsh endemics.

It is unclear what role hybridization between the Saltmarsh and Nelson's Sparrow may play in their plight against climate change driven sea level rise and habitat loss. Studies have shown increased rates of introgression throughout the zone over a 15-year period (1997 to 2013) and a southward expansion of the zone (Walsh et al., 2017b). However, species boundaries continue to be maintained despite increased admixture, with evidence for assortative mating as well as selection against mitochondrial markers and reduced survival of hybrid females (Walsh et al., 2016a; 2018b). This suggests there may be fitness consequences to hybridization, but they are still not well understood. Walsh et al. (2016a) found that in the southern end of the hybrid zone, hybrid

females had greater nesting success than Nelson's Sparrows and comparable to that of Saltmarsh Sparrows; however, broader implications of these studies were limited by unequal densities of the parental species (5:1 Saltmarsh to Nelson's) and small sample sizes for hybrids and Nelson's sparrows in the southern edge of the hybrid zone. Patterns of hybridization and introgression often vary spatially across hybrid zones (Futuyma & Shapiro, 1995); therefore, further research from locations of the hybrid zone where the two species are more equally distributed is needed to assess if fitness differences exist between parental species and their hybrids and whether these differences appear to drive patterns of introgression in an adaptive manner. If fitness trends between species hold true throughout the hybrid zone, increased genetic diversity resulting from gene flow between these species may increase the adaptive capacity of Nelson's Sparrows as they move into more coastal environments (Nicotra et al., 2015) and expand their distribution southward (Walsh et al. 2017b). Conversely, potential negative impacts on Saltmarsh Sparrows may warrant consideration in the face of ongoing population declines (Correll et al., 2017). Additional uncertainty exists about the role of adaptive nesting traits on differential reproductive success in this system. It is known that tidal flooding is a major source of nest failure and plays a critical role in determining reproductive success in these two sparrow species (Shriver et al. 2007, Ruskin et al., 2017). Tidal marsh nesting adaptations that may mitigate flooding include nest structure characteristics and female behaviors. The Saltmarsh and Nelson's Sparrows have a nesting period that is 24 days in length, which fits tightly between two 28-day lunar tide cycles and allows for the laying, hatching, and fledging of young in between two high tide flooding events (Shriver et al., 2007). However, if a nesting attempt is started as few as one or two days late, there is a high risk of nest failure due to flooding. Shriver et al. (2007) suggested that the higher nesting success of Saltmarsh Sparrows was due their greater nesting synchrony with tidal cycles, which evolved as an adaptation gained

from living in tidally inundated marshes. Walsh et al. (2016a) modeled nesting success for pure and admixed Saltmarsh Sparrow and Nelson's Sparrows in the southern end of the hybrid zone and found that the timing of nest initiation in relation to the flood tides was a consistent predictor of nesting success. They did not find, however, differences between the species and hybrids in their dataset, which was limited by small sample sizes of Nelson's sparrows and hybrids.

Structural nest characteristics, such as nest height and cover and the vegetation composition of and surrounding the nest, may also serve as adaptations to mitigate flooding. Both species have been found to have nest site and structure preferences, including nesting in areas with more *Spartina patens* (high marsh), deeper thatch, and higher elevation than random (Gjerdrum et al. 2005, Shriver et al. 2007, Ruskin et al. 2015). However, conflicting conclusions have been reached about the relationship between these nest site characteristics and nesting success within and between the species. Shriver et al. (2007) and Gjerdrum et al. (2005) found no relationship of nest characteristics and nesting success. Ruskin et al. (2015) found that nine different nest characteristics differed between Saltmarsh and Nelson's Sparrows, but a model that included all nine of these characteristics did not predict nesting success better than a null model, causing the authors to conclude that nest site selection was nonadaptive. Walsh et al. (2016a) found that Saltmarsh Sparrows had characteristics associated with mitigating nest flooding (higher nests constructed of mixed high and low marsh vegetation), but they were not correlated with nesting success, as the nests of both species experienced similarly high rates of nest flooding. Lastly, in further contrast, Benvenuti et al. (in press), with the largest dataset of any of the prior studies, found that successful Saltmarsh Sparrow nests had more canopy cover and were built higher in the vegetation and in higher elevation areas of the marsh than flooded nests. In light of this apparently conflicting evidence, further research is warranted on whether differential nesting traits that exist

between the species lead to differential nesting success and whether there are such traits that hold an adaptive advantage to nesting in tidal marshes subject to high rates of flooding. By understanding adaptive nesting strategies in relation to hybridization and resulting fitness consequences, we gain insight into the evolutionary outcomes of hybridization and what role that might play in the future persistence of these species in the face of increasing threats due to climate change.

Objectives

In this study, we aimed to determine (1) if fitness differed among pure and hybrid Saltmarsh and Nelson's Sparrow females, and (2) whether observed fitness is predicted by female tidal marsh nesting adaptations.

Predictions

(1) We predicted that female fitness is a function of genotype; such that metrics of reproductive success (fledging success, hatching success, daily nest survival rates, clutch size, nestling size) are positively associated with the proportion of Saltmarsh Sparrow alleles of individual females in the hybrid zone. We expected that hybrids will have higher reproductive success than Nelson's but lower than Saltmarsh Sparrows.

(2) We predicted that female fitness is associated with tidal marsh nesting adaptations (nest structure and timing). We expected females with nest structures and behaviors that mitigate flooding (higher nest height, deeper thatch, higher height of vegetation surrounding the nest, more high marsh vegetation at nest, greater presence of nest canopy, nest synchrony with flood tides, and rapid nest initiation after flood tide) will have higher reproductive success than those

that do not display tidal marsh nesting adaptations (fledging success, daily nest survival rates, larger clutches, larger chick sizes).

Methods

Study Area

Two field sites were selected in the current center of the hybrid zone- the marshes at Popham Beach State Park and Wharton Point on Maquoit Bay, located on the northeastern coast of the United States, between Brunswick, Maine and Phippsburg, Maine. We expected these sites to have relatively similar species abundances based on recent regional abundance estimates of the two species (Wiest et al., 2016) and high numbers of hybrids based on a peak in interspecific heterozygosity at these sites relative to locations across the hybrid zone (Walsh et al., 2016b). The two study sites also span opposite ends of a habitat gradient between coastal and inland tidal marshes and differ slightly in habitat and amount of tidal inundation. The marshes at Popham Beach State Park are located at the tip of a peninsula, directly on the coast. The area of marsh at Popham is expansive; therefore, we selected to focus on a portion of the marsh consisting of ~ 15-hectare plot. The marsh at Maquoit Bay is located more inland and is much smaller than Popham, with the selected study area (~5 hectares) about a third the size of the study plot at Popham. Popham marshes are part of an expansive coastal marsh network, while Maquoit is located in a small cove that is surrounded by mostly forest and field. Although both sites experience daily and monthly tidal inundation, the flooding rates are dampened at Maquoit, with tide heights consistently lower at Maquoit than Popham (see Chapter 1).

Demographic Data Collection/ Nest Monitoring

We conducted nest monitoring at both sites during May- August, encompassing 3 nesting cycles in 2016 & 2017. Nest searches were conducted within each site by walking the marsh systematically and looking for females to flush off of their nest. Once found, we marked and numbered each nest with a flag 3 meters away (a distance thought to be sufficient to minimize disturbance to the female and attraction of predators). We followed standardized protocols established by the Saltmarsh Habitat and Avian Research Program (SHARP; wwwtidalmarshbirds.org) for monitoring nests and determining fate (Ruskin et al., 2017). Nests were visited every 3–4 days until completed and assigned an overall fate (categorical and quantitative). Categorical assignments described the fate of the nestlings (unsuccessful or successful), while the quantitative fates summarized the factors that lead to the categorical assignment (fledged, flooded, depredated, and failed-unknown cause). A nest was considered successful/fledged if one or more nestlings reached fledging age. A nest was determined to be flooded if nest contents were found wet and cold or were found outside of the nest cup. A nest was considered depredated if there were signs of predator activity, including partial remains of nestlings/eggs, the nest cup was stretched or destroyed, or nests were missing eggs or chicks but showed no signs of flooding (Ruskin et al., 2017). A nest was considered to have failed with an unknown cause if eggs or chicks were missing but did not meet any of the previous requirements, and we were unsure of fate. We calculated date of nest initiation based on known duration of egg-laying (3–5 days), incubation (11–12 days), and chick development (8–11 days) to determine first egg date following methods developed by Shriver et al. (2007).

We collected vegetation and nest characteristic data to test predictions about nesting characteristics as drivers of reproductive success. Vegetation data was collected at 1 m^2 surrounding each nest upon its completion (fledge/fail/abandon). Measurements included: thatch

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depth, average vegetation height, and the tallest vegetation height and species composition. A paired random location on the marsh was also surveyed for the same vegetation characteristics. We recorded physical characteristics of the nests including, height above the ground (from cup lip and cup bottom to surface of the marsh), presence/absence of nest canopy (woven/domed structure that effectively covers the nest cup), percent of nest visible from above, and the species of vegetation of which the nest was made. To determine nest initiation and success in relation to the nearest flood tide, we calculated the number of days the nest was initiated after the new moon because the highest tidal amplitudes (and flooding) were on new moon dates due to lunar tidal cycles. In addition, HOBO water level loggers (ONSET, Bourne, MA) were placed at the bottom of a central channel at each study site to monitor the water levels on each day of the breeding season. These loggers measure the total pressure above their location at 15-minute intervals. With barometric pressure collected from the National Oceanic and Atmospheric Administration Stations nearest the study site locations, a compensation was made using HOBOware Pro software to determine water level seen at each marsh in 15-minute intervals throughout the entire three-month breeding season.

From each nest, nestlings were banded with a USGS aluminum leg band and a single site-specific color band when they were 6 days old. Standard morphological measurements were taken including: weight, tarsus length, bill length, head length, and wing cord. A blood sample (few drops on a filter card) was also collected from the medial metatarsal vein of each nestling for genotyping and hybrid identification. We also collected any eggs that had floated out of the nest or were destroyed by other means to use in genetic analyses. To determine the identity of females associated with each nest, we conducted targeted mist-netting to capture females off of their nests during incubation or brooding. Once caught, each female was banded with a USGS

aluminum band, a site-specific color band, and a Passive Integrated Transponder (PIT) tag (Biomarker HTP 12 tag) that was modified to a color band for non-invasive detection of re-nesting attempts. PIT tags use Radio Frequency Identification (RFID) technology to transmit a signal between a tag and a scanner, to positively identify animals remotely. PIT tags were glued to a Darvic color band using an epoxy, with one end of the tag sticking out very slightly from the bottom of the band. The color band with the PIT tag was placed on the bird's tarsus with a second Darvic band placed below to fill the gap between the bird's tarsus and the PIT tag. A small piece of electrical tape (~8mm in length and exactly the width of the two bands -not extending past them) was wrapped around the bands and PIT tag, ensuring no gaps between bands. After the first breeding cycle (i.e., once females had been captured and PIT-tagged), a PIT tag reader was placed at each nest for 30 minutes to determine the identity of the female, if it was a re-nesting attempt from an already tagged female. If no female was detected by the PIT tag reader (i.e., it was not previously PIT-tagged), targeted mist-netting was then employed to capture and identify the female directly off of its nest. We collected standard morphological measurements from females at capture, in addition to recording presence/absence of brood patch. Blood samples from adult females were drawn from the cutaneous ulnar vein and stored on blood filter strips at room temperature for later genetic analysis.

ddRAD Library Preparation

Samples of adult females, nestlings, and salvaged chicks or eggs from the two field seasons were used to prepare double digest restriction site associated DNA (ddRAD) sequencing libraries. In addition, we also used 30 samples each from allopatric Nelson's Sparrow and allopatric Saltmarsh Sparrow populations from previous sampling of the hybrid zone (Walsh *et al*., 2015) for developing a hybrid index. DNA was extracted from blood samples using the

Qiagen DNeasy Blood or Tissue kit (Qiagen, Valencia, CA), following manufacturer protocol. We determined the concentration of resulting DNA samples using Qubit fluorometer Broad Range double-stranded DNA assay kit (Life Technologies, NY, USA). We targeted a DNA concentration of 5–25 ng/ul. Samples below 10ng/ul after initial extraction were vacuum centrifuged to concentrate to within the target range. Samples that were above 25 ng/ul were diluted down to 25 ng/ul. A small number of samples below 5 ng/ul were included and grouped into one index group to ensure the best results. ddRADtags were created using the protocol described in Peterson et al. (2012). DNA was digested with SbfI and MspI, and ligated to P1 and P2 adapters using T4 DNA ligase (30min at 37 ºC and 60min at 20 ºC, held at 10 ºC; Peterson et al., 2012). Samples were pooled into index groups by their unique P1 adapter and cleaned using 1.5x Agencourt AMPure XP beads. Using BluePippin (Sage Science, MA, USA), fragments were size selected between 400–700 bp in length. Low cycle PCR reactions were then performed to incorporate the Illumina TruSeq primer sequences into the library, as well as a final clean up using AMPure XP beads. Libraries were visualized on a fragment Bioanalyzer to ensure desired fragment size/distribution and index groups pooled. Resulting libraries were sequenced across three Illumina HiSeq 2500 lanes and one HiSeq 2500 rapid run lane (read length 100 bp) at the Cornell University Institute for Biotechnology (Genomics Facility Research Center).

Bioinformatic Data Processing & SNP Detection

Sequences were initially evaluated for overall quality using FastQC, then trimmed and filtered using FASTX-Toolkit. Specifically, reads were trimmed on the 3' end to 97 bp and eliminated if the Phred quality scores were below 10 or if 95% of the bases had Phred quality scores below 20. Using STACKS (version 1.48), we demultiplexed the remaining sequences. We used the process_radtags command with the following conditions: any reads not meeting

Illumina's chastity/purity filter and of low quality were discarded, data were cleaned such that any read with an uncalled base was removed, reads with mismatches in the adapter sequence >1 were removed, and reads were only processed if the sequence had an intact SbfI RAD site and one of the unique barcodes. Subsequently, fastx_trimmer was used to trim all sequences to the length of the shortest sequences. Reads were aligned to the Saltmarsh Sparrow reference genome (Walsh et al., 2017a) using STACKS (version 1.48). Minimum stack depth for a read to be assembled into a catalog was 6. The number of mismatches allowed between sample loci was set at 5. We filtered catalog loci based on the mean log likelihood of the catalog locus in the population, with the minimum log likelihood set at -300. These filtering steps resulted in the recovery of 5,391 SNPs.

We used the program Populations to subset a panel of SNPs for use in calculating a hybrid index. We chose only one SNP per locus and required that a SNP be present in a minimum of 50% of all individuals, with a minimum stack depth of 6, for it to be called. Subsequently, VCFtools (Danecek et al., 2018) was used to group individuals into 3 populations: 1) all individuals sampled in this study from the center of the hybrid zone, 2) allopatric Nelson's Sparrows, and 3) allopatric Saltmarsh Sparrows. We then calculated the fixation index (F_{st}) for each SNP using VCFtools and subsetted the panel further to only fixed SNPs ($F_{st} = 1$) between Nelson's and Saltmarsh Sparrows of allopatric populations. This resulted in a panel of 135 fixed SNPs that we used for the development of a hybrid index to classify pure and hybrid sparrows by genotypic class.

Calculating Hybrid Index & Genotypic Classes

Sparrows were assigned to genotypic classes using methods of Milne and Abbot (2008), as in Walsh et al. (2015). Using this method, which combines hybrid index and interspecific heterozygosity, we placed each individual into genotypic classes consisting of: pure Nelson sparrow, backcrossed Nelson, F1/F2, backcrossed Saltmarsh, or pure Saltmarsh sparrow. Hybrid index was defined as the proportion of alleles inherited from the Saltmarsh Sparrow (0 = pure Nelson's Sparrow and 1 = pure Saltmarsh Sparrow), based on the 30 allopatric Saltmarsh and Nelson's sparrows. Interspecific heterozygosity was defined as the proportion of genotypes that are heterozygous across the species for the parental alleles (0 = all homozygous genotypes, found only in one parental species, and 1 = all heterozygous genotypes across species). Individuals with intermediate hybrid index (0.25–0.75) and high heterozygosity (>0.3) were considered recent generation hybrids (F1 or F2), and individuals with very low or high hybrid index (0.05–0.25 or 0.75–0.95) and low heterozygosity (<0.3) were considered backcrossed. Pure individuals were defined as a hybrid index of 0–0.05 (Nelson's Sparrow) or 0.95–1 (Saltmarsh Sparrow). The Introgress package in R was used for calculating the hybrid index and interspecific heterozygosity (Gompert & Buerkle 2010). Analyses do not distinguish between F1 and F2 individuals and these were grouped together into single recent-generation hybrid category, used throughout.

Nest Success Modeling

To identify the drivers of nest success, Program MARK (Dinsmore, 2002; White & Burnham, 1999) was used to generate daily nest survival and failure rates using nest monitoring data in relation to a set of predictor covariates. Prior to analyses, we censored the data by removing any nests that were active for only one visit or were missing covariate values. We used a multi-stage modeling approach where we worked hierarchically through three categories of

covariates additively, with no interaction terms. The categories of covariates were: 1) nest structure measurements, 2) female genotype and 3) nest timing and environmental measurements. Nest structure covariates included height of nest (bottom of nest to ground in cm), percent of nest visible from above, presence of nest canopy, depth of thatch at nest center, average vegetation height surrounding the nest, and percent of high marsh vegetation surrounding the nest. Genotype covariates were hybrid index and genotypic class. Nest timing covariates were days since new moon (measure of nest initiation date post flood tides) and the daily maximum tide height calculated from water level loggers deployed at Popham Beach field site. We used the maximum daily water level from Popham site alone, because it was highly correlated with the water level data collected at the Maquoit site (cor. 0.60, t 9.933, $P < 2.2^{-16}$). The pattern and timing of the high tides was the same between sites, however, the magnitude of the tides differed between the sites, such that Popham had higher tide levels than Maquoit (Figure 1).

When working through the multi- stage approach, we modeled each set of covariates separately for group 1 (nest structure) and retained the models that had strong support (delta AIC<2 and covariate beta estimate CIs not spanning zero). We subsequently added group 2 (genotype) covariates independently to the retained model and identified models that had strong support (delta AIC<2 and covariate beta estimate CIs not spanning zero). Using all informative covariates retained from both the previous groups, models with all combinations of covariates from group 3 (nest timing and environment) were constructed to find the best supported models across the 3 groups. Beta estimates of covariates from informative models (delta AIC <2) in the final round were checked for informative power (confidence interval does not span zero). Because year was found to be a significant covariate influencing daily nest survival, we also

worked through the same multi-stage modeling approach for each breeding season (2016 & 2017) separately.

Evaluating Relationships between Female Fitness and Nesting Adaptations across Genotypic Classes

To determine if female fitness is a function of genotype, we evaluated reproductive success for each female and compared it among genotypic classes. We tested for differences among three broad genotypic classes of Saltmarsh-like birds (pure and backcrossed), Nelson's-like birds (pure and backcrossed), and recent generation hybrids (F1/F2). We subsequently determined if there was a relationship between observed reproductive success and our predicted tidal marsh nesting adaptations. In addition to daily nest survival (modeled above), we also calculated five other fitness/reproductive success metrics: hatching success (# eggs /nest that hatched), fledging success (# nestlings successfully fledged/nest), clutch size, average chick weight, and maximum chick weight in a nest. We evaluated the relationship of these fecundity measures with the same nesting adaptation metrics used for daily survival estimates.

First, we tested for relationships between the five nesting success metrics and genotype to determine if females of differing genetic makeup along the Saltmarsh-Nelson's Sparrow species gradient differ in fitness. We used an ANOVA to compare nesting success among the genotypic classes (categorical variables) and performed Tukey Post Hoc tests on any significant ANOVA results. We also performed a linear regression for each fitness metric to determine the relationship between nesting success and hybrid index. Poisson regression was used in the cases of fledging success, clutch size, and hatching success, due to the fact that these were count-data and did not meet assumptions of linear regression. We then tested for a relationship between the nesting adaptations and genotype to determine if individuals of differing genetic makeup differ

in their nesting habits and characteristics. As above, we used ANOVA to compare nesting adaptations among the genotypic classes and a linear regression to determine the relationship between the same nesting adaptations and hybrid index. If differences were found at among the three broad genotypic classes, we further tested for differences among the five specific genotypic classes (pure Saltmarsh, backcrossed Saltmarsh, recent-generation hybrids, backcrossed Nelson's and pure Nelson's). Finally, we tested for relationships between the nesting adaptations and the five metrics of fitness using linear regression to determine if these nesting characteristics had an influence on fitness. All covariates were tested for collinearity before analyses. We did not use the thatch or vegetation height measurements around the nest because they were found to be positively correlated with nest height (data not shown). Nest height was the strongest predictor; therefore, we chose to use that measurement instead. Any fitness metric that differed significantly among genotypes and was influenced by our predicted nesting adaptations was modeled using generalized linear models (GLM). We created a set of candidate models using significant variables derived from the previous analyses and evaluated them using Akaike Information Criterion (AIC).

Results

We captured, banded, and calculated the genotype for a total of 104 females and 301 nestlings/collected eggs across the two sites and years. We monitored 201 nests of pure and admixed Saltmarsh and Nelson's Sparrows across the two sites in the 2016 and 2017 breeding seasons. Of the 201 nests, 31% of nesting attempts were successful, while 69% were not successful (19% depredated, 34% flooded, 16% failed for unknown reasons). Nesting success was extremely similar for the two sites. At the inland site, Maquoit, we monitored 30 nests across the two years; 70% of them failed and 30% fledged. At the coastal site, Popham Beach,

we monitored 172 nests, 68% of which failed and 32% fledged. Of the nests with known female genotype (79%), we had 9 pure Nelson's Sparrow nests (56% failed, 44% fledged), 6 pure Saltmarsh sparrow nests (50% failed, 50% fledged), 48 backcrossed Nelson's Sparrow nests (69% failed, 31% fledged), 79 backcrossed Saltmarsh sparrow nests (58% failed, 42% fledged), and 17 recent generation hybrid nests (71% failed, 29% fledged; Figure 2). When grouped into three broad genotypic classes, Saltmarsh and backcrossed Saltmarsh Sparrows had 58% (49 failed, 36 fledged) raw nest success rate, Nelson's and backcrossed Nelson's Sparrows had 33% (38 failed, 19 fledged) raw success rate, and hybrids had 29% (12 failed, 5 fledged) raw success rate.

Modeling Daily Nest Survival Across Years

To evaluate drivers of nesting success, we modeling daily nest survival in program MARK with a three-stage modeling approach with covariates of nest structure, female genotype, and nesting timing. From the nest structure category, nest height (B 0.06 ± 0.03, range CI: 0.004–0.110) was the only covariate that resulted in a significant model of daily nest survival across the two breeding seasons, with all other models having delta AIC > 2 (AIC weight = 0.43; Table 1). The average height of the vegetation at the nest center and the average vegetation height surrounding the nest (averaged across 4 cardinal points in 1square meter radius) both provided models that were better than the null model; however, both models had delta AIC >2 (Table 1). All the other nest structure measurements lead to models that were less supported than the null model of constant daily nest survival (Table 1).

The addition of the second category of genotype predictors resulted in no additional supported models than the one with nest height carried over from the first model group. The

model with additive effects of nest height and hybrid index was within 2 delta AIC of the top model, with nest height alone; however, the beta estimate 95% CI for hybrid index spanned zero (B 0.34 ± 0.26, range CI: -0.17–0.86), suggesting it was not an informative covariate; therefore, we did not include this parameter in the next step of modeling (Table 2). The model including genotypic class and nest height was no better supported than the null model of constant daily nest survival (Table 2).

With the addition of the nest timing and environmental covariates, there were two resulting supported models (delta AIC <2). The best supported model included covariates of site (B 0.54 ± 0.29), year (B -0.53 ±0.22), nest initiation post new moon (B -0.03 ± 0.01), daily maximum tide height (B -1.05 ± 0.22) and nest height (B 0.04 ± 0.03; Table 3). The second supported model included all of the same covariates, except site. The 95% confidence intervals for the beta estimates of nest height (range CI: -0.02–0.09) and site (range CI: -0.04–1.11) were overlapping zero; therefore, were not informative parameters. The coefficients for daily maximum tide height (range CI: -1.48 to -0.62) nest initiation post new moon (range CI: -0.05 to -0.002), and year (range CI -0.97 to -0.10) did not overlap zero and were therefore considered to be informative parameters for daily nest survival rates across the two years (Table 4). Nest initiation post new moon (in days) had a negative relationship with daily nest survival (Figure 3), such that daily nest survival decreased with an increase in the number of days past the new moon that a nest was initiated. Daily maximum tide height had a negative relationship with daily nest survival, with large dips in daily nest survival seen across the nesting season in relation to the peak in maximum daily tide height, corresponding with new moon flooding events (Figure 4). Finally, year was also an important factor for daily nest survival. Maximum tide height as well as daily nest survival estimates were lower and had less fluctuation in 2016 than 2017 (Figure 4).

Modeling Daily Nest Survival Within Years

Due to the above finding of yearly differences in nesting success, we subsetted the data by year to determine the drivers of nest success for each year separately, following the same three-stage modeling process as above. We found different outcomes for the two years.

In 2016, nest height (B 0.11 ± 0.05, range CI: -0.01 to - 0.20) was the only covariate that resulted in the highest supported model of daily nest survival from the first category of nest structure measurements (AIC 227.6, delta AIC 0.00, AICw 0.53; Table 5). The addition of the second category of genotype predictors resulted in a single supported model (AIC <2) including nest height and hybrid index (AICw 0.88; Table 6). This lead to a total of four best supported models when nest timing and environmental covariates were added in the third tier of modeling (Table 7). The parameters included in those models were: nest height (B 0.06 ± 0.05), tide height (B -1.66 ± 0.43), hybrid index (B 1.26 ± 0.47), nest initiation post new moon (B -0.05 ± 0.03), and site (B 0.59 ± 0.46); however, the only informative parameters that did not have beta coefficient confidence intervals overlapping zero were hybrid index (range CI: 0.34–2.18) and tide height (range CI: -0.82 to -2.50; Table 8). Similar to the results for the two years combined, we found that tide height had a strong relationship to daily nest survival (Figure 4). Daily nest survival rates across the 2016 breeding season tracked closely with the lunar tide cycles, such that large observed drops in daily nest survival corresponded to new moon events that caused tidal marsh flooding (Figure 4). Overall, daily nest survival rates and maximum tide height had less fluctuation in 2016 than in 2017. We also found genetic makeup of the nesting female to have an effect on daily nest survival, but this was not seen in both years. In 2016, there was a positive relationship between hybrid index of the female and nest survival, such that daily nest survival increases with the proportion of alleles from the Saltmarsh Sparrow (Figure 5).

For the models with the 2017 breeding season data, there were no informative covariates from models with either of the first two, nest structure or genotype, categories of predictors (Table 9, Table 10). There were a number of competing models that had delta AIC < 2, with the covariates of nest height, thatch depth, nest canopy, percent high marsh vegetation, vegetation height around the nest, and hybrid index. However, none of the models did any better at predicting daily nest survival than the null model of constant daily nest survival and were therefore not found to be informative. After adding in the nest timing category of predictors, we found one informative model that included daily maximum tide height only (B -2.03 ±0.23, range CI: -2.47 to -1.58; Table 11). This model was strongly supported, with the next best model having a delta AIC of greater than 70. Similar to what was seen across years and in 2016 alone, daily nest survival was largely influenced by daily maximum tide height. Similar to patterns across years and in 2016, temporal trends in nest survival across the breeding season tracked the lunar tide cycle (Figure 4). The magnitude of tides differed between the sites and years, Popham had higher daily maximum tides than Maquoit in both years (Figure 1), and tide heights were higher in 2017 than 2016 at both locations, with maximum tide levels per day being much closer in value between the sites in 2016 than 2017.

Relationships between Female Fitness and Nesting Adaptations across Genotypic Classes

In addition to modeling daily nest survival, we looked at five additional metrics of fitness and tested to see if they differed across the genotypic classes of pure and hybrid sparrows. Both parental groups (pure and backcrossed Saltmarsh as well as pure and back-crossed Nelson's) fledged more offspring than hybrids (F1/F2), although the difference was marginally significant (ANOVA followed by Tukey Post Hoc test; F = 2.62 P = 0.08). Hybrids fledged the fewest offspring (0.588 ± 0.37), followed by Nelson's (backcrossed & pure, 0.909 ± 0.43), and

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Saltmarsh Sparrows (backcrossed & pure, 1.365 ± 0.41; Table 12) respectively. Hatching success also differed among the genotypic classes, with Saltmarsh/backcrossed Saltmarsh Sparrows (3.882 ± 0.20) having significantly higher hatching success than Nelson's/backcrossed Nelson's sparrows (3.527 ± 0.21, ANOVA followed by Tukey Post Hoc test; F = 3.88, P = 0.02; Table 12). Hatching success did not differ significantly between hybrids and either Nelson's/backcrossed Nelson's or Saltmarsh/backcrossed Saltmarsh. Hybrid index had a significant effect on fledging success (Poisson regression; B 0.45 ± 0.15, z 2.18, P = 0.03), such that fledging success was positively associated with the amount of Saltmarsh sparrow alleles of females (Table 14). There was no relationship between hybrid index and hatching success (Poisson Regression; B 0.12 ± 0.11, z 1.1, P = 0.27) or clutch size (Poisson regression; B 0.05 ± 0.11, z 0.50, P = 0.63; Table 14).

Genotype did not influence clutch size (ANOVA; F = 1.53, P = 0.22) but had an effect on maximum chick weight per nest (ANOVA, F = 4.82, P = 0.01) (Table 12). Maximum chick weight was higher in Saltmarsh (pure & backcrossed, 13.6g ± 0.70; Tukey Post Hoc test; P = 0.0095) than Nelson's Sparrows (pure & backcrossed, 12.2 g ± 0.74; Table 12; Figure 6). Hybrids had intermediate weight (12.6 ± 0.65) between the two species and were significantly different than either parental species. Because we found a difference in chick weight at the level of the broad genotypic classes, we also tested for differences among the five specific genotypic classes: pure Nelson's Sparrow, backcrossed Nelson's Sparrow, F1/F2, backcrossed Saltmarsh sparrow, and pure Saltmarsh sparrow. Backcrossed Saltmarsh Sparrows had significantly higher maximum chick weights (13.5 g ± 0.49) per nest (ANOVA followed by Tukey Post Hoc test; F = 2.68, P = 0.04) than backcrossed Nelson's Sparrows (12.1 g ± 0.41), with hybrids being intermediate (12.7 g ± 0.65) – although not significantly (Table 13; Figure 6). Average chick

weight did not differ among the genotypic classes (ANOVA; F = 1.34, P = 0.26) Table 12; Table 13). We found a positive relationship between hybrid index and chick weight, such that chick weight was positively correlated with the amount Saltmarsh sparrow alleles of females (linear regression; B 0.05 ± 0.002, t 2.3, P = 0.03) as well as maximum weight (linear regression; B 0.06 ± 0.02, t 3.08, P = 0.003; Table 14).

ANOVA showed no significant differences in nesting adaptations among the five genotypic classes (Table 15). When looking at the five genotypic classes, we see that pure species (both Nelson's and Saltmarsh) initiate nests earlier than any of the introgressed genotypes, although the difference was not statistically significant. Nelson's initiate a nest ~8 days after a flood tide and Saltmarsh Sparrows initiate a nest ~6 day after a flood tide on average, while all the other genotypes initiated 9 or more days after a flood tide. We found no relationship between nest height (linear regression; B 0.001 ± 0.003, t 0.41, P = 0.68, nest initiation post new moon (linear regression; B 0.002 ±0.003, t 0.42, P = 0.68), or percent of the nest visible from above (linear regression; B -0.002 ± 0.001, t -1.75, P = 0.08) and hybrid index. There was a significant negative relationship between percent high marsh vegetation (S. patens) surrounding the nest (linear regression; B -0.002 ± 0.001, t -2.22, P = 0.03) and hybrid index (Table 16), such that percent high marsh vegetation around the nest decreased with the proportion of Saltmarsh sparrow alleles of the nesting female. Nelson's Sparrows had a higher proportion of high marsh vegetation in their nesting location, while Saltmarsh sparrows had more of a mix of low and high marsh vegetation.

We found that none of the nesting adaptations were good predictors of hatching success, clutch size, average chick weight per nest, or maximum chick weight per nest (Table 17).

However, nest height (Poisson regression; B 0.05 ± 0.01, z 3.80, P = 0.0002) and nest initiation post new moon (Poisson regression; B -0.003 ± 0.001, z -2.9, P = 0.004) were significant predictors of fledging success (Table 17).

Modeling Fledging Success

Based on the above relationships, we modeled predictors of fledging success (as measured by number of offspring fledged in a nest; the only fitness metric with significant predictor variables) using GLM. We created a set of candidate models that included every combination of significant covariates from the prior univariate analyses: hybrid index, nest height, and nest initiation post new moon. We also included site and year as independent models. This analysis resulted in only one highly supported model of fledging success (AICw 0.73) that included nest height (B 0.05 ± 0.02, range CI: 0.1–1.02), hybrid index (B 0.47 ±0.20, range CI: 1.07–2.39), and initiation post new moon (B -0.03 ± 0.01, range CI: 0.95–1.10) (Table 18). All covariates had beta estimates that did not overlap zero and were therefore considered informative to fledging success (Table 19). Hybrid index had a positive relationship with fledging success (Poisson regression; B 0.47 ±0.20, z 2.29, P = 0.02), such that number of offspring fledged increased with increasing amount of Saltmarsh sparrow alleles (Figure 9). Nest height (Poisson regression; B 0.05 ± 0.02, z 3.23, P = 0.001) also had a positive relationship with fledging success, such that the higher the nest was built off of the ground, the higher the number of offspring fledged (Figure 8). Conversely, nest initiation post new moon had a negative relationship with fledging success (Poisson regression; B -0.03 ± 0.01, z -2.4, P = 0.02): the number of offspring fledged decreased with increasing number of days the nest was initiated after the new moon (Figure 7).

Discussion

Concurrent with known population trends (Correll et al., 2017), Nelson's, Saltmarsh, and hybrid individuals all had low nesting success, with over half of nests failing, primarily due to flooding, regardless of genetic makeup. Despite low success overall, we observed differential reproductive success among female Saltmarsh and Nelson's Sparrows and their hybrids, as well as across a continuum of hybrid index values for multiple metrics of fitness. Saltmarsh Sparrow females had higher fitness than Nelson's Sparrow females in the form of raw nesting success, fledging success and hatching success. Hybrid females were intermediate in some fitness metrics, while lower than both parental species in others. In addition, fledging success across years and daily nest survival in 2016 increased with hybrid index values – with daily nest survival and the number of offspring fledged both positively associated with Saltmarsh Sparrow genotypes.

Saltmarsh Sparrows and backcrossed Saltmarsh Sparrows also had larger maximum chick weights per nest than Nelson's and backcrossed Nelson's Sparrows, and although chick weight may be a predictor of female fitness, this relationship we observed across species may be an artifact of differing morphology between the two species in nestling growth and size (Nelson Sparrows tend to be smaller than Saltmarsh Sparrows in bill length and weight; Greenlaw, 1993; Shriver et al., 2005). Larger and faster growing chicks may have an advantage over those that are smaller during a flood tide, where larger and more mobile chicks may be able to climb up on vegetation and avoid drowning, but this speaks little to overall fitness of female parent and more to the nestling individual.).

Our findings of differential reproductive success between Saltmarsh and Nelson's Sparrows is consistent with findings from previous work in the southern portion of the hybrid zone (Shriver et al., 2007; Walsh et al., 2016a). Because our study was performed in the center of the hybrid zone, we were able to eliminate the confounding factor of differing species densities and small sample sizes of Nelson's Sparrows. This suggests fitness trends between species are consistent throughout the hybrid zone – with Saltmarsh Sparrows having higher reproductive success than Nelson's Sparrows. Hybrid females did not show a clear pattern of differential fitness relative to the parental species in this study (some fitness metrics were intermediate, while others lower than both species), perhaps due to small sample sizes in relation to the other genotypic classes. Additionally, we found that nesting success generally increased positively with hybrid index values, suggesting that fitness increases linearly along a gradient of increasing Saltmarsh Sparrow alleles.

Although hybrid index showed a significant influence on fledging success across both years of the study and on daily nest survival in 2016, we did not find any differences in daily nest survival across Saltmarsh, Nelson's or hybrid females for the combined 2016 & 2017 nesting seasons. This finding suggests that differential fitness may be year dependent. Variable environmental factors can cause fluctuating selection pressures that favor hybrids or parental forms on the short term and between years (Grant, & Grant, 1992). During the 2016 breeding season, genotype had a significant influence on daily nest survival, however daily maximum tide height had an even stronger relationship. In 2017, the only significant predictor of daily nest survival was daily maximum tide height. Tides were much more intense and higher surrounding the new moon events in 2016 than 2017. The new moon flooding events were dampened in 2016 compared to 2017: with max tide heights reaching 7.3 m in 2016 and 8.7 m in 2017. Precipitation

and climatological factors have been shown to affect marsh systems and coastal water levels (Childers et al., 1990). Lower maximum tide levels in 2016 are likely due to very low levels of precipitation during the nesting season. Low precipitation led to lower baseline water levels on the marsh, and subsequently lower tide heights during monthly flooding events. This suggests that during dry years on the marsh (when water levels are low), potential adaptive differences between the species may manifest in differential fitness, but in wetter years (with higher water levels), nest failure rates due to flooding are similarly high for both species, masking any potential genotype effect. There may be some threshold tide height above which sparrows of both species (and hybrids) reproduce consistently poorly and the only driver of nest success is the tide height (as was seen in 2017).

Based on these findings and those of previous researchers in this system, we hypothesize that Saltmarsh and Nelson's Sparrows are currently experiencing conditions (water levels) that differ from those in which they evolved (Bayard & Elphick, 2011). The relatively low flood tide levels in 2016 may be more similar to conditions to which Saltmarsh Sparrows are adapted. Conditions we see today, characterized by high rates of flooding-associated nest failure, may explain inconsistencies in prior studies in detecting adaptive nesting differences between Saltmarsh and Nelson's Sparrows (Gjerdrum et al., 2005; Shriver et al., 2007; Ruskin et al., 2015; Walsh et al., 2016a; Benvenuti et al. (in press). Sea-level-rise associated increases in tidal water levels on the marsh and frequency of days in which the marshes are flooded (monthly flooding and increased stochastic events) are reducing the window for successful nesting of tidal marsh birds (Bayard & Elphick, 2011). Accordingly, with respect to fitness consequences, although the genetic makeup of the nesting female appears to be an important predictor of success, its influence is secondary to predictors that capture the risk of flooding in this system.

Two tidal marsh nesting adaptations that mitigate the effect of nest flooding were subsequently found to be good predictors of fitness for all sparrows, regardless of genotype: nest height and nest initiation post new moon. Nest height had a positive relationship with daily nest survival and fledging success, while nest initiation post new moon had a negative relationship with those same fitness metrics. Nest height intuitively relates to flooding risk, with nests closer to the marsh surface having a higher risk of being inundated with tidal water. However, nest height is also a balance between building the nest low enough to be concealed from predators, yet high enough to withstand tidal water level on the marsh (Greenberg et al., 2006). A positive relationship with nest height and success for these birds suggests nest flooding presents a larger threat than predation in this system, at the northern latitudes of this study (Ruskin et al., 2017). While females can increase their chances of avoiding nest flooding by placing their nests sufficiently high in the vegetation, they can also benefit from synchronizing their nesting with the tidal cycles. Specifically, timing nest initiation soon after the new moon is a behavioral adaptation to mitigate flooding (Shriver et al., 2007). The sooner the female is able to initiate a nest after a new moon flooding event, the longer amount of time and better chance she will have of completing the 24-day nesting cycle before the next flooding event. Nesting adaptations that mitigate flooding, including nest height and nest initiation post new moon, are important predictors of success in a system where tidal marsh flooding is the major source of nest failure.

Conditions we see today, characterized by high water levels and nest flooding failure, may also account for discrepancies in drivers of reproductive success observed among recent studies. We found tidal marsh nesting adaptations (nest height, nest initiation) to be reliable predictors of nest success. Our results support the studies of Walsh et al. (2016a) and Shriver et al. (2007), who also found nest initiation in relation to the flood tides was a consistent predictor

of nesting success, and Benvenuti et al. (in press), which found successful nests were placed higher in the vegetation than flooded nests. Our findings also contrast other studies in this system that found no relationship between nesting characteristics and nest success (Gjerdrum et al., 2005, Ruskin et al., 2015). Rather than nesting behaviors lacking an adaptive benefit (Ruskin et al. 2015), we hypothesize that benefits of tidal marsh nesting adaptations fail to be realized under current environmental conditions because they have changed outside the range of conditions to which tidal marsh nesting birds have adapted.

Although we found nest height and nest synchrony were strong predictors of fitness, these traits did not differ among pure species and hybrids. We did find evidence that pure females of both species initiate nests after flood tides three or more days sooner than admixed females, with Saltmarsh Sparrows having the earliest initiation post new moon. This suggests an adaptive advantage to pure species, and especially Saltmarsh Sparrows; however, these results were limited by small numbers of pure individuals in our study. Further, we also found limited support for differences in nesting traits of Saltmarsh and Nelson's Sparrows remnant of their evolutionary histories with the tidal marsh. The percent of high marsh vegetation around the nest varied with hybrid index, such that more Saltmarsh Sparrow-like females had more of a mix of high and low marsh vegetation around the nest, while more Nelson's Sparrow-like females had less vegetative diversity, with the immediate nesting area consisting of mostly of *S. patens*. Walsh et al. (2016a) hypothesized that nest structure increases with a higher diversity of vegetation including a mixture of both high and low marsh vegetation. A mixture of *Spartina patens* (high marsh) and, *S. alterniflora* (low marsh) may allow nests to withstand flooding better than nests built primarily in the less rigid *S. patens*. Benvenuti et al. (in press) found support for this hypothesis in Saltmarsh Sparrows, as successful nests were within more mixed vegetation

than failed nests. Despite differences observed between the species in their vegetation nest structure, we did not find that vegetation diversity was a consistent predictor of nest success.

Conclusions

We found differential nesting success and adaptation to tidal marsh environments between the Saltmarsh and Nelson's Sparrows. Saltmarsh Sparrows had higher reproductive success than Nelson's Sparrows, and although genotype had an effect on reproductive success, the strongest and most consistent predictors of fitness in this system were daily maximum tide height and nest initiation post new moon, as a measure of synchrony with lunar tide cycles. Two tidal marsh nesting adaptations that mitigate the effect of nest flooding were subsequently found to be good predictors of fitness for all sparrows, regardless of genotype: nest height and nest initiation post new moon. Increased genetic diversity resulting from gene flow between these species may increase the fitness capabilities of Nelson's Sparrows as they move into more coastal environments (Nicotra et al., 2015) and expand their distribution southward (Walsh et al. 2017b). Introgression, however, does not appear to provide a fitness benefit for female Saltmarsh Sparrows, which have higher reproductive success than Nelson's and hybrids, and in most cases backcrossed Saltmarsh Sparrows. High levels of introgression between the species may warrant increased conservation concern for the Saltmarsh Sparrow as the spread of Nelson's alleles into the Saltmarsh may disrupt local adaptation and further exacerbate ongoing population declines (Correll et al., 2017). Fitness differences could drive patterns of hybridization between the Saltmarsh and Nelson's Sparrows (Walsh et al., 2016a); however, current tide levels and observed alteration in precipitation regimes due to sea-level rise are leading to overall low nest success, and monthly flooding events could be swamping out any observed effect hybridization on differential fitness between the species. Although hybridization and continued gene flow

between these two sister-species still has the power to influence the evolutionary trajectory and future persistence of the Saltmarsh and Nelson's Sparrow, it does not appear to be the largest driver of nest success in this system. Sea-level rise due to climate change, rather than hybridization outcomes, are more likely to determine the future course of these two threated tidal marsh endemics.
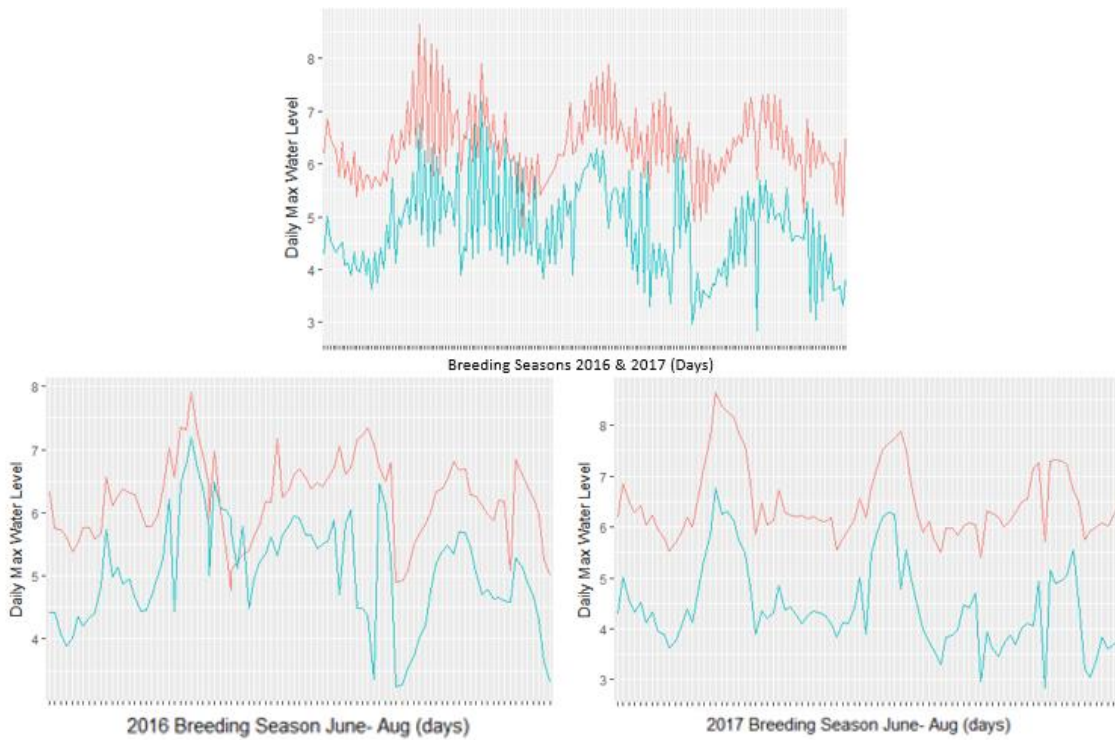
Figure 2.1: Daily maximum tide levels as measured by water-level loggers at the two study sites in the center of the Saltmarsh-Nelson's Sparrow hybrid zone. The red line shows water level at Popham and the blue line shows water level at Maquoit. Top graph shows all days in both breeding seasons, while the bottom two panels are split out by year (2016 &2017).
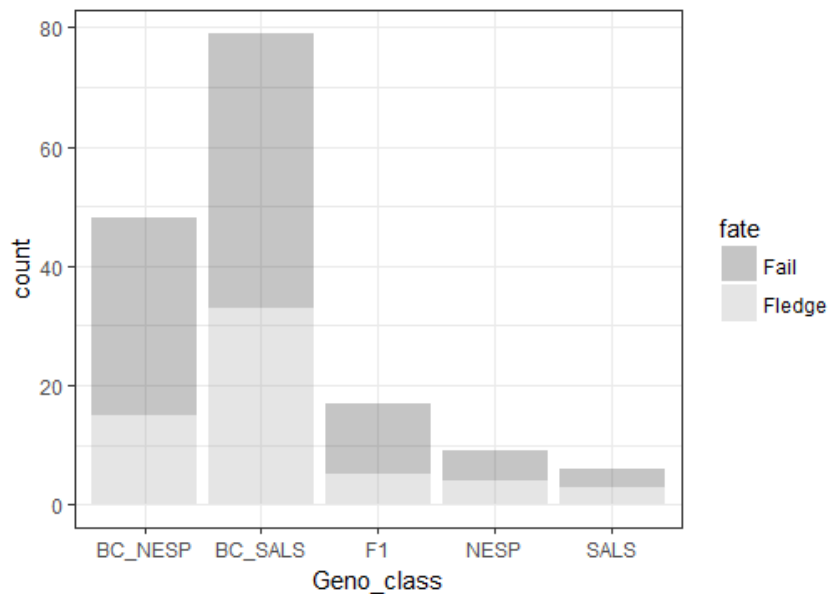
Figure 2.2: Raw nesting success by genotypic class for pure and hybrid females at two sites in the Saltmarsh and Nelson's Sparrows hybrid zone across 2016 & 2017 breeding seasons. Labels are as follows: Backcrossed Nelson's (BC_NESP), Backcrossed Saltmarsh (BC_SALS), 1st/2nd Generation Hybrids (F1), Pure Nelson's (NESP), and Saltmarsh (SALS)

Table 2.1: Daily nest survival modeling results from group 1 predictors of nest structure measurements across 2016 & 2017 breeding seasons.

Model	K	AICc	Delta AICc	AICc Weight	Model Likelihood	Deviance
{B0} + Nest Height[1]	2	603.279	0.00	0.44	1.00	599.27
{B0} + Veg. Height at nest[2]	2	605.978	2.70	0.11	0.26	601.97
{B0} + Veg. Height avg.[3]	2	606.080	2.80	0.11	0.25	602.07
{B0}	1	606.104	2.82	0.11	0.24	604.10
{B0} + Nest canopy[4]	3	606.879	3.60	0.07	0.17	600.87
{B0} + Thatch depth avg.[5]	2	607.779	4.50	0.05	0.11	603.77
{B0} + Thatch depth at nest[6]	2	607.877	4.60	0.04	0.10	603.87
{B0} + Percent high marsh veg.[7]	2	608.073	4.79	0.04	0.09	604.07
{B0] + Percent Visible[8]	2	608.105	4.83	0.04	0.09	604.10

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Average height of vegetation at location directly above the nest
3 Average height of vegetation (cm) across measurements taken at 4 cardinal directions surrounding the nest (1 square meter sampling radius)
4 Presence of nest canopy (full, partial, or none)
5 Average depth of thatch (cm) of measurements taken at 4 cardinal directions surrounding the nest (1 square meter sampling radius)
6 Depth of thatch below the nest (cm)
7 Percent of spartina patens in the square meter sampling plot surrounding the nest
8 Percent of the nest visible to the observed from a point directly above the nest

Table 2.2: Daily nest survival nest modeling results from group 2 predictors of genotype across 2016 & 2017 breeding seasons.

Model	K	AICc	Delta AICc	AICc weight	Model Likelihood	Deviance
{B0} + Nest Height[1]	2	603.279	0.00	0.44	1.00	599.27
{B0} + Nest Height + Hybrid Index[2]	3	603.622	0.34	0.37	0.84	597.61
{B0}	1	606.104	2.82	0.11	0.24	604.10
{B0} + Nest Height + Genotypic Class[3]	6	606.879	3.60	0.07	0.17	594.84

1 Number of centimeters bottom of nest cup is from the marsh surface
2. Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)
3. Five genotypic classes: Backcrossed Nelson's (BC_NESP), Backcrossed Saltmarsh (BC_SALS), 1st/2nd Generation Hybrids (F1), Pure Nelson's (NESP), and Saltmarsh (SALS)

Table 2.3: Daily nest survival modeling results from group 3 including nest timing and environment predictors across the 2017 & 2017 breeding seasons.

Model	K	AICc	Delta AICc	AICc Weight	Model Likelihood	Deviance
{B0} + Nest Height[1] + Tide Height[2] + Post Moon[3] + Year[4] + Site[5]	6	578.975	0.00	0.48	1.00	566.94
{B0} + Nest Height + Tide Height + Post Moon + Year	5	580.044	1.07	0.28	0.59	570.02
{B0} + Nest Height + Tide Height + Year	4	582.250	3.28	0.09	0.20	574.23
{B0} + Nest Height + Tide Height + Post Moon + Site	5	583.396	4.42	0.05	0.11	573.37
{B0} + Nest Height + Tide Height + Site	4	583.907	4.93	0.04	0.09	575.89
{B0} + Nest Height + Tide Height + Post Moon	4	583.998	5.02	0.04	0.08	575.98
{B0} + Nest Height + Tide Height	3	585.021	6.05	0.02	0.05	579.01
{B0} + Nest Height + Post Moon	3	602.866	23.89	0.00	0.00	596.85
{B0} + Nest Height + Post Moon + Site	4	602.962	23.99	0.00	0.00	594.94
{B0} + Nest Height + Site	3	603.022	24.05	0.00	0.00	597.01
{B0} + Nest Height	2	603.279	24.30	0.00	0.00	599.27
{B0} + Nest Height + Post Moon + Year + Site	5	603.318	24.34	0.00	0.00	593.29
{B0} + Nest Height + Post Moon + Year	4	603.381	24.41	0.00	0.00	595.36
{B0} + Nest Height + Year + Site	4	604.081	25.11	0.00	0.00	596.06
{B0} + Nest Height + Year	3	604.475	25.50	0.00	0.00	598.46

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Daily maximum water-level (m) on the marsh (as measured by water-level loggers)
3 Number of days the nest was initiated post the monthly new moon
4. Year: 2016 & 2017 nesting seasons
5 Site: Inland (Mquoit) & Coastal (Popham)

Table 2.4: Beta coefficients and upper and lower 95% confidence intervals (CI) for covariates in the best supported model from the third tier of multi-stage hierarchical daily nest success modeling across the 2016 & 2017 breeding seasons.

Parameter	Beta	Beta SE	2.5% CI	97.5% CI
Nest Height[1]	0.037	0.027	-0.016	0.090
Tide Height[2]	-1.050	0.218	-1.478	-0.622
Post Moon[3]	-0.028	0.013	-0.053	-0.002
Year[4]	-0.534	0.221	-0.967	-0.102

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Daily maximum water-level (m) on the marsh (as measured by water-level loggers)
3 Number of days the nest was initiated post the monthly new moon
4. Year: 2016 & 2017 nesting seasons
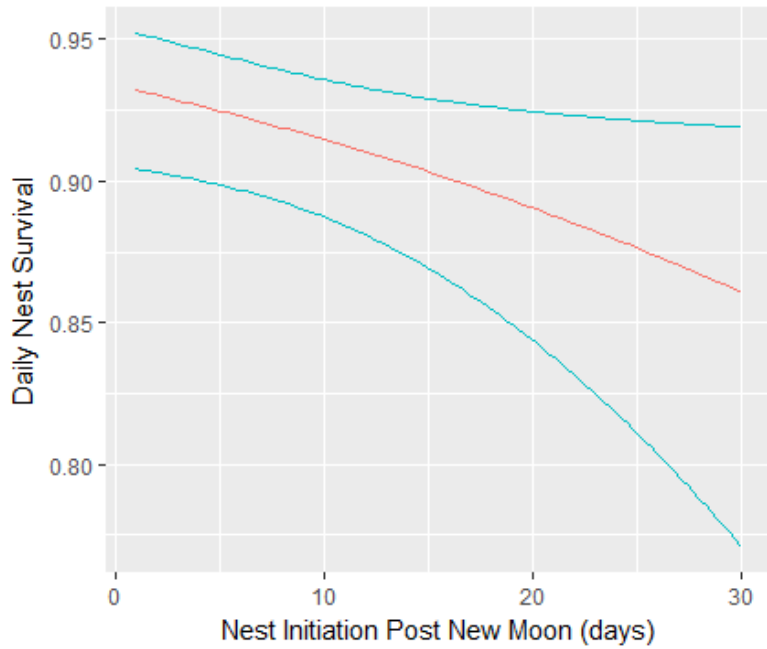
Figure 2.3: Estimated daily nest survival and number of days a nest was initiated past the new moon. Red line is the estimated daily nest survival in relation to nest initiation across the 2016&2017 breeding season, with blue lines representing 95% confidence interval.
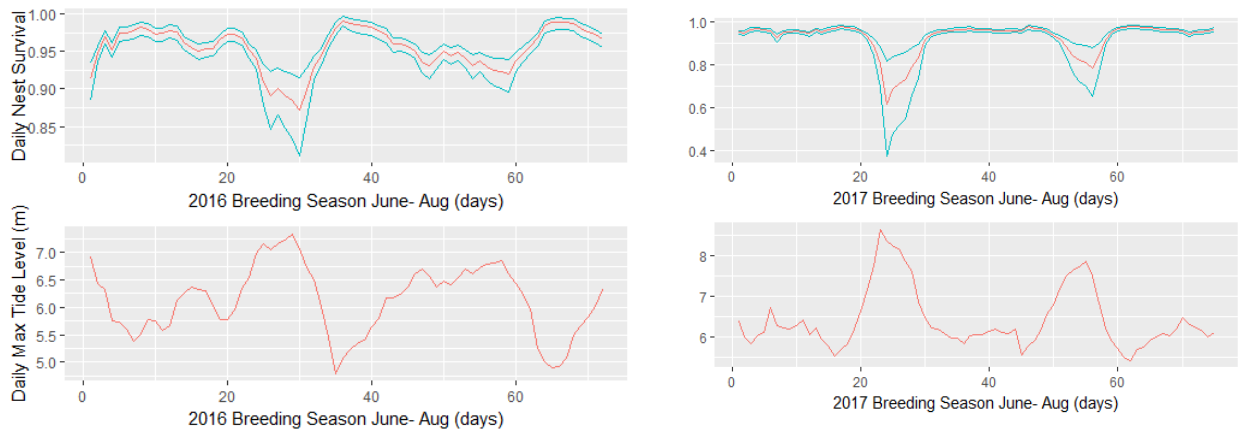
Figure 2.4: Estimated daily nest survival for the 2016 & 2017 breeding seasons with 95% confidence intervals in blue (top 2 plots). Daily maximum tide level on the marsh as measured by water-level loggers for the 2016 & 2017 breeding seasons (bottom 2 plots).

Table 2.5: Daily nest survival modeling results from group 1 (nest structure measurements) in 2016.

Model	K	AICc	Delta AICc	AICc weight	Model Likelihood	Deviance
{B0} + Nest Height[1]	2	227.617	0.00	0.53	1.00	223.60
{B0} + Veg. Height at nest[2]	2	230.171	2.55	0.15	0.28	226.16
{B0} + Nest canopy[3]	3	230.739	3.12	0.11	0.21	224.71
{B0}	1	231.238	3.62	0.09	0.16	229.23
{B0} + Thatch depth at nest[4]	2	233.015	5.40	0.04	0.07	230.00
{B0] + Percent Visible[5]	2	233.145	5.53	0.03	0.06	229.13
{B0} + Percent high marsh veg.[6]	2	233.224	5.61	0.03	0.06	229.11
{B0} + Thatch depth avg.[7]	2	233.241	5.62	0.03	0.06	229.22

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Average height of vegetation at location directly above the nest
3 Presence of nest canopy (full, partial, or none)
4 Depth of thatch below the nest (cm)
5 Percent of the nest visible to the observed from a point directly above the nest
6 Percent of spartina patens in the square meter sampling plot surrounding the nest
7 Average depth of thatch (cm) of measurements taken at 4 cardinal directions surrounding the nest (1 square meter sampling radius)

Table 2.6: Daily nest survival modeling results including group 2 (genotype metrics) in 2016.

Model	K	AICc	Delta AICc	AICc weight	Model Likelihood	Deviance
{B0} + Nest Height[1] + Hybrid Index[2]	3	222.884	0.34	0.88	1.00	216.85
{B0} + Nest Height + Genotypic Class[3]	5	226.253	3.37	0.14	0.13	216.93
{B0} + Nest Height	2	227.617	4.73	0.07	0.09	223.60
{B0}	1	231.238	8.35	0.01	0.02	229.23

1 Number of centimeters bottom of nest cup is from the marsh surface
2. Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)
3. Five genotypic classes: Backcrossed Nelson's (BC_NESP), Backcrossed Saltmarsh (BC_SALS), 1st/2nd Generation Hybrids (F1), Pure   Nelson's (NESP), and Saltmarsh (SALS)

Table 2.7: Daily nest survival final modeling results including group 3 of nest timing and environment covariates from 2016.

Model	K	AICc	Delta AICc	AICc weight	Model Likelihood	Deviance
{B0} + Nest Height[1] + Hybrid Index[2] + Tide Height[3] + Post Moon[4]	5	205.162	0.00	0.39	1.00	195.08
{B0} + Nest Height + Hybrid Index + Tide Height	4	206.014	0.85	0.25	0.65	197.96
{B0} + Nest Height + Hybrid Index + Tide Height + Site[5]	5	206.503	1.34	0.20	0.51	196.42
{B0} + Nest Height + Hybrid Index + Tide Height + Site + Post Moon	6	206.886	1.72	0.16	0.42	194.77
{B0} + Nest Height + Hybrid Index + Post Moon	4	221.799	16.64	0.00	0.00	213.74
{B0} + Nest Height + Hybrid Index	3	222.884	17.72	0.00	0.00	216.85
{B0} + Nest Height + Hybrid Index + Site	4	223.162	18.00	0.00	0.00	215.11
{B0} + Nest Height + Hybrid Index + Site + Post Moon	5	223.457	18.30	0.00	0.00	213.37
{B0}	1	231.238	26.08	0.00	0.00	229.23

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)
3 Daily maximum water-level (m) on the marsh (as measured by water-level loggers)
4 Number of days the nest was initiated post the monthly new moon
5 Site: Inland (Mquoit) & Coastal (Popham)

Table 2.8: Beta coefficients of covariates and 95% confidence intervals within the top models of daily nest survival in 2016

Parameter	Beta	Beta SE	2.5% CI	95% CI
Nest Height[1]	0.055	0.047	-0.037	0.146
Hybrid Index[2]	1.257	0.047	0.335	2.178
Initiation Post New Moon[3]	-0.052	0.029	-0.108	0.005
Site[4]	0.588	0.458	-0.309	1.485
Tide Height[5]	-1.657	0.427	-0.821	-2.494

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)
3 Number of days the nest was initiated post the monthly new moon
4 Site: Inland (Mquoit) & Coastal (Popham)
5 Daily maximum water-level (m) on the marsh (as measured by water-level loggers)
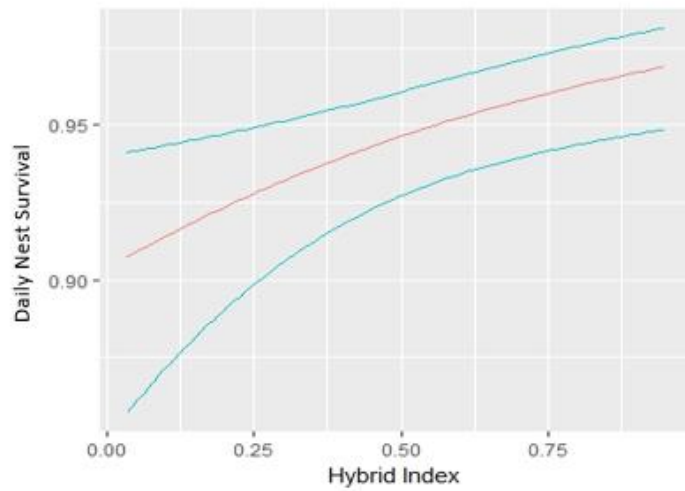
Figure 2.5: Daily nest survival estimates from 2016 in relation to hybrid index (0-1). Red line is the estimated daily nest survival in relation to hybrid index across the 2016&2017 breeding season, with blue lines representing 95% confidence interval.

Table 2.9: Daily nest survival group 1 (nest structure measurements) modeling results from 2017.

Model	K	AICc	Delta AICc	AICc weight	Model Likelihood	Deviance
{B0}	1	353.22	0.00	0.21	1.00	351.22
{B0} + Nest Height[1]	2	353.745	0.53	0.16	0.77	349.74
{B0} + Avg Depth Thatch[2]	2	345.447	1.23	0.11	0.54	350.44
{B0} + Nest canopy[3]	3	354.615	1.40	0.10	0.50	348.59
{B0} + Veg. Height at nest[4]	2	354.927	1.71	0.09	0.43	350.92
{B0} + Percent high marsh veg.[5]	2	354.93	1.71	0.09	0.43	350.92
{B0} + Avg Veg. Height around nest[6]	2	355.019	1.80	0.09	0.41	351.01
{B0} + Thatch depth at nest[7]	2	355.136	1.92	0.08	0.38	351.13
{B0] + Percent Visible[8]	2	335.227	2.01	0.08	0.37	351.22

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Average depth of thatch (cm) of measurements taken at 4 cardinal directions surrounding the nest (1 square meter sampling radius)
3 Presence of nest canopy (full, partial, or none)
4 Average height of vegetation at location directly above the nest
5 Percent of spartina patens in the square meter sampling plot surrounding the nest
6 Average height of vegetation (cm) across measurements taken at 4 cardinal directions surrounding the nest (1 square meter sampling radius)
7 Depth of thatch below the nest (cm)
8 Percent of the nest visible to the observed from a point directly above the nest

Table 2.10: Daily nest survival modeling results including group 2 predictors (genotype) in 2017 breeding season.

Model	K	AICc	Delta AICc	AICc weight	Model Likelihood	Deviance
{B0}	1	353.220	0.00	0.624	1.00	351.22
{B0} + Hybrid Index[1]	2	354.565	1.35	0.32	0.51	350.55
{B0} + Nest Height[2] + Genotypic Class[3]	5	358.012	4.79	0.06	0.09	347.96

1 Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)
2 Number of centimeters bottom of nest cup is from the marsh surface
3 Five genotypic classes: Backcrossed Nelson's (BC_NESP), Backcrossed Saltmarsh (BC_SALS), 1st/2nd Generation Hybrids (F1), Pure   Nelson's (NESP), and Saltmarsh (SALS)

Table 2.11: Daily nest survival final model results from 2017 including nest timing and environmental covariates.

Model	K	AICc	Delta AICc	AICc weight	Model Likelihood	Deviance
{B0} + Tide Height[1]	2	267.062	0.00	1.00	1.00	263.05
{B0} + Post Moon[2]	2	345.314	78.25	0.00	0.00	341.30
{B0}	1	353.220	86.16	0.00	0.00	351.22
{B0} + Site[3]	2	355.190	88.13	0.00	0.00	351.18

1 Daily maximum water-level (m) on the marsh (as measured by water-level loggers)
2 Number of days the nest was initiated post the monthly new moon
3 Site: Inland (Mquoit) & Coastal (Popham)

Table 2.12: Group means and standard error for fitness metrics compared among three broad genotypic classes (Pure & Backcrossed Nelson's, F1/F2 Hybrids, and Pure & Backcrossed Saltmarsh). Letters denote significance between groups from ANOVA followed by Tukey Post Hoc test.

Fitness Metrics	Nelson's (Pure & Backcrossed)	F1/F2	Saltmarsh (Pure & Backcrossed)
Fledging Success	0.909 ± 0.43 A	0.588 ± 0.37 B*	1.365 ± 0.41 A
Hatching Success	3.527 ± 0.21 A	3.588 ± 0.19 AB	3.882 ± 0.20 B
Clutch Size	3.745 ± 0.19 A	3.588 ± 0.17 A	3.882 ± 0.19 A
Average Chick Weight	11.620 ± 0.73 A	12.100 ± 0.63 A	12.600 ± 0.68 A
Maximum Chick Weight	12.180 ± 0.74 A	12.630 ± 0.65 AB	13.570 ± 0.70 B

*significant at the P< 0.1 confidence level (P= 0.08)

Table 2.13: Group means and standard error for fitness metrics compared among five genotypic classes (Pure Nelson's, Backcrossed Nelson's, F1/F2 Hybrids, Pure Saltmarsh, and Backcrossed Saltmarsh). Letters Denote significance between groups from ANOVA followed by Tukey Post Hoc test.

Fitness Metrics	Pure Nelson's	Backcrossed Nelson's	F1/F2	Backcrossed Saltmarsh	Pure Saltmarsh
Fledging Success	1.625 ± 0.59 A	0.787 ± 0.22 A	0.588 ± 0.43 A	1.342 ± 0.28 A	1.667 ± 0.66 A
Hatching Success	3.750 ± 0.30 A	3.489 ± 0.11 A	3.588 ± 0.23 A	3.886 ± 0.14 A	3.833 ± 0.33 A
Clutch Size	3.750 ± 0.23 A	3.745 ± 0.10 A	3.588 ± 0.20 A	3.886 ± 0.13 A	3.833 ± 0.31 A
Average Chick Weight	11.970 ± 0.11 A	11.570 ± 0.40 A	12.100 ± 0.75 A	12.550 ± 0.48 A	13.180 ± 0.98 A
Maximum Chick Weight	12.800 ± 1.13 AB	12.090 ± 0.41 A	12.630 ± 0.77 AB	13.500 ± 0.49 B	14.350 ± 1.00 AB

Table 2.14: Beta estimates, standard error and p - values for results of regression analyses of five fitness metrics and hybrid index. Poisson regression was used for fledging & hatching success as well as clutch size (associated z statistic shown), while linear regression was used for average and maximum chick weight per nest (associated t statistic shown).

(Response, Predictor)	Beta est.	SE Beta	t / z stat	P
Fledging Success ~ Hybrid Index	0.44650	0.15370	2.185	0.0289*
Hatching Success ~ Hybrid Index	0.11931	0.10762	1.109	0.2680
Clutch Size ~ Hybrid Index	0.05063	0.10583	0.478	0.6320
Average Chick Weight ~ Hybrid Index	0.05100	0.02246	2.271	0.0259*
Maximum Chick Weight ~ Hybrid Index	0.06400	0.02085	3.075	0.0029*

Table 2.15: Group means and standard error results comparing tidal marsh nesting adaptations among five genotypic classes (Pure Nelson's, Backcrossed Nelson's, F1/F2 Hybrids, Pure Saltmarsh, and Backcrossed Saltmarsh) and three genotypic classes (with backcrossed birds group with their parental species). No significant differences in nesting adaptations were found among any of the genotypic classes.

		Nelson's			Saltmarsh		
Nesting Adaptations	Pure Nelson's	Backcrossed Nelson's	(Pure & Backcrossed)	F1/F2	(Pure & Backcrossed)	Backcrossed Saltmarsh	Pure Saltmarsh
Nest Height[1]	11.81 ± 1.71	7.82 ± 0.65	8.40 ± 1.23	9.47 ± 1.10	8.57 ± 1.21	8.6 ± 0.83	8.25 ± 1.94
Nest Initiation Post New Moon[2]	7.5 ± 3.02	9.53 ± 1.15	9.24 ± 2.20	9.35 ± 1.20	9.92 ± 2.10	10.23 ± 1.46	5.83 ± 3.43
Percent Visible from above[3]	14.38 ± 9.18	21.04 ± 3.50	20.07 ± 6.84	13.24 ± 5.80	14.13 ± 6.35	14.39 ± 4.42	10.67 ± 10.4
Percent High Marsh Vegetation[4]	60.0 ± 12.02	55.21 ± 4.60	55.91 ± 8.67	56.41 ± 7.60	44.84 ± 8.30	44.70 ± 5.80	46.67 ± 13.63

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Number of days the nest was initiated post the monthly new moon
3 Percent of the nest visible to the observed from a point directly above the nest
4 Percent of spartina patens in the square meter sampling plot surrounding the nest
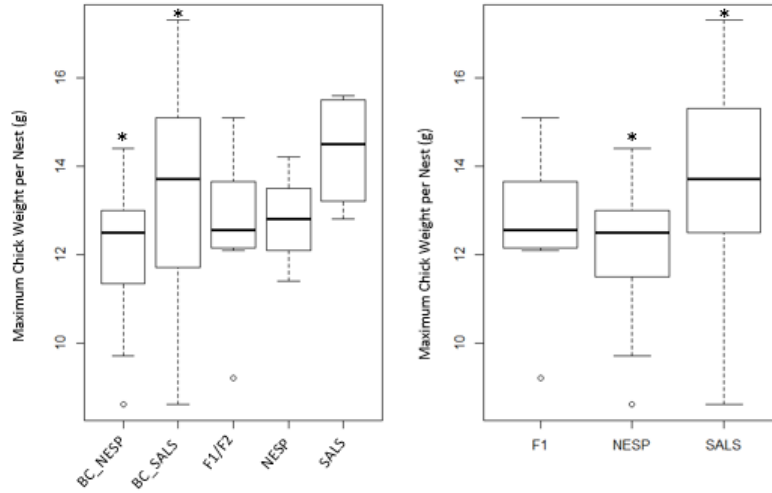
Figure 2.6: Mean maximum chick weight per nest compared among broad (left panel) and specific (right panel) genotypic classes. Asterisks denote significantly different groups. Labels are as follows for the left plot: Backcrossed Nelson's (BC_NESP), Backcrossed Saltmarsh (BC_SALS), $1^{st}/2^{nd}$ Generation Hybrids (F1/F2), Pure Nelson's (NESP), and Pure Saltmarsh (SALS). Labels are as follows for the right plot: $1^{st}/2^{nd}$ Generation Hybrids (F1), Backcrossed and Pure Nelson's (NESP), and Backcrossed and Pure Saltmarsh (SALS).

Table 2.16: Beta estimates, standard error, t statistic, and p - values for linear regression tests between nesting adaptations and hybrid index. Asterisk denotes significance.

(Response ~ Predictor)	Beta	SE B	t. stat	P
Nest Height[1] ~ Hybrid Index[2]	0.00140	0.00330	0.409	0.6832
Nest Initiation Post New Moon[3] ~ Hybrid Index	0.00167	0.00340	0.419	0.6760
Percent High Marsh Vegetation[4] ~ Hybrid Index	-0.00220	0.00097	-2.22	0.0279*
Percent Visible from above[5] ~ Hybrid Index	-0.00230	0.00130	-1.75	0.0814

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)
3 Number of days the nest was initiated post the monthly new moon
4 Percent of spartina patens in the square meter sampling plot surrounding the nest
5 Percent of the nest visible to the observed from a point directly above the nest

Table 2.17: Beta estimates, standard error and p-values for regression tests between nesting adaptations and five fitness metrics. Poisson regression was used for fledging & hatching success as well as clutch size (associated z statistic shown), while linear regression was used for average and maximum chick weight per nest (associated t statistic shown).

Fledging Success

Predictors	Beta	SE Beta	z stat	P
Nest Height	0.0540	0.01430	3.80	0.000145***
Nest Initiation Post New Moon	-0.0031	0.01138	-2.90	0.00358**
Percent High Marsh Vegetation	-0.0005	0.00240	-0.23	0.8210
Percent Visible from above	-0.0037	0.00350	-1.07	0.2860

Hatching Success

Predictors	Beta	SE Beta	z stat	P
Nest Height	-0.0045	0.00929	-0.49	0.625
Nest Initiation Post New Moon	-0.0079	0.00548	-1.45	0.147
Percent High Marsh Vegetation	0.0001	0.00132	0.06	0.949
Percent Visible from above	-0.0012	0.00178	-0.65	0.516

Clutch Size

Predictors	Beta	SE Beta	z stat	P
Nest Height	-0.0059	0.00923	-0.64	0.524
Nest Initiation Post New Moon	-0.0069	0.00539	-1.28	0.200
Percent High Marsh Vegetation	0.0001	0.00130	0.09	0.928
Percent Visible from above	-0.0006	0.00174	-0.33	0.740

Average Chick Size

Predictors	Beta	SE Beta	t stat	P
Nest Height	0.0181	0.04485	0.40	0.687
Nest Initiation Post New Moon	-0.0238	0.02643	-0.90	0.372
Percent High Marsh Vegetation	0.0059	0.00651	0.91	0.365
Percent Visible from above	-0.0073	0.00865	-0.85	0.399

Maximum Chick Size

Predictors	Beta	SE B	t stat	P
Nest Height	0.0228	0.04711	0.48	0.630
Nest Initiation Post New Moon	-0.0273	0.02775	-0.98	0.329
Percent High Marsh Vegetation	0.0040	0.00686	0.58	0.562
Percent Visible from above	-0.0097	0.00906	-1.07	0.288

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Number of days the nest was initiated post the monthly new moon
3 Percent of spartina patens in the square meter sampling plot surrounding the nest
4 Percent of the nest visible to the observed from a point directly above the nest

Table 2.18: GLM results from modeling predictors of fledging success across the 2016 & 2017 breeding seasons.

Model	K	AICc	Delta AICc	AICc Weight	Cum Weight
{B0} + Nest Height[1] + Nest Initiation Post Moon[2] + Hybrid Index[3]	4	516.15	0.00	0.73	0.73
{B0} + Nest Initiation Post New Moon + Nest Height	3	519.53	3.38	0.14	0.87
{B0} + Hybrid Index + Nest Height	3	520.43	4.28	0.09	0.96
{B0} + Nest Height	2	523.35	7.21	0.02	0.98
{B0} + Hybrid Index + Nest Initiation Post New Moon	3	523.46	7.31	0.02	1.00
{B0} + Nest Initiation Post New Moon	2	526.76	10.61	0.00	1.00
{B0} + Hybrid Index	2	531.35	15.21	0.00	1.00
{B0} + Year[4]	2	532.66	16.51	0.00	1.00
{B0}	1	534.27	18.12	0.00	1.00
{B0} + Site[5]	2	534.68	18.53	0.00	1.00

1 Number of centimeters bottom of nest cup is from the marsh surface
2 Number of days the nest was initiated post the monthly new moon
3 Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)
4 Year: 2016 & 2017 nesting seasons
5 Site: Inland (Mquoit) & Coastal (Popham)

Table 2.19: Beta coefficients of covariates from best-supported model of fledging success across the 2016&2017 breeding season.

Covariates	Beta Estimate	Std. Error	z value	2.5% CI	97.5% CI	P
Initiation Post New Moon[1]	-0.02720	0.01130	-2.40	0.952	1.095	0.0165
Nest Height[2]	0.04720	0.01463	3.23	0.995	1.019	0.0013
Hybrid Index[3]	0.46943	0.20459	2.29	1.071	2.388	0.0218

1 Number of days the nest was initiated post the monthly new moon
2 Number of centimeters bottom of nest cup is from the marsh surface
3 Percent of alleles from Saltmarsh Sparrow (0-1 scale; 0 = Pure Nelson's Sparrow, 1 = Pure Saltmarsh Sparrow)

Figure 2.7: Relationship of fledging success, as measured by the number of offspring fledged from the nest (Y axis), and the timing of nest initiation relative to the high tides, as measured by the number of days post the new moon that the nest was initiated (X axis). Gray shading around the trend line (y = -0.03x +0.40) represents 95% confidence interval across the 2016 & 2017 breeding seasons.

Figure 2.8: Relationship of fledging success, as measured by the number of offspring fledged from the nest (Y axis), and the nest height, as measured by the number of centimeters from the ground to the bottom of the nest cup (X axis). Gray shading around the trend line (y = 0.05x -0.40) represents 95% confidence intervals across the 2016 & 2017 breeding seasons.

Figure 2.9: Relationship of fledging success, as measured by the number of offspring fledged from the nest (Y axis) and hybrid index of the nesting female (0-1, 0 = pure Nelson's Sparrow and 1 = pure Saltmarsh sparrow; X axis). Gray shading around the trendline (y= 0.45x -0.12) represents 95% confidence intervals across the 2016 & 2017 breeding seasons.

CHAPTER 3

PRE AND POST COPULATORY SEXUAL TRAITS PREDICT DIFFERENTIAL FITNESS

BETWEEN MALE SALTMARSH AND NELSON'S SPARROWS[3]

Abstract

Mating signals in the form of primary and secondary male sexual traits can influence

mating behaviors and interspecific interactions of hybridizing populations, yielding fitness

consequences and either promoting or impeding gene flow. In this study, we evaluate relative

fitness in relation to competitive ability and overall condition of males of two species of

hybridizing tidal marsh endemics: Saltmarsh (*Ammospiza caudacutus*) and Nelson's sparrows

(*A. nelsoni*). We assessed the role of male sexual traits, including cloacal protuberance

(CP)volume, fat and muscle scores, and body size in determining male fitness (number of

offspring sired). Relative fitness was then compared among Nelson's, Saltmarsh and hybrid

sparrows in relation to these measured sexual condition characteristics to inform potential

outcomes of hybridization in this system. We found differential male fitness, with Saltmarsh

Sparrows siring more offspring than Nelson's Sparrows (ANOVA; $F = 3.81$, $P = 0.04$) and

hybrids being intermediate in fitness, although more similar to Nelson's Sparrows. CP volume (a

proxy for sperm competition) and mass were significant predictors of interspecific fitness,

providing evidence that both pre and post copulatory sexual selection may be driving mating

patterns and interspecific competition. None of the measured sexual traits were informative

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[3] Co-authored manuscript prepared for submission to publication

predictors of intraspecific fitness within the Nelson's Sparrow; however, mass was a significant predictor of intraspecific fitness within Saltmarsh Sparrows, resulting in differential male fitness. Competitive asymmetries between these two species holds the potential to drive patterns of gene flow towards the Saltmarsh Sparrow by outcompeting Nelson's Sparrows and hybrids; however, observed patterns of introgression and high rates of assortative mating (Chapter 1) suggest mechanisms exist to maintain species boundaries in the face of gene flow and could include other unmeasured sexual signals or reduced fitness of hybrid females.

Introduction

Sexual signals are known to influence mating behaviors and interspecific interactions of hybridizing populations, either promoting gene flow between species, or impeding it through reproductive isolation (Irwin & Price, 1999). This role of sexual selection on hybridization and speciation is often framed in the context of female mate choice and male- male competition, whereby females, due to higher investment in gametes, choose mates based on inferred reproductive benefits or consequences of hybridization, while males maximize fitness by mating frequently with multiple females and are therefore not choosy if they mate intra or interspecifically (Andersson 1994; Grant & Grant, 1997; Parker & Partridge, 1998). When hybridization is maladaptive, reinforcement may occur – whereby divergence in sexually selected traits evolves to aid in species recognition and avoidance of between-species mating (Servedio & Noor, 2003; Schumer et al., 2017). Although this explains mechanisms by which female mate choice can drive divergence, Darwin's second mechanism of sexual selection, male-male competition, can also play a role (Darwin 1871). Male-male competition is a form of intrasexual selection, in which interactions within the male sex drive sexual selection due to competitive access to females (Darwin 1871; Andersson 1994). It can generate strong selection

that favors divergent phenotypes between species, and competition for mating resources which can in turn drive speciation via natural selection (Lackey et al., 2018). Additionally, more attention is beginning to focus on broadening this dichotomy to include female-female competition and interactions between female choice and male competition in the discussion of divergence and speciation in the face of gene flow (Doorn et al., 2004; Wong et al., 2005; reviewd in Lackey et al., 2018; reviewed in Lipshutz, 2018). Female-female competition and interactions between female choice and male competition has been understudied, and therefore it is not well understood when these factors influences divergence and speciation in secondary contact (Lackey et al., 2018; Lipshutz, 2018).

Mate competition in secondary contact may promote reproductive isolation in the form of reproductive or agonistic character displacement, where divergence in competitive traits or mating signals reduce interspecific interactions (Lipshutz, 2018). However, interspecific interactions in secondary contact are not limited to reducing gene flow through reproductive isolation, rather these interactions can also promote hybridization in some circumstances (Veen et al., 2001; as reviewed Lipshutz, 2018;). Interspecific reproductive competition may occur when species compete for limited space in relation to mate attraction and reproduction (Grether et al., 2009; Martin et al., 2017). This can lead to increased introgression if one male species is more dominant/competitive than the other (Pearson, 2000; Krosby & Rohwer, 2010;). Competitive asymmetry can promote directional hybridization, and in some cases generate asymmetric introgression from the competitively superior species to the inferior (While et al., 2015).

Intersexual mate choice and competition for mates can lead to variance in fitness that drives selection *(*Fisher et al., 2016), and is often responsible for the evolution of numerous kinds of male secondary sexual traits or sexual signals (Andersson, 1994). These traits can be static, like body size, but can also be more plastic, such as behavior (Hill et al., 1999; Hagelin, 2002). Avian sexual characteristics involved in competition include ones directly used in fighting, including body size, as well as traits important in signaling dominance, such as song, or mate guarding (Andersson 1994). Body size, dominance in mating or courtship display, age, and aggressive behavior have been found to result in higher mating success in a variety of taxa, including birds (Wagner et al., 1996; Hasselquist, 1998;; Mateos & Carranza, 1999; Hagelin, 2002). Body condition has also been found to correlate with reproductive fitness in several bird species (Chastel et al., 1995; Dyrcz et al., 2005; Sanchez-donoso et al., 2018). As a result of Darwin's and Fisher's theory of sexual selection, ornamentation and coloration have often been the focus of many avian studies of mate choice (Yezerinac & Weatherhead, 1997; Hagelin, 2002; Loyau et al., 2005), with links drawn between ornamentation as "honest" traits that may also provide signal of overall health/body condition in addition to social dominance, especially in the form of coloration as a result of carotenoids or melanins (Berglund & Pilastro, 1996; Hagelin, 2002; Jawor & Breitwisch, 2003).

Even once mating has occurred, there is room for post-copulatory sexual selection to act in the form of sperm competition or cryptic female choice. Sperm competition, an intense form of post-copulatory male-male competition, occurs when sperm from males compete for the fertilization of eggs within a female (Parker, 1970), and increases the relative variance in male mating success (Moller & Ninni, 1998). Higher sperm production may lead to higher male fitness because this can allow males to copulate more often or release more sperm per ejaculate,

while increased sperm size/motility might enhance individual male's competitive ability after copulation to fertilize eggs (Laskemoen et al., 2010). However, direct effects of sperm morphology may be masked by cryptic female choice effects, when females are able to influence fertilization success (Birkhead, 1998). Primary sexual characters can often be hard to study in natural populations, without invasive sampling or controlled experiments; however, in birds there are some morphometric cues that can help determine the role of sperm competition in mate success. The intensity of sperm competition is one factor known to determine variation in the size of male reproductive organs in birds (Sax & Hoi 1998). Accordingly, the size (volume) of the cloacal protuberance (CP) has been found as a proxy for sperm production, sperm velocity, and fertilization success (Tuttle et al., 1996; Peer, et al., 2000; Laskemoen et al., 2008; Laskemoen et al., 2010;), and in this way serves as a good proxy for sperm competition.

In this study, we evaluated relative male fitness in relation to competitive ability and overall condition between two species of hybridizing tidal marsh sparrows: Saltmarsh (*Ammospiza caudacutus*) and Nelson's sparrows (*A. nelsoni*). These sister species are tidal marsh endemics that are restricted to a narrow strip of habitat across the northeastern coast of the United States. Nelson's Sparrows breed in marshes from the Canadian Maritimes to Massachusetts, while the Saltmarsh Sparrow's breeding range extends from southern Maine to Virginia (Nocera et al. 2007, Greenlaw & Woolfenden 2007). The two species are currently in secondary contact, and hybridize where their populations co-occur from South Thomaston, Maine, USA to Plum Island in Newburyport, Massachusetts, USA (Hodgman et al., 2002; Shriver et al., 2005; Walsh et al., 2015a).

Previous work in the Saltmarsh – Nelson's Sparrow hybrid zone documented high promiscuity and reproductive skew in both Saltmarsh and Nelson's sparrow males (Walsh et al., 2018b). Hybrid males exhibited significantly lower reproductive success than pure species; however, because this work was performed in the southern range margins of the hybrid zone, species densities were highly skewed (~5:1 Saltmarsh to Nelson's) and sample sizes for Nelson's Sparrows were low. As such, mating behaviors and relative fitness of Nelson's Sparrow and hybrid males may not be reflective of reproductive strategies in sympatric populations near the center of the hybrid zone where the densities of the two species are more equal. Further, the role of male sexual characteristics in driving patterns of mating, fitness, and consequently, hybridization between these two species is still unknown.

Closely related species often show more divergence in secondary sexual characteristics than other phenotypic traits (Allender et al., 2003). Although they are closely related sister species, Saltmarsh and Nelson's Sparrows show slight differences in morphology and behavior. Morphologically, Nelson Sparrows tend to be smaller than Saltmarsh Sparrows (bill length, weight), and have paler and less discrete plumage characteristics (Greenlaw, 1993; Shriver et al., 2005). Both sparrows exhibit a unique, promiscuous mating system in which males are non-territorial and provide no parental care to young (Greenlaw, 1993; Shriver et al., 2007). Despite high levels of multiple paternity in both species, they have different mating behaviors (Hill et al., 2010, Walsh et al., 2018b). Saltmarsh Sparrow males engage in scramble-competition access to females. Conversely, Nelson Sparrows may guard females during their fertile period and exhibit flight displays (Greenlaw, 1993; Shriver et al., 2007, 2010). The two species also differ in their song as well as the frequency with which males' sing, with Nelson's Sparrows singing for longer periods of time. Sperm competition is higher in males belonging to birds with polygynous

mating systems, as measured by things such as testes lengths, CP volume, as well as ejaculate quality and quantity (Briskie 1993, Moller 1988). Due to the nature of the scramble polygyny mating system shared between the Saltmarsh and Nelson's sparrows, one could predict that post-copulatory processes may be acting to drive patterns of fertilization. It is reasonable to hypothesize that these processes are acting in conjunction with pre-copulatory sexual selection in the form of male competitive ability and female choice to influence male fitness and subsequent patterns of gene flow in Saltmarsh and Nelson's Sparrows.

Objectives

In this study we aimed to determine (1) the relative fitness of Saltmarsh Sparrow, Nelson's Sparrow, and hybrid males in a sympatric population within the center of the hybrid zone and subsequently, (2) if male condition and competitive ability are positively correlated with fitness.

Predictions

1). We expect short-term relative fitness to be a function of genotype such that either:

a) Male hybrids will have higher fitness than Nelson's Sparrows but lower fitness than Saltmarsh Sparrows due to differences in size and behavior, such that males with higher proportion of Saltmarsh Sparrow alleles will be more successful in a scramble competition for mates and/or have an advantage through direct or indirect female choice.

b) Male hybrids will be less fit than Nelson's and Saltmarsh Sparrows due to intermediacy in mating behaviors and morphological characteristics between the species; therefore, hybrids will not perform well in either mating strategy or be a successful competitor for mates.

2). We expect four sexual characteristics (CP volume, mass, fat and muscle scores) of males across all genotypic classes to be positively correlated with reproductive success (number of offspring sired) due to sexual selection.

Methods

Study Area

Two field sites were selected in the current center of the hybrid zone- the marshes at Popham Beach State Park and Wharton Point on Maquoit Bay, located on the northeastern coast of the United States, between Brunswick, Maine and Phippsburg, Maine. We expected relatively similar species abundances (based on recent abundance estimates; Wiest et al., 2016) and subsequent high number of hybrids individuals based on and a peak in interspecific heterozygosity across the hybrid zone (Walsh et al., 2016b). The two study sites also span opposite ends of a habitat gradient between coastal and inland tidal marshes and differ slightly in habitat and amount of tidal inundation. The marshes at Popham Beach State Park are located at the tip of a peninsula, directly on the coast. The area of marsh at Popham is expansive; therefore, we selected to focus on a portion of the marsh consisting of ~15-hectare plot. The marsh at Maquoit Bay is located more inland and is much smaller than Popham, with the selected study area (~5 hectares) about a third the size of the study plot at Popham. Popham marshes are part of an expansive coastal marsh network, while Maquoit is located in a small cove that is surrounded by mostly forest and field.

Field Data Collection

To collect male condition data, we sampled the population of sparrows at both sites during the 2016 & 2017 breeding seasons. We followed standardized protocols established by the Saltmarsh Habitat and Avian Research Program (SHARP; wwwtidalmarshbirds.org). We performed systematic as well as opportunistic netting, using 2–6 12-m mist nets, throughout the breeding season to sample as many resident adult males as possible. Males were banded with a USGS aluminum band and a site-specific color band. A blood sample was drawn from the cutaneous ulnar vein and stored at room temperature on blood filter strips for later genetic analysis. Standard morphological measurements were taken, in addition to measurements used to assess competitive ability of males. The size of the cloacal protuberance was collected from each male (measured by depth along the axial plane anterior to posterior and width at the widest point along the sagittal plane superior to inferior at the widest section of the CP). CP volume was then calculated following Schut et al. (2012) adapted from Mulder & Cockburn (1993) via volume of a barrel ($\pi \times radius2 \times height$). Radius was calculated as 0.5 of the width at the widest point. We also estimated abdominal and furcular fat scores (0–6), as well as pectoral muscle scores (0–6). Fat and muscle scoring was based on SHARP protocols (wwwtidalmarshbirds.org). Fat scores were an average between abdominal and furcular scores. If an individual was captured more than once, muscle scores, fat scores and CP measurements were averaged.

To determine the number of offspring sired from each male, we sampled as many offspring as possible to reconstruct parentage. We conducted nest monitoring at both sites during May–August, encompassing approximately 3 nesting cycles (see Chapter 2 for further methodological details). From each nest, nestlings were banded with a USGS aluminum leg band and a single site-specific color band when they were 6 days old. A blood sample (a few drops on a filter card) was also collected from the medial metatarsal vein of each nestling for genotyping

and hybrid identification. We also collected any eggs or unbanded chicks that had floated out of the nest following a flooding event or failed to hatch for other reasons to use in genetic analyses. To determine the identity of females associated with each nest (as needed for parentage analyses), we conducted targeted mist-netting to capture females off of their nests during incubation or brooding. Once caught, each female was banded with a USGS aluminum band, a site-specific color band, and a PIT tag that was modified to a color band for non-invasive detection of re-nesting attempts A blood sample was drawn from the cutaneous ulnar vein and stored at room temperature on blood filter strips for later genetic analysis.

ddRAD Library Preparation

Samples of adult females, nestlings, and salvaged chicks or eggs from the two field seasons were used to prepare double digest restriction site associated DNA (ddRAD) Sequencing libraries. DNA was extracted from blood samples using the Qiagen DNeasy Blood or Tissue kit (Qiagen, Valencia, CA), following manufacturer protocol. We determined the concentration of resulting DNA samples using Qubit fluorometer Broad Range double-stranded DNA assay kit (Life Technologies, NY, USA). We targeted a DNA concentration of 5–25 ng/ul. Samples below 10ng/ul after initial extraction were vacuum centrifuged to concentrate to within the target range. Samples that were above 25 ng/ul were diluted down to 25 ng/ul. A small number of samples below 5 ng/ul were included and grouped into one index group to ensure the best results. ddRADtags were created using the protocol described in Peterson et al. (2012). DNA was digested with SbfI and MspI, and ligated to P1 and P2 adapters using T4 DNA ligase (30min at 37 ℃ and 60min at 20 ℃, held at 10 ℃; Peterson et al., 2012). Samples were pooled into index groups by their unique P1 adapter and cleaned using 1.5x Agencourt AMPure XP beads. Using BluePippin (Sage Science, MA, USA), fragments were size selected between 400–700 bp in

length. Low cycle PCR reactions were then preformed to incorporate the Illumina TruSeq primer

sequences into the library, as well as a final clean up using AMPure XP beads. Libraries were

visualized on a fragment Bioanalyzer to ensure desired fragment size/distribution and index

groups pooled. Resulting libraries were sequenced across three Illumina HiSeq 2500 lanes and

one HiSeq 2500 rapid run lane (read length 100 bp) at the Cornell University Institute for

Biotechnology (Genomics Facility Research Center).

Bioinformatic Data Processing & SNP Detection

Sequences were initially evaluated for overall quality using FastQC, then trimmed and

filtered using FASTX-Toolkit. Specifically, reads were trimmed on the 3' end to 97 bp and

eliminated if the Phred quality scores were below 10 or if 95% of the bases had Phred quality

scores below 20. Using STACKS (version 1.48), we demultiplexed the remaining sequences. We

used the process_radtags command with the following conditions: any reads not meeting

Illumina's chastity/purity filter and of low quality were discarded, data were cleaned such that

any read with an uncalled base was removed, reads with mismatches in the adapter sequence >1

were removed, and reads were only processed if the sequence had an intact SbfI RAD site and

one of the unique barcodes. Subsequently, fastx_trimmer was used to trim all sequences to the

length of the shortest sequences. Reads were aligned to the Saltmarsh Sparrow reference genome

(Walsh 2018a) using STACKS (version 1.48). Minimum stack depth for a read to be assembled

into a catalog was 6. The number of mismatches allowed between sample loci was set at 5. We

filtered catalog loci based on the mean log likelihood of the catalog locus in the population, with

the minimum log likelihood set at -300. These filtering steps resulted in the recovery of 5,391

SNPs. We used the program Populations to subset a panel of SNPs for use in paternity analyses.

For the paternity panel we again chose only one SNP per locus and required that a SNP be

95

present in a minimum of 95% of the individuals with a minimum stack depth of 6. This resulted in a 589 SNP paternity panel.

Assigning Genotypic Classes

A panel of fixed SNPs (135) between the species was used to assigned sparrows to genotypic classes (see Chapter 1 for further methodological details). Briefly, this method combines hybrid index and interspecific heterozygosity to place each individual into genotypic classes consisting of: pure Nelson sparrow, backcrossed Nelson, F1/F2, backcrossed Saltmarsh, or pure Saltmarsh sparrow. Hybrid index was defined as the proportion of alleles inherited from the Saltmarsh Sparrow (0 = pure Nelson's Sparrow and 1 = pure Saltmarsh Sparrow). Interspecific heterozygosity was defined as the proportion of genotypes that are heterozygous across the species for the parental alleles (0 = all homozygous genotypes, found only in one parental species, and 1 = all heterozygous genotypes across species). Individuals with intermediate hybrid index (0.25–0.75) and high heterozygosity (>0.3) were considered recent generation hybrids (F1 or F2), and individuals with very low or high hybrid index (0.05–0.25 or 0.75–0.95) and low heterozygosity (<0.3) were considered backcrossed. Pure individuals are defined as a hybrid index of 0–0.05 (Nelson's Sparrow) or 0.95–1(Saltmarsh Sparrow). The Introgress package in R was used for calculating the hybrid index and interspecific heterozygosity (Gompert & Buerkle 2010). Analyses do not distinguish between F1 and F2 individuals and are grouped together into an overall recent-generation hybrid category, used throughout.

Paternity Analyses

To determine the number of offspring sired from each male, we conducted paternity analyses of nestlings using genotype data from the SNP paternity panel to reconstructed mating pairs. Candidate fathers were assigned using the approaches implemented in CERVUS (Marshall et al., 1998) and COLONY V2.0 (Jones & Wang, 2010). The maximum likelihood approach of CERVUS uses simulated genotypes from provided data to create a log-likelihood confidence level in true parentage assignments but does not account for unsampled males in the population. To address this problem, we used the full likelihood approach in COLONY, which can determine the number of sires for each nest, even if the true father was not among the sampled males. For both methods, we used a genotyping error rate of 1%, 95% of loci typed, and candidate father sampling of 70%. We assumed the proportion of sampled mothers to be 95% given the targeted netting identification of females off of their nests. For each site and year, a list of candidate fathers was developed. For 2016, all sampled adult males were included, and for 2017, all males that were sampled in that year, as well as any males from 2016 (adults and offspring as determined from molecular sexing) were included to account for any hatch years that may have returned to their natal site, as well as any returning adult males that may have evaded capturing in 2017. For each offspring, we determined the most likely father as assigned by CERVUS (delta trio value ≥95%). This was then compared to the paternity assignment made in COLONY. For any discrepancies on confident paternity assignments (>95%) between the two programs, we compared the number of loci mismatches, delta pair confidence, and overall loci typed to identify the best male assignment.

Assessing Male fitness & Condition

To determine if male reproductive success was a function of genotype, we evaluated number of offspring sired and genotypic class of each male across the two breeding seasons. Due

to the small sample sizes of pure individuals, we categorized all individuals into three broad

genotypic classes: backcrossed and pure Nelson's Sparrows, backcrossed and pure Saltmarsh

Sparrows, and recent generation hybrids. We used a Kruskal Wallis rank sum test to identify

differences in male reproductive success (number of offspring sired) among the three groups of

males (categorical variables) followed by a pairwise Wilcox test with Bonferroni adjustment.

Due to the non-normal nature of the data, we used Poisson regression to test for an association

between male reproductive success and the hybrid index (continuous variable). To determine if

there was reproductive skew between the species, the number of males that produced offspring in

the population and their corresponding genotypic class was determined. We also determined the

number of offspring per male and compared across hybrids, Nelson's and Saltmarsh Sparrows.

We looked for differences in levels of multiple paternity between the three broad genotypic

classes of backcrossed and pure Nelson's Sparrows, backcrossed and pure Saltmarsh Sparrows

and hybrid individuals. The number of nests from which each individual's total offspring

originated was compared across genotypic class using a Kruskal Wallis Rank Sum Test,

followed by a pairwise Wilcox test with Bonferroni adjustment. We also tested for an association

between the number of nests from which a male's offspring were sired and hybrid index using

Poisson regression (due to the count-nature of the data).

To determine if male fitness differed in relation to size and condition of the male for

competitive access to females, we tested for correlation between four predictors of male

competitive ability and the number of offspring sired (fat score, muscle score, weight, CP

volume). Poisson regression was used to determine if a relationship exists between the measured

male condition predictors and reproductive success. This test was performed for the full dataset

of all males, to determine interspecific drivers of success, and also for each genotypic class

separately, to determine intraspecific drivers of success. In this way, we could determine if patterns seen within species boundaries mirrored those seen across both species and hybrids. Further, we tested to see if species differed significantly in body condition, using categorical variable of genotypic class and continuous variable of hybrid index. Linear regression was used to test for association between hybrid index and all body condition metrics (fat score, muscle score, weight, CP volume). ANOVA was used to compare CP volume across genotypic classes, and a Kruskal Wallis rank sum test followed by a pairwise Wilcox test with Bonferroni adjustment was used for fat, muscle and weight, which were not normally distributed.

Results

Across our two study sites, we genotyped a total of 120 adult male birds. We monitored a total of 201 nest across the 2016 and 2017 breeding seasons and sampled 326 nestlings/collected eggs, with 301 that we were able to genotype. Using the hybrid index and interspecific heterozygosity, we determined that 35% of the adult males were backcrossed Nelson's Sparrows, 44% were backcrossed Saltmarsh Sparrows, 14% were recent generation hybrids (F1/F2), 5% were pure Nelson's Sparrows, and 2% were pure Saltmarsh Sparrows. We assigned paternity to 274 of the 301 offspring (91%) genotyped. Paternity assignments were in 100% agreeance between COLONY and CERVUS at the Maquoit Site. At Popham there were 13 cases in which COLONY and CERVUS did not agree. For all 13 of these instances, CERVUS had either high loci mismatches or low delta pair confidence levels, and COLONY provided higher confidence; therefore, we used the COLONY assignments for these cases.

We found that the number of offspring sired differed significantly among genotypic classes. Pure Saltmarsh Sparrows and backcrossed Saltmarsh Sparrows sired more offspring

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(mean = 3.8 offspring/male) than pure and backcrossed Nelson's Sparrows (mean = 2.5;

ANOVA followed by Tukey HSD; F = 3.81, P =0.04), while hybrids sired an equal number of

offspring to Nelson's Sparrows (mean = 2.5; Figure 1). At a finer scale, the number of offspring

sired among the five genotypic classes (pure Saltmarsh Sparrow, backcrossed Saltmarsh

Sparrow, hybrids, pure Nelson's Sparrow, and backcrossed Nelson's Sparrow) was positively

associated with the proportion of Saltmarsh Sparrows alleles; however, there were very small

samples of both pure Nelson's (4 individuals) and Saltmarsh Sparrows (1 individual), precluding

strong conclusions about the reproductive success of genotypically pure males (Figure 2).

We observed a large number of sampled males that produced no offspring during the

duration of the study (30%). By broad genotypic class, over half of the hybrid (F1/F2) adult

males (53%), 33% of Nelson's Sparrows (pure and backcrossed), and 18% of Saltmarsh Sparrow

(pure and backcrossed) males sired no offspring. (Table 1). Of all the males that sired at least one

offspring (61%), 52% were backcrossed Saltmarsh Sparrow, 33% were backcrossed Nelson's

Sparrow, 9% were recent generation hybrids (F1/F2), 5% were pure Nelson's Sparrow, and 1.2%

were pure Saltmarsh Sparrow. Generally, we found that Saltmarsh Sparrow males sired more

offspring and had more variation in the number of offspring sired across males (reproductive

skew) than Nelson's and hybrids (Figure 3). Of all the birds that sired at least one offspring, the

majority of hybrids (62%) and Nelson's Sparrows (66%) sired 1–2 offspring, while 67% of

Saltmarsh Sparrows sired 3 or more offspring. All genotypes had a high proportion of males that

sired no offspring across the two breeding seasons. Thirteen male Saltmarsh Sparrows (28%)

produced 5 or more offspring (with a maximum of 12), while the majority of males produced

only 1–3 offspring over the two years. Although a third of male Nelson's Sparrows sired no

offspring over the two years, reproductive success was less skewed among the successful males, with most individuals siring 1 or 2 offspring and very few males (34%) siring more than that.

We observed high levels of multiple paternity across all genotypic classes. After excluding nests that had only 1 chick (leaving 80 nests in total), we found that 28 nests (35%) had a different father for each chick, while only 15 (19%) of nests had only one father. Over half of all nests (54%) had two fathers, and 15 (19%) had three fathers. Of the 28 nests with a different father for each chick, 15 (54%) were Saltmarsh Sparrow female nests, 8 (29%) were Nelson's Sparrow female nests, and 5 (18%) were hybrid female nests. Saltmarsh Sparrows sired offspring from more nests than Nelson's Sparrows (pairwise Wilcox test; $H(2) = 8.74$, $p = 0.01$; Figure 4).

We found interspecific differences in male condition. Three of the four male condition metrics differed significantly among genotypic classes. Overall, Nelson's Sparrows had smaller CP volumes (ANOVA; $F = 5.16$, $p = 0.007$), lower average muscle scores (pairwise Wilcox test; $H(2) = 6.16$, $p = 0.04$), and smaller overall mass (pairwise Wilcox test; $H(2) = 14.87$, $p = 0.0007$) than Saltmarsh Sparrows (Figure 5). Hybrids showed intermediate levels in all three of these categories, however, did not differ significantly from either of the parental species. Average fat scores did not differ among the genotypic classes (pairwise Wilcox test; $H(2) = 1.15$, $p = 0.56$; Figure 5). These same condition metrics also showed a significant relationship with hybrid index, such that mass (linear regression; $B = 0.08$, $t = 3.27$, $p = 0.002$), CP volume (liner regression; $B = 0.01 \pm 0.0003$, $t = 0.58$, $p = 0.003$), and muscle scores (linear regression; $B = 0.20 \pm 0.07$, $t = 2.91$, $p = 0.005$) were positively correlated with hybrid index score, meaning the values increased linearly with increasing frequency of Saltmarsh Sparrow alleles (Figure 6).

There was no relationship with average fat score and hybrid index (linear regression; B = 0.07, t = 0.73, p = 0.47). Further, we found that three of the four male condition metrics were predictive of the number of offspring sired. The number of offspring sired increased with both CP volume (Poisson regression; B = 0.001, z = 2.31, p = 0.02) and mass (Poisson regression; B = 0.07, z = 2.25, p = 0.025) and decreased with the average fat score (Poisson regression; B = -0.40, z = -2.96, p = 0.003; Figure 7). Average muscle score was not a good predictor of number of offspring sired (Poisson regression; B = -0.03, z = -0.28, p = 0.78). We finally tested the strength of the relationships between CP volume, mass, and hybrid index with number of offspring sired using a correlation test to determine if one trait was a stronger predictor of fledging success than the others. We found that hybrid index ($r^2 = 0.26$, t = 2.48, p = 0.01), CP volume ($r^2 = 0.22$, t = 1.92, p = 0.05), and mass ($r^2 = 0.23$, t = 1.81, p = 0.07) all had relatively equal strength of correlation with number of offspring sired.

Finally, we investigated intraspecific differences in male condition metrics. We evaluated the male condition metrics in relation to offspring sired within each of the broad genotypic classes using Poisson regression. For all groups, offspring sired had a positive relationship with CP volume (Figure 8); however, it was not significantly associated with intraspecific fitness, such that CP did not correlate significantly with the number of offspring sired in Saltmarsh Sparrows (Poisson regression; p = 0.23), Nelson's Sparrows (Poisson regression; p = 0.46), or hybrids (Poisson regression; p = 0.76).The relationship between mass and the number of offspring sired was not consistent across genotypic classes, with a positive relationship found in Saltmarsh Sparrows and hybrids (stronger in Saltmarsh Sparrows) and a negative relationship in Nelson's Sparrows (Figure 8). Intraspecific effects of mass were found for Saltmarsh Sparrows,

with a significant positive association with offspring sired and mass of Saltmarsh Sparrow males (Poisson regression; B = 0.21, z = 3.13, p = 0.002), but not for Nelson's Sparrows or hybrids.

Discussion

Differential Fitness

Reproductive fitness varied among male Saltmarsh Sparrows, Nelson's Sparrows, and their hybrids within a sympatric population in the center of the hybrid zone. Saltmarsh Sparrows sired more offspring with a greater number of females than Nelson's Sparrows or hybrids. The number of offspring sired, was correlated with hybrid index, which suggests that male fitness levels vary along a continuum according to genotypic composition, with highest levels found in males with Saltmarsh Sparrow--like genotypes. While F1/F2 hybrids had intermediate levels of fitness to either the backcrossed or pure parental groups, when compared across the three genotypic classes, hybrids sired the same amount of offspring on average as the combined Nelson's Sparrow and backcrossed Nelson's Sparrow group. Our findings are similar, although not completely, to those of prior work in the southern range of the hybrid zone, where hybrid males were found to sire lower numbers of offspring than either parental type (Walsh et al., 2018b). Here, in the center of the hybrid zone, where densities of the two species are relatively similar, hybrid males had similar or slightly higher success rates than Nelson's Sparrows.

Saltmarsh Sparrow genotypes have higher levels of reproductive output than both Nelson's Sparrows and hybrids in sympatry, suggesting a competitive advantage in interspecific interactions. Interspecific reproductive competition may occur when species in secondary contact compete for limited space or mates (Grether et al., 2009; Martin et al., 2017). If one male species is more dominant than the other, competition between the two can lead to increased introgression

(Pearson, 2000; Krosby & Rohwer, 2010), directional hybridization or even asymmetric

introgression from the competitively superior species to the inferior (While et al., 2015).

Observed reproductive differences between Saltmarsh and Nelson's males suggests that

interspecific reproductive competition may occur between the species. This could have direct

implications of extent and directionality of hybridization. Interspecific competition may increase

rates of hybridization and cause asymmetric introgression towards the inferior Nelson's

genotypes. This may also explain observed hybrid zone movement (Walsh et al., 2017b).

Competitive interactions may lead to geographic or genetic displacement of the inferior

competitor -Nelson's Sparrows, causing the hybrid zone to appear more Nelson's-like over time,

as illustrated in hermit (*Setophaga occidentalis*) and Townsend's (*S. townsendi*) warbler hybrid

zone (Pearson, 2000). However, directional hybridization can also be adaptive in certain

environments where inheritance of competitive traits from the dominant parent may provide a

selective advantage and drive patterns of hybridization. This is seen in hybrid tadpoles (*S.

bombifrons and S. multiplicate*), where females are more likely to mate with one parental species

in certain environmental conditions to which that parental species has a competitive advantage

(Pfennig & Simovich 2002). Hybridization may increase competitive ability of Nelson's

Sparrows in this system and subsequently drive introgression in the Saltmarsh Sparrow direction,

and this may be especially true in coastal locations where Saltmarsh Sparrows have higher

nesting success than Nelson's (Chapter 2). Although interspecific competition may occur

between Saltmarsh and Nelson's Sparrows, we have evidence that successful interspecific

offspring production is relatively rare, suggesting that assortative mating occurs or selection

against interspecific offspring (Chapter 1).

Similar to findings of Walsh et al. (2018b) for the southern end of the hybrid zone, we also observed high levels of multiple paternity across both parental species and hybrids. Although high, the levels of multiple paternity observed in this study were lower than those found by Walsh et al. (2018b) for both species, with more nests in this study having a single father per nest (19%), as well as lower observed levels of nests that had a different father for each chick (35%). Higher levels of multiple paternity in Saltmarsh Sparrows was also documented by Hill et al. (2010), where 95% of nests exhibited multiple paternity with one third of nests having a different father for each chick. However, Saltmarsh Sparrows sired offspring from significantly more nests than Nelson's Sparrows. This variation in levels of multiple paternity is consistent with differences in mating behavior between the species. Nelson's Sparrow males are known to mate guard and therefore likely have mating opportunities with fewer females than Saltmarsh males, which exhibit scramble competition polygyny (Greenlaw, 1993; Shriver et al., 2007, 2010). Saltmarsh Sparrows search for and attempt to mate with many females, without any mate guarding (Greenlaw 1993; Shriver 2007, 2010; Greenlaw & Post 2012). These divergent mating behaviors also provide a basis for variation in reproductive skew among male Saltmarsh, Nelson's, and hybrids. Many males of each genotypic class were found not to sire any offspring across the two-year study period; however, variation in the number of offspring sired among males differed in magnitude across the species. Saltmarsh Sparrows had the lowest number of unsuccessful males at 18%, while 33% of Nelson's males produced no offspring, and 53% of hybrid males produced no offspring over the two-year study period.

Although Saltmarsh Sparrows were more successful at siring at least one offspring than hybrids or Nelson's, there was large variation in the number of offspring sired by successful male Saltmarsh Sparrows, with 13 (28%) individuals siring 5 or more offspring each, accounting

for over half (52%) of all Saltmarsh Sparrow-sired offspring. The maximum number of offspring sired from one Saltmarsh Sparrow male was 12. This suggests that there were a few Saltmarsh Sparrow males that were much more successful than the majority, which produced 0–3 offspring across the two years. This reproductive skew, where a small number of males produce high numbers of offspring, is indicative of scramble competition polygyny, where there are clear winners and losers in access to mates and/or fertilization rates (Andersson 1994). Conversely, the majority of Nelson's and hybrid sparrows were marginally successful, with 66% and 62% of males siring 1–2 offspring respectively, and very few birds siring a large number of offspring. The maximum number of offspring sired by one hybrid (n = 6) was half of the maximum sired by a single Saltmarsh Sparrow. Similarly, the maximum number of offspring sired by one Nelson's male was 7. This suggests a more even distribution of reproductive success among Nelson's and hybrid males than Saltmarsh Sparrow males. When the two species mating strategies exist together in sympatry, the scramble competition mating behavior of Saltmarsh Sparrow males makes them better competitors overall. The larger size, as well as more active patrolling for mates may allow Saltmarsh Sparrows to outcompete smaller Nelson's Sparrows, which may spend less time actively searching for mates (Greenlaw & Post, 2012; Shriver et al., 2007). The mating strategy for Nelson's Sparrow appears to be at a disadvantage in sympatric populations; however, it may be more effective in allopatric populations where there is no competition with Saltmarsh Sparrows. As such, mate guarding may be a reinforcing behavior to these species contributing to assortative mating in sympatry.

Interspecific Predictors of Fitness

Across Saltmarsh, Nelson's and hybrid males, we found that male reproductive success increased with body weight. This suggests pre-copulatory sexual selection may be contributing

to patterns of gene flow and hybridization between Saltmarsh and Nelson's Sparrows. Competition is an important determinant of mating success, especially for individuals with polygynous mating systems where reproductive success is skewed toward dominant individuals (Clutton-Brock, 2007; Moller, 1988). Male-male competition between Saltmarsh and Nelson's Sparrows could come in the form of aggressive behavior between males that may allow for the dominant bird to copulate with more females (Darwin 1871; Andersson 1994). It may also come in form of time spent searching or patrolling for mates, which may allow for a male to copulate with a female before others and gain a competitive edge (Hasselquist & Bensch 1991; Schwagmeyer & Woontner 1986). Male–male competition will often select for large body size (Greenlaw 1993, Andersson 1994). Additionally, body size is known to be to be important for avian competition (Andersson 1994), and has been found to correlate with reproductive success in numerous birds species (Chastel et al., 1995; Dyrcz et al., 2005; Sanchez-donoso et al., 2018). Weight significantly differed between Saltmarsh and Nelson's Sparrows, and also correlated with reproductive success, providing further evidence that weight/body size may be important in determining mate success within this system and could be acting as a sexual signal influencing the extent of interspecific mating events. The larger Saltmarsh Sparrow males may out-compete smaller Nelson's male or hybrids for access to mates. It is unlikely that one sex controls mate choice entirely—interactions between male and female choice may ultimately determine mate success. Females may solicit competition among males and make choices based on displayed dominance (Andersson 1994). Indeed, Saltmarsh females have been known to solicit mating during nest building as well as prevent forced mountings by males by fighting or aggressive calls (Greenlaw & Post, 2012), suggesting some female control exists in this system. Females may

also be actively choosing to accept matings with Saltmarsh Sparrow or hybrid males that have higher mass and are larger in body size.

Average fat and muscle scores do not appear to be acting as sexual traits informing mate choice between the Saltmarsh and Nelson's Sparrows. We were surprised to observe the number of offspring sired decrease with fat content across Saltmarsh, Nelson's and hybrid sparrows; however, one possible explanation is that the most successful males are likely expending more energy finding, competing for and copulating with females (Vehrencamp et al., 1989), which could lead to reduced fat stores. We also found that muscle scores were significantly higher in Saltmarsh Sparrows than Nelson's Sparrows; however, this was not a consistent predictor of reproductive success between the species. There also may be other secondary sexual characteristics driving sexual selection that were not accounted for in this study. In particular, known differences in male behavior, song, frequency of mating display/singing, as well as melanin content and brightness of plumage between Saltmarsh and Nelson's Sparrows should also be considered (Greenlaw 1993; Shriver et al., 2005, 2007, 2010).

Across males of all genotypic classes, reproductive success increased with male CP volume, providing evidence that post-copulatory sexual selection is acting on patterns of gene flow between the species. Fertilization success can be influenced by either male-male competition or female choice post-copulation, in the form of sperm competition and cryptic female choice (Parker, 1970; Birkhead, 1998). Sperm competition is higher in males belonging to birds with polygynous mating systems, as measured by things such as testes lengths, CP volume, as well as ejaculate quality and quantity (Briskie 1993, Moller 1988). Further, CP volume has been found as a proxy for sperm production, sperm velocity, and resulting

fertilization success in birds (Peer, et al., 2000; Laskemoen et al., 2008; Laskemoen et al., 2010).

Larger CP volumes in Saltmarsh Sparrows than Nelson's Sparrows, as well as a relationship

between CP volume and proportion of Saltmarsh Sparrow alleles, suggests that sperm

competition is likely a factor contributing to fertilization rates and male reproductive success in

this system. If Saltmarsh Sparrows have higher sperm production or velocity than that of

Nelson's Sparrows, eggs may be preferentially fertilized by Saltmarsh Sparrow males out

competing sperm of Nelson's. Hybrids may be superior sperm competitors to Nelson's but not

Saltmarsh Sparrows. This competitive advantage could subsequently increase rates of

hybridization or drive patterns of introgression. Despite any competitive advantage, we saw few

hybrids and nearly equal backcrossing in both the Saltmarsh and Nelson's direction in the center

of the hybrid zone (Chapter 1), suggesting mechanisms exist to limit hybridization and minimize

asymmetric introgression towards one parental species or the other. Females may also have

control over post-copulatory mate choice. Since we did not account for any cryptic female choice

in this study, we therefore cannot eliminate the effect of female sparrows biasing fertilization

rates between males of differing genetic makeup.

Intraspecific Predictors of Fitness

Traits that determined interspecific success were consistent with those found to predict

intraspecific mating success; however, the relationships were less strong. Mass was the only

significant predictor of reproductive success in Saltmarsh Sparrows. The relationship of body

size and reproductive success in Saltmarsh Sparrows supports the observed reproductive skew in

this population. In a scramble polygyny mating system, larger males may outcompete smaller

males for access to females causing certain males to sire more offspring than others. Pre-

copulatory actions (mating) may drive patterns of mate success in Saltmarsh Sparrows more than

post-copulatory (sperm competition). CP volume was not a good predictor of success within Saltmarsh Sparrows; however, all observed Saltmarsh Sparrow males had large CP volumes suggesting competition may play a role. Although CP measurements in the field were able to illustrate interspecific differences in size, the measurements may not be precise enough to accurately pick up on size differences within species. Additionally, the large sizes of CPs in Saltmarsh Sparrows in comparison to other species suggests that sperm competition may be an important factor in this system despite the fact that we did not find a direct relationship with volume and number of offspring sired (Greenlaw & Post, 2012).

Within Nelson's Sparrows, neither CP volume nor weight were good predictors of reproductive success. Although not significant, there was a trend for a negative relationship between mass and the number of offspring sired. This suggests that the smaller Nelson's males may have a reproductive advantage over larger males. This may be explained by their mating strategy, whereby size may not matter as much due to active mate guarding of females. Smaller body size may also provide advantages in acrobatic performance in their characteristic flight displays to attract females (Walsh et al., 2015a, 2018b). Because we did not find weight or CP volume to be predictors of reproductive success with Nelson's Sparrows, this suggests there is some unmeasured sexual trait that may lead to success of some males over others. Mating behavior such as length of singing period or active display period may be more important to Nelson's Sparrows than overall size in attracting females (Greenlaw, 1993; Shriver et al., 2007, 2010). Additionally, the frequency and length of mate guarding may be important in predicting success. This may act as a way to ensure their sperm does not get replaced by another male and increase their odds of fertilization.

Conclusion

We found differential male fitness and reproductive skew in a sympatric population of Saltmarsh and Nelson's Sparrows within the center of the hybrid zone. Saltmarsh Sparrows sired more offspring than Nelson's Sparrows and hybrids. Although hybrid individuals showed intermediate fitness, it was much closer to that of Nelson's. Reproductive success between Saltmarsh, Nelson's, and hybrid sparrows was related to CP volume and body weight across all individuals; however, the only significant predictor of intraspecific male fitness was body size for Saltmarsh Sparrows. CP volume and mass may represent primary and secondary sexual characteristics driving patterns of interspecific gene flow and fitness in sympatric populations of Saltmarsh and Nelson's Sparrows. The competitive advantage of Saltmarsh Sparrows in interspecific mating interactions could lead to competitive asymmetry between the species, driving rates of hybridization and causing asymmetric introgression towards the inferior Nelson's genotypes. However, directional hybridization may also be adaptive for the Nelson's Sparrows and drive introgression in the Saltmarsh Sparrow direction, along a patchy habitat gradient throughout the hybrid zone. Introgression was observed heavily in both directions in our study (Chapter 1), suggesting another mating signals may exist to lead to assortative mating between the species or a role of reduced fitness of hybrid females including song, behavior, or plumage.
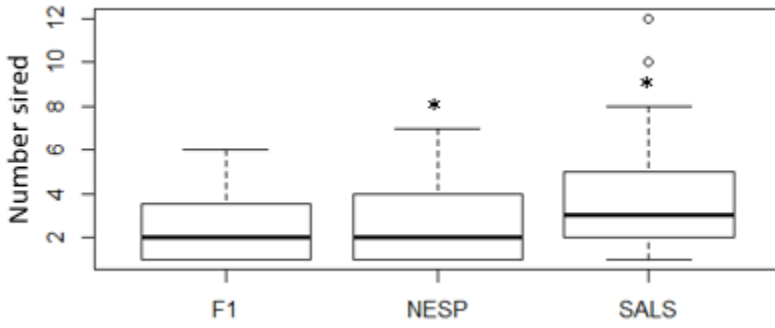
Figure 3.1: The number of offspring sired by males of 3 genotypic classes across 2016 and 2017 breeding seasons: recent generation hybrids (F1), backcrossed/ pure Nelson's Sparrows (NESP), and backcrossed/pure Saltmarsh Sparrows (SALS). Asterisk denotes significance.
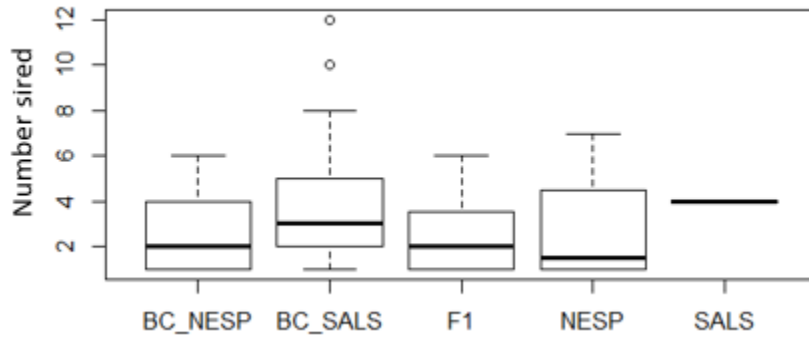
Figure 3.2: The number of offspring sired by males of 5 genotypic classes across 2016 and 2017 breeding seasons. Labels are as follows: backcrossed Nelson's Sparrows (BC_NESP), backcrossed Saltmarsh Sparrows (BC_SALS), F1/F2 individuals (F1), pure Nelson's Sparrow (NESP), pure Saltmarsh Sparrow (SALS).
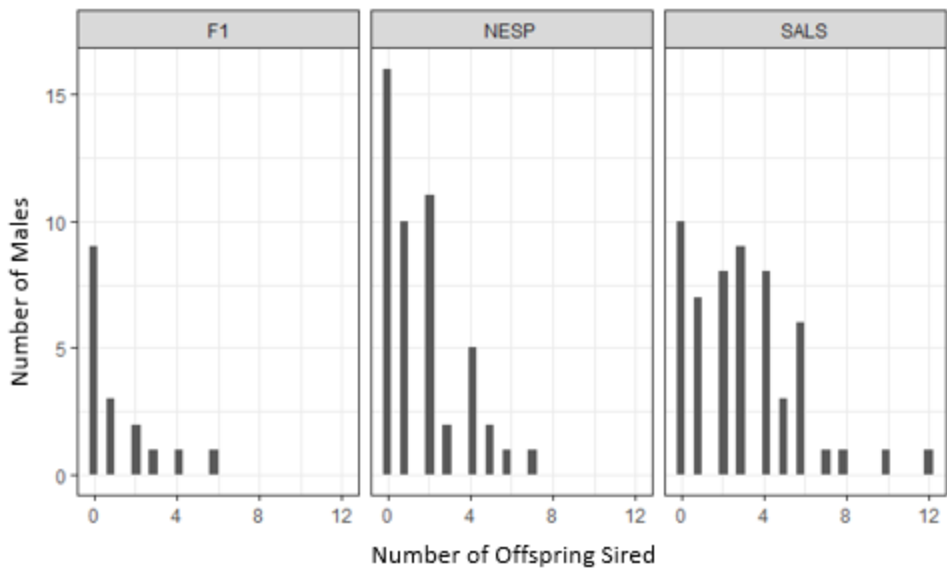
Figure 3.3: Variance in reproductive success for males of the three genotypic classes: recent generation hybrids (F1), pure and backcrossed Nelson's Sparrows (NESP), and pure and backcrossed Saltmarsh Sparrows (SALS). Each panel shows frequency distribution of the number of offspring sired across the two breeding seasons (2016 and 2017).
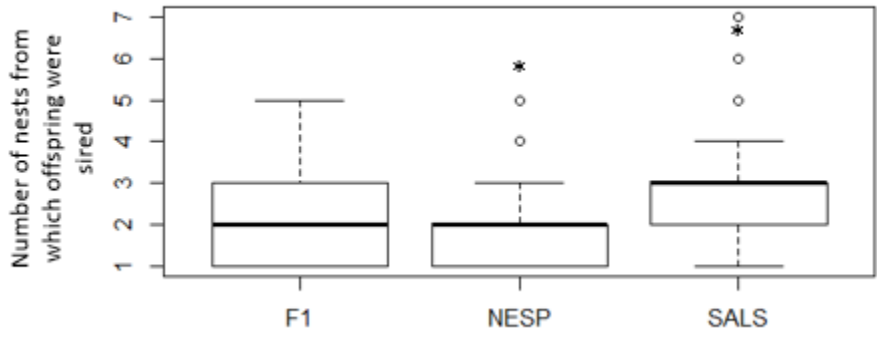
Figure 3.4: The average number of nests from which offspring were sired by males of three genotypic classes: recent generation hybrids (F1), pure and backcrossed Nelson's Sparrows (NESP), and pure and backcrossed Saltmarsh Sparrows (SALS).

Table 3.3: Number of males that sired zero, one, two, three, four, or greater than or equal to five offspring in 2016 and 2017 breeding seasons across each of three genotypic classes. SALS = Saltmarsh Sparrow & NESP = Nelson's Sparrow.

	Sired 0 offspring	Sired 1 offspring	Sired 2 offspring	Sired 3 offspring	Sired 4 offspring	Sired 5+ offspring
Hybrids (F1/F2)	53% (9)	18% (3)	12% (2)	6% (1)	6% (1)	6% (1)
SALS (backcrossed & pure)	18% (10)	13% (7)	15% (8)	16% (9)	15% (8)	24% (13)
NESP (backcrossed & pure)	33% (16)	21% (10)	23% (11)	4% (2)	10% (5)	8% (4)
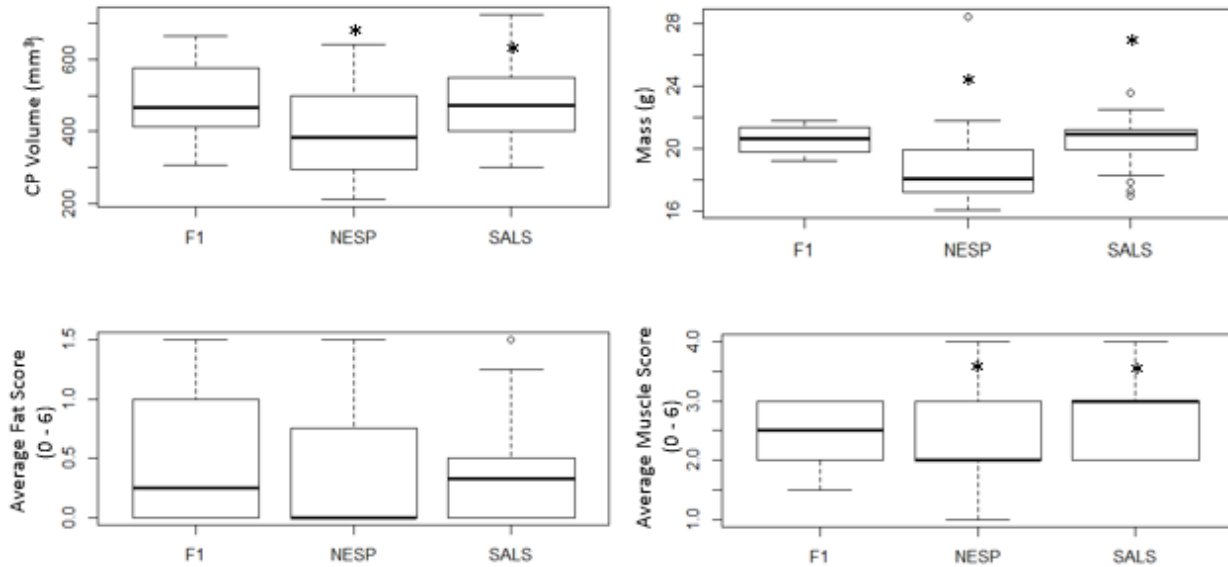
Figure 3.5: Average male condition metrics across three genotypic classes. The top left panel is CP volume, the top right panel is mass, the bottom left panel is average fat score, and the bottom right panel is average muscle score. Across all panels the genotypic classes are as follows: recent generation hybrids (F1), pure and backcrossed Nelson's Sparrows (NESP), and pure and backcrossed Saltmarsh Sparrows (SALS). Asterisk denotes significance.
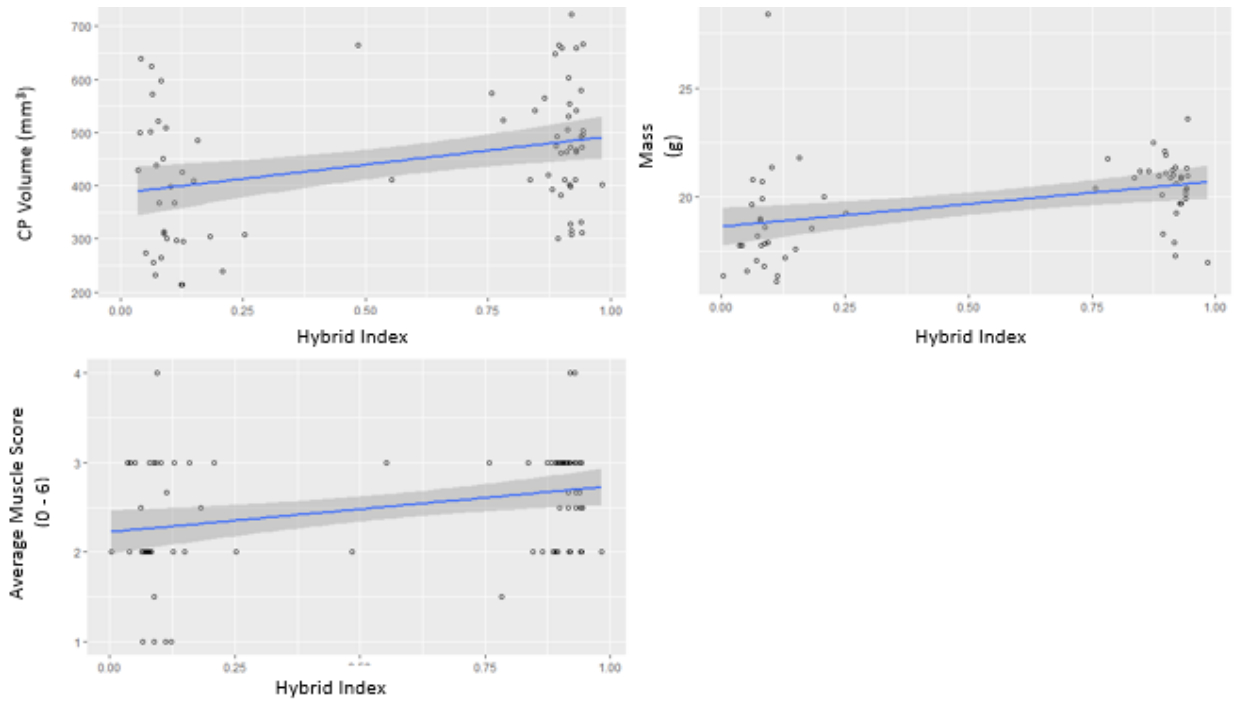
Figure 3.6: Three male condition metrics across male hybrid index values. The top left panel is CP volume, the top right panel is mass, and the bottom left panel is average muscle score.
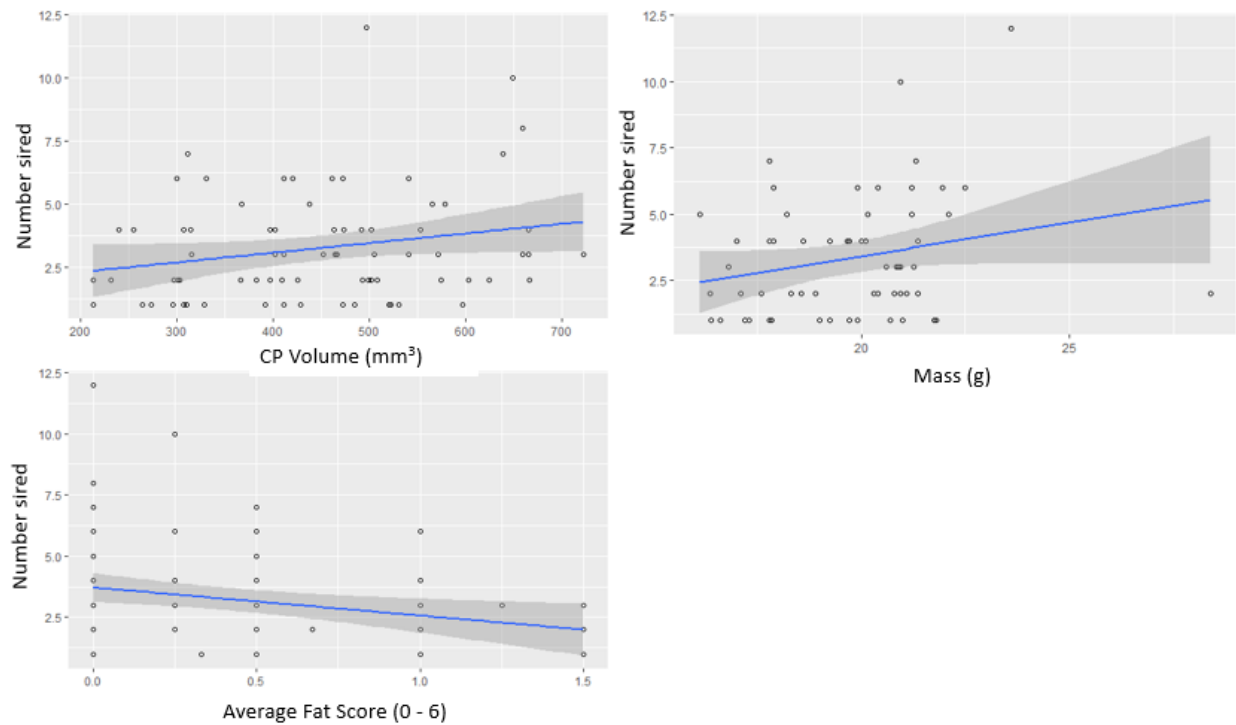
Figure 3.7: The number of offspring sired by male Saltmarsh and Nelson's Sparrows and their hybrids as predicted by 3 male condition metrics. The top left panel is CP volume, the top right panel is mass, and the bottom left panel is average fat score.
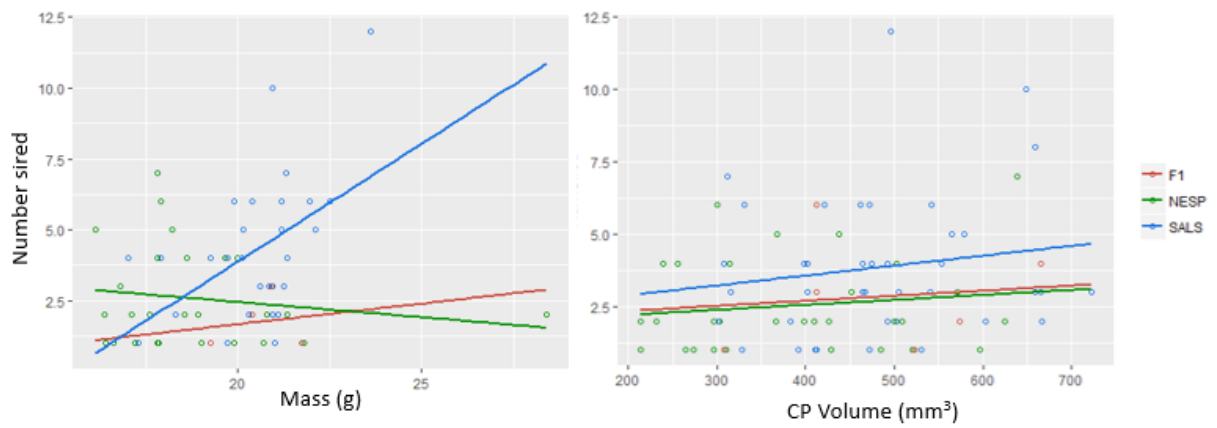
Figure 3.8: Number of offspring sired by males of three genotypic classes in relation to 2 predictive male condition metrics -- CP volume, left panel and mass, right panel. Blue represents pure and backcrossed Saltmarsh Sparrows (SALS), green represents pure and backcrossed Nelson's Sparrows (NESP), and red represents recent generation hybrids (F1/F2). Note, the only significant relationship within genotypic classes is that of number sired and mass within Saltmarsh Sparrows (Blue line in the left panel).

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CONCLUSION

Endogenous and exogenous drivers of hybrid zone structure are operating at multiple spatial scales within the Saltmarsh-Nelson's Sparrow hybrid zone. Fitness differences among parental species and hybrids, relative population densities and species distributions, differential adaptation to local environments, and pre-zygotic and post-zygotic reproductive isolating mechanisms all play a role in the dynamics of this hybrid zone. Relative population densities differed on a large scale between the center and the south of the hybrid zone and influenced patterns of introgression, with more backcrossing towards the abundant parent species (Saltmarsh Sparrow) in the south of the zone and relatively equal backcrossing in the center of the zone where there were more equal species densities. On a small scale, local site-specific characteristics of the two study locations influenced the distribution of genotypes and patterns of introgression across a tidal marsh habitat gradient.

Increased genetic diversity resulting from gene flow between these species may increase the fitness capabilities of Nelson's Sparrows as they move into more coastal environments (Nicotra et al., 2015) and expand their distribution southward (Walsh et al., 2017). Interspecific gene flow does not, however, appear to benefit the Saltmarsh Sparrow, which has higher reproductive success than Nelsons and admixed sparrows. Although female fitness levels and adaptive nesting differences exist between the Saltmarsh and Nelson's Sparrows (Walsh et al., 2016), high water levels and associated high rates of nest flooding appear to be swamping out any observed differential fitness between these two birds. Hybridization and continued gene flow between these two sister-species has the power to influence the evolutionary trajectory and future

persistence of the Saltmarsh and Nelson's Sparrow, it does not appear to be the largest driver of nest success in this system.

Pre and post-copulatory sexual selection appears to be acting on male sexual signals between the Saltmarsh and Nelson's Sparrows leading to observed differences in reproductive success and reproductive skew. Cloacal Protuberance (CP) volume (as a proxy for sperm competition) and mass represent primary and secondary sexual characteristics driving patterns of interspecific gene flow and fitness in sympatric populations of Saltmarsh and Nelson's Sparrows. Competitive asymmetry may cause asymmetric introgression towards the inferior Nelson's Sparrow genotypes and hybrid zone movement throughout the range. However, directional hybridization may also be adaptive for the Nelson's Sparrows and drive introgression in the Saltmarsh Sparrow direction, especially in more coastal marshes within the patchy mosaic of habitats characterized by the hybrid zone. Despite these mating asymmetries and potential for reproductive dominance of Saltmarsh Sparrows, assortative mating is prevalent, resulting in relatively few interspecific matings. Recent generation hybrids are infrequent and there is evidence for reduced survival of hybrid females. Hybridization dynamics appear to remain stable between the Saltmarsh and Nelson's Sparrows, and species boundaries continue to be maintained

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APPENDIX A

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

31-May-2016

Kovach, Adrienne I
Natural Resources & The Environment
Rudman Hall Rm G87
Durham, NH 03824-2618

IACUC #: 160503
Project: Ecology, Genetics and Conservation of Tidal Marsh Sparrows
Approval Date: 17-May-2016

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are used.* The IACUC made the following comment(s) on this protocol:

1. The investigator is responsible for obtaining any necessary permits for capturing animals as proposed in the study.
2. All principal investigators/instructors are responsible for knowing about zoonotic diseases, safety issues, laws, and regulations applicable to the proposed field study activity, taking appropriate precautions, instructing/informing project personnel and students ahead of time about pertinent issues accordingly, and ensuring project personnel review the collection permit before capturing/trapping/handling any animals. Please contact the UNH Office of Environmental Health & Safety (603/862-4041) with any questions.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jill A. McGaughy, Ph.D.
Chair

cc: File

APPENDIX B

Sample	Site Name	Location	Coordinates		Year	Collector	Reference
198_nesp	Hobart Stream	ME	-67.204266	44.889845	2015	M. Conway	M. Conway Dissertation (in prep)
201_nesp	Hobart Stream	ME	-67.204266	44.889845	2015	M. Conway	M. Conway Dissertation (in prep)
205_nesp	Hobart Stream	ME	-67.204266	44.889845	2015	M. Conway	M. Conway Dissertation (in prep)
300_nesp	Hobart Stream	ME	-67.204266	44.889845	2015	M. Conway	M. Conway Dissertation (in prep)
8200_nesp	Hobart Stream	ME	-67.204266	44.889845	2015	M. Conway	M. Conway Dissertation (in prep)
SALS_1241-89105	Sawmill Creek	NY	-74.191103	40.610062	2012	A. Kocek	SHARP Sampling Site
SALS_1241-89167	Sawmill Creek	NY	-74.191103	40.610062	2012	A. Kocek	SHARP Sampling Site
SALS_1241-89173	Idlewild	NY	-73.745247	40.648212	2012	A. Kocek	SHARP Sampling Site
SALS_1361-54712	Idlewild	NY	-73.745247	40.648212	2014	A. Kocek	SHARP Sampling Site
SALS_1361-54720	Idlewild	NY	-73.745247	40.648212	2014	A. Kocek	SHARP Sampling Site
SALS_1361-54733	Idlewild	NY	-73.745247	40.648212	2014	A. Kocek	SHARP Sampling Site
SALS_1361-54759	Marine Nature Center	NY	-73.622075	40.621339	2014	A. Kocek	SHARP Sampling Site
SALS_1361-54856	Marine Nature Center	NY	-73.622075	40.621339	2014	A. Kocek	SHARP Sampling Site
SALS_1601-37004	Shirly	NY	-72.893452	40.769611	2007	J. Walsh	Walsh et al. 2012
SALS_1601-37007	Shirly	NY	-72.893452	40.769611	2007	J. Walsh	Walsh et al. 2012
SALS_2281-67180	Shirly	NY	-72.893452	40.769611	2007	J. Walsh	Walsh et al. 2012
SALS_2281-67191	Shirly	NY	-72.893452	40.769611	2007	J. Walsh	Walsh et al. 2012
SALS_2281-67197	Shirly	NY	-72.893452	40.769611	2007	J. Walsh	Walsh et al. 2012
SALS_2511-17270	Sachuset	RI	-71.247571	41.486677	2011	E. King	SHARP Sampling Site
SALS_2511-17272	Sachuset	RI	-71.247571	41.486677	2011	E. King	SHARP Sampling Site
SALS_2511-17274	Sachuset	RI	-71.247571	41.486677	2011	E. King	SHARP Sampling Site
SALS_2511-17279	Sachuset	RI	-71.247571	41.486677	2011	E. King	SHARP Sampling Site
SALS_2511-17347	Sachuset	RI	-71.247571	41.486677	2011	E. King	SHARP Sampling Site
SALS_2541-59170	Barn Island	CT	-71.8627481	41.337466	2014	C. Field/A. Borowske	SHARP Sampling Site
SALS_2571-82303	Barn Island	CT	-71.8627481	41.337466	2014	C. Field/A. Borowske	SHARP Sampling Site
SALS_2581-97070	Barn Island	CT	-71.8627481	41.337466	2014	C. Field/A. Borowske	SHARP Sampling Site
SALS_2581-97281	Barn Island	CT	-71.8627481	41.337466	2014	C. Field/A. Borowske	SHARP Sampling Site
SALS_2661-46831	Idlewild	NY	-73.745247	40.648212	2013	A. Kocek	SHARP Sampling Site
SALS_2661-46912	Marine Nature Center	NY	-73.622075	40.621339	2014	A. Kocek	SHARP Sampling Site
SALS_2661-46932	Sawmill Creek	NY	-74.191103	40.610062	2014	A. Kocek	SHARP Sampling Site
SALS_2661-46933	Sawmill Creek	NY	-74.191103	40.610062	2014	A. Kocek	SHARP Sampling Site
SALS_2661-46952	Marine Nature Center	NY	-73.622075	40.621339	2014	A. Kocek	SHARP Sampling Site
SALS_2661-46953	Marine Nature Center	NY	-73.622075	40.621339	2014	A. Kocek	SHARP Sampling Site
SALS_2661-46986	Sawmill Creek	NY	-74.191103	40.610062	2014	A. Kocek	SHARP Sampling Site

Sample	Site Name	Location	Coordinates		Year	Collector	Reference
SALS_2691-08432	Barn Island	CT	-71.8627481	41.337466	2014	C. Field/A. Borowske	SHARP Sampling Site
upnarr_221_nesp	Upper Narraguagus	ME	-67.913004	44.570516	2015	M. Conway	M. Conway Dissertation (in prep)
upnarr_222_nesp	Upper Narraguagus	ME	-67.913004	44.570516	2015	M. Conway	M. Conway Dissertation (in prep)
upnarr_224_nesp	Upper Narraguagus	ME	-67.913004	44.570516	2015	M. Conway	M. Conway Dissertation (in prep)
upnarr_225_nesp	Upper Narraguagus	ME	-67.913004	44.570516	2015	M. Conway	M. Conway Dissertation (in prep)
upnarr_227_nesp	Upper Narraguagus	ME	-67.913004	44.570516	2015	M. Conway	M. Conway Dissertation (in prep)
upnarr_228_nesp	Upper Narraguagus	ME	-67.913004	44.570516	2015	M. Conway	M. Conway Dissertation (in prep)
wolf_764_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_765_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_766_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_767_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_768_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_769_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_770_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_771_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
wolf_772_nesp	Wolfville	Nova Scotia	-64.213541	45.53034	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_748_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_749_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_750_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_752_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_753_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_754_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_756_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_759_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_763_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)
yarm_Y1_nesp	Yarmouth	Nova Scotia	-66.7258	43.501485	2015	Walsh/ Kovach	Walsh et al. 2018 (in press)