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RESEARCH ARTICLE

The role of divergent mating strategies, reproductive success, and compatibility in maintaining the Saltmarsh–Nelson’s sparrow hybrid zone

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ABSTRACT

Understanding inter- and intra-specific variation in mate compatibility and reproductive success can offer insight into the factors driving sexual selection, behavioral dynamics, and isolating mechanisms across natural populations. This information is particularly relevant when trying to understand the patterns that shape the causes and outcomes of hybridization in natural systems. We evaluated mating patterns and male reproductive success in a hybrid zone between the Saltmarsh (*Ammodramus caudacutus*) and Nelson’s sparrow (*A. nelsoni*). Specifically, we investigated variation in male reproductive success between pure Saltmarsh and pure Nelson’s sparrows and between pure and hybrid males, while testing for assortative vs. random mating. DNA samples were collected from adults ($n = 342$) and nestlings ($n = 348$), and paternity analyses and assignment of individuals to pure and hybrid classes were conducted using 11 microsatellite loci. We documented high promiscuity and reproductive skew in Saltmarsh and Nelson’s sparrow males, with greater skew in Saltmarsh Sparrows. Across Nelson’s Sparrow and hybrid individuals, we documented a significant correlation between reproductive success and genetic heterozygosity. F1–F2 hybrid males exhibited significantly lower reproductive success (number of offspring sired) compared with pure individuals, and Saltmarsh and Nelson’s sparrows exhibited strong patterns of assortative mating. We conclude that differences in reproductive success among pure and hybrid individuals, coupled with strong assortative mating, are shaping hybrid zone dynamics in this system and may be important for maintaining species boundaries.

Keywords: *Ammodramus caudacutus*, *Ammodramus nelsoni*, hybrid fitness, assortative mating, mating behavior

El rol de las estrategias de apareamiento divergente, del éxito reproductivo y de la compatibilidad en mantener la zona híbrida de *Ammodramus caudacutus* y *A. nelsoni*

RESUMEN

Entender la variación inter- e intraespecífica en la compatibilidad de la pareja y el éxito reproductivo puede ofrecer información sobre los factores que determinan la selección sexual, las dinámicas de comportamiento y los mecanismos de aislamiento a través de las poblaciones naturales. Esta información es particularmente importante cuando se trata de entender los patrones que determinan las causas y los resultados de la hibridación en los sistemas naturales. Evaluamos los patrones de apareamiento y el éxito reproductivo del macho en una zona híbrida entre *Ammodramus caudacutus* y *A. nelsoni*. Específicamente, investigamos la variación en el éxito reproductivo del macho entre individuos puros de *A. caudacutus* y *A. nelsoni*, y entre machos puros e híbridos, mientras evaluamos apareamiento selectivo versus aleatorio. Las muestras de ADN fueron colectadas a partir de adultos ($n = 343$) y polluelos ($n = 348$); los análisis de paternidad y asignación de individuos a las clases pura e híbrida fueron realizados usando 11 loci de microsatélites. Documentamos alta promiscuidad y sesgo reproductivo en los machos de *A. caudacutus* y *A. nelsoni*, con mayor sesgo en *A. caudacutus*. Documentamos una correlación significativa entre el éxito reproductivo y la heterocigosidad genética para los individuos de *A. nelsoni* y los híbridos. Los machos híbridos F1/F2 mostraron un éxito reproductivo significativamente más bajo (número de descendientes engendrados) comparados con los individuos puros, y los individuos de *A. caudacutus* y *A. nelsoni* mostraron fuertes patrones de apareamiento selectivo. Concluimos que las diferencias en el éxito reproductivo entre individuos puros e híbridos, acopladas con un fuerte apareamiento selectivo, están dando forma a las dinámicas de la zona híbrida en este sistema y pueden ser importantes para mantener las fronteras de las especies.

Palabras clave: aptitud biológica híbrida, *Ammodramus caudacutus*, *Ammodramus nelsoni*, apareamiento selectivo, comportamiento de apareamiento

INTRODUCTION

The structure of mating systems can have substantial impacts on sexual selection, influencing the evolution of both behavior and secondary sexual characteristics. Thus, understanding the factors driving variation in the intensity of sexual selection in different species and in populations of the same species remains an important topic in biology (Emlen and Oring 1977). Differences between hybridizing species in mating behaviors, such as vocalizations or displays, can be a critical determinant of reproductive isolation in hybrid zones (Randler 2002, Muñoz et al. 2010). Behavioral mechanisms, including male–male competition and female mate choice, have been shown to both inhibit (Sætre et al. 1997, Veen et al. 2001) and facilitate (Moore 1987, Pearson 2000) interbreeding and asymmetrical pairing. In avian systems, females are the choosier sex as their parental investment is typically higher than that of males (Andersson 1994, Randler 2002). Thus, females are predicted to choose conspecific mates to avoid hybrid incompatibilities and reduced reproductive success (Andersson 1994, Wirtz 1999, Randler 2002, Schumer et al. 2017, Semenov et al. 2017). However, there are hybrid zones in which females more commonly choose hetero-specific males (McDonald et al. 2001, Ronsenfield and Kodric-Brown 2003, Pfennig 2007). Understanding inter-specific mate compatibility and variation in reproductive success can thus offer important insight into the evolutionary trajectory of a hybrid zone.

In intraspecific interactions, the intensity of sexual selection, or the degree of polygamy within populations, is thought to be driven by the ability of individuals to monopolize and control access to mates (Emlen and Oring 1977, Weir et al. 2011). The degree to which mates can be monopolized, in turn, has been linked to ecological factors, with local environmental conditions considered to be an important driver of polygamy (Emlen and Oring 1977). Polygamy is expected to be more prevalent in environments in which one sex is freed from parental care, either due to minimal parental care requirements or due to readily available and abundant food resources that allow for uniparental care (Trivers 1972, Emlen and Oring 1977). The conditions that allow for uniparental care also favor polygamy by providing more opportunities for male mate competition as well as increasing the importance of female choice (Andersson 1994, Randler 2002). In these scenarios, females may assess male quality through body size and/or condition (Pujolar et al. 2005) or through dominant behaviors during male–male competition (Tiira et al. 2006). In the absence of these cues, females may choose to mate with multiple males to increase the genetic diversity shared with their offspring (Yasui 1998). In addition, the spatial and temporal arrangement of available mates is an

important driver of variability in reproductive success among males. When females are spatially clumped, the opportunity arises for a small percentage of males with superior competitive ability to monopolize available mates and sire a high proportion of the offspring produced (Emlen and Oring 1977). Alternatively, when females become receptive at the same time, it reduces the potential for a given male to mate with multiple females; monopolization of available mates is predicted to increase with mating asynchrony (Emlen and Oring 1977). Differences in mating systems between hybridizing taxa can facilitate or inhibit heterospecific pairings; thus, studying intra- vs. inter-specific variation in reproductive success can broaden our understanding of how mating systems affect hybridization dynamics.

Here, we evaluate the influence of inter- and intra-specific variation in genetic compatibility and reproductive success on hybridization patterns between 2 avian tidal marsh endemics, the Saltmarsh Sparrow (*Ammodramus caudacutus*) and Nelson's Sparrow (*A. nelsoni*). In the USA and maritime Canada, these 2 species are restricted to a narrow ribbon of habitat along the Atlantic seaboard. They are currently in secondary contact along the New England coast between the Weskeag River estuary in South Thomaston, Maine, USA, and Plum Island in Newburyport, Massachusetts, USA (Hodgman et al. 2002, Shiver et al. 2005, Walsh et al. 2015). Tidal salt marshes are highly productive environments, providing abundant food resources and limited competition from other species (Post and Greenlaw 2006, Greenberg and Olsen 2010), a scenario that predicts a high potential for polygamy (Emlen and Oring 1977). Furthermore, both species exhibit an unusual mating system among passerines, characterized by nonterritoriality and a lack of male parental care (Greenlaw 1993), and the ground-nesting females face the challenges of nesting in an environment shaped by regular tidal inundation (Shriver et al. 2007). In salt marshes, flooding affects nests during the highest spring tides; during this time, the entire marsh is flooded and nests can be inundated with water for multiple hours (Gjerdrum et al. 2008). Thus, females that synchronize their nesting with the tides and renest immediately after a flood tide have higher reproductive success than those that do not (Shriver et al. 2007, Walsh et al. 2016). The above characteristics likely lead to complex temporal and spatial patterns in mating opportunities for both species, which may drive inter- and intra-specific variation in reproductive success. Ecological drivers of polygamy within the tidal marsh environment, coupled with differences in male and female reproductive behaviors between species, provide an opportunity to investigate the role of mating patterns in shaping hybridization outcomes. We outline differences in mating systems between the 2 species below, and present predictions of how behavioral and ecological factors may

influence reproductive success and mating behavior across the hybrid zone.

In addition to being nonterritorial, Saltmarsh Sparrows are also highly promiscuous and exhibit a scramble competition polygynous mating system, wherein males search for and attempt to mate with multiple receptive females (Greenlaw and Rising 1994, Hill et al. 2010). The lack of territoriality in Saltmarsh Sparrows increases the chances of many females nesting near each other in optimal nesting locations. The close spacing of females may allow individual males to gain access to multiple mates, potentially leading to variability in reproductive success among Saltmarsh Sparrow males according to competitive ability. Alternatively, Saltmarsh Sparrow females have been found to have nesting cycles (~24-day nesting periods) that are highly synchronized with tidal cycles (26–28-day flooding cycles; Greenlaw and Rising 1994, Shriver et al. 2007); as a result, most females on a given marsh are receptive simultaneously for only a few days each nesting cycle. This temporal clustering of receptive females may prevent a single male from being able to monopolize all receptive females in an area and thus reproduction may be distributed across several males. While the spatial and temporal characteristics of Saltmarsh Sparrow mating and nesting behaviors may have divergent effects on reproductive skew, both have the potential to drive strong male–male competition within this species. Because males are free from holding territories and providing parental care, we predicted that competitively superior males would be able to mate with multiple females, even in the face of temporal clustering. Thus, we predicted high reproductive skew among Saltmarsh Sparrow males. On the other hand, if females play an active role in controlling mating and fertilization success through soliciting multiple matings, this may decrease the degree of skew by enabling more males to successfully sire offspring. Male–male competition should also select for large body size (Greenlaw 1993, Andersson 1994, Fairbairn and Preziosi 1994, Székely et al. 2004) and increased body condition, which can be manifested through plumage signals or as individual vigor. These traits may put males at a competitive advantage while also providing honest signals of quality to females; thus, we predicted a correlation between individual reproductive success and body size and condition (measured by plumage traits and genetic heterozygosity) in Saltmarsh Sparrow males. We hypothesized that plumage traits (particularly the darkness of the plumage) might serve as potential indicators of individual condition to females in tidal marsh environments (darker plumage has been identified as an important adaptive trait; Greenberg and Droege 1990). Lastly, we predicted that increased genetic heterozygosity, which has been linked to male condition and attractiveness (Brown 1997) and increased vigor (Mays and Hill 2004), would be

linked to increased reproductive success of males. Specifically, we predicted that the phenotype and genotype with the highest reproductive success would differ in the following ways: larger in size (weight), darker in plumage, and higher in individual genetic heterozygosity.

Nelson's Sparrow males differ from Saltmarsh Sparrow males in that they spend substantial time mate guarding and have a more distinctive song and flight display for attracting females (Greenlaw 1993, Shriver et al. 2007, 2010). Mate guarding for paternity assurance is expected to occur when sex ratios are highly skewed toward males; when females are rare in a population, a male may be less likely to search for additional females once a mate has been secured (Weir et al. 2011). This is consistent with previous findings that Nelson's Sparrow females are less synchronized with the tidal cycle than Saltmarsh Sparrow females (Shriver et al. 2007). This reduction in temporal clustering likely reduces the number of receptive females at any given time, creating increased pressure for mate guarding. Based on this mating strategy, we predicted less of a skew in the reproductive success of Nelson's Sparrow males compared with Saltmarsh Sparrow males. However, flight displays by Nelson's Sparrows may provide additional information for females to evaluate mate quality. Thus, while we predicted that the reproductive skew would be reduced in this species compared with Saltmarsh Sparrows, we expected some variation based on the display and competitive abilities of males. Along these lines, the performance of frequent flight displays by Nelson's Sparrows should select for a smaller, more acrobatic body size (Székely et al. 2004, Byers et al. 2010). The predictions outlined above are consistent with known morphological differences between the 2 species, with Nelson's Sparrows being smaller, both in body mass and structural measurements, than Saltmarsh Sparrows (Walsh et al. 2015).

Interspecific variation in body size and mating strategy may have important implications for male reproductive success in hybridizing populations (Pearson 2000, McDonald et al. 2001). In cases of interspecific male–male competition, we predicted that the larger Saltmarsh Sparrows would dominate Nelson's Sparrows and that any interspecific pairings would occur between Saltmarsh Sparrow males and Nelson's Sparrow females. Further, we predicted low reproductive success of hybrid individuals, as intermediately sized (body mass) hybrid males would be at a disadvantage both in terms of aerial displays and direct male–male competition. Lastly, uniparental female investment in both species should result in females choosing conspecific mates, particularly in light of potential reductions in hybrid fitness. This should select for assortative mating, and we thus predicted a low frequency of heterospecific pairings.

To test our above predictions, we used paternity analyses to investigate mating patterns, genetic compatibility, and

male reproductive success in hybrid populations of Saltmarsh and Nelson's sparrows. We had 2 ultimate objectives: (1) to evaluate reproductive success in pure and hybrid individuals and identify individual male traits that were correlated with increased reproductive success; and (2) to test for random vs. assortative mating and assess the relationship between inter- and intra-specific mate compatibility and reproductive success.

METHODS

Field Methods

We conducted this study during 3 breeding seasons (2011–2013) in 3 marshes located in the southern portion of the Saltmarsh–Nelson's sparrow overlap zone: Eldridge Marsh in Wells, Maine, USA (43°17.31'N, 70°34.27'W; sampled in 2011–2013), Chapman's Landing in Stratham, New Hampshire, USA (43°02.24'N, 70°55.32'W; sampled in 2011–2013), and Lubberland Creek in Newmarket, New Hampshire (43°04.29'N, 70°54.48'W; sampled in 2012–2013). Due to the small size of Chapman's Landing and Lubberland Creek (11.0 and 10.5 ha, respectively), we used the entire marsh as our study plot. For Eldridge Marsh, we focused our efforts within a 15-ha plot, which comprised only a portion of the marsh. To sample the breeding adult population, we subdivided each site into 3–5 subplots and systematically trapped adults using mist nets; a minimum of 3 netting sessions were conducted per subplot each season. Once captured, we banded adults and collected standard morphological measurements. We also collected plumage data for each individual using a plumage scoring method developed by Shriver et al. (2005) to characterize phenotypic differentiation between pure Saltmarsh and Nelson's sparrows and intermediate patterns of admixture. We sexed adults by presence or absence of a cloacal protuberance or brood patch. For adults sampled in 2012 and 2013, we drew 10–20 µl of blood from the brachial vein and transferred samples to Nobuto blood filter strips (Sterlitech, Kent, Washington, USA). For adults sampled in 2011, we pulled the 2 outer tail feathers (R1 and R6) and stored feathers for later genetic analyses.

We found nests through systematic subplot and site searches; once found, we marked nests and visited them every 3–4 days until completion of the nesting attempt to monitor their fates (Ruskin et al. 2017). Briefly, we considered a nest successful if it was found empty when at least 1 nestling would have been 10 days old; we counted a nest as successful if at least 1 chick fledged. We classified nests as flooded if they contained drowned chicks or if eggs were found outside the nest, and we considered nests depredated if nests were torn or contained broken or punctured eggs. We captured females at their associated nests to establish maternal identity; for females not captured previously during systematic netting efforts, we

took a blood sample for genetic analysis. We banded chicks at age 6–7 days and collected morphological measurements, including mass, tarsus length, and bill length, as well as pinfeathers for genetic analyses.

Molecular Methods

For adults, we extracted DNA from blood samples using a DNeasy Blood Kit (Qiagen, Valencia, California, USA) according to the manufacturer's protocol. We extracted DNA from pinfeathers collected from banded nestlings using a DNeasy Tissue Kit (Qiagen), with a minimum 24-hr incubation for the lysis stage. For adult tail feathers, we isolated the calamus and followed the same protocol as used for pinfeathers, except with the addition of 10 µl of DTT (dithiothreitol) to the lysis buffer and a 48-hr incubation. We initially genotyped individuals at 12 microsatellite loci (Hanotte et al. 1994, Bulgin et al. 2003, Hill et al. 2008), including 6 diagnostic microsatellite loci developed specifically to differentiate between Nelson's and Saltmarsh sparrows (Kovach et al. 2015). However, due to missing data, we dropped 1 of the microsatellites and all subsequent results are reported for 11 loci. We calculated allele frequencies, deviations from Hardy-Weinberg equilibrium, and null allele frequencies for each locus in CERVUS 3.0 (Marshall et al. 1998). We calculated individual heterozygosity across loci for each genotyped adult individual using the H-individual option in GENALEX (Peakall and Smouse 2006). Only nestlings with known and confirmed maternity were used for this study. To confirm maternity of the female that we trapped at each nest, we compared multilocus genotypes between the female and nestlings for each nest.

Lastly, to differentiate pure species and hybrids, we assigned individuals to genotypic classes using a combination of a hybrid index and interspecific heterozygosity (Milne and Abbot 2008, Walsh et al. 2015), calculated in package *introgress* (Gompert and Buerkle 2009, 2010) in R (R Core Team 2014). This method is similar to the approach implemented in program *NewHybrids* (Anderson and Thompson 2002) but requires fewer assumptions (i.e. markers are unlinked and not subject to selection; Milne and Abbott 2008, Hamilton et al. 2013). The hybrid index for each individual was defined as the proportion of alleles inherited from the Saltmarsh Sparrow (0 = pure Nelson's Sparrow and 1 = pure Saltmarsh Sparrow), whereas interspecific heterozygosity referred to the proportion of genotypes that were heterozygous for the parental alleles (0 = all homozygous genotypes and 1 = all heterozygous genotypes). To characterize pure individuals, we used genotypes from a total of 60 Saltmarsh and Nelson's sparrows from allopatric populations, from the dataset of Walsh et al. (2015). Using genotypes of allopatric individuals from diagnostic markers that exhibit fixed differences between parental species ensured accurate

estimation of hybrid index values for admixed individuals (Gompert and Buerkle 2009, 2010). Following the methods of Milne and Abbott (2008), individuals with intermediate hybrid index values (0.25–0.75) and high heterozygosity (>0.3) were considered recent-generation hybrids (F1, F2), and individuals with low hybrid index values (<0.25 or >0.75) and low heterozygosity (<0.3) were considered backcrossed. We considered individuals to be pure if they had a hybrid index of 0.00–0.05 (Nelson's Sparrow) or 0.95–1.00 (Saltmarsh Sparrow; Walsh et al. 2015).

Paternity Analyses

Candidate fathers were assigned using the maximum likelihood approach implemented in CERVUS (Marshall et al. 1998). CERVUS calculates the log-likelihood of each candidate parent being the true parent and uses simulated genotypes to determine the level of confidence in the parentage assignment. A list of candidate fathers was prepared for each site (Eldridge Marsh, Chapman's Landing, and Lubberland Creek). For a given year, we included all males that were adults in the year that offspring were sampled. As such, males were only excluded from the analyses if they were nestlings in the year being analyzed and were considered potential sires even if they were not captured in the same year as the sampled offspring. In this way, we accounted for adult males that may have been present on the marsh but avoided capture in a given year. We assumed a genotyping error rate of 3%, a candidate father sampling rate of 70%, and 95% for the proportion of loci typed (based on our data). Given the relatively small area of each sampling location and high recapture rates observed over the duration of the study, we considered a 70% sampling rate of the males in the population to be a conservative estimate. For each offspring, we determined the 2 most likely fathers and assigned paternity to the male that had the highest trio delta value (likelihood of a parent being the true parent relative to an arbitrary individual given the maternal, candidate paternal, and offspring genotypes) if confidence was also $>80\%$. To account for potential unsampled sires, we also conducted parentage analyses using the likelihood approach implemented in COLONY 2.0 (Jones and Wang 2010). COLONY analyzes multilocus genotypes and differs from CERVUS in that it can identify the number of sires for a given nest, even if the father is unknown. A list of candidate fathers was prepared using the methods described above and a full likelihood method was used. Males were assigned parentage when confidence was $>80\%$. For both methods, we visually compared multilocus genotypes between the nestling and the candidate father as a final confirmation; we did not assign paternity to father–nestling pairs with >3 locus mismatches. We observed no disagreement between assignments made by COLONY or CERVUS, although there were some nests for which

COLONY could make an assignment but CERVUS could not.

Inter- and Intra-specific Variation in Fitness and Predictors of Reproductive Success

To investigate differences in reproductive success between species, we used linear regression to test for a relationship between the number of offspring sired and genotype, which we expressed both as a continuous variable (hybrid index) and as a categorical variable (genotypic class). We assessed variation in reproductive success across our 5 genotypic classes. In addition, because backcrossed individuals from these study populations share a high proportion of their alleles with their associated parental species (i.e. most backcrossed individuals have a hybrid index of 0.85–0.95 or 0.05–0.15 for Saltmarsh and Nelson's sparrows, respectively), we also evaluated factors that may influence male fitness separately within 3 categories of sparrow—Saltmarsh Sparrows (pure and backcrossed; hybrid index >0.75), Nelson's Sparrows (pure and backcrossed; hybrid index <0.25), and admixed (recent-generation hybrids; hybrid index 0.25–0.75)—to focus assessment of fitness variation on the most admixed individuals. Within each of these groups, we used linear regression to test for relationships between the number of offspring sired and morphological and genetic characteristics of the male. Specifically, predictor variables included mass, 13 individual plumage traits (Shriver et al. 2005), and individual multilocus heterozygosity. To account for the likely possibility that we did not find every nest in a site, we tested for these relationships using (1) all males trapped in a study site and (2) only the males that were assigned paternity.

Mating Patterns and Compatibility

To evaluate our hypotheses about assortative mating, we tested for an influence of genetic compatibility on overall male and female fitness. One important measure of genetic compatibility is genetic similarity (Marshall et al. 2003, Tarvin et al. 2005). In relation to a hybrid zone, we would predict that more genetically similar (conspecific) pairs would have greater reproductive success than more genetically dissimilar (heterospecific) pairs. To test this hypothesis, we used multilocus genotypes to generate a dissimilarity matrix for all individuals in the dataset using package POPPR 2.7.1 (Kamvar et al. 2014, 2015) in R (R Core Team 2014). Using this matrix, each mating pair was assigned a dissimilarity score, which is expressed as a ratio of the number of observed differences to the number of possible differences (e.g., a mated pair who share half of the same alleles will have a dissimilarity score of 0.5). To test whether mating was random or assortative between Saltmarsh and Nelson's sparrows, we calculated intrapair Pearson product-moment correlation coefficients between

the hybrid index for males and females between each of the pairs. To test the prediction that genetic compatibility of mating pairs would be associated with fitness, we used linear regression to test for a relationship between the dissimilarity score and nesting success and the mass of nestlings (condition).

RESULTS

Across our 3 sampling sites, we genotyped a total of 246 males (Chapman's Landing: $n = 93$; Eldridge Marsh: $n = 121$; Lubberland Creek: $n = 32$), 96 females (Chapman's Landing: $n = 63$; Eldridge Marsh: $n = 18$; Lubberland Creek: $n = 15$), and 348 nestlings (Chapman's Landing: $n = 252$; Eldridge Marsh: $n = 59$; Lubberland Creek: $n = 37$) associated with 126 nests (Table 1). Based on the hybrid index and interspecific heterozygosity, we assigned 2 (2%) of the females as pure Nelson's Sparrow, 5 (5%) as backcrossed in the direction of Nelson's Sparrow, 8 (8%) as F1–F2 hybrids, 50 (52%) as backcrossed in the direction of Saltmarsh Sparrow, and 31 (32%) as pure Saltmarsh Sparrow. Of the 246 males, 10 (4%) were assigned as pure Nelson's Sparrow, 21 (9%) as backcrossed in the direction of Nelson's Sparrow, 33 (13%) as F1–F2 hybrids, 110 (45%) as backcrossed in the direction of Saltmarsh Sparrow, and 72 (29%) as pure Saltmarsh Sparrow.

We assigned paternity to 219 (63%) of the 348 nestlings sampled. For nests for which we were unable to assign paternity, we used COLONY to identify the number of candidate fathers, regardless of individual identity. We found that a large proportion of nests of Saltmarsh Sparrows exhibited multiple paternity. Excluding nests with only 1 chick, 13 broods (12%) had a single father for all nestlings (8 of these nests were full clutches of 2–4 chicks), 64 broods (57%) had a different father for each nestling (29 broods of 2 chicks, 23 broods of 3 chicks, 11 broods of 4 chicks, and 1 brood of 5 chicks), and the remaining 35 broods (31%) had a minimum of 2 fathers for all nestlings in the brood. We also observed multiple paternity in Nelson's Sparrow nests, although it is important to note that sample sizes were considerably lower for pure Nelson's Sparrow males. We observed no instances of a Nelson's Sparrow nest exhibiting a single father for all nestlings. Six broods (75%) had a different father for each nestling (3 broods of 2 chicks, 2 broods of 3 chicks, and 1 brood of 5 chicks). The remaining nests had a minimum of 2 fathers for all nestlings in the brood.

Inter- and Intra-specific Variation in Fitness and Predictors of Reproductive Success

Among the 5 genotypic classes, we found no significant differences in reproductive success between admixed and pure individuals ($P = 0.07$). When using 3 groups instead of 5, or, specifically, when comparing the number of offspring

sired by F1–F2 males with the number sired by Nelson's (pure and backcrossed) and Saltmarsh (pure and backcrossed) sparrows, we observed significantly lower reproductive success in F1–F2 males relative to Saltmarsh Sparrows ($F_{2,243} = 4.03$, $P = 0.02$; Figure 1). Because many of our backcrossed individuals shared a large proportion of their ancestry with their closest parental taxon, all subsequent results are reported for 3 genotypic classes (pure and backcrossed Nelson's Sparrows, F1–F2 hybrids, pure and backcrossed Saltmarsh Sparrows) to provide a more accurate assessment of hybrid (F1–F2) individuals. Among the 3 groups described above, 10 (32%) of the Nelson's Sparrow males (pure and backcrossed) sired offspring, 76 (42%) of the Saltmarsh Sparrow males (pure and backcrossed) sired offspring, and only 3 (9%) of the sampled F1–F2 males sired offspring (Table 1).

Male reproductive success was also variable within species. Across the 3 yr, of the assigned Nelson's Sparrow (pure and backcrossed) males ($n = 10$), 6 individuals (60%) sired 1–2 offspring each and 4 individuals (40%) sired 3–5 offspring each. Of the assigned Saltmarsh Sparrow (pure and backcrossed) males ($n = 76$), 43 individuals (57%) sired 1–2 offspring each, 25 (33%) individuals sired 3–5 offspring each, and 8 individuals (10%) sired 6–10 offspring each. A large proportion of the males of both species did not sire offspring (only 76 of the 182 (42%) sampled Saltmarsh Sparrows and 10 of the 31 (32%) sampled Nelson's Sparrows sired offspring; Figure 2), and, while we cannot say with certainty that we found all nests in our study sites, our results offer compelling evidence for high reproductive skew. Morphological differences among males did not predict reproductive success within a species. We found no significant relationship between the number of offspring sired and mass (all males: $P = 0.72$; assigned males: $P = 0.56$) or plumage score (all males: $P = 0.44$; assigned males: $P = 0.88$) of Nelson's Sparrows. The same was true for both Saltmarsh Sparrows (mass, all males: $P = 0.75$; assigned males: $P = 0.83$; plumage, all males: $P = 0.19$; assigned males: $P = 0.12$) and F1–F2 males (presented for all males only, due to small sample sizes; mass: $P = 0.22$; plumage: $P = 0.40$). We found a significant positive relationship between the number of offspring sired and multilocus heterozygosity across our assigned Nelson's Sparrow males (pure and backcrossed: $\beta \pm SE = -7.25 \pm 1.58$, $t = -4.59$, $P = 0.04$) and across F1–F2 males ($\beta \pm SE = 2.09 \pm 0.91$, $t = 2.29$, $P = 0.03$). However, we did not observe this pattern in Saltmarsh Sparrow males (pure and backcrossed: $P = 0.46$).

Mating Patterns and Compatibility

Although our intention was to use the genetic dissimilarity matrix to assess variation in reproductive success between heterospecific and conspecific pairs, our lack of hybrid pairs (see below) resulted in this being a largely

TABLE 1. Summary of data collected at each of the 3 monitored sites where we evaluated reproductive success of pure and hybrid male Saltmarsh and Nelson's sparrows in relation to assortative vs. random mating patterns in a hybrid zone between the 2 species. Eldridge Marsh is located in Wells, Maine, USA; Chapman's Landing is located in Stratham, New Hampshire, USA; and Lubberland Creek is located in Newmarket, New Hampshire. Shown are the number of nestlings and nests sampled, the total number of males sampled in each genotypic class, the total number of males assigned as sires in our paternity analyses, and the average number of sires per nest.

Site	Nestlings	Nests	Males sampled				Males assigned				Sires per nest
			Nelson's	Saltmarsh	Hybrid	Total	Nelson's	Saltmarsh	Hybrid	Total	
Eldridge Marsh	59	20	19	80	22	121	3	19	1	23	2.55
Chapman's Landing	252	91	11	78	4	93	7	49	2	58	2.28
Lubberland Creek	37	15	1	24	7	32	0	8	0	8	1.93
Total	348	126	31	182	33	246	10	76	3	89	–

intraspecific measure of variation. Individual pairs varied in their degree of multilocus genotypic similarity. The mean dissimilarity index was 0.54 (range: 0.13–0.81), and the dissimilarity index was skewed toward higher values for most of the sampled pairs (i.e. most pairs (74%) were different at $\geq 50\%$ of their alleles; Figure 3). The dissimilarity index did not differ between pairs with failed and fledged nests ($P = 0.42$) and was not correlated with nestling mass ($P = 0.88$). Within breeding pairs, female and male hybrid indices were highly correlated ($R^2 = 0.78$, $P < 0.001$), indicative of assortative mating (Figure 4). Al-

though we know that contemporary hybridization occurs, we found no cases in this dataset of Saltmarsh Sparrows (pure or backcrossed) paired with Nelson's Sparrows (pure or backcrossed). More specifically, in terms of hybrids, backcrossed Saltmarsh Sparrows always paired with pure or backcrossed Saltmarsh Sparrows, and backcrossed Nelson's Sparrows always paired with pure or backcrossed Nelson's Sparrows. The only nonassortative mating that we observed occurred in F1–F2 individuals. We were only able to assign sires for the offspring of one F1 female; she paired with 3 backcrossed Saltmarsh Sparrow males, 1 pure

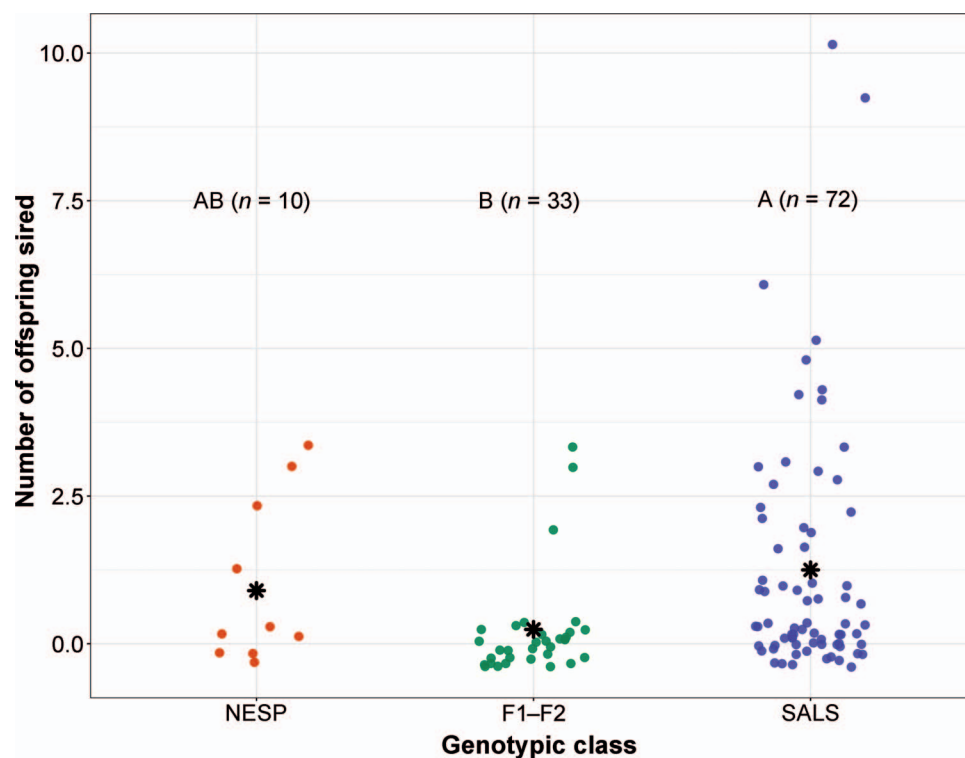


FIGURE 1. Number of offspring sired compared among male sparrows assigned to 1 of 3 genotypic classes: Nelson's Sparrows (NESP; hybrid index < 0.25), Saltmarsh Sparrows (SALS; hybrid index > 0.75), and F1–F2 hybrids (hybrid index $= 0.25–0.75$). The mean is denoted by an asterisk (*). Values with different letters are significantly different, based on a Tukey's post hoc test. Sample sizes are included for each letter comparison.

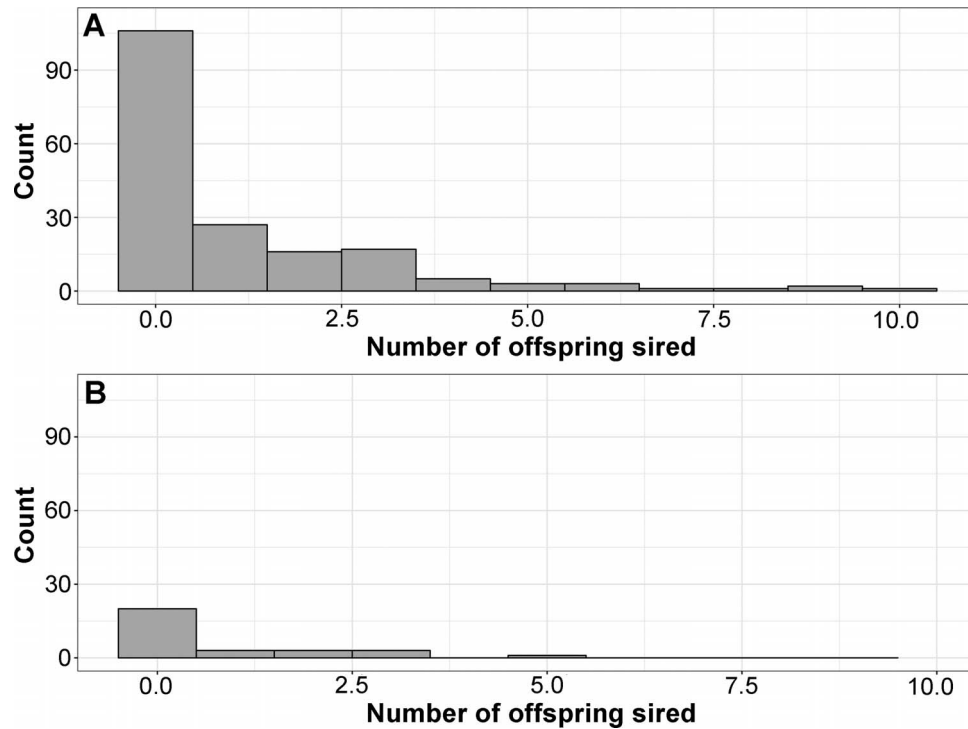


FIGURE 2. Patterns of reproductive skew in Saltmarsh and Nelson's sparrows. (A) The number of Saltmarsh Sparrow males (y axis) that sired 0–10 offspring. (B) The number of Nelson's Sparrow males (y axis) that sired 0–10 offspring.

Saltmarsh Sparrow male, and 1 Nelson's Sparrow male. Of the 3 F1–F2 males that sired offspring, 1 male paired with 2 Nelson's Sparrow females and 1 F1–F2 female, another male paired with 2 backcrossed Saltmarsh Sparrow females, and the third male paired with 2 backcrossed Saltmarsh Sparrow females and 1 pure Saltmarsh Sparrow female.

DISCUSSION

We documented high levels of multiple paternity in Saltmarsh Sparrows in the Saltmarsh–Nelson's sparrow hybrid zone, consistent with previous work on allopatric populations of this species (Hill et al. 2010). Fifty-seven percent of broods had a different father for each nestling. Also, consistent with our predictions, we observed reproductive skew in Saltmarsh Sparrow males: 57% of assigned males sired 1–2 offspring, while only 10% of assigned males sired 6–10 offspring. Although we cannot be certain that we found all nests in a given study site, if we compare the number of males that sired 6–10 offspring with the total number of sampled males, we find that only 5% of males sired >5 offspring. This suggests that the lack of territories coupled with the spatial clustering of receptive females may create an opportunity for a few males to mate with multiple females, either through mechanisms of male–male competition or female choice.

However, the temporal synchrony of females, as restricted by tidal cycles, may also limit the amount of reproductive skew possible among males. Our findings are thus consistent with our hypothesis about temporal and spatial patterns of female receptivity limiting reproductive success; a small percentage of males sired a high proportion of offspring, but the degree of reproductive skew was limited by the ability of many males to mate successfully. Additionally, high promiscuity in Saltmarsh Sparrows likely provided advantages to females by creating opportunities for increased genetic compatibility and heterozygosity (Tarvin et al. 2005). Given uniparental female care, it is likely that Saltmarsh Sparrow females exert some influence over the paternity of their offspring, potentially soliciting matings with multiple males. For example, females in one study were found to successfully resist unwanted males in 57% of recorded interactions (Greenlaw and Post 2012). Our finding that many broods had multiple fathers is consistent with female Saltmarsh Sparrows exhibiting some degree of choice or control over the paternity of their offspring, as expected given their high parental investment in their clutches.

Although we found evidence for considerable variation in male reproductive success in Saltmarsh Sparrows, we did not find support for our prediction that male condition, as measured by mass, plumage traits, and genetic heterozygosity, would be a driver of the observed

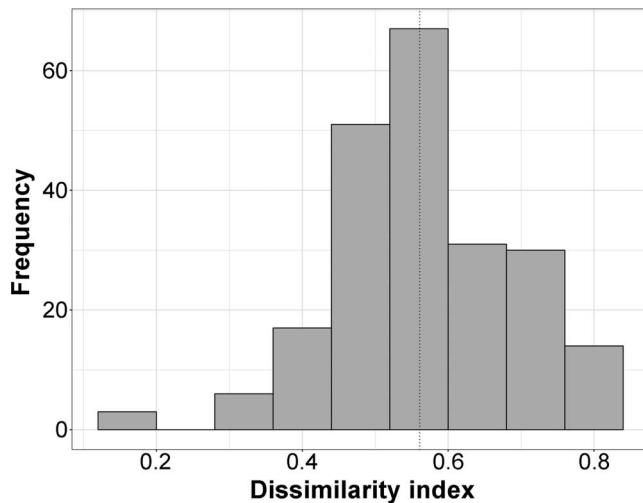


FIGURE 3. Patterns of genetic dissimilarity between males and females in mated pairs of Nelson's and Saltmarsh sparrows (as assigned through paternity data). The histogram shows dissimilarity scores between the mates of each identified pair (mated pairs that share half of their alleles would have a dissimilarity score of 0.5, pairs that share all of their alleles would have a dissimilarity score of 0.0, and pairs that share no alleles would have a dissimilarity score of 1.0), with the average dissimilarity score indicated by the vertical dashed line.

reproductive skew. Some other unmeasured individual characteristic that confers competitive ability may be driving the observed patterns. While much attention has been directed toward the impact of body size on mating success, an important (but less commonly measured) trait is mobility, as it relates to mate searching ability (Baena and Macías-Ordóñez 2015). Perching behaviors or search efficiency may drive reproductive skew, as they can be correlated with mate encounter rate (Thornhill and Alcock 1983, Schwagmeyer 1988), particularly in scramble competition polygynous strategies (Alcock 1980, Kovach and Powell 2003, Barry et al. 2011). Thus, while larger males may be better able to outcompete smaller males in direct competition, it is possible that a more influential driver of mating success in Saltmarsh Sparrows is the ability of an individual male to quickly recognize signals and respond with rapid movement (Baena and Macías-Ordóñez 2015). Search efficiency and overall male vigor appear to be critical components of reproductive success; a previous study found that male Saltmarsh Sparrows spent ~75% of their time patrolling home ranges, presumably searching for receptive females (Greenlaw and Post 2012).

Our study also provides the first view of paternity in Nelson's Sparrows. Although sample sizes were considerably smaller than for Saltmarsh Sparrows, Nelson's Sparrows appeared to have similar levels of promiscuity, with 75% of broods having a different father for each nestling. We also detected modest variation in reproduc-

tive success among Nelson's Sparrow males, with 60% of males siring 1–2 offspring each vs. 40% siring 3–5 offspring each. While, as predicted, we found a less pronounced pattern of reproductive skew in Nelson's Sparrow males vs. Saltmarsh Sparrow males, the finding of relatively high levels of multiple paternity within Nelson's Sparrow nests was unexpected, based on previous observations of mate guarding behavior (Greenlaw 1993, Shriver et al. 2010). It is important to note, however, that this study was conducted in the southern portion of the hybrid zone, where Nelson's Sparrows (males and females) are outnumbered by Saltmarsh Sparrows by ~5:1 (Walsh et al. 2016). As such, mating behaviors of Nelson's Sparrows in sympatry may not be reflective of reproductive strategies in allopatry, or even in sympatric populations near the center of the hybrid zone where the densities of the 2 species are more equal. Similar to our findings for Saltmarsh Sparrows, we found no correlation between male size or plumage and reproductive success in Nelson's Sparrows. However, we did find that the number of offspring sired was significantly correlated with individual genetic heterozygosity in Nelson's Sparrow and hybrid males. Heterozygosity is thought to be beneficial to individuals across a range of species (Avice 1994, Brown 1997, Winternitz et al. 2015, Walker et al. 2017), with links to disease resistance (Acevedo-Whitehouse et al. 2003, Reid et al. 2003, Osborne et al. 2015), condition and attractiveness in males (Brown 1997), increased reproductive success (Foerster et al. 2003, Weeks et al. 2017), survival (Coltman et al. 1998, Velando et al. 2015), and larger territory size (Seddon et al. 2004). Genetic heterozygosity could also be linked to increased vigor (Mays and Hill 2004), which would allow the more heterozygous Nelson's Sparrow males to perform more frequent flight displays and to exhibit energetically expensive activities, such as mate guarding. In this case, flight displays may provide a reliable signal of male vigor and quality to Nelson's Sparrow females and may be an important component shaping female mating strategies in this species. Small sample sizes warrant further investigation into the relationship between heterozygosity, reproductive success, and mate choice in Nelson's Sparrows, perhaps with comparisons between sympatric and allopatric populations.

In addition to variation in male reproductive success in both species, we found interspecific variation in reproductive success between pure and hybrid individuals. Only 9% of the sampled F1–F2 males in our study sired offspring (compared with 32% and 42% of the Nelson's and Saltmarsh sparrow males, respectively). Hybrids are often less fit than parental species, with the costs of hybridization expressed as reductions in fertility or survival (Lancaster et al. 2007). While we are unable to definitively

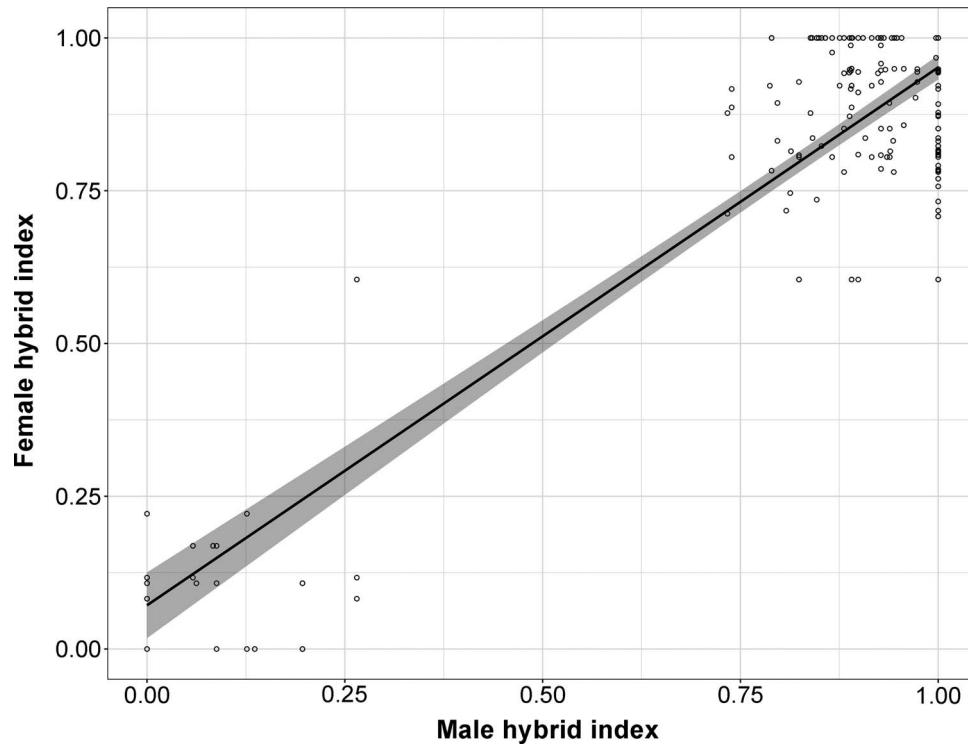


FIGURE 4. Correlation of the genetic hybrid indices (used to differentiate pure species and hybrids) for all mated pairs of Nelson's and Saltmarsh sparrows. Within breeding pairs, the female and male hybrid indices were highly correlated (line shows mean correlation and gray shaded area shows the 95% confidence interval), indicative of assortative mating.

identify the characteristics of F1–F2 males that resulted in lower reproductive success, we posit that differences in mating behaviors between the 2 parental taxa (scramble competition vs. flight displays and mate guarding) may place intermediate males at a competitive disadvantage. Reduced reproductive success in hybrid males translates into lower reproductive output compared with parental species, leading to decreased gene flow between hybridizing species over time (Neubauer et al. 2014). These results, coupled with previous work documenting reduced survival in F1–F2 females (Walsh et al. 2016), suggest fitness costs associated with hybridization. This may explain the strong patterns of assortative mating observed in this study. Although F1–F2 individuals have previously been identified in our study populations (Walsh et al. 2015, 2016), and we identified F1–F2 adults among those sampled for this study, within the pairs monitored here, we observed no occurrences of heterospecific mating. This suggests that premating barriers, such as divergent mating strategies, behavioral differences between male Saltmarsh and Nelson's sparrows, phenotypic differences (such as plumage signals), or possibly auditory cues for females, may play an important role in preventing hybridization between these species. The only nonassortative mating that we documented with our dataset was by an F1 female, which may provide further support for divergent mating behav-

iors between species being an important mechanism driving assortative mating. More data on the mating preferences of F1–F2 individuals is warranted, as it is possible that hybrid individuals choose mates less discriminately than pure individuals.

For within-species pairings, mate dissimilarity was not correlated with overall reproductive success. However, 74% of pairs were different at $\geq 50\%$ of their alleles, and, while this topic requires further investigation, our results suggest that females may choose genetically dissimilar mates, within the boundaries of their species. Female preference for dissimilar males has been documented in several systems (Potts et al. 1991, Landry et al. 2001, Freeman-Gallant et al. 2003, Marshall et al. 2003). Choosing dissimilar mates can increase the heterozygosity of offspring, avoiding deleterious effects of inbreeding depression (Keller and Waller 2002) or enhancing the immune function of offspring (Reid et al. 2003). While previous genetic studies of Saltmarsh Sparrows have not documented any indication of significant inbreeding within populations (Walsh et al. 2012), banding data from our sampled sites suggest moderate return rates to the same marshes for both adults and juveniles (A. Kovach personal observation) and the potential for slightly elevated relatedness on small marshes (Walsh et al. 2012). Thus, choosing dissimilar mates may be an

important inbreeding avoidance mechanism in this system.

Our findings offer new insight into the mating patterns and reproductive success of pure and hybrid Saltmarsh and Nelson's sparrows. We identified high levels of promiscuity and considerable variation in male reproductive success in both species, which was unexpected for Nelson's Sparrows. We observed differences in the amount of reproductive skew between the 2 species, which may have been driven by spatial and temporal patterns of female receptivity, interspecific differences in male mate guarding vs. scramble competition behaviors, and female influence on mating and fertilization success. We also documented large differences in reproductive success between pure and hybrid males and strong patterns of assortative mating in Saltmarsh and Nelson's sparrows. The low observed reproductive success of F1–F2 hybrid males suggests fitness consequences of hybridization in this system and is consistent with previous work documenting decreased survival of F1–F2 hybrid females (Walsh et al. 2016). This, coupled with a low rate of heterospecific mating, suggests that variation in reproductive success and assortative mating are important mechanisms for maintaining pure species boundaries in the face of ongoing gene flow in hybridizing populations.

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Ethics statement: Sampling was conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of New Hampshire (IACUC approval numbers 100605, 130604).

Author contributions: This study was part of the dissertation research of J.W. under the supervision of A.I.K. J.W. and A.I.K. conceived and designed the study. J.W. conducted laboratory work. L.M.M. and J.W. analyzed the data and wrote the manuscript with input from A.I.K. All authors read and approved the final manuscript.

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