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Courtship Behavior and Mechanisms of Isolation Across a Hummingbird Hybrid Zone

Author

Myers, Brian Matthew

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Courtship Behavior and Mechanisms of Isolation Across a Hummingbird Hybrid Zone

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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by

Brian M. Myers

September 2020

Dissertation Committee:
    Dr. Kevin Burns, Co-Chairperson
    Dr. Christopher Clark, Co-Chairperson
    Dr. Rulon Clark
    Dr. Alan Brelsford
    Der. Erin Rankin

The Dissertation of Brian M. Myers is approved:

Committee Co-Chairperson

Committee Co-Chairperson

University of California, Riverside
San Diego State University

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DEDICATION

To paupers and kings, princes and thieves,
singers of songs, righters of wrongs, be what you believe.
Saddle your horse and shoulder your load,
burst at the seams, be what you dream, and take to the road.

ABSTRACT OF THE DISSERTATION

Courtship Behavior and Mechanisms of Isolation Across a Hummingbird Hybrid Zone

by

Brian M. Myers

Doctor of Philosophy, Graduate Program in Evolutionary Biology
University of California, Riverside and San Diego State University, September 2020
Dr. Kevin Burns and Dr. Christopher Clark, Co-Chairpersons

In my dissertation, I focused on the discovery and evolutionary maintenance of a hybrid zone between migratory Allen's (*Selasphorus sasin*) and Rufous (*Selasphorus rufus*) Hummingbird centered in southern Oregon. Via incorporation of morphological, genomic, and behavioral data of 304 birds, my dissertation examines how natural selection, sexual selection, and biogeographic processes have affected the diversification and interactions of Allen's and Rufous Hummingbird.

In Chapter 1, I used Linear Discriminant Function Analysis (LDA), cline analysis, and a hybrid index to reveal the geographic extent of the Allen's and Rufous Hummingbird hybrid zone, classify individuals as parents or hybrids, and investigate how selection acts on a suite of behavioral and morphological characters. The contact zone extends north into the breeding range of Rufous, and south into the range of Allen's. Using geographic cline analysis, I found a role of sexual selection in shaping species barriers.

In Chapter 2, I performed behavioral sequence analysis on Allen's, Rufous, and their hybrids to assign discrete phenotypes based on the sequences of their courtship

displays. For the first time in ethology, I implemented k-mer analysis to identify how many behavioral phenotypes are present across my sampled individuals. I also described novel and transgressive courtship behaviors across the contact zone and found that hybrids are more variable than parental species. At least within Allen's and Rufous Hummingbird, the modular nature of displays shows that courtship might play a role in diversification between these two species, and possibly within *Selasphorus*.

In Chapter 3, using whole-genome data, I found evidence of gene flow from Rufous Hummingbird across the range of migratory Allen's, evidence of intergradation between non-migratory and migratory Allen's, and high gene flow across the entire non-migratory Allen's, migratory Allen's, and Rufous Hummingbird species complex. Further, I found evidence of selection on the Z chromosome in migratory Allen's and Rufous Hummingbird and estimated divergence dates of Allen's and Rufous to be further back in time than previously reported.

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INTRODUCTION OF THE DISSERTATION

Sexually selected behavioral traits are important triggers of speciation. In my dissertation, I investigated the role of courtship behavior in speciation in Allen's (*Selasphorus sasin*) and Rufous (*S. rufus*) Hummingbirds by studying their behavior, morphology, and genomes across a naturally occurring hybrid zone. Hybrid zones provide an opportunity to learn about evolutionary processes, such as effects of gene flow, how different mechanisms of speciation occur, and how alleles introgress from one species to another. Although hybrid zones have received considerable attention in biology, fewer studies have investigated how behavioral characters vary across these regions of interaction. My dissertation provides new insight into hybrid zones and the effects of hybridization on migratory Allen's Hummingbird (*Selasphorus sasin sasin*).

Allen's and Rufous Hummingbirds are difficult to differentiate because of their similar morphology, differing subtly in tail shape and back color. Tail shape is associated with sounds produced during courtship. The inclusion of a courtship display unique to Allen's Hummingbird, the pendulum display, makes field identification based on courtship behavior easier. During this display, the bird slowly flies back and forth in a shallow U-shape, producing a "chirrup" sound with the wings. Additionally, both species have courtship displays involving a male hummingbird performing an ascent and subsequent J-shaped dive, during which the male produces a species-specific sound with his tail feathers.

The displays of Allen's and Rufous are broken into distinct elements, and some dive elements are analogous to those in the pendulum display. The hybrid zone I investigated in Chapter 1, centered in northern California and southern Oregon, encompasses hybrids that exhibit displays incorporating distinct elements from each parental species. Given the behavioral differences in Allen's and Rufous, I also investigated the role of sexual selection in maintaining species barriers.

In Chapter 2, I explored the complex nature of displays between Allen's and Rufous Hummingbird and their hybrids. I broke down their courtship displays into discrete modular elements. I also implemented k-mer analysis, followed by hierarchical clustering analysis, to categorize behavioral sequences into discrete phenotypes for 304 males across parental species ranges. Display phenotypes were comprised of behavioral elements found within each species. Further, my analyses revealed several novel and transgressive courtship displays within hybrids.

In Chapter 3, I investigated how gene flow affects the genomic composition of of Allen's Hummingbird throughout its range and explored the evolutionary history of Allen's and Rufous Hummingbird. Allen's Hummingbird consists of two subspecies, one migratory (*S. s. sasin*) and one non-migratory (*S. s. sedentarius*). Non-migratory Allen's was previously endemic to the Channel Islands but is now widespread throughout southern California. Migratory Allen's is 15% smaller than non-migratory Allen's and is found from Oregon south to Santa Barbara County, CA. The two subspecies differ in their migratory behavior: migratory Allen's migrates annually, breeds along the coast from approximately Santa Barbara County, CA to southern OR, and winters in central Mexico. Non-migratory Allen's remains in southern California (including the Channel Islands off of the southern California coast) as a year-round resident. The two subspecies appear to overlap in Santa Barbara County, and may form a zone of intergradation. No previous study has investigated the genetic distinctiveness of these two subspecies or the extent to which gene flow is occurring between them.

In Chapter 3, I also investigated the effect of gene flow from the Allen's × Rufous hybrid zone on parental populations of migratory Allen's Hummingbird. Because Allen's and Rufous Hummingbird are remarkably similar in morphology, behavior, and in their genomes, I compared gene flow of the entire genome to the Z chromosome, isolated from the rest of the genome, and searched for fixed differences across the genomes of each species. I also evaluated the evolutionary history of Allen's and Rufous Hummingbird through the implementation of three scenarios: 1) the currently-described relationship, a split between Allen's and Rufous, followed by a split between migratory and non-migratory Allen's, 2) since migratory Allen's is reported to share ancestry with both non-migratory Allen's and Rufous Hummingbird, I tested for a split between non-migratory Allen's and Rufous, followed by a hybridization event between them to form migratory Allen's, a hybrid species, and 3) given the hybrid interactions of migratory Allen's and Rufous, a split between non-migratory Allen's and an ancestor to Rufous and migratory Allen's, followed by a split between Rufous and migratory Allen's Hummingbird.

My dissertation adds to the growing body of literature investigating evolutionary processes by studying genomic markers in conjunction with phenotypic data across a hybrid zone. Importantly, my study of the Rufous × Allen's hybrid zone investigates the modes of selection that separate Allen's and Rufous Hummingbird via the investigation of morphological, behavioral, and genomic data. Through the examination of how hybridization, natural selection, sexual selection, and biogeographic processes have affected the diversification and interactions of Allen’s and Rufous Hummingbird, my dissertation provides insight into fundamental evolutionary phenomena, and highlights the significant role hybridization has on species outside of areas of contact.

CHAPTER 1

Behavioral and morphological evidence of an Allen's × Rufous Hummingbird (*Selasphorus sasin* × *S. rufus*) hybrid zone in southern Oregon and northern California

INTRODUCTION

Hummingbirds (Trochilidae) have some of the highest rates of hybridization in the wild (Grant and Grant 1992, McCarthy 2006, Ottenburghs et al. 2015). Most instances of hybridization appear to be sporadic, as is the case for Costa's (*Calypte costae*) × Broad-tailed (*Selasphorus platycercus*), Rufous × Calliope (*S. calliope*), and Costa's × Black-chinned (*Archilocus alexandri*) hybrids (Banks and Johnson 1961, Lynch and Ames 1970, Graves and Newfield 1996, Graves 2006). The only species pair north of Mexico with a described hybrid zone is Black-chinned × Ruby-throated Hummingbird (*A. colubris*), which hybridize in Oklahoma (Judd et al. 2011). In the Caribbean, two Streamertail species (*Trochilus polytmus* × *T. scitulus*) form a hybrid zone in eastern Jamaica (Graves 2015, Judy 2018). In 2012, Arch McCallum sent CJC a recording of a possible hybrid Allen's × Rufous Hummingbird dive sound that had been recorded on the coast in southern Oregon. Subsequent field work in this area by CJC revealed multiple birds that seemed to have intermediate phenotypes. Here, I describe the phenotypic data that indicate these two species form a hybrid zone in Southern Oregon and Northern California.

Speculation of hybridization between Allen’s (*Selasphorus sasin*) and Rufous (*S. rufus*) Hummingbird has circulated for years. Allen’s and Rufous Hummingbird are phenotypically very similar, and differ mainly in sexually-selected characters. Identification of even pure individuals of each species has been problematic and uncertain, particularly for hatch-year birds and females (Stiles 1973). Female Allen’s and Rufous Hummingbird both have a fully green back and are virtually identical; female Rufous have a very slightly emarginated r2 tip that female Allen’s apparently lack (Stiles 1973, Pyle 1997). The primary phenotypic differences between Allen’s and Rufous Hummingbird males are reflected by sexual characters: Rufous Hummingbirds usually have a rufous colored back, while Allen's are green. Similar to the r2 of females, the male's tail-feathers have subtle differences in shape, the most prominent of which is a small notch near the tip of rectrix 2 of Rufous that is absent in r2 of Allen's; the r2 differences in males are related to courtship. Multiple authors report that a small number of adult male *Selasphorus* seemed to have tail-feathers of intermediate shapes (Newfield 1983, McKenzie and Robbins 1999, Colwell 2005). One hypothesis was that these individuals were Allen's × Rufous hybrids. These birds were sampled on migration, outside the breeding range of either species. Thus, whether they simply represented previously undescribed variation in Rufous Hummingbird morphology, or whether they were in fact hybrids, either the product of an undescribed hybrid zone, or an instance of 'one-off' (sporadic) hybridization is not clear.

In addition to morphological characters, courtship characters also differentiate male Allen’s and Rufous Hummingbird, making identification of putative hybrids an easier task. Male *Selasphorus,* along with other members of the bee hummingbird clade

(Mellisuginae), court females with acrobatic dives that include sounds made by the tail feathers. During their dives, Rufous make sound with r2 with a fundamental frequency from 0.7-0.8 kHz while Allen's Hummingbird produce sound with r3 with a fundamental frequency from 1.8-1.9 kHz (Clark et al. 2011, Clark 2014). Within Rufous Hummingbird, dozens of harmonics give the dive sound a buzzier quality than the pure tone of Allen's Hummingbird (Hurly et al. 2001, Clark 2014).

The full behavioral repertoire that a male performs for a female differs between the species (Figure 1.1). Allen's Hummingbird display sequences consist of multiple courtship behaviors. Males perform a dive display, where the bird ascends, then turns and descends in a J-shape, during which the male produces a relatively thin, high-pitched sound with r3 (Clark 2014). Dives typically end with a variable number of 'chirrups', which are short, loud pulses of wing trill produced during a behavior I term 'writhing', in which males visibly flip their tails up and down (Aldrich 1939). Allen's Hummingbird also performs a courtship behavior that Rufous Hummingbird apparently does not, the pendulum display, in which the bird flies back and forth in a shallow U-shape, 1-3 m above the female. During the pendulum display, the bird first descends and produces a loud wing trill, and after passing the female and ascending, performs writhing (Figure 1.1).

Rufous Hummingbird males also dive to females and are not reported to perform the pendulum display. Most authors report J-shaped dives, similar to Allen's Hummingbird (Figure 1.1, Hurly et al. 2001, Howell 2002). Johnsgard (1983) instead describes an O-shaped dive where the bird flies in a complete oval by ascending smoothly out of the dive, similar to the shape of Costa's Hummingbird dives (Clark and Mistick 2018). Johnsgard's source for this assertion is unclear (Howell 2002). Similar to Allen’s, Rufous Hummingbird dives also conclude with a bout of writhing with associated chirrup sounds produced by the wings. Here, I define a “bout” of display as the sequence of courtship behaviors a male performs towards his stimulus within a single courtship episode.

Males of both species include in their bouts of courtship an additional display: the shuttle display, where the male flies in a bouncy back-and-forth motion in proximity (<1 m) to the target female (or intruding male), produces sound with his wing feathers, and flares his iridescent gorget (Clark and Mitchell 2013, Clark et al. 2018). There are no reported differences between Allen’s and Rufous Hummingbird within this poorly described display. Investigation of the shuttle display within the context of an entire bout of display during courtship may be more informative in detecting differences in how Allen’s and Rufous Hummingbird and any putative hybrids.

Tail feather morphology, which produces the different dive sounds of Allen’s and Rufous Hummingbird during courtship, does so based on size and shape, and the tail feathers are likely a sexual signal involved in mate choice. If tail shape is under polygenic control, morphology of hybrids might vary continuously between Allen’s-like and Rufous-like. However, because different tail-feathers are the physical source of sound in each species, and their mode of vibration differs (Clark et al 2018), it is physically implausible for the dive sound of hybrids to vary continuously between the buzzy dive sound made by Rufous Hummingbird and the purer, more tonal sound produced by Allen’s Hummingbird. As producing sound with r2 appears to be ancestral (Clark 2014), Allen’s Hummingbird evolved to produce sound with r3 in which r3 vibrates at the (former) second harmonic of r2, meaning that the fundamental frequency of sound 'hopped' from the first to the second harmonic of r2 as the source transition from r2 to r3 (Clark 2014). This 'harmonic hopping' hypothesis makes two predictions about hybrids between Allen's and Rufous hummingbird. First, although tail morphology of hybrids might vary continuously in shape, the dive sound cannot; it is a discrete character. Thus, the possibilities for hybrids to produce sounds are that they could produce Rufous-like buzzy dive sounds (+r2, -r3), Allen's-like pure tone dive-sounds (-r2, +r3), as well as the possibility of multi-tonal sounds with both r2 and r3 (+r2, +r3), or plausibly, neither feather (-r2, -r3). Second, the harmonic hopping hypothesis predicts that the fundamental frequency of the dive sound of hybrids will not vary continuously between 0.7 kHz (Rufous) and 1.9 kHz (Allen's). Rather, it predicts that there will be a sizable gap in the distribution of sound frequency that corresponds to the fundamental frequency of the dive sound of Rufous 'hopping' to twice its value, e.g. from 0.7 kHz to 1.4 kHz, as the tail morphology crosses a morphological threshold that causes the sound source shift discretely from an r2 source to an r3 source.

Here, I explore a hybrid zone between Allen's and Rufous Hummingbird using behavioral and morphological data and investigate the role of sexual selection in courtship behavior within Allen's and Rufous Hummingbird. I also investigate the underlying behavioral elements that form courtship displays within each species.

MATERIALS AND METHODS

Life History

Allen's and Rufous Hummingbird breed within forest edges and disturbed areas along the Pacific coast (Figure 1.2, Calder 1993, Clark and Mitchell 2013). Allen's Hummingbird breeds in riparian habitats adjacent to scrub and forest edge habitat along the California coast (Figure 1.2, Jewett 1929, Calder 1993, Gilligan et al. 1994). Allen's Hummingbird has two subspecies: *Selasphorus sasin sasin* (migratory) and the slightly larger *S. s. sedentarius* (non-migratory), which has a breeding range restricted to southern California (Stiles 1972). In this chapter, all references to 'Allen's Hummingbird' refer to statements true of *S. s. sasin* only. Rufous Hummingbird breeds in Oregon and extends as far north as southeast Alaska along the coast, and inland to Idaho, western Montana, and Alberta (Calder 1993). This species breeds in open areas and riparian habitat, usually in general association with fir, spruce, and hemlock-dominated Pacific rain forests.

Sampling

I sampled along a north-south transect from southern Oregon to northern California, and from a smaller number of localities outside of the transect. A full list of localities is provided in Table S1.1. I sampled Allen's Hummingbird parental populations along the California coast in southern Humboldt, Mendocino, Monterey, and San Luis Obispo counties, which are 233 km, 354 km, 696 km, and 864 km away (respectively) from the central locality along the coastal transect in Curry County, OR. I sampled Rufous Hummingbird populations in Clatsop and Douglas counties in Oregon, which are 405 km north and 185 km east (respectively) from the central locality along the coastal transect in Curry County, OR. To complement samples collected in the field and improve power for Linear Discriminant Function Analysis (LDA), museum specimens were measured for female Allen's Hummingbird (N=29), and female Rufous Hummingbird (N=35). Museum specimens dated from March through May were used to minimize the probability of using non-breeding (i.e., migrating) birds in the dataset.

Female Allen's and Rufous Hummingbird were identified based on range (only those collected outside of the area of overlap between the two species, described above, and away from the range of non-migratory Allen's Hummingbird during the breeding season were measured) because of the difficulty in discriminating females based on morphology (Stiles 1973, Newfield 1983, Pyle 1997, Colwell 2005). Breeding males vigorously defended their territories, and typically guarded feeders placed on their territories from other birds, providing high confidence that the correct male was captured with a feeder-trap. I sampled territorial males March through May of 2014-2017, and when available, females. Males showed no signs of discriminating between heterospecific and conspecific females; further, due to the difficulty in diagnosing hybrid females in the field, I did not diagnose females captured in the field as Allen's Hummingbird, Rufous Hummingbird, or hybrid until the end of the field season.

A female was kept in a small mesh cage to elicit displays from territorial males. In rare instances in which wild-caught females were not available, I recorded males displaying to natural stimuli, such as wild females, other males, or (rarely) unknown stimuli. After a minimum of ten bouts of displays were recorded, target males were then captured. I considered an individual bout as concluded when a male ceased displaying for half of a second or longer. I recorded 183 territorial males using a Sennheiser MKH 70 shotgun microphone (Wedemark, Germany) and a Sound Devices 702 24-bit digital recorder (Reedsburg, Wisconsin, sampling rates: 44.1, 48.0, and 96.0 kHz).

Males and females were either collected and prepared as museum skins (N=127), or banded and released (N=130) after morphological data and tissue or dried blood (for future genetic research) were taken. At least one female per locality was either collected or banded and released (N=74). In total, 183 males with behavioral and morphological data, and 138 females with morphological data were included in all analyses. All specimens were deposited in either the San Diego State Museum of Biodiversity or the San Diego Natural History Museum.

Morphology

Linear measurements were collected with digital calipers to the nearest 0.01 mm. R2 measurements for males followed the four r2 measurements described by Colwell (2005): length of the inner web of r2, depth of the inner web of r2, length of the outer web of r2, and depth of the outer web of r2 (Figure S1.1). For males, back color was measured similarly to Aldrich (1956) and Colwell (2005), dorsally from the posterior margin of the nape to the upper tail coverts, and shoulder to shoulder from the upper back to the upper tail coverts (Figure 1.3E). Other morphological characters included width of the tail feathers, including r1, r2, r3, r4, and r5 (from the widest point of the feather), folded wing chord (from the wrist to the tip of the longest primary), tail length (measured from r1 base to tip), and exposed culmen length (Stiles 1973, Newfield 1983, Calder 1993, Hurly et al. 2001, Colwell 2005). For females, measurements were the same for males, except there are no back color measurements, and I only measured the length of the outer notch of r2, which is most prominent portion of the less distinct r2 in females.

Behavior

Courtship behaviors can be broken apart into their underlying elements, which I defined as discrete behavioral units that correspond to individual movements and are below the level of a fixed action pattern (i.e., elements are the 'building blocks' of displays). Analyses of Carola's Parotia (*Parotia carolae*, Scholes 2006, Scholes 2008) and *Habronattus* jumping spiders (Elias et al. 2012) provide examples of courtship display sequences made of behavioral elements that correspond to every movement contained within the display. Here, I described the courtship displays of Allen's and Rufous Hummingbirds by categorizing displays into discrete elements in order to identify display differences between parental species and hybrids.

Male Allen's and Rufous Hummingbird produce an 8.0-10.0 kHz wing trill in flight that is accentuated during courtship displays (Hunter and Picman 2005, Clark 2016). Behavioral characters presented here are produced by motions of the wings and tail, where there is a 1:1 match between motion and the ensuing sound (Clark 2009). Thus, the courtship behaviors of individual birds were straightforward to quantify from the ensuing sounds, where dives, shuttle, and pendulum displays, as well as wing trill, tail feather sound, and the presence and absence of chirruping was quantifiable (Figure 1.4, Table 1.1). Behavior was analyzed using spectrograms in Raven Pro 1.5 (Cornell Lab of Ornithology Bioacoustics Research Program 2014) using 1881, 2048, and 4096-sample FFT windows for audio recorded at 44.1, 48.0, and 96.0 kHz respectively. Whenever dive sound frequency varied over the course of the dive (i.e. as in Allen's), I analyzed the highest frequency of the sound.

The behavioral characters I analyzed included average fundamental frequency of the dive (calculated by taking the average fundamental frequency of each dive recorded for an individual), the average number of chirrups at the end of a dive, the maximum number of consecutive pendulums and the maximum number of consecutive dives a bird performed in a bout, the ratio of pendulums to dives performed by the individual, and the percentage of pendulums performed immediately following a dive. I defined a ratio of pendulums to dives as the total number of dives relative to the total number of pendulums summed across all bouts performed by one individual. I also analyzed courtship displays based on underlying behavioral elements that I describe. These elements formed the basis of every display in Allen’s and Rufous Hummingbird and were used to identify differences within the courtship display repertoire in each species.

Hybrid classification

LDA assesses which characters best differentiate two species and tests for differences among groups that are defined *a priori* (Whitmore 1983, Heaney and Timm 1985, James and McCulloch1990, Poulsen and French 1996). I used LDA to assign individuals to groups defined *a priori*, evaluate the extent of hybrid individuals across the transect, and assess how populations differ across the sampling transect (James and McCulloch 1990).

I implemented several definitions and characters to diagnose hybrids. Here, I defined a hybrid character as one that fell outside the 95% confidence interval (CI) of the variation present in the population of each parental species using the same method as Pyle (1997), where I estimated the 95% CI as ± twice the standard deviation (SD) from the mean for each character. An intermediate character was one that was in-between two parental phenotypes (for example, a dive sound of 1.1 kHz). I diagnosed hybrids using the phenotypic characters that are used in identification of each parental species, where intermediate individuals with characters representing a mosaic of each parental species' phenotypes (for example, an Allen's-like bird with a Rufous-like character) were used to identify putative hybrids, following Graves (1990). I calculated the 95% CI for morphological measurements and behavioral data from samples defined *a priori* as Allen's and Rufous Hummingbird to have a diagnostic reference for pure individuals, and compared measurements to other work (Table 1.2, S1.2, S1.3). I defined a diagnostic character as one that fell in the 95% CI of one species and out of the 95% CI of the other, beyond the interval of overlap, as calculated by the 95% CI for each character. I used the 95% CI to assign hybrid and parental characters to individuals. Characters falling outside of the 95% CI for a given species were classified as hybrid characters. A character outside of the 95% CI might not actually be hybrid, since, assuming a normal distribution, 5% of parental samples will fall outside of the 95% CI. However, if only one such character was scored for an individual out of all of the characters studied here, that individual still resided close to a pure parental based on its overall hybrid index score or LDA classification. True hybrids fell outside the 95% CI for multiple traits. Values reported in the Results are means ± SD. To classify hybrid versus parental individuals, I performed an LDA. Before performing this analysis, I used a

Cluster Analysis of Observations to determine the best number of groups to use for males and females, to assign individuals to a group *a priori*. Next, these individuals were entered into the LDA for evaluation of their placement (Minitab 17 Statistical Software 2010). Cluster analysis uses complete linkage and Euclidean distance to calculate similarity and distance measures to group user data into clusters. For cluster analysis, I standardized the data to have a mean of zero. For clustering, I used an agglomerative algorithm, where each observation starts as its own cluster, and observations are merged together into clusters with each other, based on distance levels, until only K=1 group remains. Similarity and distance levels showed that K=3 groups best fit the data. Distances between clusters were calculated using complete linkage, where the distance between clusters equals the distance of the data points in each cluster that are furthest away from each other. Based on similarity and distance levels, K=3 groups also showed the strongest support for female classification. Thus, for LDA, I used K=3 groups for male clusters, and K=3 groups for female clusters using complete linkage and Euclidean distance. I assigned each individual to a group 1-3 for males, and 1-3 for females. Using three groups, I designated each male into one of three categories: Rufous Hummingbird, hybrid, or Allen's Hummingbird. For females, morphological data for nine characters were incorporated in all analyses (Table 1.3). The three clusters designate each female into one of three categories: Rufous Hummingbird, hybrid, and Allen's Hummingbird.

I used all characters in an LDA to find the variables that maximized the separation between hybrid and pure individuals, and to predict membership of individuals into a hybrid or parental group to quantify populations across the hybrid zone (Rao 1948, James and McCulloch 1990, Poulsen and French 1996). I used LDA to evaluate classifications of individuals within each population estimated *a priori*, based on the presence of intermediate characters that partitioned individual samples into different clusters, and yielded which variables maximized the differences between each group. If any individual had at least one hybrid character, that individual was scored as a hybrid *a priori* before evaluation by the LDA. Depending on the amount of overlap in a focal character, some individuals were scored by the LDA as hybrid based on one hybrid character (if there was little to no overlap), while others required two or more characters to be scored as hybrid. For example, a Rufous-like bird performing a pendulum display was scored as hybrid, as I never observed a pendulum display in parental Rufous Hummingbird (N=34). For females, individuals with one or more hybrid characters were also scored as hybrids *a priori*, and then evaluated by the LDA.

To search for a relationship between tail feather morphology and the fundamental frequency of the dive sound made by each male, I performed a geometric morphometric analysis on 29 landmarks for each tail feather (r1 through r5) for each individual (Figure 1.5A). I digitized landmarks for each tail feather and performed a Procrustes superimposition of all tail feathers for each individual and analyzed the entire tail and its role in dive sound production. With this dataset, I implemented a Principal Components Analysis in MorphoJ v1.06d (Klingenberg 2011). PC1 represented a continuum of Allen's-like to Rufous-like tail shape. I regressed PC1 against the resultant fundamental frequency of the dive sound made by each individual during courtship; fundamental frequency of the dive was calculated and represented on this plot.

Cline analysis and phenotypic hybrid index

To map a cumulative character cline, I designed a hybrid index incorporating all 20 characters for males, which represents the overall proportion of parental characters contained within an individual, on a scale from 0-1, with the most intermediate individuals scoring in the middle (Anderson 1949, Hatheway 1962, Hubbard 1969, Anderson and Daugherty 1974, Mettler and Spellman 2009, Abbott and Brennan 2014). I incorporated the hybrid index and all male characters individually into a geographic cline analysis to compare patterns of phenotypic characters among males across the coastal transect using the statistical package HZAR (Derryberry et al. 2014) in R v.3.4.3 (R Core Team 2018), which uses the Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm to estimate cline parameters. I used the Akaike information criterion (AIC, Akaike 1974) to test different cline models: one with no tails (Model 1), symmetrical tails on both sides of the cline (Model 2), and asymmetrical tails on both sides of the cline (Model 3). In comparison to sigmoid clines, tailed cline models allow for modeling of "stepped" patterns of abrupt change near the center of a cline and gradual shifts away from the cline center (Szymura and Barton 1986). Independent models of each tail of stepped clines allow for asymmetries between each parental species. In addition to cline center and cline width, tailed clines allow for investigation of tau and delta, parameters that estimate the shape of decay for each cline. Tau is the slope from the cline center to each tail, while delta indicates the distance from the cline center to each tail. The best-fit model for the majority of the clines was Model 3. Thus, in order to enable comparison across all character clines, I fit all characters to clines for Model 3. HZAR calculated cline center and width for each character using the MCMC. I ran 100,000 iterations for each cline parameter with a burn-in of 10,000 generations. I used two-unit likelihood support limits as a measure of confidence in the parameter estimates (Barton and Gale 1993) to search for any significant differences in parameter estimates between clines for different characters.

RESULTS

Courtship displays

All displays across all populations were split into three types: dives, shuttles, and pendulums (Table 1.1). Within these displays, there were multiple types of pendulum displays: regular pendulums and a previously undescribed display which I call "half pendulums". I found that dive displays were split into two types typified by each parental species: "Allen's dive" and "Rufous dive", as described in Table 1.1, Figure 1.4A, and Figure 1.4B; see also below. To understand how these displays were behaviorally assembled into display bout sequences, the difference between a regular pendulum and a “half pendulum”, and the two kinds of dives, I subdivided dives and pendulum displays into individual elements. These elements included 'short descent', 'short ascent', 'writhing', 'long ascent', and 'long descent'; definitions of each are provided in Table 1.1 below. I also described detailed elements of courtship displays in male Allen’s and Rufous Hummingbird that I sampled (Figure 1.4, 6, Table 1.1).

All of the following display elements were common to both male Allen's Hummingbird and Rufous Hummingbird. For example, the short descent, where a bird descended about 2-3 m in a shallow half-U shape, occurred in the beginning of the dive in both species, but also in the beginning of a pendulum display in Allen's Hummingbird (Figure 1.4). During a short ascent, the male ascended 2-3 m in a shallow half-U shape; this element occurred during the dive ascent, after the dive descent, and during a pendulum in Allen’s Hummingbird, after the dive descent in Rufous Hummingbird, and in the half pendulum in both species (Figure 1.4). Writhing consisted of individual ‘chirrup’ sounds made by the wings (see, Clark 2016). Writhing occurred during the dive, half pendulum, and pendulum displays, and the number of chirrups differed in each display in Allen’s Hummingbird and Rufous Hummingbird (see below, Figure 1.4). During a long ascent, a bird ascended about 20 m for a dive; long ascents only occurred during dive displays in both species. A long descent followed a long ascent during the dive in both species and entailed a male tracing (in reverse) a similar path as the ascent, where he spread his tail feathers and produced a dive sound (Figure 1.4A, 4B). Finally, shuttle segments were individual left-to-right or right-to-left motions; multiple shuttle segments formed shuttle displays (Figure 1.4C). All display elements are further described in Table 1.1 and Figure 1.4.

The difference between the two species is that behavioral elements were assembled in a different order to form species-diagnostic displays (Figure 1.6, Table 1.1). For example, the elements of the pendulum display, which were found in Allen's and absent in Rufous, were not unique *per se* to the pendulum display or Allen's Hummingbird. Rather, the pendulum display assembled the elements in an *order* not observed in Rufous Hummingbird (short descent, short ascent with writhing, Figure 1.4).

Dive displays

The dives performed by Allen's Hummingbird (hereafter, “Allen’s dive”) were (usually) comprised of the following display elements (see also Figure 1.4): (a) short descent, (b) short ascent, (c) writhing, (d) long ascent, (e) long descent, where the male traced back a similar path as the ascent, spread his tail feathers, and made a dive sound, then another short ascent (b) and ended with (c) another bout of writhing. Three elements in the Allen’s dive appeared kinematically similar to the pendulum display: an (a) initial short descent and (b) a short ascent followed by (c) a bout of writhing (Figure 1.4A, 4E, Table 1.1).

The dives performed by Rufous Hummingbird (hereafter, "Rufous dive”) were kinematically similar to that of Allen’s Hummingbird, with one key difference: the bird performed (a) an initial short descent followed by (d) a long ascent, i.e., skipping the short ascent following by writhing (usually) observed in Allen’s Hummingbird; the rest of the dive was comprised of (e) a long descent, then another short ascent (b), and a bout of writhing (c, Figure 1.4B). Individuals of both species reached approximately 20 m in height during a dive display (Figure 1.4A, 4B, Table 1.1). Kinematically, the two dives differed only in the presence and absence of writhing during the ascent for a dive (Allen’s and Rufous dives, respectively), and in the bouts of writhing at the end of the dive, which differed in chirrup number between Allen’s Hummingbird and Rufous Hummingbird (see below).

Allen’s and Rufous Hummingbird also differed in quantitative characters. The bout of writhing upon conclusion of the dive for Rufous Hummingbird averaged 7.9 ± 1.1

individual chirrup sounds made by the wings (N=34, Figure 1.4B, Table 1.1). Before writhing after the dive was performed, Rufous Hummingbird made a sound with the tail feathers towards the end of the descent of the dive, averaging 0.8 ± 0.0 kHz (N=34). Allen's Hummingbird also made sound with the tail feathers towards the end of the descent of the dive, averaging a fundamental frequency of 1.8 ± 0.1 kHz (N=27). The bout of writhing at the end of the dive consisted of an average of 4.6 ± 1.4 individual chirrups (N=27, Figure 1.4A, Table 1.1). Allen's Hummingbird performed writhing on the ascent for the dive 90.5% of the time (Table 1.1, 1.2). Although a few male Allen's Hummingbird occasionally performed individual dives without writhing on the ascent, I sampled at least ten bouts of display per male, and every male Allen's Hummingbird observed in this study eventually performed at least one dive with writhing on the ascent.

Shuttle display

A shuttle display was comprised of individual "shuttle segments", which occurred as individual right-to-left or left-to-right motions, with sound made by the wings (Figure 1.4C). I did not detect any differences within Allen's Hummingbird, Rufous Hummingbird, or any hybrid populations in how males performed this display. Thus, I did not focus any of my detailed analyses on this display.

Pendulum display

In the pendulum display, a male performed a short descent (element a), followed by a short ascent (2 m high, element b), and a bout of writhing (element c) to a target stimulus (Figure 1.4E, Table 1.1, 1.2). On average, Allen's Hummingbird performed 10.2 ± 2.8 pendulums in sequence before ascending for a single dive (N=27), although Allen's

Hummingbird was observed to dive after as few as two consecutive pendulums. Allen's Hummingbird usually performed series of pendulums followed by a single dive, although some Allen's Hummingbird were observed to perform a maximum of two consecutive dives in a bout (1.1 ± 0.3, N=27). Rufous Hummingbird never performed a pendulum display and performed as many as 10 dives in a row (3.6 ± 1.1, N=34).

A previously undescribed display, the 'half pendulum' display, was present in both Allen's and Rufous Hummingbird. In both species, when this behavior occurred it always followed a shuttle display (Figure 1.4D, Table 1.1). Within the half pendulum display, the individual flew upward, consistent with the motion of an upward-moving pendulum and ended with a bout of writhing. The difference between this display and a regular pendulum display was that the male began the display next to the female, and thus, did not do the short descent that comprised the first half of a regular pendulum display (i.e., the behavioral element sequence of the half pendulum was b, c). At the end of a half pendulum display, bouts of writhing from individual Rufous Hummingbird averaged 12.3 ± 2.6 individual chirrups (N=34, Figure 1.4D, Table 1.1, 1.2) and Allen's Hummingbird averaged 8.0 ± 0.5 individual chirrup sounds (N=27, Figure 1.4D, Table 1.1, 1.2).

Back Color

Out of 27 Allen's Hummingbird males, 23 had a 90-100% green back. Of 34 Rufous Hummingbird males analyzed here, only one had a 50% green back, while most (N=20) had a 10-20% green back (Figure 1.3A, Table 1.2, S1.1).

Evidence of hybridization

Of the 183 breeding males sampled from the hybrid zone, most behavioral and morphological characters were clinal (Table 1.2). There was no sharp boundary that demarcated the limit between two sympatric species. Instead, birds showed continuous variation in several characters across the contact zone. Putatively highly admixed individuals expressed a suite of characters representative of each parental species (Figure 1.2).

LDA revealed a gradual north-south shift from a Rufous-like to an Allen's-like phenotype (Figure 1.2). Across the hybrid zone, there was a shift in tail feather morphology and the resultant fundamental frequency of the dive sound (Figure 1.3, 1.4A-D) with some putative hybrids producing intermediate dive sounds (Figure 1.3). Sequences of courtship behavior also differed along the transect, with a shift from a Rufous-like to Allen's-like behavioral phenotype (Figure 1.7).

During courtship displays, sounds produced by the tail feathers of Allen's Hummingbird, Rufous Hummingbird, and putative hybrids varied based on morphology. PC1 for tail feather shape explained 68% of the variance. All groups were significantly different from each other ($P < 0.05$), with some overlap in PC1 between Allen's Hummingbird and some putative hybrid individuals (Figure 1.5B). PC1 was uncorrelated with fundamental frequency for Rufous Hummingbird, then fundamental frequency showed a sudden, discontinuous break between 0.8-1.1 kHz, as predicted by the 'harmonic hopping' hypothesis. Above this break, PC1 was strongly correlated with hybrid fundamental frequency, and uncorrelated with Allen's Hummingbird fundamental frequency. Acoustic quality of the dive-sounds on either side of this break was striking: dive-sounds of hybrids usually either sounded "Rufous-like" or "Allen's-like". Rufous-like dive-sounds were buzzy as the result of dozens of acoustic harmonics, whereas the dive-sounds that were intermediate in fundamental frequency resembled Allen's Hummingbird in that they were higher-pitched (though not as high-pitched as Allen's), and tonal, without the buzzy quality of Rufous Hummingbird. I did not detect any birds that made separate (multitonal) sounds with both r2 and r3, nor any birds that failed to produce any dive-sound at all. Qualitatively, some hybrids produced dive-sounds that seemed much fainter than the parental species. I did not attempt to quantify loudness, since this acoustic parameter is difficult to measure in the field.

Individuals sampled within the northern and southern boundaries of the hybrid zone closely resembled the parental species to which they were geographically closest. The northernmost transect locality, in Lane County, OR, was almost fully phenotypically Rufous Hummingbird. The southernmost hybrid population, in Humboldt County, CA, was relatively Allen’s-like, although some individuals performed intermediate dive sounds as a result of mixed tail feather morphology. The LDA prediction probability for the *a priori* grouping, expressing the level of confidence in group membership for males, was 94% for Rufous Hummingbird, 92% for Allen’s Hummingbird, 90% for hybrid, (i.e., there was 94% confidence that individuals that were classified *a priori* as Rufous Hummingbird were correctly categorized), reflecting effective classifications of individuals using all phenotypic characters.

The characters with the highest LDA scores, indicating the characters that best diagnosed the three classes of male individuals, were the inner and outer depths of r2, tail feather width (r1-r5), folded wing chord, exposed culmen, and post-dive chirrups (Table 1.2, S1.4, S1.5).

Similar morphological characters best-separated females as well (Table S1.4, S1.6). The characters with the highest LDA scores, indicating the characters that best diagnosed across the three classes of female individuals, were folded wing chord, tail length, exposed culmen, and tail feather width. For 138 females (including museum specimens and females captured in the field), LDA diagnosed hybrid individuals spanning from Lane County, OR, as far south as Humboldt County, CA (Table S1.6). Most females identified as hybrid were found in Coos and Curry County, OR. LDA prediction probabilities for each grouping, expressing the level of confidence in each classification for females, were as follows: 87% for Rufous Hummingbird, 76% for hybrid, and 81% for Allen's Hummingbird (i.e., 87% of individuals that were classified *a priori* as Rufous Hummingbird were correctly categorized), showing that while there was still success in classification of individuals, fewer available characters (some of which, including tail length, exposed culmen, and folded wing chord, overlap to some extent based on the 95% CI and prior work) made diagnosis of female individuals more troublesome than for males.

Cline analysis

Cline parameters varied across the character suite (Table 1.4). Most clines centered between Port Orford, OR (Curry County), and Bandon, OR (Coos County,

Figure 1.8, S1.2, S1.3). For eight of the remaining 15 characters, the position of the cline centers were within 20 km of Bandon, OR (km 950 on the transect, relative to km 0-1,127, which spanned the length of the transect). The average cline center for all courtship behaviors was 957.2 km, while the average cline center for all morphological characters was 950.3 km. HZAR indicated that cline widths ranged from 4.5 (post-shuttle chirrups) to 152.7 km (folded wing chord, Figure 1.8E, S1.3E).

Based on the interval bound by two log-likelihood values, several characters were different in cline center and width, and behavioral courtship characters generally had significantly different cline widths than morphological characters (Table 1.4). Cline widths for all courtship behaviors were under 100 km wide, with the ratio of pendulums to dives, post-shuttle chirrups, post-dive chirrups, and occurrence of pendulums after dives under 28 km wide. Four morphological characters were over 100 km wide, while five were under 100 km wide. The average cline width for all courtship behaviors was 38.4 km, while the average cline width for all morphological characters was 89.4 km.

Tau and delta differed across characters as well (Table 1.4). Generally, decay was lower on the left tail of the cline center (towards Allen's range) than on the right side of the cline center (towards Rufous Hummingbird range). Generally, decay was lower for courtship behaviors than for morphological characters across both sides of the cline center.

DISCUSSION

The data indicated that Allen's and Rufous Hummingbird form a previously undescribed hybrid zone in coastal southern Oregon and northern California, spanning 310 km from Lane County, OR in the north into Humboldt County, CA, in northern California to the south (Figure 1.2). The center of the coastal transect is in-between Bandon (Coos County) and Port Orford (Curry County), where cline analysis suggests the most admixed, diverse phenotypes occur (Brumfield et al. 2001, Brelsford and Irwin 2009). Idiosyncratic sampling of populations off of the coastal transect shows putative inland hybrid populations at least as far inland as 95 km from the coast in Seiad Valley, CA, implying that the hybrid zone also has an east-west component along the Klamath River, for which I did not have samples to include in the cline analyses presented here (Siskiyou County, Figure 1.2). Although geographic clines for phenotypic characters are often concordant in spatial position and width, there are documented exceptions to this (Baldassarre et al. 2014, Semenov et al. 2017). The full extent of the hybrid zone reported here will be further documented in the future with genetic data.

Morphology

Some characters, such as back color, present difficulties in identification due to overlap between species. Although male Rufous Hummingbirds typically have an all-rufous back, a small number of individuals that otherwise key out as Rufous have a partially to completely green back: the so-called "green-backed Rufous" (Patterson 1988, Jones 1992, Williamson 2002). Based on the 95% CI, most Rufous Hummingbird I sampled had 10-20% green on the back, while most Allen’s Hummingbird had 90-100%

green on the back (Figure 1.3A, 3D). One hypothesis for these green-backed Rufous was that they are actually of hybrid origin. However, it appears likely that this hypothesis can be rejected: observations that up to 10% of adult male Rufous Hummingbirds in Alaska have entirely green backs (G. Baluss, pers comm; CJC unpubl.) imply that green-backed Rufous Hummingbirds are unlikely to be of hybrid origin, unless this is a phenotypic character that has introgressed far into the range of Rufous.

Continuous variation in the underlying morphology may nonetheless produce discontinuous variation in a sexual signal, when the signal itself is threshold-dependent (Clark 2014, Clark et al. 2018). Although hybrid tail feather morphology varied continuously, as the dive-sound is either produced with r2 (Rufous) or r3 (Allen's) as the source, hybrids appeared to recapitulate this pattern, with Rufous-like hybrids producing buzzy dive-sounds that were not different in pitch from Rufous, while Allen's-like hybrids produced dive-sounds that varied in pitch, with the very lowest frequency sounds having a fundamental frequency of ~1.1 kHz (Figure 1.5B). In between the Rufous-like and Allen's-like portions of the dive-sound is the frequency gap predicted by the 'harmonic hopping' hypothesis (Clark 2014): no hybrids made sound between ~0.8-1.1 kHz. I predict birds on the upper side of this gap produce sound primarily with r3 as a source; any influence of r2 is secondary, while birds on the lower side of this gap produce sound primarily with r2 as the source, with r3 possibly contributing to harmonic frequencies. I did not detect any polymorphic birds that produced separate sound with both r2 and r3 separately. Because these two feathers are adjacent and aerodynamically coupled, it may be physically impossible for r2 and r3 to flutter at different frequencies. I also did not detect any cases of birds that produced no dive sound (i.e. neither feather as a source); although qualitatively, some hybrids seemed to produce rather faint dive sounds. I did not quantify dive sound loudness; it might be that some hybrids approximate this condition (neither feather as a source) by producing a faint dive-sound.

Behavior

I did not find evidence of any populations of Rufous Hummingbird that perform O-shaped dives (Figure 1.4B), contrary to Johnsgard's (1983) description. Birds I sampled in Clatsop County, OR, and the Umpqua National Forest, OR, show that Rufous Hummingbird in these southerly populations perform a J-shaped dive similar to descriptions of Rufous Hummingbird from Alberta, Vancouver, and Seattle, described by Hurly et al. (2001).

Here, I described the presence of a previously undescribed display, the ‘half pendulum' display, which is present in both Rufous and Allen's Hummingbird (Figure 1.4D). Hurly et al. (2001) provided a brief, ambiguous description of Rufous Hummingbird performing something, but it is not entirely clear which display they described. Hurly et al. (2001) describe this display as a “waggle-flight” (synonymous to the bouts of writhing described here), that “was sometimes used as a *separate* display before dives”, and when it occurred, “the waggle at the completion of the dive appeared to follow the same path as the original waggle-flight”. This can be interpreted as either a pendulum display (i.e. the display otherwise only reported from Allen’s Hummingbird), or as the 'half pendulum' I describe here. Half pendulums always followed the shuttle display in all Rufous Hummingbird and Allen’s Hummingbird populations I studied, contra Hurly et al.'s (2001) description of it as a 'separate' display. I interpret Hurly et al.'s (2001) account as referring to the half pendulum display (Table 1.1), as it is parsimonious to assume that they saw the same display I found to be widespread within all populations of Rufous Hummingbird I studied, rather than a display I observed only in Allen’s Hummingbird.

All of the courtship displays described here comprise a set of discrete behavioral elements, common to both Rufous and Allen's Hummingbird (Figure 1.4, Table 1.1, Barlow 1968, Barlow 1977). These elements are not performed in isolation and are below the level of a 'fixed action pattern' (i.e. multiple elements together comprise a fixed action pattern). The same elements are found within the different courtship displays of each species; the difference is the order in which they appear (or are absent) in each display (Figure 1.4). An example of courtship behaviors differing due to a rearrangement of common elements found within Rufous and Allen's Hummingbird is present within the half pendulum display of both species. The half pendulum is comprised of a short ascent and bout of writhing, while the pendulum display, only found in Allen’s Hummingbird, includes a short descent, short ascent, and bout of writhing (Figure 1.4D, 1.4E, Table 1.1). The half pendulum display only follows a shuttle display in each species and does not occur consecutively. The pendulum display of Allen’s Hummingbird can occur independently of other displays and is usually repeated several times in succession (Figure 1.6A, 1.6B). Taken further, the element sequence of the pendulum display is embedded within the first three elements of the sequence of an Allen's dive (Figure 1.4A, 1.4E, Table 1.1).

The similarities between the pendulum and dive displays suggest a hypothesis: the pendulum display might be a modified dive. No similar display to the pendulum is found in the nearest eight outgroups (Clark et al. 2018). Thus, its evolutionary origin as a distinct behavioral character is of interest: it may be a true evolutionary novelty (*sensu* Wagner 2014), a behavioral innovation. To explain its existence, I hypothesize that, deeper in the bee hummingbird phylogeny, dives evolved multiple types, and that in the ancestor of Allen's Hummingbird, one of these types then became modified into the pendulum display. Formally assessing this hypothesis, as well as further description of display sequences using behavioral sequence analysis techniques, will be the topic of future work. In Chapter 2, I classify putative hybrids into discrete behavioral phenotypes based on the sequences of their behavioral elements and identify several unique (transgressive) combinations of elements not found in either parental species.

The displays often occur in a typical order (Figure 1.6, 7A, 7D). For example, if Rufous Hummingbird performs a dive, it is likely to perform multiple consecutive dives. If Allen's performs a series of pendulums, it often ends the series of pendulums with a dive (Figure 1.7A, 7D). The orders in which these displays occurred was homogenous across the sampled populations of Rufous Hummingbird and Allen's Hummingbird. Males across the hybrid zone performed displays that were variable along the spectrum between Allen's and Rufous Hummingbird. For example, putative hybrids, on average, performed fewer consecutive pendulums than Allen's Hummingbird, and often concluded displays with single pendulum displays, not dives, a phenotype rarely expressed in Allen's Hummingbird and never in Rufous Hummingbird (Figure S1.3).

The order in which putative hybrids performed displays often differed from each parental species. For instance, Allen's Hummingbird rarely ended a display with a single pendulum following a dive; in certain putative hybrids, this occurred frequently (Figure 1.7B, 7D). Alternatively, some putative hybrids performed a series of pendulums, similar to that of Allen's Hummingbird, then performed a series of multiple dives (with no writhing on the ascent), the latter of which does not usually occur within Allen's Hummingbird but is characteristic of Rufous Hummingbird (Figure 1.7A, 7C, 7D).

Selection

Although many hybrid zone studies incorporate morphological data, courtship behavior may also vary across hybrid zones, and similar to morphological traits, behavior can act as a species barrier. Courtship displays seem to play a larger role in reproductive isolation across the Allen's × Rufous Hummingbird contact zone than morphological traits, as reflected by the steep cline width within some courtship-related characters such as post-shuttle chirrups, the ratio of pendulums to dives, and the frequency of pendulums after dives (Table 1.4). Such behavioral differences could be caused by postzygotic selection against hybrids expressing unusual behavioral phenotypes. Alternatively, sexual selection and prezygotic isolation might cause these differences, because sexually selected characters, including courtship displays, are among the most rapidly diverging traits (Uy and Borgia 2000). Similarly, courtship behaviors serve as barriers to gene flow across a White-collared Manakin (*Manacus candei*) and Golden-collared Manakin (*Manacus vitellinus*) contact zone (Uy and Borgia 2000, McDonald et al. 2001).

Several hybrid zone studies, from arthropods to birds, have found an important role of behavior in reproductive isolation (Doherty and Storz 1993, Delmore et al. 2015, Lipshutz et al. 2017). A comprehensive study of song, color, and morphology between sister pairs of North American migratory birds found that pairs with migratory divides were more likely to stay reproductively isolated, even when they were similar in other phenotypic traits (Delmore et al. 2015). Across some of these hybrid zones, females discriminated based on song, as was the case in field cricket (*Gryllus firmus* and *G. pennsylvanicus*) and White-crowned Sparrow (*Zonotrichia leucophrys*) hybrid zones (Doherty and Storz 1993, Lipshutz et al. 2017). Alternatively, across a Black-capped (*Parus atricapillus*) × Carolina Chickadee (*P. carolinensis*) hybrid zone, females did not base mating decisions on the song repertoire of prospective mates, because they may have had a difficult time discriminating between conspecific and heterospecific males (Robbins et al. 1986, Reudink et al. 2005, Curry et al. 2007).

Hybrid zones are often found in intermediate habitats, where admixed individuals are sometimes able to compete for resources and/or access to mates as well as (or better than) parental species (Grabenstein and Taylor 2018). There is a mosaic of suitable Allen's and Rufous Hummingbird habitat in the center of the hybrid zone that is present as a result of late Pleistocene shifts in vegetation (see below). This might suggest that habitat isolation outside of the area of contact acts as a prezygotic barrier for these species, although further investigation is warranted.

I detected a substantial portion of individuals with an intermediate phenotype in the center of the hybrid zone. I will address the actual frequency of hybridization across the Allen's and Rufous Hummingbird hybrid zone with genetic data in a subsequent study. Further, in conjunction with the phenotypic data presented here, genetic data will tease apart whether individuals across the hybrid zone resulted from high rates of interbreeding, or whether they arose due to late-generation backcrossing. Correct phenotype classifications are prone to some error (Meyer et al. 2017). For instance, LDA reveals some populations in which the females were all putatively Rufous, but the males were putatively hybrid (Table S1.5, S1.6). While this could be the product of sex bias in dispersal, I find it more likely that the LDA has mis-classified some individuals (most likely, females).

Cline analysis

Narrow cline widths imply strong selection, while wider clines imply relaxed selection (Barton and Hewitt 1985). With cline widths ranging from 4.5 km (relatively narrow; post-shuttle chirrups) to 152.7 km (relatively wide; folded wing chord) selective forces seem to be acting across the Allen's × Rufous Hummingbird hybrid zone (Figure 1.8E, S1.3E).

My results indicate clinal variation across the suite of phenotypic characters for males, especially when comparing behavior and morphology. Wide confidence intervals for tail length and exposed culmen were likely due to the amount of overlap between Allen's and Rufous Hummingbird in these two traits, making it unclear why the LDA strongly loaded culmen length to discriminate between groups (Figure S1.2A, Table S1.4). Further sampling outside of the hybrid zone might better clarify any clinal relationships these characters have between species and across the hybrid zone.

Characters such as "pendulums after dives" were not clinal; they were transgressive, as hybrids differed from both parental species (Figure 1.8). The differing cline widths between behavioral and morphological characters imply that sexual selection is acting on these birds, where courtship-related behavioral traits are more restricted than morphological characters (Figure 1.8, S1.2, S1.3, Table 1.4). Furthermore, shorter cline decay for courtship behaviors compared to morphology on both sides of the clines implies stronger selection on courtship behaviors (Szymura and Barton 1986). Selection is stronger (decay is lower) towards Rufous Hummingbird's range than towards Allen's Hummingbird's range). The strength of selection across hybrid zones can have implications for hybridizing species, as traits under weak selection may introgress into the range of another species. For example, hybridization between Hawaiian crickets *Laupala paranigra* and *L. kohalensis* resulted in an introduction of intraspecific variation in song from *L. kohalensis* into the range of *L. paranigra* (Shaw 1996).

Direct comparison of clines of characters such as plumage, morphology, and behavior helps tease apart modes of selection and relative introgression of each character type. Although there is a growing body of work on the role of behavior in reproductive isolation across hybrid zones, direct comparisons of morphological and behavioral traits are uncommon (Robbins et al. 1986, Doherty and Storz 1993, Shaw 1996, Curry et al. 2007). Here, geographic cline analysis suggests stronger selection on courtship behaviors relative to morphological traits. I also found evidence of stronger selection of behavior towards the range of Rufous Hummingbird than towards Allen's Hummingbird.

Knowledge of the mechanisms of reproductive isolation provides information on how hybrid zones are maintained. Postzygotic isolation maintains a Swainson's Thrush (*Catharus ustulatus*) hybrid zone, where subspecies have different migratory routes and wintering areas (Ruegg 2012). This thrush hybrid zone is an example of a tension zone, which is a hybrid zone that is maintained by a balance of selection against hybrids and dispersal of parental forms into the contact zone. Behavioral clines are narrow in width due to migratory arrival time, breeding, and song, demonstrating how behavior might contribute to reproductive isolation (Ruegg 2012). Additionally, differences in migratory routes distinguished subspecies more effectively than song, morphology, and color (Delmore and Irwin 2014). Another hybrid zone driven by postzygotic isolation is exemplified by a Western (*Larus occidentalis*) and Glaucous-winged (*L. glaucescens*) Gull hybrid zone, where hybrids show better hatchling and fledgling success than Glaucous-winged Gulls at the edge of the hybrid zone (Good et al. 2000). This hybrid zone fits the bounded hybrid superiority model, where hybrids are more successful than parental species within the area of contact. Incorporation of genetic cline analysis in future work will corroborate my findings and explore whether a tension zone or hybrid superiority model describe the Allen's × Rufous Hummingbird hybrid zone described here.

Hybrid zone origin

The Klamath-Siskiyou region spans northwest California and southwest Oregon, and is a biodiversity hotspot (Bury and Pearl 1999). The highest elevations in the Klamath-Siskiyou region were glaciated during the Pleistocene, which likely forced movement of animal and plant species to more suitable areas (Whittaker 1960).

California plant taxa experienced extreme climatic change and a southern migration of many plant species found along the Pacific Northwest coast, with some groups moving into northern California, creating a mosaic of habitat (Wanket and Anderson 2007, Schierenbeck 2014). Specifically, within the last 2,500-6,000 years, Sitka spruce (*Picea sitchensis*) and Douglas fir (*Pseudotsuga menziesii*), two coniferous trees within the temparate rainforest ecosystem Rufous Hummingbird is usually associated with, have advanced south, into the extreme northern California coast, contributing to the diverse landscape as a result of wetter winters and cooler annual temperatures (Heusser 1960, Wanket and Anderson 2007).

Most hybrid zones originate through secondary contact of allopatric, previously isolated species, although this phenomenon are difficult to tease apart from primary differentiation, where speciation occurs in situ (Barton and Hewitt 1985). This change in climate and habitat in northern California may have led to a southern expansion of the breeding range of Rufous Hummingbird, leading to secondary contact, interbreeding, and gene flow with the already-present Allen's Hummingbird. Thus, this Klamath-Siskiyou region, which has among the most complex landscapes and vegetative communities in western North America, likely supported the formation of the Allen's × Rufous Hummingbird hybrid zone presented here (Bury and Pearl 1999).

The Klamath-Siskiyou region is a suture zone, where multiple contact zones occur within a single geographic area, within the mosaic habitat in northern California and southern Oregon between southerly and northerly distributed taxa, where the center of the Allen's × Rufous Hummingbird hybrid zone resides (Remington 1968, Swenson and

Howard 2005). This area is also a hot spot for phylogeographic breaks, as genetic clusters of much of the vegetation of the Pacific Northwest, despite having a continuous geographic distribution, group together from Alaska to central Oregon, and from central Oregon to northern California (Swenson and Howard 2005). Post-glaciation influence on forest communities in the Pacific Northwest has been shown in several plant species (*Pinus monticola*, *Erythronium montanum*, *Ribes bracteosun*, *Alnus rubra*), rainbow trout (*Onchorynchus mykiss*), and spotted frogs (*Rana pretiosa*, Thorgaard 1983, Green et al. 1996, Soltis et al. 1997). Furthermore, some hybrid zones, including a Red-naped (*Sphyrapicus nuchalis*) × Red-breasted (*S. ruber*) Sapsucker hybrid zone, and a contact zone between Douglas iris (*Iris douglasiana*) and Del Norte County iris (*I. innominata*), occur in northern California and southern Oregon, and share some overlap along the inland transect of the Allen's × Rufous Hummingbird hybrid zone presented here (Young 1996, Billerman et al. 2016).

Hybrid zones arising via secondary contact are characterized by sets of concordant cline widths and centers, while those originating from primary differentiation often exhibit non-concordant clines. Although I did not observe such concordance in the data, I still posit this contact zone may have arisen via secondary contact, with selection acting differently across the suite of phenotypic characters over time, leading to the non-coincident clines exhibited here (Barton 1979). Genetic data from a future cline analysis, as well as ecological niche models comparing late Pleistocene estimates to current species distributions for both species will test this hypothesis. If the Allen's × Rufous Hummingbird contact zone did originate as a result of secondary contact, in addition to a geographic model of isolation, I expect elevated linkage disequilibrium and genetic diversity in the center of the zone, which is to be expected when two divergent populations make contact (Durrett et al. 2000, Chavez-Galarza et al. 2015).

CONCLUSIONS

In Chapter 1, I described a newly discovered hybrid zone between Allen's and Rufous Hummingbird using courtship behavior and morphology. I found that the hybrid zone between migratory Allen's (*Selasphorus sasin*) and Rufous (*Selasphorus rufus*) Hummingbird is centered in northern California and southern Oregon, and spans from Humboldt County, CA, to Lane County, OR. I used Linear Discriminant Function Analysis, cline analysis, and a hybrid index to reveal the geographic extent of the hybrid zone, classify individuals as parental species or hybrids, and investigate how selection acts on the 20 behavioral and morphological characters I used for male individuals (nine morphological characters for females). As narrow as three km, clines for sexually selected courtship behaviors were far narrower than others (as wide as 300km), showing the strength of sexual selection in driving separation of Allen's and Rufous Hummingbird.

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FIGURES AND TABLES

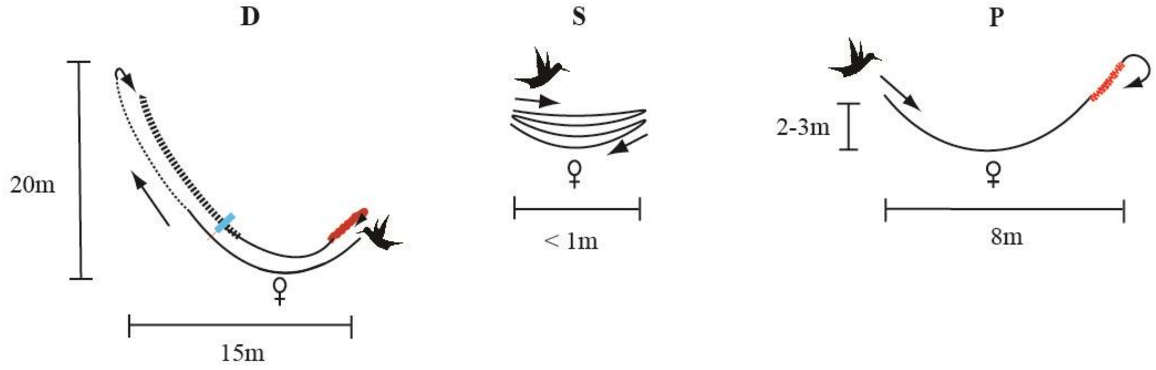

Figure 1.1 – **Courtship displays of male Allen's Hummingbird (*S. s. sasin*) and male Rufous Hummingbird (*S. rufus*).** Bird silhouette is not to scale. ♀: approximate position of the female during the male's courtship dive. During the dive in both species **(D)**, the bird ascends for the dive, turns around, and descends for the dive, emitting the dive sound with the tail feathers (blue hashmark). Red lines: a bout of writhing, in which the tail is flipped up and down while a 'chirruping' sound is produced by the wings (Clark 2016). Dotted and hashed lines represent the long ascent and long descent, respectively. During the shuttle display **(S)**, the bird flies back and forth in front of and in close proximity to (< 1 m from) the female with a variable horizontal trajectory. During the pendulum display **(P)**, several meters above the target female, the bird performs a short descent, followed by a short ascent and bout of writhing (performed only by Allen's Hummingbird). See also Figure 1.4.

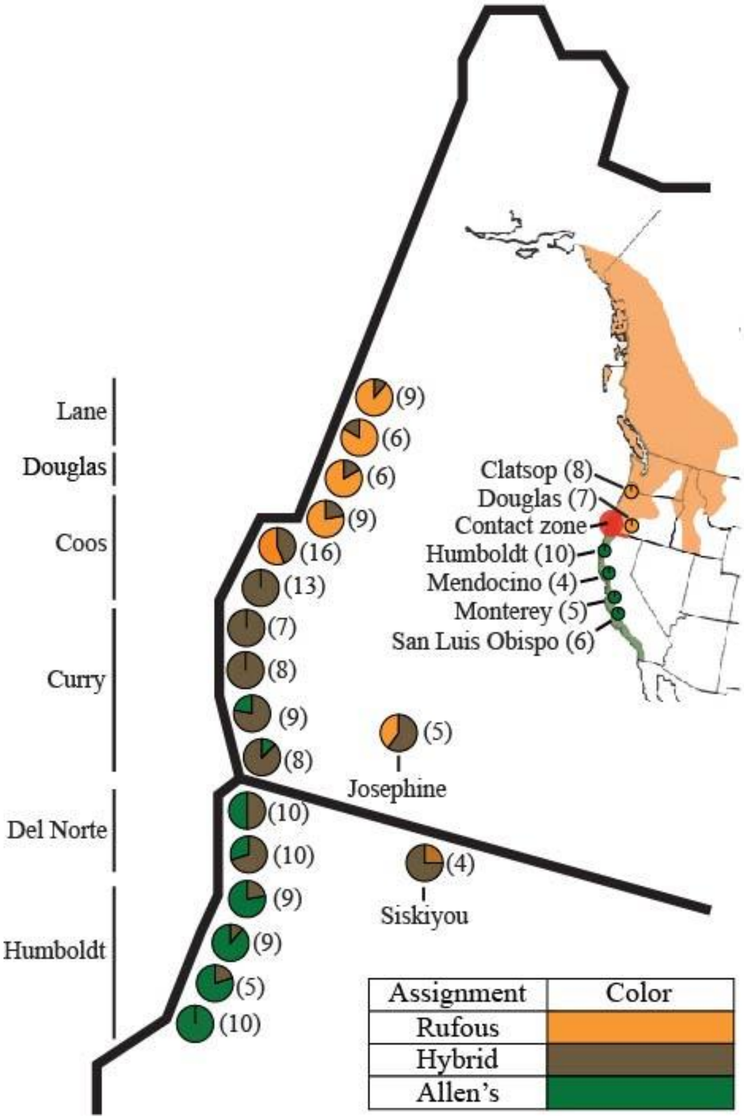

Figure 1.2 – **Approximate breeding ranges of Allen's (*S. s. sasin*) and Rufous (*S. rufus*) Hummingbird (inset map) and Linear Discriminant Function Analysis (LDA) of phenotypic characters along a coastal transect and other localities for Rufous Hummingbird, Allen's Hummingbird, and putative hybrid males.** Note: only migratory Allen's Hummingbird (*S. s. sasin*) is addressed here. For breeding ranges and sampling of reference parental species outside the hybrid zone (inset map): orange: Rufous Hummingbird breeding range, green: Allen's Hummingbird breeding range, red: sampling area, from Lane County (north) to Humboldt County (south, Calder 1993, Clark and Mitchell 2013). Each pie represents the proportion of the designated phenotype in that population, as determined by LDA.

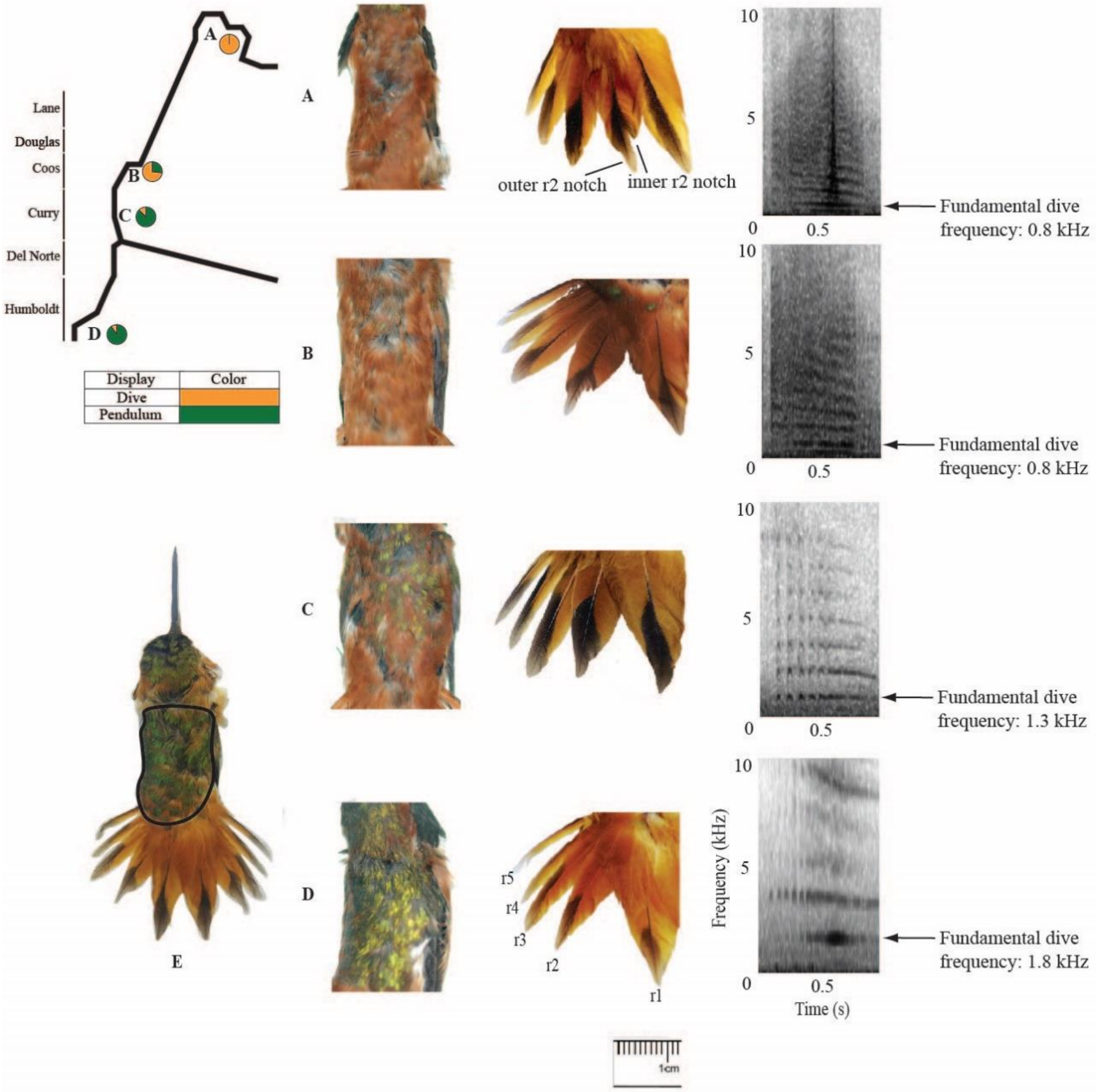

Figure 1.3 – **Back, rump, tail, fundamental frequency of the dive sound, and the proportion of pendulums to dives along the coastal gradient. (A)** Rufous Hummingbird with a 10% green back (Clatsop State Forest, Clatsop County, OR; CJC 382), **(B)**, a Rufous-like hybrid with a 0% green back; (Sunset Bay State Park in Coos County, OR; SDSU 3074), **(C)**, an Allen's-like hybrid with a 50% green back; (Alfred A. Loeb State Park, Curry County, OR; SDSU 2999), **(D)**, an Allen's Hummingbird with a 100% green back, (Humboldt Redwoods State Park, Humboldt County, CA; SDSU 2989). Along the north to south gradient, the rectrix two notches (on both the inner and outer webs of the feather) become less prominent; all of the tail feathers become thinner, back color transitions from rufous to green, the ratio of pendulums to dives increases, and the fundamental frequency of the dive sound increases from north to south (A-D). Brightness and contrast edited in Adobe Photoshop. Back color **(E)** was measured from the top of the upper back to the bottom of the lower back, down to the upper rump, not including the sides of the lower back.

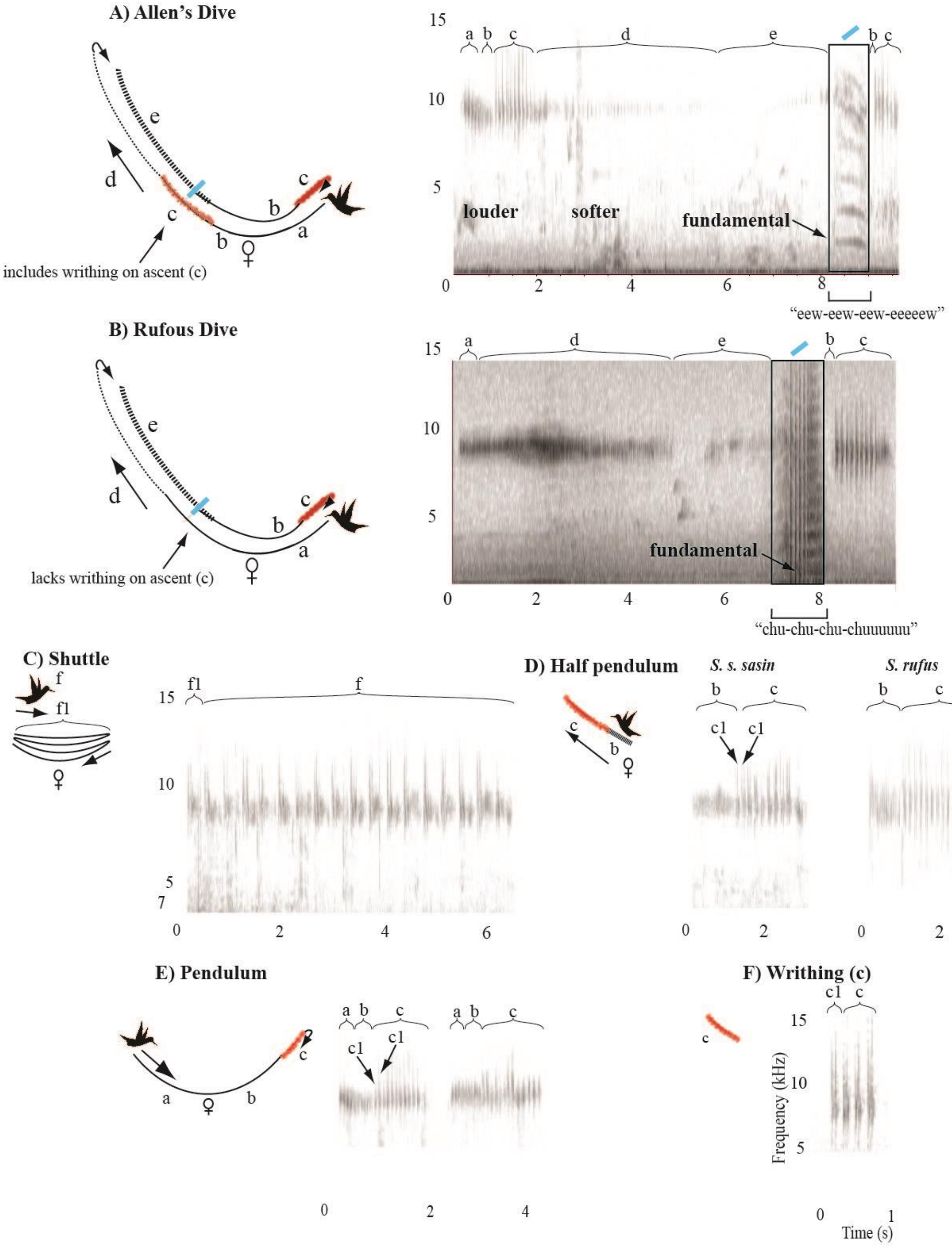
A) Allen's Dive
e
d
c
c
b
b
a
♀
includes writhing on ascent (c)
15
10
5
0
a b c d e b c
louder
softer
fundamental
0 2 4 6 8
"eew-eew-eew-eeeeew"
B) Rufous Dive
e
d
c
b
a
♀
lacks writhing on ascent (c)
15
10
5
a d e b c
fundamental
0 2 4 6 8
"chu-chu-chu-chuuuuuu"
C) Shuttle
f
f1
♀
f1 f
15
10
5
7
0 2 4 6
D) Half pendulum
c
b
♀
S. s. sasin
b c
c1 c1
0 2
S. rufus
b c
0 2
E) Pendulum
a
♀
b
c
a b c
c1 c1
a b c
0 2 4
F) Writhing (c)
c
c1 c
15
10
5
Frequency (kHz)
0 1
Time (s)

Figure 1.4 **– Courtship display kinematic sequences and sounds of male Allen's (*S. s sasin*) and Rufous (*S. rufus*) Hummingbird.** Lower case letters refer to display elements, as defined in Table 1.1 and as follows: a=short descent, b=short ascent, c=bout of writhing (c1=a single chirrup, c denotes the rest of the bout), d=long ascent, e=long descent, f=shuttle display (f1 a single shuttle segment, f denotes the rest of the bout), blue hashmark=dive sound, with the fundamental frequency of the dive boxed near the bottom of the appropriate spectrogram. Red lines represent a bout of writhing. As display sounds are produced by the wings and tail, there is a direct correspondence between display kinematics (left) and sound spectrograms for each display (right). Recorded at 48.0 kHz, shown with 2048-sample FFT Hann windows. **A)** Allen's Dive, which includes 'writhing in the ascent' (c; red squiggle). The tail sound is a thin, high-pitched "*eew-eew-eew-eeeeew*". The bird makes a short descent (a), followed by a short ascent (b), a bout of writhing (c), followed by a long ascent (d). Next, the bird turns around and descends (e), emitting the dive sound with the tail feathers through the late descent and the bottom of the dive (blue hashmark), then performs another short ascent (b), and usually ends with a bout of writhing (c). **B)** Rufous Dive, which does not include writhing in the ascent, where the tail sound is a lower-pitched, buzzier "*chu-chu-chu-chuuuuuu*. The bird makes a short descent (a), followed by a long ascent (d). Next, the bird turns around and descends (e), emitting the dive sound with the tail feathers (blue hashmark). The bird then performs a short ascent (b) and ends with a bout of writhing (c). **C)** Shuttle, common to both Allen's and Rufous Hummingbird. The bird performs multiple shuttle segments (f; the first one, f1, is individually labeled; subsequent shuttle segments not individually labeled) while facing the female and flashes his iridescent gorget. **D)** Half pendulum, common to both Allen's Hummingbird and Rufous Hummingbird, where the bird makes a short ascent (b) and finishes with a bout of writhing (c). Half pendulums performed by Allen's Hummingbird and Rufous Hummingbird, each of which end the display with a different number of chirrups (Table 1.2). **E)** Pendulum, found only in Allen's Hummingbird, begins with a short descent (a), followed by a short ascent (b), and is followed by a bout of writhing (c). **F)** Writhing (display element "c"), found within the dive and half pendulum displays of Allen's and Rufous Hummingbird, and in the pendulum display of Allen's Hummingbird, in which males visibly flip their tails up and down, and make individual "chirrup" sounds with the wings.

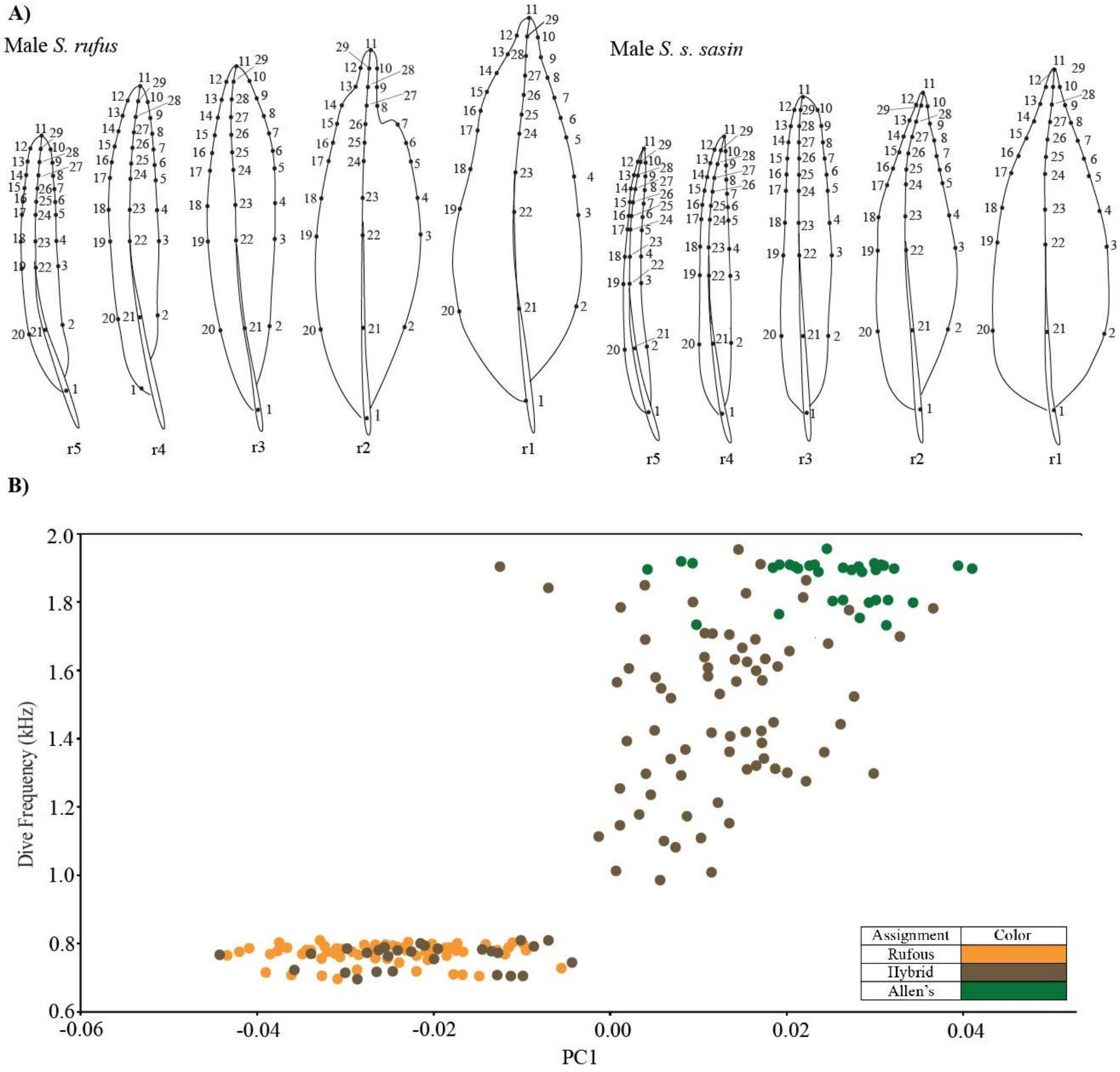

Figure 1.5 – **Geometric morphometric landmarks (A) and analysis (B) of one set of five tail feathers for individuals spanning the hybrid zone. (A)** For one set of tail feather rectrices (r1, r2, r3, r4, and r5) for each individual, 29 landmarks per tail feather were taken. **(B)** Morphometric data suggest differences in tail shape drive differences in the fundamental frequency (kHz) of the sound made towards the end of a dive. Allen's Hummingbird generates sound with rectrix three, while Rufous Hummingbird makes sound with rectrix two, which has a notched tip that Allen's Hummingbird lacks. Putative hybrids produce intermediate sounds based on mixed tail feather morphology.

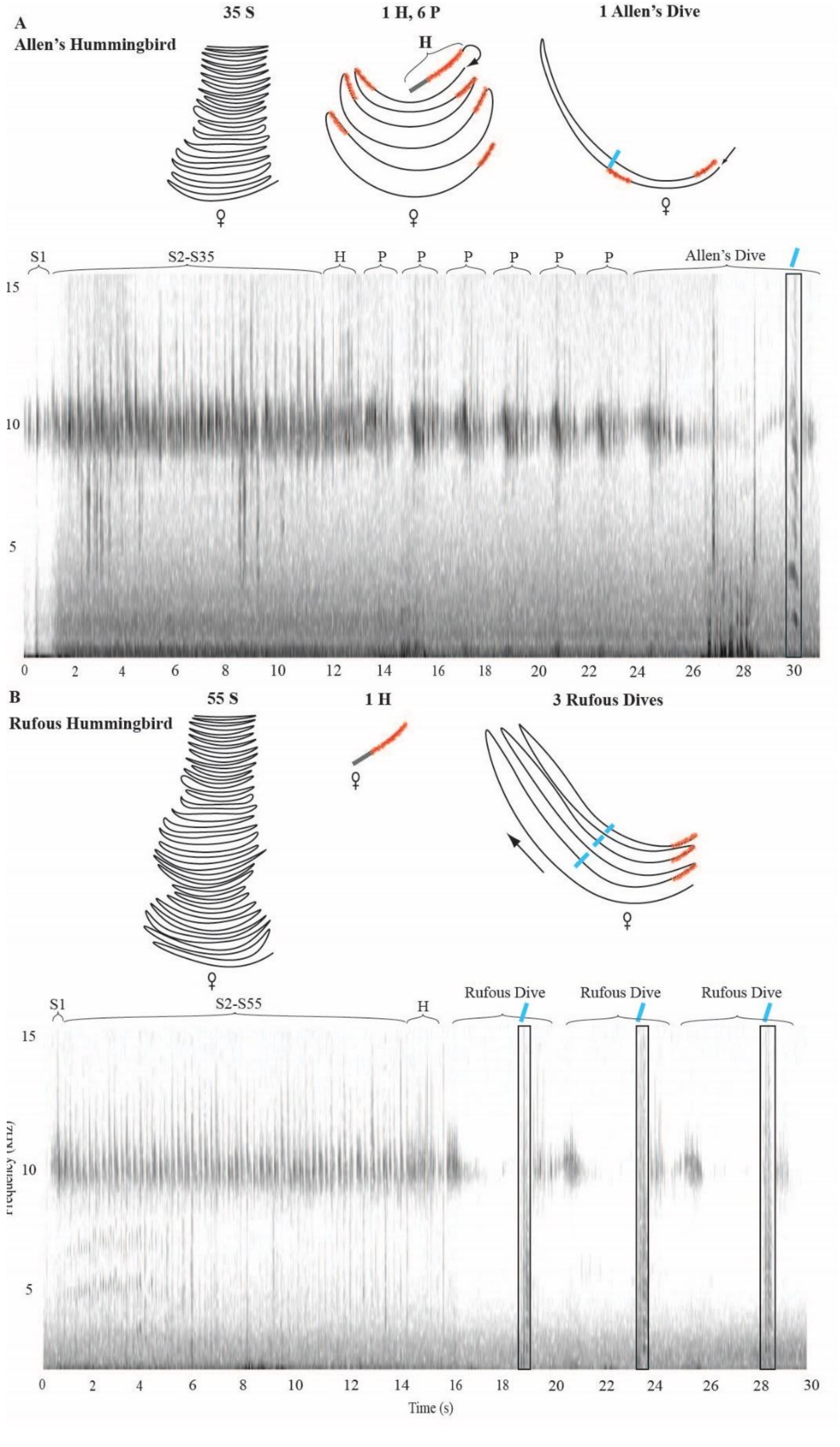
A
Allen's Hummingbird
35 S
1 H, 6 P
H
1 Allen's Dive
♀
♀
♀
S1
S2-S35
H
P
P
P
P
P
P
Allen's Dive
15
10
5
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
B
Rufous Hummingbird
55 S
1 H
♀
3 Rufous Dives
♀
♀
S1
S2-S55
H
Rufous Dive
Rufous Dive
Rufous Dive
15
Frequency (kHz)
10
5
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (s)

Figure 1.6 – **Typical courtship display sequences in male Allen's (*S. s. sasin,* A) and Rufous Hummingbird *(S. rufus*, B), from populations on the coastal transect.** Display elements for kinematics (top) and spectrograms for each display (bottom), as in Figure 1.4: a=short descent, b=short ascent, c=bout of writhing (c1: a single chirrup sound, c denotes the rest of the bout), d=long ascent, e=long descent, f=shuttle display (f1: a single shuttle segment), blue hashmark=dive sound, with the dive sound boxed near the bottom of the appropriate spectrogram. Red lines: a bout of writhing. **(A)** Allen's Hummingbird often performs a series of shuttle segments (35S = 35 segments), a half pendulum (1H = 1 half pendulum), several pendulums (6P = 6 pendulums), and concludes with a single dive (D) with writhing on ascent (Allen's dive). **(B)** Rufous Hummingbird often performs a series of shuttle segments (55S = 55 segments), a half pendulum (1H = 1 half pendulum) and concludes with three consecutive dives with no writhing on the ascent (Rufous dives). The red line indicates a bout of writhing.

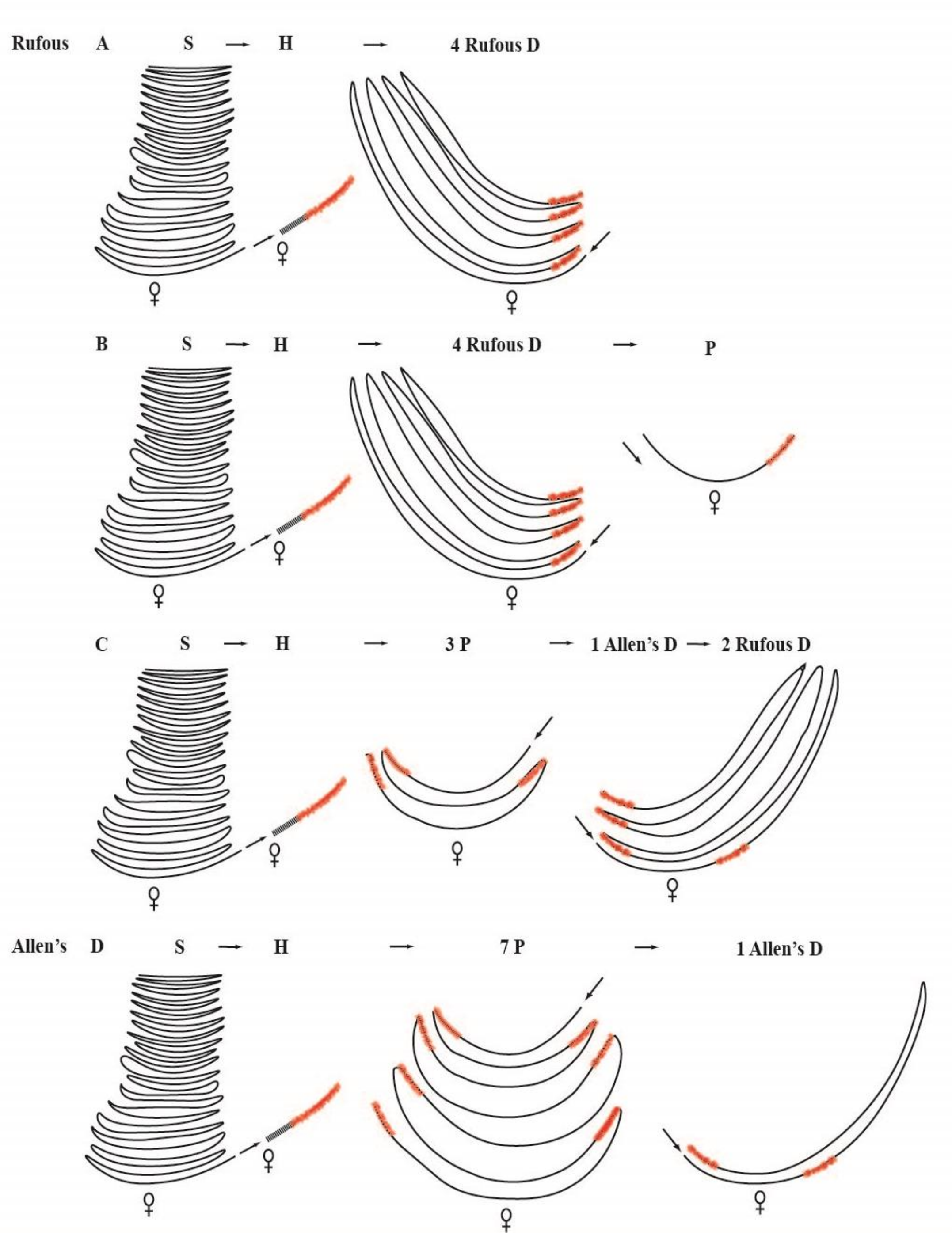

Figure 1.7 – **Typical courtship display sequences in males across the hybrid zone, from (A) north in Rufous Hummingbird, and (B) south into a Rufous-like hybrid population, (C) an Allen's-like hybrid population, and (D) an Allen's Hummingbird population.** From north to south, there is a transition from Rufous-like to Allen's-like sequences in courtship behavior, with behaviors designated as follows: shuttle=S, half pendulum=H, Allen's dive (D), Rufous dive (D), pendulum=P. The red line indicates a bout of writhing. Note: Dive orientation (left or right) is arbitrary.

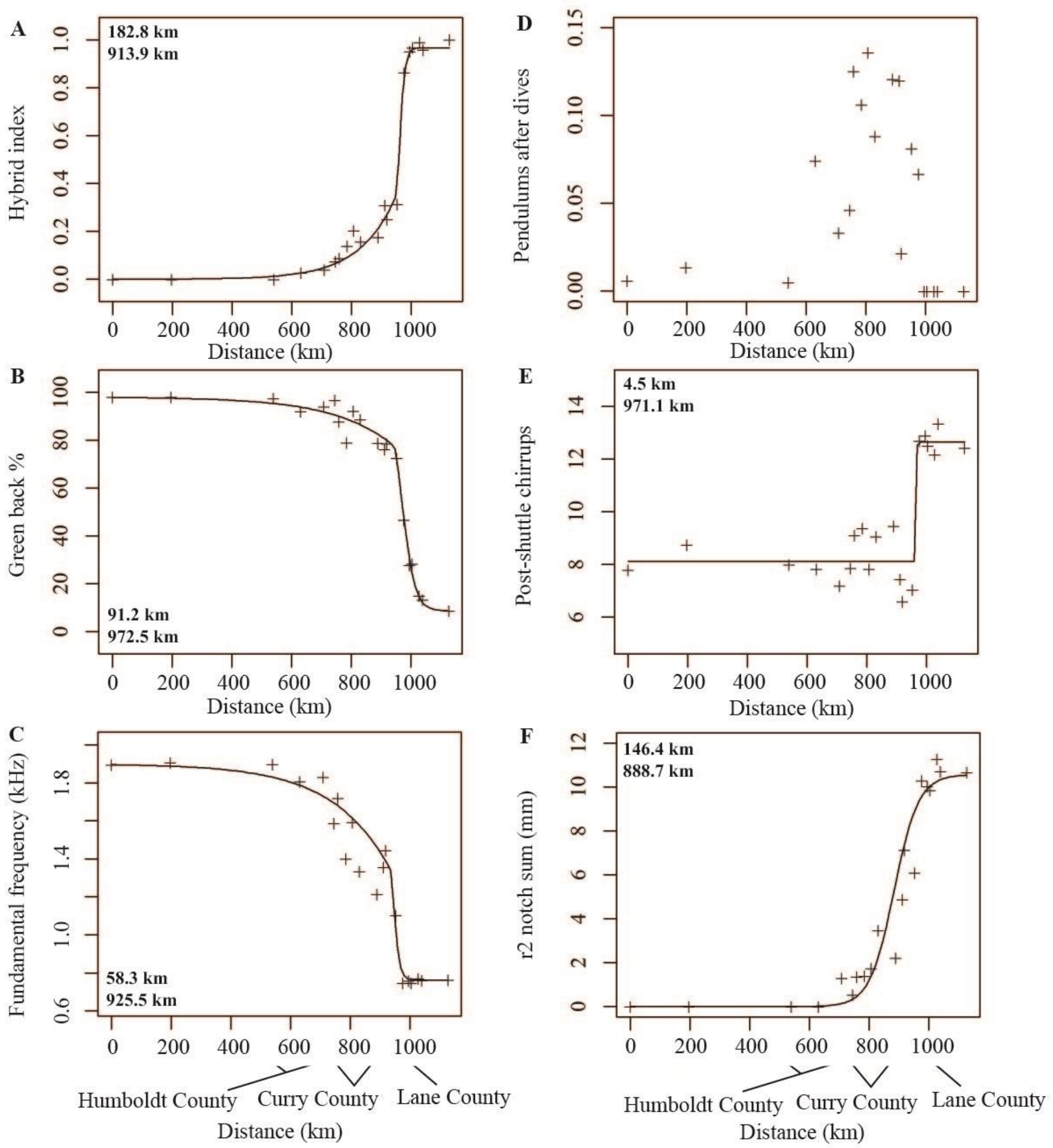

Figure 1.8 – **Cline analysis for males across the coastal, north-south transect for the following characters based on population means: (A) the hybrid index based on all characters, (B) percent green back, (C) fundamental dive frequency (kHz), (D) proportion of pendulums after dives, (E) post-shuttle chirrups, and (F) the sum of the four measurements for rectrix two (mm) based on Colwell (2005).** Cline and associated data for proportion of pendulums after dives not included because variation was not clinal. The southernmost Allen's Hummingbird (*S. s. sasin*) population (San Luis Obispo County, CA) was starting point for distance (zero km), with the northernmost Rufous Hummingbird (*S. rufus*) population (Clatsop County, OR) 1200 km away from the southernmost Allen's Hummingbird population. Within each plot, top distance is cline width (km), bottom distance designates cline center (km).

Table 1.1 – **Definitions of display behaviors and elements; ID refers to display elements illustrated in Figure 1.4.**

Display element	ID	Description of element
Short descent	a	A descent of about 2-3m in first half of a shallow half-U shape.
Short ascent	b	An ascent of about 2-3m in second half of a shallow half-U shape.
Writhing	c	Consists of individual chirrup sounds made by the wings; number of chirrups differs between dive, half pendulum, and pendulum displays in Allen's and Rufous Hummingbird.
Long ascent	d	During a dive display, an ascent of about 20m. Follows either a short descent (during a Rufous dive) or short ascent (during an Allen's dive).
Long descent	e	During a dive display, after completing long ascent, male turns around, descends, tracing similar path as ascent, spreads tail feathers near end of descent, producing dive sound; finishes descent, continuing in the same direction, performs (b), making a slight ascent with writhing.
Shuttle segment	f	Occurs within a shuttle display, as a left-to-right or right-to-left motion, with sound made by the wings. Shuttle displays are composed of multiple shuttle segments.

Display behavior	ID	Description of behavior
Allen's dive (writhing on ascent)	D (Allen's)	Male performs short descent, then short ascent with writhing, then performs long ascent for dive, turns around, performs long descent, tracing similar path as ascent; spreads tail feathers near end of descent, producing dive sound; finishes descent, makes slight ascent with writhing. Dive is ~20m high, 15m wide.
Rufous dive (no writhing on ascent)	D (Rufous)	Male performs short descent, then performs long ascent for dive, turns around, performs long descent, tracing similar path as ascent; spreads tail feathers near end of descent, producing dive sound; finishes descent, makes slight ascent with writhing. Dive is ~20m high, 15m wide.
Shuttle	S	Male flashes gorget, flies with variable vertical trajectory in overall left/right motion; within 1-2m of female, .25m high; consists of individual shuttle segments.
Half pendulum	H	Male performs short ascent, concluding with writhing; 2-3m high, 2-4m wide. Similar to pendulum display, except missing short descent. Nearly always follows a shuttle display.
Pendulum	P	Male performs a short descent followed by a short ascent that concludes with writhing; 2-3m high, 8m wide.

Table 1.2 – **Morphological and behavioral characters of male Allen's (*S. s. sasin*) and Rufous (*S. rufus*) Hummingbird.** Reference populations are Humboldt, Mendocino, Monterey, and San Luis Obispo counties (Allen's Hummingbird), and Clatsop, Douglas, and Lane Counties (Rufous Hummingbird); all measurements are in mm.

Character	Allen's (N=27)		Rufous (N=34)	
Morphology	95% CI	Mean ± SD	95% CI	Mean ± SD
Tail length	23.4-27.0	25.2 ± 0.9	26.2-30.2	28.2 ± 1.0
Folded wing chord	36.0-40.4	38.0 ± 1.0	37.9-41.5	39.7 ± 0.9
Exposed culmen	15.2-17.2	16.2 ± 0.5	14.9-17.3	16.1 ± 0.6
*r1 width	7.0-9.0	8.0 ± 0.5	8.0-10.0	9.0 ± 0.5
*r2 outer depth	0.0	0.0 ± 0.0	0.1-1.3	0.7 ± 0.3
*r2 outer distance	0.0	0.0 ± 0.0	2.8-4.4	3.6 ± 0.4
*r2 inner depth	0-0.3	0.1 ± 0.1	0.4-1.6	1.0 ± 0.3
*r2 inner distance	0.0-0.0	0.0 ± 0.0	4.4-6.4	5.4 ± 0.5
*r2 width	4.5-6.5	5.5 ± 0.5	6.8-8.4	7.6 ± 0.4
*r3 width	2.6-3.8	3.2 ± 0.3	4.6-5.8	5.2 ± 0.3
*r4 width	1.6-2.8	2.2 ± 0.3	3.1-3.9	3.5 ± 0.2
*r5 width	1.0-2.6	1.8 ± 0.4	2.2-3.0	2.6 ± 0.2
Percent green back (%)	82.0-100.0	95.6 ± 7.0	0.0-39.7	13.5 ± 13.2
Behavior				
Post-shuttle chirrups	6.0-10.0	8.0 ± 1.0	7.1-17.5	12.3 ± 2.6
Post-dive chirrups	1.8-7.4	4.6 ± 1.4	5.7-10.1	7.9 ± 1.1
Fundamental frequency of dive (Hz)	1,670-1,950	1,810 ± 70	690-840	770 ± 35
Maximum consecutive dives	0.5-1.7	1.1 ± 0.3	1.8-6.2	4.0 ± 1.1
Maximum consecutive pendulums	4.6-15.8	10.2 ± 2.8	0.0	0.0 ± 0.0
Pendulums to dives (ratio)	2.7-32.3	17.5 ± 7.4	0.0	0.0 ± 0.0
Percent of pendulums after dives	0.0-0.14	0.0 ± 0.07	0.0	0.0 ± 0.0

*r1-r5 designate tail rectrix measurements.

Table 1.3 – **Morphological characters of adult female Allen's (*S. s. sasin*) and Rufous Hummingbird (*S. rufus*).** Sampled reference populations are Humboldt, Mendocino, Monterey, and San Luis Obispo counties (Allen's Hummingbird), and Clatsop, Douglas, and Lane Counties (Rufous Hummingbird); all measurements are in mm.

Character	Allen's(N=29)		Rufous (N=36)	
	95% CI	Mean ± SD	95% CI	Mean ± SD
Tail length	22.5-26.9	24.7 ± 1.1	24.2-27.8	26.0 ± 0.9
Folded wing chord	38.7-43.9	41.3 ± 1.3	40.5-45.7	43.1 ± 1.3
Exposed culmen	14.7-17.9	16.3 ± 0.8	15.4-17.8	16.6 ± 0.6
*r2 notch length	0.0-0.9	0.1 ± 0.4	0.2-2.6	1.4 ± 0.6
*r1 width	8.3-9.3	7.3 ± 0.5	7.4-9.4	8.4 ± 0.5
*r2 width	4.3-6.7	5.5 ± 0.6	5.5-8.3	6.9 ± 0.7
*r3 width	3.3-4.5	3.9 ± 0.3	4.2-6.6	5.4 ± 0.6
*r4 width	2.3-3.5	2.9 ± 0.3	3.3-5.3	4.3 ± 0.5
*r5 width	2.0-2.6	2.2 ± 0.2	2.4-4.4	3.4 ± 0.5

*r1-r5 designate tail rectrix measurements from widest point of the feather.

Table 1.4 – **Cline parameters for each male character, including center, width, tau L, tau R, delta L, and delta R; the 2-unit likelihood support limits for each included in parentheses.**

Character	Center (km)	Width (km)	Tau L	Tau R	Delta L	Delta R
Phenotypic hybrid index	913.9 (912.8-915.1)	182.8 (182.4-183.6)	0.7 (0.7-0.8)	0.5 (0.5-0.6)	1152.4 (1140.4-1164.4)	1309.1 (1301.4-1316.7)
Rectrix one width (mm)	944.2 (918.4-959.5)	19.5 (0.0-68.3)	0.4 (0.0-1.0)	0.6 (0.0-1.0)	1093.7 (0.0-1429.4)	681.8 (2.8-1424.7)
Rectrix two width (mm)	944.9 (940.2-953.7)	138.4 (26.6-161.4)	0.2 (0.1-0.4)	0.6 (0.6-0.6)	0.7 (0.7-8.6)	46.3 (36.0-1384.1)
Rectrix three width (mm)	947.1 (946.2-957.1)	65.9 (54.5-81.5)	0.1 (0.1-0.2)	0.6 (0.6-0.6)	0.7 (0.7-8.6)	46.3 (36.0-1384.1)
Rectrix four width (mm)	951.5 (947.0-956.0)	43.9 (27.8-59.9)	0.1 (0.1-0.2)	0.6 (0.6-0.8)	1.8 (0.0-4.10)	273.3 (63.3-480.3)
Rectrix five width (mm)	937.9 (932.6-963.6)	56.8 (37.8-96.4)	0.0 (0.0-1.0)	0.6 (0.0-1.0)	0.6 (0.0-1423.8)	325.9 (2.4-1336.9)
Rectrix two notch sum (mm)	888.7 (871.4-890.3)	146.4 (141.3-156.7)	0.9 (0.3-1.2)	0.1 (0.0-0.1)	316.4 (274.4-358.0)	185.8 (1.1-1329.8)
Green back percentage	972.5 (963.3-979.0)	91.2 (54.2-128.3)	0.1 (0.1-0.2)	0.6 (0.0-1.0)	26.1 (11.1-46.5)	1311.3 (3.1-1429.9)
Folded wing chord (mm)	1015.55 (975.7-1065.1)	152.7 (101.5-274.8)	0.3 (0.0-1.0)	0.1 (0.0-1.0)	674.9 (24.2-1381.8)	16.5 (2.1-1228.4)
Ratio of pendulums to dives	950.1 (946.1-956.5)	33.2 (29.1-35.3)	0.0 (0.0-0.1)	1.0 (0.0-1.0)	1.2 (0.0-3.9)	1129.9 (193.1-1424.5)
Post-shuttle chirrups	971.1 (954.3-975.8)	4.5 (0.0-19.9)	0.3 (0.0-1.0)	0.5 (0.0-1.0)	82.1 (0.9-1429.2)	191.3 (0.2-1429.1)
Post-dive chirrups	970.8 (954.2-975.5)	19.4 (0.0-51.7)	0.4 (0.0-1.0)	0.2 (0.0-1.0)	934.8 (2.2-1426.3)	168.5 (1.1-1425.4)
Maximum consecutive pendulums	959.7 (955.4-961.3)	42.4 (40.6-45.1)	0.6 (0.6-0.7)	0.6 (0.6-0.7)	225.9 (221.0-447.4)	34.0 (6.4-729.6)
Maximum consecutive dives	965.9 (956.9-969.2)	54.5 (32.6-92.0)	0.1 (0.1-0.2)	0.0 (0.0-1.0)	12.7 (5.5-31.2)	78.8 (3.7-1406.1)
Fundamental frequency of the dive (kHz)	925.5 (921.7-929.4)	58.3 (53.3-63.3)	0.1 (0.1-0.1)	0.2 (0.2-0.3)	2.1 (0.8-3.4)	270.0 (260.9-279.1)

CHAPTER 2

Behavioral sequence analysis of Allen's Hummingbird (*Selasphorus sasin*), Rufous Hummingbird (*S. rufus*), and their hybrids reveals novel and transgressive phenotypes

INTRODUCTION

Within species, repetitive courtship display sequences are often predictable and relatively invariant from one sequence to the next. Thus, courtship displays are one of the quintessential examples of the somewhat-controversial concept of a fixed action pattern, an "inherited relatively complex movement pattern within instinctive behavior, which is as characteristic of the species or group as are structural features" (Thorpe 1951). During the courtship of many animals, including jumping spiders, fish, birds of paradise, ducks, and bowerbirds, males perform stereotyped sequences of behaviors in the course of attempting to convince a female to mate with them, exemplifying the fixed action pattern (Borgia 1995, Sharpe and Johnsgard 1996, Frith and Beehler 1998, Elias et al. 2012).

Fixed action patterns are hierarchical and modular. Once a fixed action pattern begins, the entire behavior is typically completed. Further, a fixed action pattern tends to be stereotyped, meaning that it is described as a predictable pattern of underlying motor acts or individual elements that comprise them (Tinbergen 1952, Barlow 1967, Barlow 1977).

Courtship displays, when stereotyped, may be categorized into discrete, quantifiable modules, or "elements". Here, I describe courtship displays comprised of underlying elements as individual fixed action patterns, as they are relatively invariant. These individual elements are organized within various stereotyped displays in each parental species (i.e., a specific sequence of elements forms each display). Sequences of courtship displays are organized into a “bout” (the entire courtship display, from start to finish) as the entire sequence of displays that an individual performs towards his stimulus within a single courtship episode. Here, displays are hierarchical (sequential elements form displays, sequential displays form bouts of display) and modular (the behavioral elements that form displays are discrete, identifiable units, Figure 2.1, Table 2.1).

Display behaviors are described at varying hierarchical levels to help understand their complexity (Tinbergen 1959). The hierarchical and modular nature of behavioral elements and the displays they form allows them to be analyzed at multiple levels, including at the level of individual behavioral elements, or at the level of individual fixed action patterns within some sort of larger unit of organization, such as a courtship display (Scholes 2006, Scholes 2008, Elias et al. 2012). The comparison of modules across related taxa permits the investigation of homology and novelty within related taxa (Scholes 2008).

I define homology as a correspondence of characters from different species that derive from a common ancestor with the same trait. A familiar example of a homologous trait is found in tetrapod limbs. Although a similar underlying skeletal structure is found in aquatic and terrestrial tetrapods, aquatic species have modified limbs that are adapted for aquatic life; these modified limbs distinguish them from their terrestrial counterparts (Wagner 2014). Definitions of homology are also applicable to behavioral traits, as exemplified by tail wagging movements in two species of *Tilapia* fish, which are considered homologous because they occur in the same place in the courtship of both species (Baerends 1958).

Quantifiable behavioral sequences present the opportunity to investigate homology of behavior, which is diagnosable via Remane's criteria of homology (Remane 1952, Wenzel 1992). The first of Remane's criteria is position, where there is a comparison of the position of behaviors relative to other behaviors in a sequence. The criterion of position is applicable to the present study because the sequences of elements that comprise displays are tractable. The position criterion is also testable: a reorganization of a sequence of behavioral elements via hybridization would form a separate display, which I define as a unique, underlying sequence of behavioral elements in which a male attempts to attract a mate, that violates this criterion. Remane's second criterion is special quality, where complex behaviors are homologous if they are innate and performed in the same context. This criterion is not appropriate for my hierarchical dataset; derivations on courtship displays that I observe are variations on existing displays, based on rearrangements of underlying behavioral elements, namely, the pendulum, half pendulum, and dive displays (see Results). Thus, the variations in displays reported here do not adhere to the special quality criterion. The third criterion, connection through intermediate forms, identifies homology when several taxa express behaviors that are intermediate between the original and derived taxa (Remane 1952). Patterson's conjunction test, a means in which to identify connection through intermediate forms, rejects homology based on a single taxon expressing both intermediate and original behaviors together (Patterson 1982). However, since behavioral evolution does not require complete replacement of underlying elements and displays evolve from the rearrangement or modification of elements, the same taxon may incorporate both the original and derived behavior in its repertoire (Wenzel 1992). This criterion is not appropriate for the data presented here because a) it is developed for broad, interspecific comparisons, while the present study is focused on two species and their hybrids, b) the same set of behavioral elements are incorporated into displays of both parental species and hybrids, and hybrid displays incorporate both original and derived behaviors. Thus, every individual hybrid repertoire that includes displays intermediate between Allen's and Rufous *and* a display identical to Allen's and/or Rufous would be considered non-homologous, i.e., nearly every hybrid I sampled. Here, I apply Remane's criterion of position, where a sequence of elements within a display that differs from what is described in either parental species is non-homologous. The position criterion was applied implicitly to identify novelty within the courtship displays of *Parotia* (Scholes 2006).

Behavioral sequence data and DNA sequence data should not be treated the same way. While single changes in a DNA sequence may be neutral, changes in behavioral sequences, when genetically controlled, are reflections of different underlying genetic architecture that present opportunities for selection. Thus, while the position criterion is not reliable for DNA sequence data, it is applicable to behavioral sequence data. As stated by Soltis (2013), "new alleles, generated via recombination of parental alleles, may have unique properties that confer novel phenotypes".

There are several conflicting definitions of novelty described throughout the literature, which are based on non-homology, process, and evolvability (Brown 2014).

Character-based definitions of novelty based on non-homology (Arthur 2000, West-Eberhard 2003) include the turtle shell, which is a character that appears discontinuous with all characters before it, making it a novel trait (Gilbert et al. 2001). Alternatively, novelty is also defined based on process, where a novel trait is generated by a transition from one adaptive peak to another after overcoming a previous developmental constraint (Hallgrímsson et al. 2012). Another definition to identify novelty is based on evolvability, which is the potential for a new trait to enable future variation and diversification (Mayr 1963, Brigandt 2007). All of these definitions are subject to criticism because they either fail to identify discontinuities in the tree of life (process-based), are too reliant on the already widely-debated concept of homology (character-based), or rely on unquantifiable future events (evolvability, Brown 2014). However, these varying definitions of novelty need not be in conflict. As suggested by Brigandt and Love (2012), different research questions require different concepts of novelty, and no single definition can capture the widespread roles played by novelty in evolutionary biology.

Investigation of novelty is applicable to behavioral traits. Researchers use the identification of the elements that comprise specific behaviors to assess how these elements are organized into the courtship displays of related species. The modular nature of these elements allows them to facilitate the origin of novelty through their reorganization, where the reordering or reuse of underlying elements differentiates taxa, as within Carola's Parotia (*Parotia carolae*), where various behavioral elements are employed differentially and re-organized; an element may be present or absent within a given display (Scholes 2006). Elias et al. (2012) broke down courtship displays of 11 species of *Habronattus* jumping spiders into different types of displays and the corresponding elements that comprise them. Similar to *Parotia*, males within *Habronattus* perform modular, stereotyped displays comprised of discrete behavioral elements, and these elements may be rearranged or lost across displays or species. Further, Prum (1990) identified homology and novelty via the position and modification of movements and postures within display sequences that helped distinguish 21 species of Neotropical manakins (family Pipridae) using phylogenetic analysis. In these systems, behavioral elements and the displays they form make up the courtship phenotype and are thought to be prominent drivers of diversification (Prum 1990, Scholes 2008, Elias et al. 2012). These examples highlight the relationship between homology and novelty when display behaviors within a study system are quantifiable.

An understanding of homology sheds light on the origin of evolutionary novelty. Here, I consider a novel trait as one which is not homologous to any trait in the ancestor or outgroups (Müller and Wagner 1991, West-Eberhard 2003, Pigliucci 2008, Wagner 2014). Thus, a novel trait is one which violates Remane's criterion of position (Remane 1952, Wenzel 1992).

In regard to species that interbreed, novel traits arise from the combination of existing genetic variation via hybridization (i.e., via recombination); mutation is not required (Jiggins et al. 2008). From the genetic reorganization that results from hybridization, novel traits arise to specialize within an already-existing function (Jiggins et al. 2008, Wagner 2014). Such a trait is novel because, theoretically, it is the origination of a new homolog that is modifiable through adaptation. Alternatively, hybrids sometimes express pre-existing traits differently than either parental species, although these traits are not necessarily novel. One such case is exemplified by transgressive behavior.

Transgressive segregation occurs when hybrid offspring exhibit extreme versions of traits found in either parental line due to the recombination of alleles from different parental populations. Specifically, I define a transgressive trait as one which is beyond the range of either parental species. This phenomenon is a mechanism thought to play a prominent role in the formation of novel and transgressive traits observed in hybrids (Rieseberg et al. 1999). Such traits typically occur through epistasis and the segregation of complementary alleles (Rieseberg et al. 1999). Most described transgressive traits are morphological (Rieseberg et al. 1999, Rieseberg et al. 2003, Mallet 2007, Hiadlovska et al. 2012, Dittrich-Reed and Fitzpatrick 2013). Behaviors may also be transgressive as well, as reported from separate instances of hybridization between hummingbirds.

Hummingbird hybrids that perform displays resembling both parental species are documented in four cases. Generally, hybrids are phenotypically intermediate between parental species. For example, an Anna's (*Calypte anna*) × Allen's (*S. s. sedentarius*) Hummingbird hybrid ascended for a dive in a similar way to Anna's, made an Allen's-like dive sound (1.75 kHz), and never sang during a dive (an Allen's-like behavior, Wells and Baptista 1979). Further, a hybrid male Peruvian Sheartail (*Thaumastura cora*) × Chilean Woodstar (*Eulidia yarrellii*) song was intermediate between both parental species (Clark et al. 2013).

A possible transgressive courtship trait is reported in the dive display of a male Broad-tailed (*Selasphorus platycercus*) × Black-chinned (*Archilocus alexandri*)

Hummingbird hybrid and Anna's × Costa's (*Calypte costae*) hybrids (Wells et al. 1978, Clark et al. 2012). Based on an alternative definition of a transgressive trait as one beyond the 95% confidence interval observed in either parental species, another potentially transgressive trait is documented in hummingbirds: in the case of the Anna's × Costa's hybrids, the inclusion of an additional element (where the male flew in a horizontal circle before diving) before a dive was performed with a higher frequency in hybrids than either parental species: this element is absent in Anna’s Hummingbird, rare in Costa’s Hummingbird, and common in hybrids. However, in each case sample sizes were small, and the behavior of each parental species is poorly documented (N=1, N=11, Wells et al. 1978, Clark et al. 2012).

A critical assumption of my study is that the courtship behaviors in Allen’s and Rufous Hummingbird are innate, rather than socially learned. If a behavior is innate, I expect this intermediate genome to express behaviors representative of both parental species. Such behavior was reported in the previous examples of intermediate hybrid Peruvian Sheartail × Chilean Woodstar, Broad-tailed × Black-chinned Hummingbird, and Anna's × Costa's displays in the previous paragraph (Wells et al. 1978, Clark et al. 2012, Clark et al. 2013). These hummingbird hybrid courtship displays are a mosaic of each parental species, showing that hybrids do not tend to behave like one parental or the other (Wells et al. 1978, Wells and Baptista 1979, Clark et al. 2012, Clark et al. 2013). In comparison, hybrid passerines exhibit vocal learning: when they sing, they typically perform songs similar to one parental species or the other as a result of socially learning song from one parental species (Haller 1940, Ficken and Ficken 1967, Gill and Murray

1972, Lemaire 1977). This is not the pattern observed in hybrid hummingbird displays; thus, I posit that hummingbird displays are innate.

The behavioral phenotype is not static through time. Because of the variable nature of behavior, sampling of multiple bouts is required (Carosi and Visalberghi 2002, Dias and Rangel-Negrín 2009). This is especially true for hybrids, where recombination may create more variation in their bouts of display. I describe the innate displays of Allen's and Rufous Hummingbird and their hybrids below, which form the foundation of the current study, and will be studied at four hierarchical levels: the element, display, the bout of display, and the display repertoire of each individual.

Allen's and Rufous Hummingbird behavior

Allen's and Rufous Hummingbird use the same behavioral elements within its courtship repertoire, though the order in which the elements appear in each species is distinct (Myers et al. 2019). Here, the behavioral phenotype is hierarchical, as it can be studied at several levels of integration (i.e., behavioral elements form each display, and displays are performed sequentially to form a bout of display, Barlow 1967, Barlow 1977).

When courting a female, males perform multiple consecutive, varied displays; these displays, and the bouts of display they form within male Allen's, Rufous, and hybrids, are the focus of the present study. Representative examples of typical display bouts found in Allen's and Rufous Hummingbird are illustrated in Figure 2.1. In the case of each species, males perform these courtship displays in bouts where the displays are repeated a variable number of times. The typical displays contained within the repertoire of Allen's and/or Rufous are the pendulum (Allen's only), the shuttle (both species), the half pendulum (both species), and the dive. The dives of the two species include a critical, sexually selected difference: Allen's performs a dive with writhing on the ascent (Allen's dive), and Rufous performs a dive without writhing on the ascent (Rufous dive, Myers et al. 2019, Figure 2.1, Table 2.2). Writhing is a behavior in which males visibly flip their tails up and down and produce short, individual pulses of wing trill, termed "chirrups" (Aldrich 1939, Myers et al. 2019). In both species, on average, bouts of writhing consist of more chirrups during a half pendulum than during the dive display, and Rufous performs writhing with more chirrups than Allen's during both the half pendulum and dive display (Myers et al. 2019, Table 2.3).

The sequences of displays in a bout are variable in Allen's and Rufous. Allen's tends to perform several pendulum displays in a row (10.2±2.8), while Rufous Hummingbird usually performs several consecutive Rufous dives in a given bout (4.0±1.1). In contrast, there is usually an absence of repetition of the Allen's dive in Allen's Hummingbird (1.1±0.3, Myers et al. 2019). Thus, the repetition of displays within a bout helps form the phenotypic identity of each species. Individuals across the hybrid zone violate the signature patterns of repetition found in both parental species. For example, individuals across the hybrid zone perform fewer consecutive pendulum displays than Allen's Hummingbird (Myers et al. 2019).

To quantify display phenotypes within Allen's, Rufous, and hybrids, I implement k-mer analysis. A k-mer is a "word" of DNA that is "k" letters long. For example, if k=4, the DNA sequence AGCTCCGGC has six four-letter k-mers, and is separated into six discrete characters: AGCT, GCTC, CTCC, TCCG, CCGG, and CGGC. The k-mer method is historically used to characterize DNA sequences to address a wide array of questions, including taxonomic classification of individuals based on sequence data (Wood and Salzberg 2014). Just as k-mer analysis is well-suited for DNA sequence data, it is equally applicable to discrete, behavioral sequence data to classify behaviorally variable individuals into phenotypic groups (Figure 2.2).

In the present study, I break down higher-level displays into lower-level elements, using k-mer analysis to classify individuals that perform sequences of these elements into discrete, diagnosable phenotypes. Specifically, I address the following questions: (1) how are hybrid phenotypes structured in terms of their display sequences? (2) how do display sequences within hybrid phenotypes vary from each other and from parental phenotypes? and (3) do any hybrid phenotypes comprise novel or transgressive behaviors?

MATERIALS AND METHODS

Sampling

I gathered courtship data from 304 male hummingbirds within and outside of the hybrid zone (comprising localities between Humboldt County, CA and Lincoln County, OR, Table S2.1) from March-June of 2014-2018. I sampled Allen's Hummingbird across its breeding range along the California coast, from southern Humboldt County to San Luis Obispo County, CA (Jewett 1929, Calder 1993, Gilligan et al. 1994). In all analyses here, I specifically studied the northern, migratory subspecies of Allen’s Hummingbird (*Selasphorus sasin sasin*). I sampled Rufous Hummingbird, which breeds from Oregon to southeast Alaska, along the coast from Lincoln County, OR to Juneau, AK (Calder 1993).

All individuals were identified as Rufous, Allen's, or hybrid via Linear Discriminant Function Analysis, as performed in Chapter 1.

A female *Selasphorus* hummingbird in a mesh cage was used to elicit displays from males. Infrequently, wild-caught females were not available; in these cases, I recorded males displaying to natural stimuli.

I obtained audio recordings of courtship displays. Within Allen's and Rufous Hummingbird, audio recordings were suitable for behavioral sequence analysis because they provided a quantitative, repeatable method of behavioral analysis, as there is a 1:1 match between the motion of the bird and the ensuing sound (Clark 2009, Myers et al. 2019). Thus, dives, shuttles, half pendulum, and pendulum displays were quantifiable from sound recordings. Males also create tractable sounds with their wings (wing trill) during flight and during bouts of writhing, making individual chirrups countable on a spectrogram. For a bout of display to be included in analysis, each display had to be audible in a recording. Displays described here were identified and quantified on a spectrogram via audio analysis software. Uncommon displays required a verbal description because they lacked the predictable form on a spectrogram of other displays.

I recorded displays for each individual until I fulfilled all of the following criteria: the male performed at least one shuttle, one half pendulum, one dive display, and at least 10 bouts of display. As no Allen's Hummingbird lacked the pendulum display in its repertoire, when in Allen's Hummingbird breeding range, I recorded displays for each individual until I obtained recordings of at least one shuttle, one half pendulum, one pendulum, and one dive display. Males often performed multiple bouts of display in rapid succession; I defined a bout of display as a sequence of displays that concluded when a male ceased to display for at least half of a second (usually much longer). To account for this, displays had to extend for a minimum of two seconds to be counted as a bout.

Ten bouts of display were chosen as a compromise between the need to sample each male's behavioral phenotype as much as possible, the need to acquire a large sample size, and the practicality that many males became acclimated to the female in the cage after roughly 7-10 displays. While a number of males acclimated to the female in the cage (and ceased displaying to her) in less than 10 displays, most of these males then performed displays to wild stimuli often enough that I was able to record a combination of elicited and natural display bouts. Similarly, certain males did not acclimate and continued to readily display to the female in the cage. Under these circumstances, I usually continued recording display bouts to a maximum of 35 displays for an individual.

I used a Sennheiser MKH 70 shotgun microphone (Wedemark, Germany), an Audio-Technica AT875R shotgun microphone (Stow, Ohio), a Tascam DR-05 portable recorder (Tokyo, Japan, sampling rate 44.1 kHz), a Tascam DR-60DmkII audio recorder (Tokyo, Japan, sampling rate 44.1 kHz), and a Sound Devices 702 24-bit digital recorder (Reedsburg, Wisconsin, sampling rates: 44.1, 48, or 96 kHz). I analyzed audio using 1881, 2048, or 4096-sample FFT windows (respectively, in Raven Pro 1.5, Cornell Lab of Ornithology Bioacoustics Research Program 2014). All analyses of behavioral elements, displays, and bouts of display were performed in R Studio v3.4.3 (R Studio Team 2019), R v3.5.0 (R Core Team 2018), and Raven Pro 1.5 (Cornell Lab of Ornithology Bioacoustics Research Program 2014).

Behavioral Nomenclature

Elements shared by Allen's and Rufous that comprised their repertoire of displays were as follows: *a*) short descent, where the male descended in a shallow half-U shape about 2-3 m, *b*) short ascent, where the male ascended in a shallow half-U shape about 2-3 m, *c*) writhing, *d*) long ascent, where the male ascended about 20 m for a dive, *e*) long descent, where, following a long ascent, after turning around, the male traced a similar path as the long ascent, spread his tail feathers, and produced a dive sound, and *f*) shuttle segments, where the male made individual left-to-right or right-to-left motions while flashing his iridescent gorget (Myers et al. 2019, Figure 2.1, Table 2.1).

When transitioning from one display to the next, the focal male usually turned around. In this study, I added "turn-around" (behavioral element "*g*") as an element to account for when a male changed direction during a bout (Figure 2.1, Table 2.1). Turn-around was assumed to be present in all cases unless otherwise noted. For example, when Allen's Hummingbird performed two consecutive pendulum displays, the bird turned around when transitioning from the first pendulum to the second. Thus, the sequence of elements for both pendulum displays was as follows: *a, b, c, g, a, b, c, g* (short descent, short ascent with writhing, turn-around, short descent, short ascent with writhing, turn-around, Figure 2.1, Table 2.2).

Both species performed the shuttle display, where individual shuttle segments (display element *f*) were repeated, and in-between each shuttle segment, the individual typically turned around (display element *g*). Shuttle displays were often followed by a half pendulum, comprised of display elements *b*, *c*, *g* (short ascent with writhing, turn-around, Myers et al. 2019, Figure 2.1, Table 2.2). The shuttle display included a highly variable number of shuttle segments. Because I did not detect differences within this display between Allen's, Rufous, and their hybrids, I treated every shuttle display as a single, discrete unit, regardless of the number of shuttle segments within each shuttle display.

K-mer Analysis

Through implementation of k-mer analysis, I separated individuals into discrete clusters of individuals and identified Allen’s, Rufous, and hybrid phenotypes along the Allen’s-Rufous phenotype continuum. I symbolized each display with capital letters and concatenated all 10 to 35 bouts of display into a single sequence for each individual. While lowercase, italicized letters (described above) designated the underlying elements of a display, capital letters designated a display, as follows: pendulum=P, half pendulum=H, shuttle=S, Rufous dive=R, Allen’s dive=A. To quantify which displays birds began and ended bouts with, and to quantify transitions from one bout of display to the next, I added “X” to denote the start of the display bout and “Z” to indicate the end of a bout and/or a transition from one sequence to another (Table 2.2). Thus, for a bird with two recorded sequences, the first complete bout of display comprising displays “S-H” (shuttle display, half pendulum display) and the second bout, two dives without writhing on the ascent (“R-R”), is expressed as “X-S-H-Z-R-R-Z” (Figure 2.2). An alternative approach, where I grouped k-mer transitions by behavioral elements rather than displays, failed, because some displays were comprised of several elements (i.e., eight elements within an Allen’s dive), which made it impossible to analyze transitions from one set of elements (display) to the next with a computationally-feasible value of k.

I used a k-mer length of k=3. One reason was practical: this was the maximum k-mer length that was computationally feasible for the dataset, since the number of k-mers is an exponential function of k (i.e., the number of k-mers = N^k). Given the alphabet size (N) of 23 (see below), k=3 yields 12,167 possible k-mers, while k=4 yields 279,841. The other reason was empirical: k=3 was sufficient to differentiate between Allen's and Rufous by accounting for repetitions of pendulum and dive displays (i.e., P-P-P and R-R-R), as well as fine-scale differences present within hybrids (see Results). K=3 was not computationally possible with an alphabet of 23. Thus, to make k=3 computationally feasible, I temporarily omitted rare display variants that few individuals performed, reducing the alphabet from 23 to 11. Individuals who performed rare display variants, which I define as relatively uncommon variations of a typical display (for example, a P without writhing at the end), were omitted from the original analysis. For example, an individual which performed a rare variant of P (P_X, P_W, P_D, see Results) was temporarily removed from the dataset. Analyses revealed that a valid assumption (see Results and Figures S2.1, S2.2) is that individuals who performed one display, for example, P (bird A), and those who did not perform P (bird B) were never placed in the same phenotype. Thus, if it were computationally possible to analyze a k-mer alphabet of 23, I assume that individuals who performed rare display variants would have been assigned their own unique phenotype by the analyses based on the presence of unique 3-mers in their repertoire. Operating under this assumption, after statistical analyses (see below), these rare display variants were manually reassigned their own unique phenotypes. Individuals who incorporated these rare variant displays would have been classified into their own phenotypes based on the presence of unique 3-mers in their repertoire by the clustering analysis. Thus, manual assignment of rare variants did not affect my results.

From each concatenated sequence that represented a single male's phenotype, I made a k-count matrix (a matrix of counts of all 3-mers within the individual's sequence) in the "k-mer" package (Wilkinson 2018) in R v3.5.0 (R Core Team 2018) and R Studio v1.2.5 (R Studio Team 2019), then imported each matrix into Mesquite (Maddison and Maddison 2018), where each 3-mer was treated as a discrete character. I sought to determine the displays (and transitions between displays within a bout) present within the repertoire of discrete phenotypes. Once a display was detected within an individual, the display was considered a part of its phenotype. When assigning individuals to display phenotypes, the displays in their repertoire, rather than how often they performed particular displays, were the targets of my analyses. A bias introduced via the incorporation of count data (rather than presence/absence) often placed individuals into clusters that were clearly incorrect (i.e., a hybrid that rarely performed single P but was lumped with Rufous). Thus, I converted counts of 3-mers to presence/absence (0/1) characters. Next, I produced an unrooted, unweighted pairwise distance group method with arithmetic mean (UPGMA) dendrogram to cluster individuals into phenotypes in a hierarchical fashion (in PAUP*), from the most similar to the most different (Swofford 2003).

Markov Chain Modeling

A first-order MC presents the probability of the next behavioral state based only on the current state (Patterson et al. 2008, Kershenbaum et al. 2014). I implemented a first-order, discrete-time MC to assess how common display transitions across phenotypes identified by from the 3-mer character matrix were using the "markovchain" package (Spedicato et al. 2017) in R v3.5.0 (R Core Team 2018) and R Studio v1.2.5 (R Studio Team 2019). This analysis allowed quantification of how rare specific transitions of displays and display variants were within each phenotype, and incorporated all displays I observed, including rare variants (Table S2.2). For this analysis, the 3-mer character matrix counts were not treated as presence/absence to enable calculation of transition probability within each phenotype. These transition probabilities are visualized in transition matrices of each phenotype (Table S2.2).

I performed an unpaired, two-tailed, two sample t-test to determine whether transitions between displays in Allen's and Rufous were significantly different between Allen's and Rufous. To ensure data were normally distributed, I performed a Shapiro-Wilk normality test (Shapiro and Wilk 1965). The distribution of the data were not significantly different from the normal distribution ($P > 0.05$). I performed an F-test to test for homogeneity in variance and found no difference in variance between the data for Allen's and Rufous ($P > 0.05$). The preceding statistics were calculated in R v3.5.0 (R Core Team 2018) and R Studio v1.2.5 (R Studio Team 2019).

Sampling the Repertoire

When gathering courtship data, as sample size increased, I expected the number of new displays to approach a ceiling. To estimate the number of display bouts necessary to sufficiently sample the phenotype of each individual (to approach the ceiling), I constructed 3-mer accumulation curves based on the number of new sequences of 3-mers each individual accumulated as sampling of each bout of display increased. Accumulation curves were constructed using the VEGAN package (Oksanen et al. 2019) in R v3.5.0 (R Core Team 2018) and R Studio v1.2.5 (R Studio Team 2019). I plotted curves for each individual using 100 permutations of each bout of display in a random order to avoid temporal biases in sampling effort using the Coleman method, which found the expected accumulation curve based on an estimation of the total extrapolated number of 3-mers within each individual. The curves were constructed using the equation asymptote=a/b, where a=the slope of the beginning of the curve, and b=the shape of the accumulation curve and solved for b using the estimateR function (Coleman et al. 1982). I estimated the amount of sampling required to observe 90% of the repertoire of each individual using the equation $q/[b(1-q)]=t$, where q=the percent of the repertoire sampled (q=0.9), and t=the number of display bouts required to reach the desired q. All values and equations were based on the Clench Equation, as reported by Soberón and Llorente (1993). The Clench Equation is based on the assumption that the probability of adding new behavioral sequences improves (up to a ceiling) as more time is spent sampling (Clench 1979, Soberón and Llorente 1993). All values reported in the results are means ± two SD, which approximates the 95% confidence interval.

RESULTS

Elements as building blocks of displays

Allen's and Rufous Hummingbird performed different displays that adhered to the definition of a fixed action pattern and were based on a common set of underlying behavioral elements (Figure 2.1). The set of display elements shared by Allen's and Rufous

Hummingbird were: *a*) short descent, *b*) short ascent, *c*) writhing, *d*) long ascent, *e*) long descent, *f*) shuttle segments (individual left-to-right or right-to-left motions), and *g*) turn-around (Figure 2.1, Table 2.1, Myers et al. 2019). These elements formed the following displays: the pendulum (P; elements *a*, *b*, *c*, *g*), the shuttle (S; elements *f*, *g*), half pendulum (H; *b*, *c*, *g*), Allen's dive (A; elements *a*, *b, c, d, g, e, b, c, g*), and Rufous dive (R; elements *a, d, g, e, b, c, g*, Figure 2.1, Table 2.2). Underlying elements were reorganized to form the variation in sequences across parent species and hybrids (Figure 2.3).

Seventeen hybrid individuals implemented bouts of writhing that exceeded the upper limit of the range observed in either parent species, which was 16 post-shuttle chirrups and 12 post-dive chirrups (95% CI for parental species and hybrids provided in Table 2.3 for reference). During post-shuttle chirrups, 13 individuals implemented writhing that extended beyond the described upper limit of the range of either parent species (Figure 2.4, Table 2.3). One individual performed 24 post-shuttle chirrups during a bout. Six individuals implemented writhing beyond the upper limit of the range of either parent species when performing post-dive chirrups. One individual performed up to 14 post-dive chirrups during a bout.

Display variants

I observed 16 display variants of S, H, P, A, and R (Figure 2.3, Table 2.1). These 16 display variants, combined with the start and end states and the five previously described displays, formed the k-mer alphabet of 23. The repertoire of behaviors within all hybrid phenotypes included the same underlying elements as those found in each parental species, but sometimes in different orders (and sometimes with specific elements omitted or duplicated). For example, some hybrids performed a P with no writhing (P_{X}), and these individuals performed element sequence *a, b, g*, rather than *a, b, c, g*, as in a typical P (Figure 2.3A, Table 2.2). Eleven of the 16 display variants were performed by fewer than ten individuals. Some of these 11 variants, such as the aborted dive (A_{A}, R_{A}), where *e, b, c*, and *g* were omitted from the end of the dive, were observed within parental species. Variants only observed in hybrids, such as P_{X}, may have been present but were undetected (Figure 2.3, Table 2.2).

The following display variants are illustrated in Figure 2.3. There were three display variants of P: P_{X}, P_{W}, and P_{D}. During P_{W}, the bird performed two bouts of writhing at the end of a pendulum display (*a, b, c, b, c, g*). During P_{D}, the bird performed two consecutive P without a turn-around between them (*a, b, c, a, b, c, g*, Figure 2.3A, Table 2.1, 2.2). There were four display variants of H, which were H_{XT}, H_{X}, H_{W}, and H_{E} (Table 2.2). In H_{XT}, the male performed an H that was not followed by a turn-around *(b, c*), and this was always followed by A. In H_{X}, the male performed an H without writhing (*b, g*). During H_{W}, the bird did an H with two instances of writhing (*a, b, a, b, g*). During H_{E}, the male performed an H with extended writhing (a bout of writhing lasting beyond the 95% CI of either parental species, *b*, *c*, *g*, Figure 2.3B, Table 2.3). I describe four display variants of A, including A_{A}, A_{X}, A_{M}, and A_{L}. During A_{X}, the male performed an A without writhing at the end of the dive (*a, b, c, d, g, e, b, g*). During A_{M}, an Allen's mini-dive, the bird performed a long and short ascent smaller in amplitude than normal and produced a faint or inaudible dive sound (*a, b, c, d, g, e, b, c, g*). During A_{L}, the bird performed an A that lacked the initial short descent (*b, c, d, g, e, b, c, g*, Figure 2.3C, Table 2.1, 2.2). There were also five display variants of R (Table 2.2), which included R_A, R_X, R_M, R_L, and R_E. During R_X, the bird performed R without writhing after the dive (*a, d, g, e, b, g*). When performing R_M, a Rufous mini-dive, where the bird completed a long and short ascent lower in amplitude than normal and produced a faint or inaudible dive sound (*a, d, g, e, b, c, g*). During R_L, the individual performed an R that lacked the initial short descent (*d*, *g*, *e*, *b*, *c*, *g*). Finally, I observed males performing R_E, an R with extended writhing (*a, d, g, e, b, c, g*, Figure 2.3D, Table 2.1, 2.3).

K-mer phenotype assignment

Behavioral elements formed the building blocks of courtship displays within Allen's and Rufous Hummingbird; however, the courtship displays they comprised were not completely invariant. The above display variants were the result of the addition and/or subtraction of elements within displays. Thus, the fact that the fixed action pattern was not entirely fixed necessitated classification of individuals into discrete phenotypes with k-mer analysis. The transition probabilities between displays within each phenotype, calculated by the MC, and identified by the k-mer and clustering analyses, are visualized in Table S2.2.

Considering only the 11 most common displays and display variants, out of a theoretically possible 1,331 3-mers, there were 186 unique 3-mers present across all individuals. Hierarchical clustering analysis separated the 304 individuals in my dataset into 29 hybrid phenotypes. Upon incorporation of individuals who performed rare displays omitted from the 3-mer matrix, I identified 39 hybrid phenotypes, shown in a dendrogram in Figure 2.5. 3-mers that regularly occurred across Allen's, Rufous, and hybrids were X-S-H, S-H-Z, and S-H-dive (Allen's or Rufous, Figure 2.6, S2.1, S2.2).

I identified eight Allen's Hummingbird display phenotypes and eight Rufous Hummingbird display phenotypes (Figure 2.5). Displays were sometimes ambiguous: Allen's and Rufous Hummingbird males occasionally concluded a display bout due to an external interruption of the display from a non-display target. These non-typical interruptions occurred in various forms, such as wind and interference of the display (for instance, by a rival male who entered the focal male's territory and interrupted the focal male's dive, or by a rival male who interrupted a focal male's pendulum display).

Some displays were likely misrepresented in my samples: certain rare phenotypes were likely underrepresented across my samples, because during recording of displays I sometimes lost visual track of a bird, which caused me to mistakenly score certain rare displays as similar sounding, more common displays.

Allen's Hummingbird phenotypes

Display data from 80 individuals characterized the phenotypes of Allen's Hummingbird (Figure 2.5). Common 3-mers of Allen's Hummingbird included X-P-P, P-P-P, P-P-A, P-A-Z, and S-H-P (Figure 2.6). Defining displays and transitions within Allen's Hummingbird phenotypes included the presence and repetition of P, the presence of A, and usually (with the exception of phenotype six) the absence of A-A (no Allen's ever performed the 3-mer A-A-A, Figure 2.6, Table 2.4). Within a given bout, Allen's performed an average of 10.5±2.9 P in a row (N=80 individuals). Allen's performed S and H in a similar manner to Rufous. Transition frequencies between various displays within

Allen's Hummingbird are displayed in Table 2.4. Allen's phenotype one was most common (S2.1). Display variants and sequences utilized within individuals belonging to other Allen's Hummingbird phenotypes were A_X, A_A, A-A, R, and R_x (Figure 2.3C, 2.3D, 2.6, S2.1).

Rufous Hummingbird phenotypes

Display data from 82 individuals characterized the phenotypes of Rufous Hummingbird (Figure 2.5, 2.6). Common 3-mers of Rufous Hummingbird were X-R-R, R-R-R, R-R-Z, and S-H-R (Figure 2.6, Table 2.4). Defining display transitions of Rufous phenotypes included the repetition of R, and the initiation of bouts of display with an R (S-R, Table 2.4). The most common display of Rufous was R; within all Rufous phenotypes, bouts of display usually began with R (55% of the time), and individuals performed 4.1±1.0 R in a row (N=82 individuals). Rufous never performed a P (N=82 individuals). Rufous phenotype one was most common (Figure S2.1). Display variants and sequences utilized within individuals grouped in other Rufous Hummingbird phenotypes were R_A, R_X, R_L, R_E, H_E, and H_X (Figure 2.3B, 2.3D, 2.5 2.6, Table S2.2).

Transition frequencies to compare and contrast display transitions within and between Allen's and Rufous Hummingbird are displayed in Table 2.4. Based on unpaired, two-tailed, two sample t-tests, I found that transitions between displays were only significantly different ($P < 0.05$) when they included a display unique to either Allen's or Rufous (i.e., either P, R, and/or A was present in a transition, Table 2.4).

Hybrid phenotypes

Among 142 hybrid individuals were 39 clusters of phenotypes. Hybrid phenotypes identified by the k-mer analysis exhibited more-or-less continuous variation during bouts of display between the Allen's and Rufous Hummingbird display phenotypes (Figure 2.5, S2.1). The order within these bouts of display separated a given phenotype from others (Figure S2.2). Most of the 39 hybrid phenotypes were formed by small clusters of 1-10 individuals (Figure 2.5, S2.2).

Several 3-mers differentiated hybrids from both Allen's and Rufous. Some of these differentiating 3-mer sequences were those which included a display variant (i.e., S-H_X-P, P-P-P_W, R-R-R_M), a single P within a 3-mer (i.e., P-R-R versus P-P-P), a single R within a 3-mer (i.e., P-P-R versus P-R-R), A-A-A, conclusion of a bout with a single P (i.e., R-P-Z), and 3-mers including transitions of A to R and R to A (Figure 2.6). Repetition of R and a fewer number of P shifted individuals towards Rufous-like phenotypes, while repetition of P and single dives (either A or R) shifted individuals towards Allen's-like phenotypes (Figure 2.6). Combinations of consecutive P, R, and A tended to place hybrids in the middle of the Allen's and Rufous phenotypes on the dendrogram (Figure 2.5, 2.6). The 3-mer analysis detected fine-scale display bout differences, such as with hybrids that performed only single P (compared to multiple consecutive P in Allen's, and single dives (compared to multiple consecutive dives in Rufous). Although Allen's Hummingbird never began a bout with an A (bouts always began with a P or S), some hybrid phenotypes did (Figure 2.6, Table S2.2). Hybrids also performed fewer consecutive P (3.2±2.5, N=142 individuals) than Allen's Hummingbird (10.5±2.9, N=80 individuals). Additionally, although it was rare that Allen's Hummingbird ended a bout with a single P (<1% of the time), individuals across the hybrid zone consistently ended courtship displays with a single P (14% of the time). Transitions within 3-mers that characterized each behavioral phenotype at the level of the display (i.e., the displays the behavioral elements comprise) are described in Figures S2.1 and S2.2.

All display and element variants were present in at least one hybrid phenotype (Figure S2.2). Hybrid phenotypes incorporated the individual displays found within each parental species. For example, several hybrid phenotypes performed both R and A or R and P (Figure S2.2). Aborted dives occurred in 3% of all displays within hybrids (N=142 individuals), while they occurred <1% of the time in either parental species (N=162 individuals, Figure 2.3C, 2.3D, S2.4, Table 2.4). Many hybrid phenotypes also incorporated display variants and display transitions not observed in either parental species in bouts of display (Figure 2.7).

Repertoire coverage

I used 3-mer accumulation curves to estimate repertoire size and the number of bouts of display required to adequately sample the repertoire of each individual (Figure 2.8). An individual curve that asymptoted implied that the repertoire of the focal individual was completely sampled, while curves that did not asymptote were under sampled. Most Rufous and some Allen's individuals asymptoted after 10 bouts of display, although most hybrids did not (Figure 2.8).

Hybrid individuals were generally estimated to require the most sampling of bouts to sample 90% of their repertoire (21.6±10.5, N=142 individuals), while Allen's required more sampling (17.4±8.7, N=80 individuals) than Rufous (8.4±3.5, N=82 individuals). However, there was substantial variation across hybrids, as some individuals asymptoted within the expected interval of Rufous, Allen's, and beyond, and several hybrids never asymptoted (Figure 2.8). Overall, while 10 recordings of display bouts from Rufous Hummingbird were adequate to sample 90% of the repertoire, it was not adequate for Allen's Hummingbird or hybrids.

DISCUSSION

Allen's and Rufous Hummingbird varied in their courtship display sequences. Allen's and Rufous were comprised of eight discrete phenotypes each, while hybrids formed 39 phenotypes based on the sequences of their courtship displays. Hybrids were more variable within and between individuals relative to Allen's and Rufous (Figure 2.8). Individuals within hybrid phenotypes performed a mixture of displays between Allen's and Rufous within their bouts of display, and sometimes performed displays not observed within either parental species. Of the 16 variant displays I discovered, eight were found only in hybrids. Hybrids were also more variable than individuals from either parental species and required more data to effectively sample their repertoire than Allen's or Rufous.

Species phenotype differences

Allen's and Rufous Hummingbird have similar courtship displays formed from the same underlying behavioral elements; the differences in their courtship displays arise from re-ordering of sequences of these elements (Figure 2.1). Both species perform dives, with a single kinematic difference: A includes writhing on the ascent, while R does not. The repetition of each dive differs (Rufous usually performs several dives in a row, Allen's does not, Figure 2.6, Table 2.4). The k-mer and clustering analyses differentiated phenotypes based on the repetition of displays such as P-P-A versus A-A-A, as well as the presence and absence of a given display (Figure 2.6). For example, Allen's performed P, while Rufous did not, and some hybrid phenotypes performed P and R without A, while others incorporated P, R, and A (Figure S2.1, S2.2).

The concept of the fixed action pattern has provided a framework for animal behaviorists to study instinctive behavior (Russell et al. 1954). Although Allen’s and Rufous Hummingbird courtship displays are examples of a fixed action pattern (they are predictable and independent motor events), these displays were not completely invariant, as underlying elements were sometimes reorganized to form alternative versions of displays, especially in hybrids (Figure 2.3). Thus, the behavioral phenotype was a hierarchy of organization, where the order of elements determined the execution of the higher-level displays (Barlow 1967).

Hybrid phenotype differences

Offspring produced in the first generation (F_1 individuals) have equal proportions of their genomes from each parental species, but after multiple rounds of recombination from additional hybridization, individuals with varying amounts of genetic admixture are produced (Cortés-Ortiz 2017). The phenotypic variation exhibited by in displays individuals in the Allen’s × Rufous hybrid zone presumably reflects extensive recombination, which I will assess in future work.

Unlike with morphology, where quantifiable characters typically remain static with time, hybrid individuals performed sequences of behaviors which exhibited variation from one display to the next, and incorporated displays from one or both parents within a single bout. I found that hybrids tended to be more variable than either parent. Hybrids often interchanged performance of a typical or variant P, dive, or H (Figure 2.6). Allen's and Rufous performed bouts of display that were more repetitive than hybrids, although parental species uncommonly performed variant displays within a bout (Figure 2.6).

Hybrids performed bouts of display that were intermediate between each parental species and tended to be more plastic in their behavioral repertoires. During courtship, hybrids may combine elements from each parental species into a single bout of display. Across a Black-capped (*Poecile atricapillus*) × Carolina (*P. carolinensis*) Chickadee hybrid zone, some individuals sang mixed songs and combined elements representative of each parental species (Curry et al. 2007). Laboratory crosses and backcrosses between *Peromyscus* mice (*P. maniculatus* × *P. polionotus*) demonstrated that backcrossed individuals exhibited burrowing behavior more closely related to *P. maniculatus* (the parental they were backcrossed with), although behaviors characteristic of *P. polionotus* were still present (Weber et al. 2013). Importantly, these burrowing behaviors were innate and genetically controlled. Here, hybrid individuals performed bouts of display comprised of combinations of one or more parental displays. For example, individuals often combined Allen's and Rufous dives into a single bout of display (Figure 2.6). Similarly, many individuals performed P and R during a single bout.

When hybrids backcross with either parent, the genomes of backcrossed individuals are more representative of one parental species than the other (Weber et al. 2013). Under the assumption that courtship behavior in Allen's and Rufous Hummingbird is genetically controlled, hybrid phenotypes that incorporate particular displays from a parental species less often than what is reported in that parental species may be due to backcrossing of hybrids. Within the typical Allen's phenotype a P was followed with another P 83% of the time (N=30 individuals); in hybrid phenotypes, P was repeated less often and appeared only within select 3-mers (i.e., some hybrids only performed S-H-P or H-P-Z, lacking the variety present in Allen's, while adding unique 3-mers such as P-P-R, Figure 2.6). The transition probabilities provided by the MC were also helpful to observe how often displays and their variant displays occurred within Allen's, Rufous, and hybrid phenotypes found by k-mer analysis (Table S2.2).

The integration of individual courtship displays by hybrids from either parental species reflects the modular nature of courtship in Allen's and Rufous Hummingbird. The ordering of lower-level display elements formed the basis of differences in displays, and the sequences of these displays within a bout made up the phenotypes found in the dataset. Hybridization produced a reorganization of elements in individuals across the Allen's × Rufous hybrid zone, which formed displays not observed in either parental species. Further, hybrids performed sequences of displays undocumented in either parental species within their bouts (Figure 2.6).

Several processes contribute to the presence of greater phenotypic diversity in hybrids, including transgressive segregation, simple mutation, and recombination of preexisting genetic variation (Tobler and Carson 2010). I observed that hybrids performed displays representative of both parental species in addition to previously undocumented displays. There was increased behavioral diversity in hybrids, documented by hybrid 3-mer accumulation curves that often failed to asymptote (Figure 2.8). As with Carola's Parotia and *Habronattus* jumping spiders, behavioral modularity may be a driver of the evolution of courtship behavior in Allen's and Rufous Hummingbird (Huxley 1914, Scholes 2006, Elias et al. 2012, Weber et al. 2013).

Novel displays within the hybrid zone

The individuals observed across the Allen's × Rufous hybrid zone performed displays that were comprised of the same behavioral elements found in each parental species, although the order in which they occurred differed from that of Allen's and Rufous. Six variant displays were comprised of different behavioral elements than previously documented in either parental species, violating Remane's position criterion (Remane 1952). Thus, these variant displays, P_X, P_W, P_D, H_{XT}, H_W, and A_L, are novel (Figure 2.6, S2.3). Non-novel display variants included A_X, A_A, A_M, R_A, R_X, R_L, R_E, R_M, H_E, and H_X. Mini-dives, where males performed Allen's or Rufous dives with a long ascent and long descent with a relatively low amplitude, are not novel because the same behavioral elements were present, in the same order, as in the dives of both parents (Figure 2.6, 2.7).

Transgressive behaviors within the hybrid zone

Transgressive phenotypes might occur due to recombination and the interaction of genes that are responsible for different traits (Hegarty 2012). Four individuals incorporated transgressive displays into their bouts of display, and all of these individuals performed transgressive displays at least twice, demonstrating these traits were repeatable parts of the display repertoire (Figure 2.6, 2.7). The Allen's and Rufous mini-dives (A_M, R_M) are transgressive because, although the same underlying elements occurred in the same order (there was no rearrangement of behavioral elements, as with novel characters) as either parental species, the expression of these displays was never observed in either parental species. The long ascent and long descent in both types of mini-dives were noticeably lower in amplitude than what was observed in either parental species (Figure 2.3, 2.7, Table 2.2). The amplitude of the long ascent and long descent were low enough to prevent the focal bird from ascending high enough before descending to reach the critical velocity required to make the dive sound audible (Figure 2.3, Table 2.2). Thus, as the position of elements that comprised some variant displays made them novel, the extreme expression of A_M and R_M made them transgressive (Figure 2.7).

A widespread transgressive transition *between* displays occurred within hybrid populations: the proportion of pendulums occurring after dives. This transition was observed consistently across the hybrid zone (14%, N=142 individuals), and was considerably higher than pendulums after dives observed in Allen's (2%, N=80 individuals) and Rufous (0%, N=82 individuals) populations (Figure S2.4). This transition is transgressive because it was expressed more often in hybrids than either parent species. No Rufous Hummingbird phenotype incorporated P into any 3-mer; thus, it did not perform P after dives. When Allen's did perform a P after a dive, it performed several P before ascending for another dive (A-P-P, then P-P-P, then P-P-A). I rarely observed Allen’s Hummingbird perform the 3-mer A-P-Z (the conclusion of a bout of displays with a P immediately after a dive, <1%, N=80) across its breeding range. Individuals across the hybrid zone consistently performed the 3-mer A-P-Z. In one population (N=15 individuals), 26% of the P that occurred followed a dive, and most of these concluded a bout of courtship displays, exceeding any parental Allen’s Hummingbird individual or population.

Finally, 17 individuals performed transgressive chirrup numbers within a display by exceeding the upper limit of the range observed in Allen’s and Rufous Hummingbird, which was 16 post-shuttle chirrups and 12 post-dive chirrups (Table 2.3). These transgressive chirrup numbers occurred in 11 individuals during H (post-shuttle chirrups), where one individual performed up to 24 chirrups. Six individuals performed a transgressive number of chirrups during a dive (post-dive chirrups).

Other potentially trangressive traits identified in hummingbird courtship displays were in the dive displays of a male Broad-tailed × Black-chinned Hummingbird hybrid and Anna's × Costa's hybrids, although small sample sizes and undersampling of parental species behavior cannot extinguish the possibility that the behaviors of these hummingbird crosses are not transgressive (Wells et al. 1978, Clark et al. 2012). Here, these caveats do not apply; the patterns observed here are backed up with robust sample sizes of both the parental forms as well as hybrids.

Display function

Courtship displays function to attract potential mates, and are a form of communication (Mulder 1997, Hurly et al. 2001). Likely, the displays of Allen’s and

Rufous Hummingbird are shaped by female choice. One courtship display—the shuttle display—seemed to be co-opted with courtship to serve as a source of aggression when a rival male entered a focal male's territory (in many hummingbirds, aggressive displays and courtship displays can be difficult to differentiate: Skutch 1972, Mobbs 1982). When an intruding male appeared in a focal male's territory, and the focal male noticed, he had two responses: he would chase the intruder out of his territory or perform a shuttle to the intruder, following the intruder until he left the territory.

Although there are no described differences in S between Allen's and Rufous, when recording a focal male displaying to wild stimuli, such as another male intruding upon the territorial male or the presence of a wild female, S seemed to vary in length to wild stimuli compared to when displays are directed at the caged female. Because the duration of S was not a character I investigated, this phenomenon did not bias my results, with the exception that I omitted "low effort" S displays from my dataset.

With the exception of S, wild stimuli versus the caged female also did not affect my results. If a male lost interest in a caged female, he generally either stopped performing bouts of display to her entirely or performed low effort S, which were omitted. I observed displays and display variants whether the female target was wild or captive. Finally, most displays were to the caged female, so although captive females are unnatural stimuli, the effects of any undetected differences between displays to wild versus captive stimuli were negligible.

Biological applications of behavior

Due to the stereotyped, innate nature of behaviors across each parental species' range, and the widely variable behaviors observed in hybrids, the courtship displays within Allen's, Rufous, and their hybrids are probably genetically controlled (Weber et al. 2013). The different displays in Allen's and Rufous may have arose via duplication and rearrangement of behavioral elements, which Scholes (2008) proposed may be responsible for much of the behavioral diversity within the bird-of-paradise family *Parotia*.

When a modular trait is duplicated, the duplicated trait might become a paramorph, which is a trait that is duplicated, diverges, and then attains its own function relative to other traits. In this case, trait A is duplicated (A → AA), followed by the modification of the duplicated trait A that differentiates into trait B (AA → AB), a novel character. For example, the multiple digits of the hand are duplicate paramorphs and are a source of morphological diversity (Müller and Wagner 1991, Wagner 2014).

Members of *Selasphorus* perform courtship dives and shuttle displays for prospective mates (Clark 2014, Clark et al. 2018). Further, there are kinematic similarities between A and P: A consists of the element sequence *a, b, c, d, g, e, b, c, g*, while P consists of the element sequence *a, b, c, g* (Figure 2.1). Thus, the entire element sequence of P is embedded within A, and prompts a fundamental biological question: how did the apparent differences between two traits (P and A) evolve at the genetic level?

Genetic homology is defined in terms of shared ancestry, where single gene copies are transmitted from parent to offspring from one generation to the next (Wagner 2014). Deviations from genetic homology occur as a result of a speciation event, when a gene diverges but conserves its main function (an orthologous gene), or as a result of a gene duplication event (a paralogous gene, Müller and Wagner 1991, West-Eberhard 2003, Pigliucci 2008, Wagner 2014).

With a definition of novelty based on non-homology, novelty may arise through the duplication of a modular trait into paramorphs, followed by the differentiation of these paramorphs relative to each other, and the formation of a novel trait (Müller and Wagner 1991, Wagner 2014). These paramorphs might be caused by one or more paralogous genes, where a gene gives the identity of a trait (gene *a*), followed by a gene duplication (gene *a*, gene *a*), and subsequent differentiation of one gene copy relative to the other (gene *a*, gene *b*). In this case, the paralogous gene (gene *b*) might give rise to a new trait identity.

Working within preceding framework of the formation of novelty, I hypothesize that the pendulum display in Allen's Hummingbird is a modified dive that became an independent character through duplication and differentiation, making it a paramorph (A -> AA -> AP), and thus an evolutionarily novel trait that is not documented in Rufous Hummingbird or any outgroup. This could be due to a) the ancestral paralog hypothesis, where P is given identity by an allele of a gene that is present in both species and is paralogous to another gene present in both species, b) the recent paralog hypothesis, where P is given identity by a paralogous gene present in Allen's and not Rufous, or c) the outside gene hypothesis, which is not based on paralogy, where P is produced by a gene that was formerly (i.e., in Rufous) not involved in production of the display phenotype.

CONCLUSIONS

In Chapter 1, I classified individuals as hybrids or parental Allen's and Rufous Hummingbird based on existing and newly discovered diagnostic behavioral and morphological traits. In Chapter 2, I incorporated these classifications and diagnostic behavioral traits into behavioral sequence analysis to distinguish sequences of courtship displays in hybrids that differed from those performed by Allen's and Rufous. Because individuals were classified *a priori*, it was known whether the phenotypes of individuals identified in Chapter 2 belonged to Allen's, Rufous, or their hybrids. Additionally, identification of individuals before display sequence phenotype classification ensured that individuals who performed uncommon displays, for example aborted dives, in parental Allen's and Rufous, were not clustered as part of a hybrid phenotype based on the performance of these uncommon displays. I analyzed the courtship sequences of Allen's and Rufous Hummingbird outside of the hybrid zone, and Allen's x Rufous individuals within the hybrid zone. I assigned individual Allen's, Rufous, and Allen's x Rufous behavioral phenotypes based on the sequences of their courtship displays by implementing k-mer analysis for the first time in animal behavior to identify 39 behavioral phenotypes. I also identified numerous novel and transgressive courtship displays across the contact zone. Hybrids were also more variable and required more display bouts on average (16 bouts) to sample 90% of their repertoire in comparison to both parental species (11 bouts for Allen's, nine bouts for Rufous). Given that I usually sampled about 10 bouts of display per individual, several additional phenotypes likely exist beyond what I described in this chapter. Finally, the modular nature of the displays within Allen's and Rufous

Hummingbird shows that the reorganization of behavioral elements during courtship might play a role in diversification between these species.

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FIGURES AND TABLES

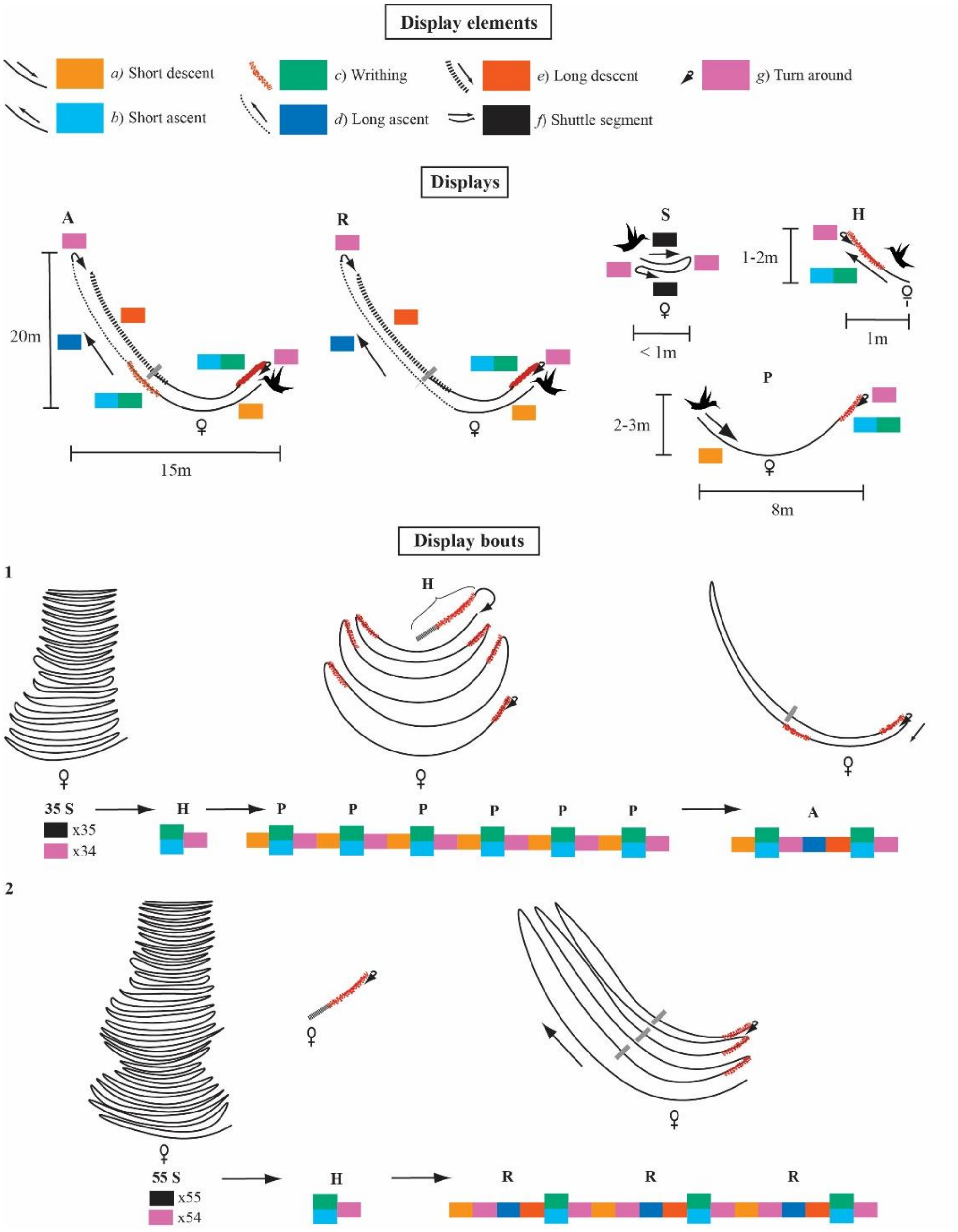
Display elements
a) Short descent
b) Short ascent
c) Writhing
d) Long ascent
e) Long descent
f) Shuttle segment
g) Turn around
Displays
A
20m
♀
15m
R
♀
S
♀
< 1m
H
1-2m
♀
1m
P
2-3m
♀
8m
Display bouts
1
♀
H
♀
♀
35 S
x35
x34
H
P
P
P
P
P
P
A
2
♀
♀
♀
55 S
x55
x54
H
R
R
R

Figure 2.1 – **Behavioral elements, courtship displays, and typical bouts of courtship displays of Allen's and Rufous Hummingbird, represented as both line drawings (actual flight trajectory during display), and as colored boxes representing individual elements.** Lowercase, italicized letters and colored blocks refer to display elements, as defined in Table 2.1 and as follows: *a* (orange block)=short descent, *b* (light-blue block=short ascent*,* *c* (green block)=bout of writhing, *d* (blue block)=long ascent, *e* (red block)=long descent, *f* (black block)=shuttle display, *g* (purple block)=turn-around, gray hashmark=dive sound. Red lines represent a bout of writhing. The Allen's dive (**A**) contains writhing on the ascent for the dive, while the Rufous dive **(R**) does not. The shuttle (**S**) and half pendulum (**H**) displays are present in both species. The pendulum display (**P**) is not found in Rufous Hummingbird (see also Table 2.1, 2.2). **(1)** Typical Allen's Hummingbird display sequence: a series of S, H, several P, and A. **(2)** Typical Rufous Hummingbird display sequence: a series of S, H, and consecutive R.

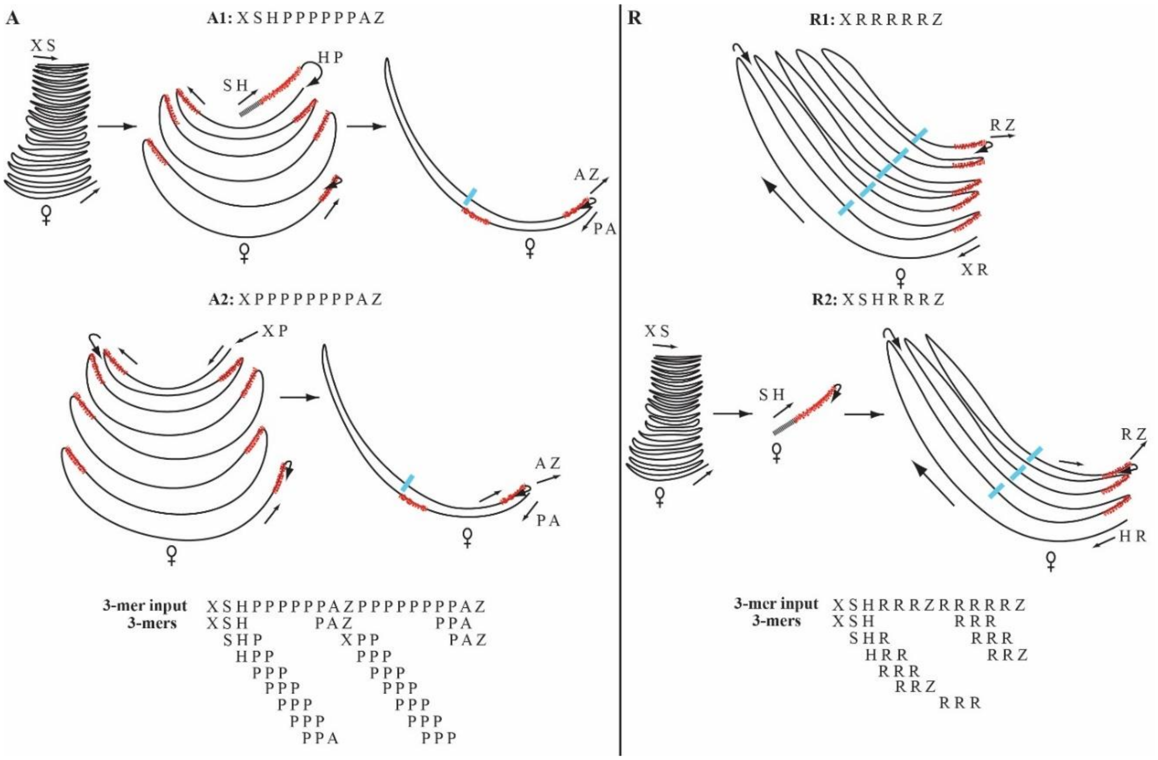

Figure 2.2 – **Example inputs for kcount matrix construction and resultant 3-mers.** In Allen's Hummingbird **(A)**, and Rufous Hummingbird **(R)**, two bouts of courtship display sequences (Allen's: A1, A2, Rufous: R1, R2) were combined into sequential bouts of courtship displays, which are comprised of underlying behavioral elements. Courtship displays are abbreviated as follows: X=start of bout, S=shuttle, H=half pendulum, P=pendulum, A=Allen's dive, R=Rufous dive, Z=end of bout and/or a transition one from display bout to the next. The two displays were concatenated into a single sequence for input into the kcount matrix, where all 3-mers in each sequence are visualized (k=3, every unique sequence of three displays).

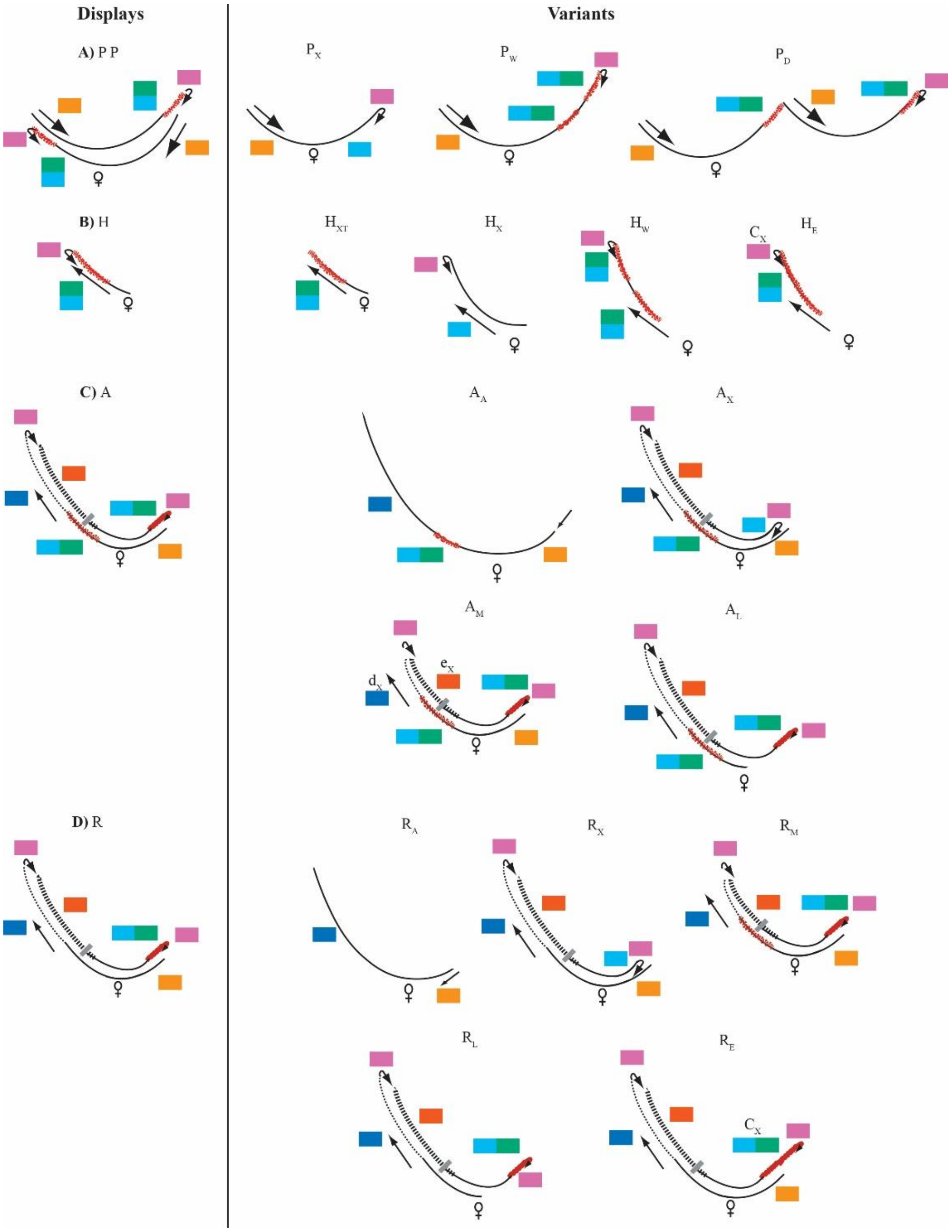
Displays
Variants
A) P P
P_X
P_W
P_D
♀
B) H
H_{XT}
H_X
H_W
C_X
H_E
♀
C) A
A_A
A_X
A_M
e_X
d_X
A_L
♀
D) R
R_A
R_X
R_M
R_L
R_E
C_X
♀

Figure 2.3 – **Courtship display variants of typical displays** (**A=pendulum, B=half pendulum, C=Allen's dive, D=Rufous dive)**. Typical versions of each display are listed as **A**, **B**, **C**, and **D** in the left column, while variants are expressed on the right column. Pendulum with no writhing (P_X), where an individual performed the 'short descent' and 'short ascent' found in the pendulum without performing 'writhing' during the 'short ascent', pendulum with double writhing (P_W), which occurred when a male performed the usual short descent, then short ascent with writhing in a pendulum that was followed by an additional short ascent with writhing in the same direction, the double pendulum (P_D), which consisted of a single pendulum followed by another pendulum in the same direction, the half pendulum display with no turn-around (H_{XT}), the half pendulum with double writhing (H_W), which involved a male performing the usual short ascent with writhing, followed by an additional short ascent with writhing in the same direction, the half pendulum with no writhing (H_X), which involved a male performing the usual short ascent in the absence of a bout of writhing, the half pendulum with extended writhing (H_E), where a male performed a bout of writhing during a half pendulum display that was beyond the 95% confidence interval of a typical bout of writhing in either parental species (Table 3), aborted Allen's dive (A_A), which included writhing on the ascent followed by a long ascent, with a subsequent turn-around and descent never taking place, Allen's dive with no writhing (A_X), where there is writhing on the ascent but no writhing after the dive, Allen's mini-dive (A_M), which was kinematically similar to the Allen's dive, except the long ascent and long descent were much shorter than usual (elements d and e respectively), resulting in a faint or inaudible dive sound, Allen's dive without an initial short descent (A_L), where the male began the display with a short ascent, followed by a long ascent, the aborted Rufous dive (R_A), with no writhing on the long ascent and a subsequent descent never taking place, Rufous dive with no writhing (R_X), where there was no writhing on the ascent or after the dive, Rufous mini-dive (R_M), which was kinematically similar to the Rufous dive, except the long ascent and long descent were much shorter than usual (elements d and e respectively), resulting in a faint or inaudible dive sound**,** Rufous dive without an initial short descent (R_L), where the male began the display with a long ascent, Rufous dive with extended writhing (R_E), where a male performed a bout of writhing following a dive display that was beyond the 95% confidence interval of a typical bout of writhing following a dive in either parental species (Table 2.3). Lowercase letters and colored blocks refer to display elements, as defined in Table 2.1 and visualized in Figure 2.1.

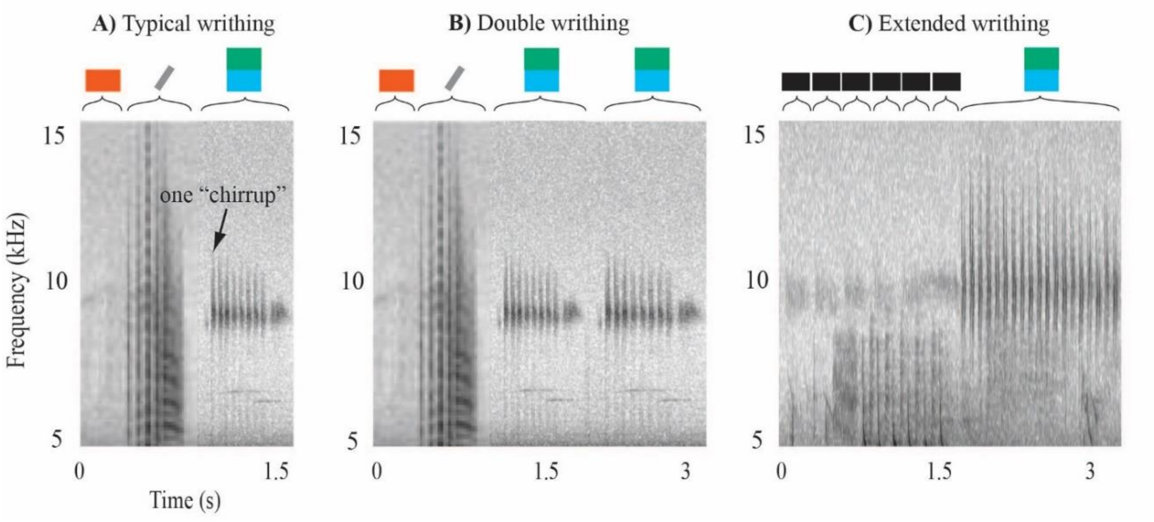

Figure 2.4 – **Forms of writhing: A) typical writhing, B) double writhing, and C) extended writhing**. **A)** writhing following long descent of a Rufous dive. **B)** double writhing (two bouts of writing interspersed with a short pause) following the long descent of a Rufous dive. **C)** extended writhing performed during a half pendulum, following a shuttle display. The 95% CI of typical writhing after a dive across Allen's and Rufous was 2.47-7.31 and 4.30-10.54 respectively, and 4.84-9.64 and 8.29-15.65 following a half pendulum (Table 2.4). There is a 1:1 match between the movement of the bird and each sound made by the bird (see Clark 2009 and Myers et al. 2019). Lowercase letters and colored blocks refer to display elements, as defined in Figure 2.1 and Table 2.1.

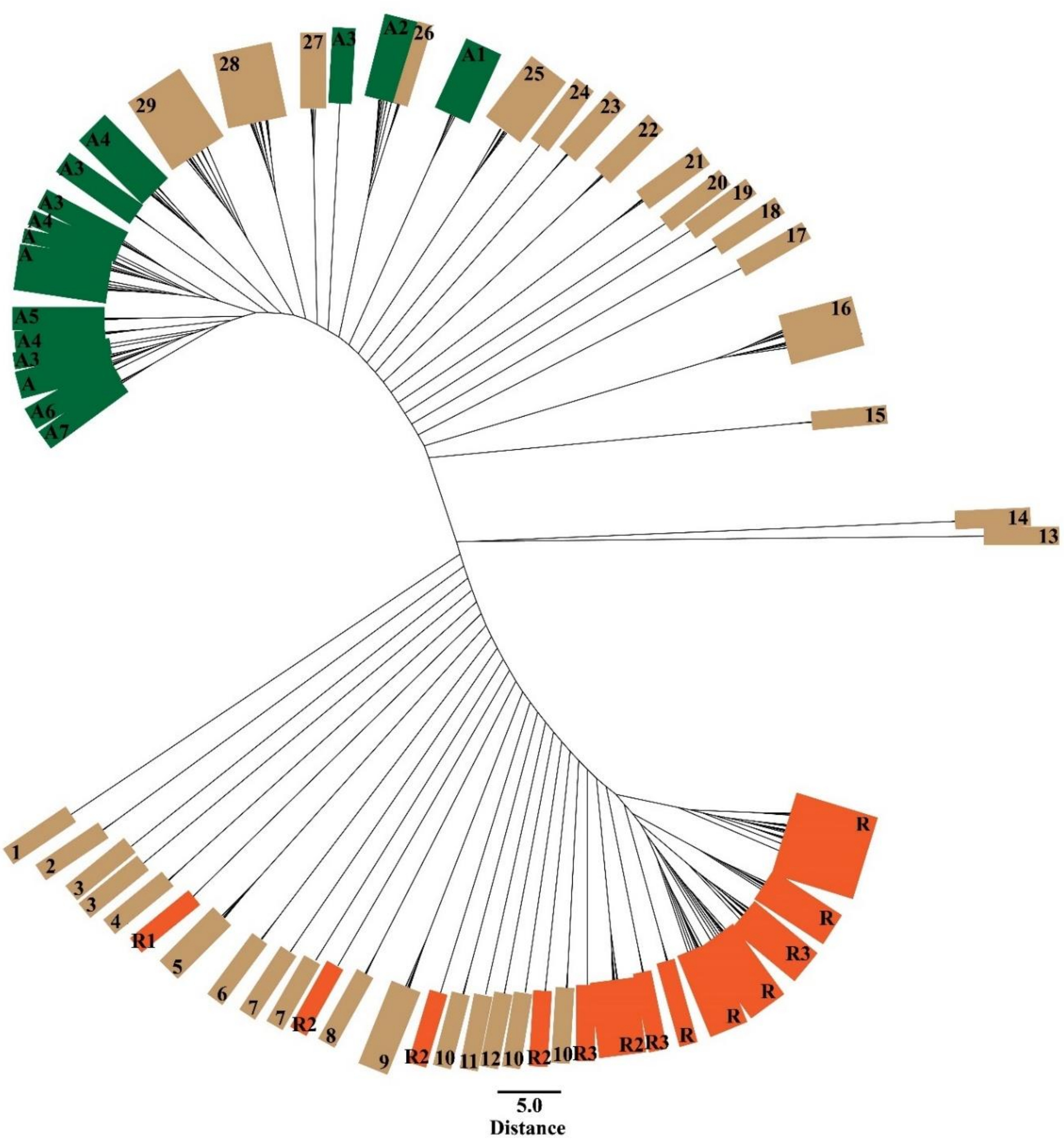

Figure 2.5 – **Unrooted dendrogram resulting from kcount matrix and hierarchical clustering analysis of 3-mers.** The analysis separated the 304 individuals in the dataset into 29 hybrid phenotypes; I found 39 hybrid phenotypes after incorporating rare displays omitted from the k-mer analysis (Figure 2.6). Brown boxes: hybrid phenotypes, orange boxes: Rufous Hummingbird phenotypes, green boxes: Allen's Hummingbird phenotypes. For Allen's and Rufous, "A" and "R" with numbers beside them denote phenotypes that are present. Phenotype 1 for each species was the most common.

Figure 2.6 **– 3-mer presence in Allen's (green), Rufous (orange), hybrids (brown), and multiple groups of phenotypes (overlap).** Bolded 3-mers indicate those present in <1% of all 3-mers within Allen's (green and brown overlap) and Rufous (orange and brown overlap) only, while bold + italicized 3-mers indicate 3-mers present in <1% of Allen's + hybrids or Rufous + hybrids. Bolded 3-mers within hybrid phenotypes were present in <1% of all 3-mers in hybrids. No 3-mer was present in a parental species that was not observed in a hybrid.

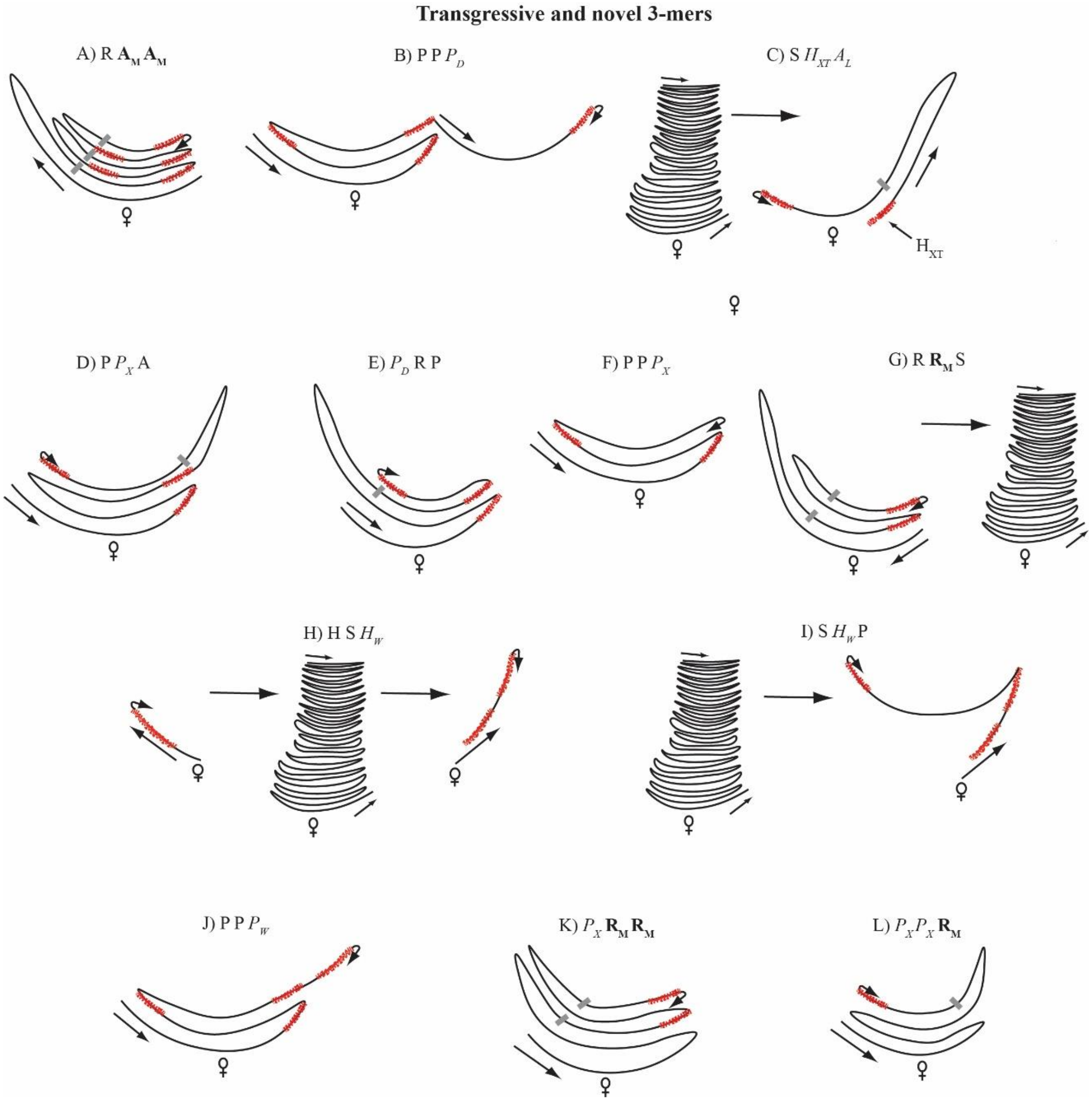

Figure 2.7 – **Line drawings of various 3-mers incorporating transgressive (bold) and novel (italicized) displays absent from both Allen's and Rufous Hummingbird.** Transgressive traits include the Rufous mini-dive (R_M) and Allen's mini-dive (A_M), while novel displays include pendulum with no writhing (P_X), P with double writhing (P_W), double P (P_D), half pendulum with no turn-around (H_{XT}), H with double writhing (H_W), and Allen's dive without the short ascent (A_L). Dive sound is designated by a gray hashmark, while red squiggles indicate writhing.

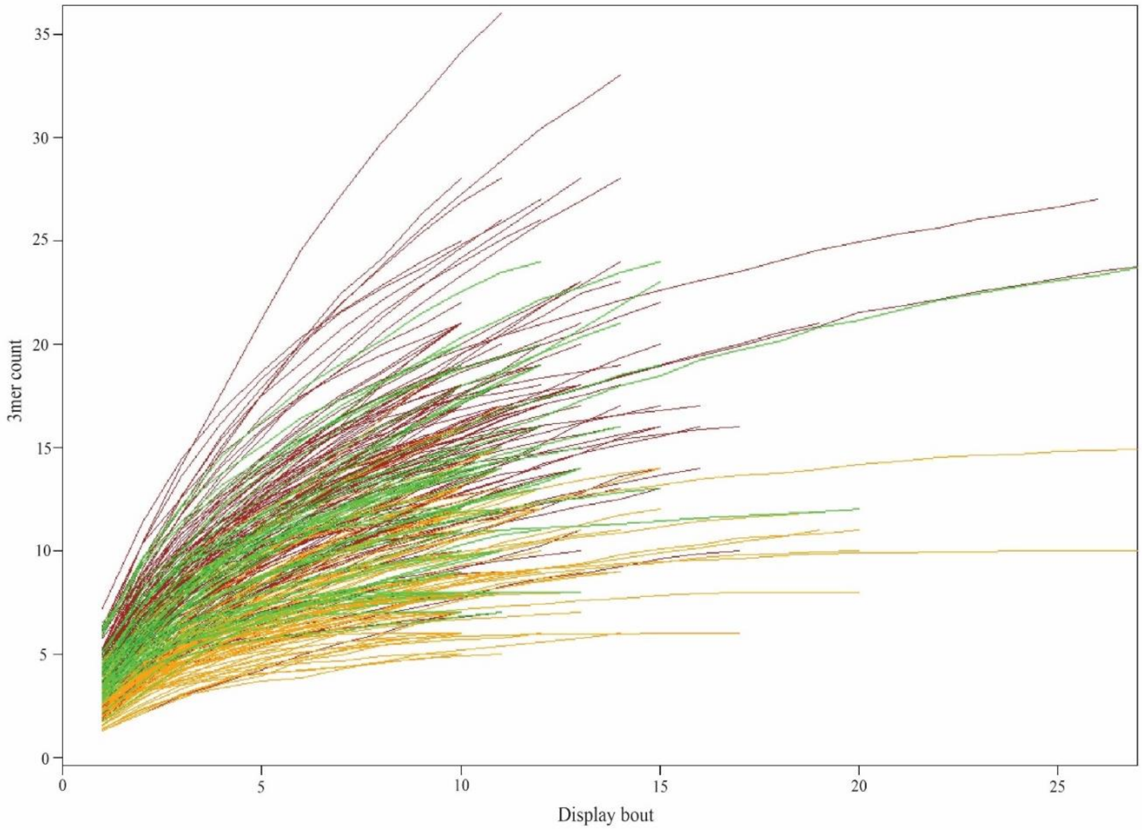

Figure 2.8 – **Expected 3-mer count accumulation curves of all Allen's (green), Rufous (orange), and hybrid (brown) individuals as sampling (in terms of display bouts) increases.** Most Rufous individuals asymptoted, many Allen's asymptoted, and many hybrids failed to asymptote. Hybrid individuals generally required more bouts of display to sample 90% of the repertoire (21.6±10.5) than Rufous (8.4±3.5) and Allen's (17.4±8.7), while Allen's generally required more sampling than Rufous.

Table 2.1 – **Definitions of behavioral elements.** ID refers to display elements in Figure 2.1. See also Myers et al. (2019).

Display element	ID	Description
Short descent	*a*	Descent of about 2-3m in a shallow half-U shape.
Short ascent	*b*	Ascent of about 2-3m in a shallow half-U shape.
Writhing	*c*	Consists of individual chirrup sounds made by the wings while the bird shudders up and down while flipping its tail.
Long ascent	*d*	During a dive display, an ascent of about 20m. Follows either a short descent (Allen's dive) or short ascent (Rufous dive).
Long descent	*e*	During a dive, after element g, male descends, traces similar path as ascent, spreads tail feathers near end of descent, produces dive sound; finishes descent.
Shuttle segment	*f*	Occurs within a shuttle display, as measured by a spectrogram; segments occur after a left-to-right or right-to-left motion, with sound made by the wings. Shuttle displays are composed of multiple shuttle segments.
Turn-around	*g*	Male turns around roughly 180 degrees, so that a subsequent movement is in the opposite direction of the previous movement.

Table 2.2 – **Definitions of display behaviors.** ID refers to displays in Figure 2.3. See also Myers et al. (2019).

Display	ID	Element sequence
Start of display bout	X	*Not applicable*
End of bout/transition to next bout	Z	*Not applicable*
Rufous dive	R	*a-d-g-e-b-c-g*
Rufous dive (no writhing at end)	R_X	*a-d-g-e-b-g*
Rufous dive (aborted)	R_A	*a-d-g*
Rufous dive (mini)	R_M	*a-d-g-e -b-c-g*
Rufous dive (no short descent)	R_L	*d-g-e-b-c-g*
Rufous dive (extended writhing)	R_E	*a-d-g-e-b-c-g*
Allen's dive	A	*a-b-c-d-g-e-b-c-g*
Allen's dive (no writhing at end)	A_X	*a-b-c-d-g-e-b-g*
Allen's dive (aborted)	A_A	*a-b-c-d-g*
Allen's dive (mini)	A_M	*a-b-c-d-g-e-b-c-g*
Allen's dive (no short descent)	A_L	*b-c-d-g-e-b-c-g*
Pendulum	P	*a-b-c-g*
Pendulum (no writhing)	P_X	*a-b-g*
Double pendulum	P_D	*a-b-c-a-b-c-g*
Pendulum (double writhing)	P_W	*a-b-c-b-c-g*
Shuttle	S	*f-g*
Half pendulum	H	*b-c-g*
Half pendulum (no writhing)	H_X	*b-g*
Half pendulum (double writhing)	H_W	*b-c-b-c-g*
Half pendulum (extended writhing)	H_E	*b-c-g*
Half pendulum (no turn-around)	H_{XT}	*b-c*

Table 2.3 – **Post-shuttle chirrups and post-dive chirrups of characters of male Allen's and Rufous Hummingbird and their hybrids.** The 95% CI is based on two standard deviations from the mean.

Character	Allen's (n = 80)	Rufous (n = 82)	Hybrids (n = 142)
Post-shuttle chirrups	4.84-9.64 (7.24 ± 1.20)	8.29-15.65 (11.97 ± 1.84)	3.43-15.27 (9.35 ± 2.46)
Post-dive chirrups	2.47-7.31 (4.89 ± 1.21)	4.30-10.54 (7.42 ± 1.56)	2.90-8.46 (5.68 ± 1.39)

Table 2.4 – **Frequency of occurrence (%) of transitions of displays found within Allen's and Rufous Hummingbird given by the First-order Markov Chain.** Starred transitions indicate transitions that were significantly different between Allen's and Rufous (P < 0.05, unpaired, two-tailed, two sample t-test).

Transition	Rufous (N=82)	Allen's (N=80)
Pendulum to Allen's dive*	0	8
Pendulum to pendulum*	0	75
Start to Allen's dive	0	0
Allen's dive to Allen's dive	0	<1
Allen's dive to pendulum to end	0	2
If Allen's dive present in bout, occurred after pendulum*	0	94
Half pendulum to pendulum*	0	63
Half pendulum to shuttle	16	6
Half pendulum to end	44	31
Half pendulum to Rufous dive*	40	0
Rufous dive to Rufous dive*	55	0
Start to Rufous dive*	60	0

CHAPTER 3

Widespread connectivity and recent evolutionary history within and between the Allen's (*Selasphorus sasin*) and Rufous Hummingbird (*S. rufus*)

INTRODUCTION

Incomplete lineage sorting and gene flow are two evolutionary phenomena that adversely affect biological interpretation. Both incomplete lineage sorting and gene flow lead to individual gene trees that disagree with the species tree (Hudson 1983, Tajima 1983, Slatkin and Maddison 1989, Rannala and Yang 2008, Leaché et al. 2014). Investigators are generally aware of, and typically address, the issue of incomplete lineage sorting (Maddison and Knowles 2006, McCormack et al. 2009, Leaché and Rannala 2011). However, the effects of gene flow have been given less attention historically despite the fact that gene flow, when unaccounted for, may also compromise results (Slatkin and Maddison 1989). When species hybridize, recent gene flow may make inferences related to deeper evolutionary history more problematic, as introgression beyond known areas of hybridization affects inferences related to phylogeny (Leaché et al. 2014). With recent gene flow, historical realities are obscured by current genomic similarity and may not accurately represent evolutionary relationships. Further, divergence time is underestimated, and effective population size overestimated (Leaché et al. 2014).

Hybridization is a prominent evolutionary force with the potential to influence populations beyond areas of overlap. In some cases, hybridization and introgression are documented to adversely affect parental species, especially in species with relatively small ranges. For example, hybridization in Lake Victoria in East Africa led to a 70% drop in species diversity in cichlid fish (Witte et al. 2013). In another case, genetic sampling across the range of the obligate estuarine Black Bream (*Acanthopagrus butcheri*) found that, due to hybridization with the migratory marine Yellowfin Bream (*A. australis*), only 5% of 970 sampled fish were parental *A. butcheri*, making hybridization and introgression a threat to its persistence (Roberts et al. 2010). Alternatively, genetic rescue provides a more optimistic outcome of gene flow, where introgression from hybrids that are better adapted to novel habitat may increase the fitness of parental populations with small ranges (Whiteley et al. 2015). For example, translocations of Pumas (*Puma concolor stanleyana*) from Texas into a Florida Panther (*P. c. coryi*) population increased genetic variation and population growth responses of the Florida Panther (Johnson et al. 2010). Gene flow can also be neutral, where introduced variants do not have any fitness effects, and thus show no effects on parental populations (Holderegger et al. 2006). Given the radically different potential outcomes of gene flow, analyses that quantify the extent of introgression in a given system as well as areas of the genome that freely introgress versus those under selection, are fundamental to evolutionary biology.

Migratory Allen's Hummingbird (*Selasphorus sasin sasin*), which breeds along the coast from approximately Ventura County in southern California to Coos County in southern Oregon (Grinnell and Miller 1944), forms a hybrid zone with Rufous Hummingbird (*S. rufus*) centered in Coos County (Figure 3.1, Myers et al. 2019). All individuals in Coos County are phenotypically hybrid or Rufous; thus, the northern extent of the breeding range of migratory Allen's is limited to Curry County, OR, where Allen's Hummingbird and hybrids are diagnosed (Myers et al. 2019).

Non-migratory Allen's Hummingbird (*S. s. sedentarius*) was formerly endemic to the Channel Islands of southern California (Grinnell 1939, Grinnell and Miller 1944), but apparently colonized the mainland on the Palos Verdes Peninsula around 1966 (Wells and Baptista 1979). Subsequently, it has rapidly expanded its range on the mainland and now occurs as far south as northern Baja California, north to Santa Barbara County, and east to Riverside County (Figure 3.1, Wells and Baptista 1979, Unitt 2004, Clark 2017). As a result, the two subspecies of Allen's Hummingbird have recently come into contact in Santa Barbara or Ventura County, but the extent of interaction and gene flow between the two subspecies is unknown. Thus, the limited range of migratory Allen's, coupled with the possibility of hybridization on the northern (with Rufous) and southern (with non-migratory Allen's) end of its range, warrants investigation of the extent of introgression by Rufous and potential intergradation between non-migratory and migratory Allen's populations.

If gene flow across the migratory Allen's, non-migratory Allen's, and Rufous Hummingbird complex is high, previous inferences of the evolutionary history of this group may be flawed. Here, by incorporating individuals known to lack admixture with Rufous Hummingbird into my analyses (see Methods section), I evaluate the evolutionary history of migratory Allen's, non-migratory Allen's, and Rufous Hummingbird by testing three scenarios. 1) The null hypothesis, that non-migratory and migratory Allen's are each other's closest relative, with Rufous Hummingbird as the outgroup. 2) Due to the large proportion of the genome shared by migratory Allen's, non-migratory Allen's, and Rufous (see Results), I evaluate the hypothesis that migratory Allen's is a hybrid taxon of Rufous Hummingbird and non-migratory Allen's. 3) Given that migratory Allen's and Rufous form a large hybrid zone and that previous work failed to resolve the relationship between migratory Allen's and Rufous Hummingbird (Licona-Vera and Ornelas 2017), I also test the hypothesis that migratory Allen's and Rufous are each other's closest relative, with non-migratory Allen's as their most recent ancestor. I estimate divergence dates of Allen's and Rufous Hummingbird, which have been variably estimated at 5,000-100,000 years ago (Battey 2019) and about 300,000 years ago for the Allen's and Calliope (*S. calliope*) Hummingbird ancestor from Rufous (McGuire et al. 2014).

Given the potential effects of hybridization on surrounding parental populations and on historical inference of evolutionary history, I explore the effects of hybridization on parental Allen's Hummingbird populations. The limited range and high instances of hybridization reported within Allen's Hummingbird motivates the current study, which includes data spanning the range of migratory and non-migratory Allen's Hummingbird and includes sporadic Rufous Hummingbird populations for reference. My dataset prompts several questions. (1) Do migratory and non-migratory Allen's intergrade in southern California? (2) What are the effects of gene flow from non-migratory Allen's and Rufous on the genome of migratory Allen's Hummingbird? Do any regions of the genome serve as barriers to gene flow? (3) Has recent gene flow obscured previous inference of the evolutionary history of Allen's and Rufous Hummingbird?

METHODS

Sampling

I sampled tissue (N=66) and blood samples (N=38) from 103 individuals along a north-south transect from southern California to northern Oregon, and from a number of counties outside of the transect (California: San Diego, Riverside, Los Angeles, Santa Barbara, San Luis Obispo, Monterey, Mendocino, Humboldt, Del Norte, and Siskiyou counties, Oregon: Curry, Coos, Douglas, Lane, Clatsop counties, Figure 3.1, Table S3.1). I supplemented sampling with 29 tissues from museum collections (total N=133, Figure 3.1, Table S3.1). Collection of samples in the field occurred from March through May (2014-2018) and museum specimens dated from March through May were used in my dataset. To address gene flow and evolutionary history, I incorporated several Rufous Hummingbird (N=9, Clatsop County, OR) and hybrids (N=66, Curry, Coos County, OR, and Humboldt, Del Norte, Siskiyou County, CA) into my analyses. The 103 individuals I gathered data from were diagnosed based on their occurrence within or beyond the extent of the phenotypic hybrid zone, which was described using morphological and behavioral data using measurements described in Myers et al. (2019).

DNA Extraction and Whole Genome Sequencing

I extracted genomic DNA from tissues and dried blood spots using a Qiagen DNeasy Blood and Tissue Kit, following the recommendations of the manufacturer (Qiagen, Valencia, California, USA). Library preparation was based on a modified version from Baym et al. (2015). I sequenced whole genomes of all individuals using an Illumina NextSeq 500 at the University of California, Riverside Genomics Core or

Illumina HiSeq X at Novogene, Inc. with an average depth of 4x per sample (Table S3.2). Reads were aligned to the Anna's Hummingbird reference available on NCBI using the software package BWA v0.7 (Burrows-Wheeler Aligner, Li and Durbin 2009, NCBI Resource Coordinators 2018). I called variants using SAMtools v1.9 and BCFtools v1.9 (Li et al. 2009, Narasimhan et al. 2016). I filtered and retained variants with a minimum depth of one, that were successfully genotyped in at least 50% of individuals, had a minimum mapping quality score of 30, and a minor allele frequency of 0.05 using VCFtools v1.16, resulting in 1,770,572 SNPs in the dataset (Danecek et al. 2011).

Population Structure

I investigated population structure by implementing a principal component analysis (PCA) on the genomic data. I used PCA, a model-free method based on variation in allele frequencies, to detect patterns of genetic structure using ggplot2 (Wickham 2016) in R v3.5.0 (R Core Team 2018) and R Studio v1.2.5 (R Studio Team 2019). To ensure the data input into the PCA was independent (there were no spurious correlations among genomic variants), I pruned the dataset of linked variants by setting an r^2 threshold of 0.1 in PLINK v1.9. Specifically, I pruned variables with an ^{2} greater than 0.1 within 50-SNP windows to remove SNPs with an R^2 greater than 0.1 that were located close together on a given chromosome (Purcell et al. 2007). I extracted PC coordinates for each individual using PLINK v1.9 and plotted the results in the tidyverse package in R v3.5.2 (Purcell et al. 2007, Hadley 2017).

To estimate potential admixture and further investigate population structure present within the dataset, I used ADMIXTURE to estimate the overall genomic structure present (Alexander et al. 2009). Geographic cline analysis in previous work suggested selection on courtship behaviors in migratory Allen's and Rufous Hummingbird (Myers et al. 2019). Thus, I analyzed the ADMIXTURE plot for the entire genome and compared this plot to the Z chromosome only, the sex chromosome in birds. I evaluated clusters of K=1-7, and the K value with the lowest cross-validation error compared to other values of K was chosen.

To address the questions raised here, we organized individuals into two sets: a set of "parental" groups and a set of "admixed + parental" groups. The parental set included three groups of individuals representing: 1) the historic breeding range of non-migratory Allen's, 2) migratory Allen's, and 3) Rufous Hummingbird. These groups were used to evaluate demography and evolutionary history. To achieve these groupings, we removed hybrids (individuals with admixture levels of over 10%, as determined by ADMIXTURE analyses) from the dataset. Removal of admixed individuals trimmed each parental group size to the following: migratory Allen's (N=7 individuals), non-migratory Allen's (N=11 individuals), and Rufous (N=9 individuals). The second set, the admixed + parental set (N=132 individuals), was used to evaluate population genetic dynamics between the Channel Islands and the mainland, between migratory Allen's and Rufous, and between migratory and non-migratory Allen's. This set included seven groups: 1) non-migratory Allen's on a northern Channel Island, Santa Cruz Island (N=7 individuals), 2) non-migratory Allen's on two southern Channel Islands (San Clemente, N=1 individual, and Santa Catalina Island, N=2 individuals), 3) mainland non-migratory Allen's (San Diego County through Santa Barbara County, CA, N=13 individuals), 4) the southern portion of the historic range of migratory Allen's (San Luis Obispo through Monterey County, CA, N=11 individuals), 5) the northern portion of the historic range of migratory Allen's (San Francisco County through Mendocino County, CA, N=24 individuals), 6) the hybrid zone of the migratory Allen's × Rufous hybrid zone based on phenotypic data (N=66 individuals), and 7) Rufous Hummingbird's historic range (N=9 individuals).

Using the admixed + parental groups, I performed pairwise comparisons of F_{ST} in Arlequin v3.5.2.2 (Nei 1987, Excoffier et al. 2005). I evaluated significance of pairwise F_{ST} estimates by implementing 10,000 permutations of the data. I also investigated whether isolation-by-distance, where genomic differences between populations increase with geographic distance, is present across the dataset by calculating the Pearson correlation coefficient (R) from the pairwise genetic distance (F_{ST}) between the seven admixed + parental groups and their geographic distances from each other using the *R* package ggpubr (Kassambara 2020). Next, I squared the coefficient (R^2) to find the coefficient of correlation. Pairwise distances of the seven admixed + parental groups were calculated based on the average distances of the sampled localities within one group from the average distances of the sampled localities in the other group.

Migration Estimates

Non-migratory Allen's Hummingbird appear to have colonized the mainland from (most likely, based on geographic proximity) the southern Channel Islands 60 years ago (Bradley 1980, Allen et al. 2016). The most likely source of the population, based on geographic proximity, is the southern Channel Islands. I investigated whether there was evidence for founder effects in non-migratory Allen's, where a small founding population preceded population expansion, by calculating the Tajima's D statistic in VCFtools v1.16 using a 50 kbp sliding window for the mainland individuals in the dataset (Tajima 1989, Danecek et al. 2011). A D-value below zero would support a founder effect from the colonizing population of non-migratory Allen's Hummingbird, followed by a population expansion, as hypothesized by Clark (2017).

To estimate migration rates and investigate directional gene flow between populations, I implemented a coalescent-based approach in LAMARC v2.1.1 (Kuhner 2006). LAMARC uses Bayesian inference to estimate the parameter *M*, which is the mutation-scaled migration rate (since divergence) between populations and assumes there has been enough time since divergence that migration and drift have a greater effect on current relationships than shared ancestry. If this assumption is violated, in the worst-case scenario, *M* may be overestimated in the event of large population sizes and recent divergence and/or colonization. However, overall patterns, including comparisons of directionality of introgression within a given dataset, are still reliable (Peter Beerli pers. comm.). LAMARC allows for asymmetrical gene flow, hybridization, and differences in effective population size across populations. For estimates of *M*, I used the admixed + parental group to investigate gene flow across seven groups.

I set the following parameters for estimation of *M*: I performed Bayesian inference analyses under the SNP model. I set each analysis to run as one long chain with an increment of 100, sampling of 1,000,000, burn-in of 100,000, and replicated this process four times with a different random number seed. For the first run, I estimated *M* from the uniform distribution, and subsequent runs used the mean of the posterior distribution from the previous run. I used Metropolis-Hastings sampling and four heated chains (static heating) with temperatures of 1.00, 1.50, 3.00, and 10,000 to improve estimation of the marginal likelihood. To assess effects of unequal sample sizes, I re-ran analyses by a) trimming all groups to equal sizes (N=7 individuals per group) and removing the southern Channel Islands (N=3 individuals), and b) trimming the hybrid group to 20 individuals. Results were within the 95% confidence interval of original admixed + parental group findings, showing that unequal sample sizes did not affect the results. Thus, I do not present results of these alternate analyses. All values reported in the results are means ± two SD, which approximate the 95% confidence interval.

Speciation History

I analyzed the parental groups to calculate the number of fixed differences (SNPs where F_{ST}=1) across the genomes of migratory Allen's and Rufous, and migratory and non-migratory Allen's using VCFtools v1.16 on a per-SNP basis. I also inferred the speciation history of non-migratory Allen's, migratory Allen's, and Rufous using DIYABC v2.1.0, which is based on approximate Bayesian computation (ABC, Cornuet et al. 2014). DIYABC enables comparison of competing historical/demographic scenarios and accounts for ongoing and historic hybridization between taxa, compared to traditional phylogenetic methods, which gives confounding results due to ancient and recent hybridization (McDade 1992, McVay et al. 2017). Using Anna's Hummingbird as an outgroup, I explored three scenarios: 1) a split between Allen's and Rufous, followed by a split between migratory and non-migratory Allen's, 2) a split between non-migratory Allen's and Rufous, followed by a hybridization event between them to form migratory

Allen's, a hybrid species, and 3) a split between non-migratory Allen's and an ancestor to Rufous and migratory Allen's, followed by a split between Rufous and migratory Allen's Hummingbird (Figure 3.2). I calculated a total of 3,000,000 simulated datasets (1,000,000 per scenario) and used the 50,000 sets closest to the observed data (according to the resultant summary statistics) for parameter estimation. To estimate divergence times, I converted generations to years using an average estimated generation time of 2.75 years, the average of generation times proposed for other hummingbird species, based on the observation that maturity begins one year after hatching and the average assumed survival rates of four hummingbird species, which range from 0.30 to 0.52 (Hilton and Miller 2003, Ruiz-Gutiérrez et al. 2012, Da Cruz Rodrigues et al. 2013, Ornelas et al. 2016). Based on Lande et al. (2003), average generation time (T) $=a+[s/(1-s)]$, where a is the time to maturity and s is the adult annual survival rate. Based on this, estimates for T range from 2.43 to 3.08 years, with an average of 2.75 years.

RESULTS

Group Structure

ADMIXTURE indicated three clusters (K=3, CV=0.49) had the lowest cross-validation score, and three groups were consistent with the approximate historic described range of migratory Allen's, non-migratory Allen's, and Rufous Hummingbird (Figure 3.3). K=2 (0.68), K=4 (0.53), and K=5 (0.55) were not as well-supported and showed less correspondence to known phenotypic groups (Figure S3.1). K=2 clustered migratory Allen's and Rufous together, separate from non-migratory Allen's, K=4 separated migratory Allen's into two separate, highly admixed clusters, and separate clusters for non-migratory Allen's and Rufous, while K=5 split non-migratory Allen's into a Santa Cruz Island cluster and mainland + southern Channel Island cluster, followed by two separate (highly admixed) clusters of migratory Allen's Hummingbird, and a Rufous Hummingbird cluster. For K=3, there was a genomic signature of hybridization throughout the extent of the migratory Allen's × Rufous Hummingbird hybrid zone from Coos County, Oregon to Humboldt County, California, with a long tail of introgression of Rufous Hummingbird alleles present in Allen's Hummingbird populations south of Humboldt County (Figure 3.3B).

There was also evidence of intergradation between migratory and non-migratory Allen's in southern California, centered in Santa Barbara County (Figure 3B). Introgression from migratory Allen's into the range of non-migratory Allen's is evident into Los Angeles County, and sporadically inland and further south in southern California (Grinnell and Miller 1944).

Analysis of the Z chromosome, isolated from the rest of the genome, showed patterns different from the entire genome. Introgression of Rufous was mostly restricted to the phenotypic hybrid zone and did not extend into the breeding range of migratory Allen's. Introgression was present, but more limited, between migratory and non-migratory Allen's (Figure 3.3A).

PCA of whole genome data revealed additional fine-scale population structure (Figure 3.4). Within non-migratory Allen's, PC1 (7.4% of the variation) separated the southern Channel Islands (Santa Catalina and San Clemente Island) from all other non-migratory Allen's populations, although a Santa Catalina Island bird did cluster with a single mainland bird from San Diego County, which may be a recent arrival on the mainland from the southern islands (see below for *M* rates, Figure 3.4). PC3 (5.7% of the variation) strongly differentiated Santa Cruz Island from all other populations, with separation also present between the southern Channel Islands and the mainland (Figure 3.4B). Migratory Allen's, Rufous, and their hybrids were differentiated most strongly by PC1 and PC2 (5.8% of the variation). Generally, within PC1 and PC2, Rufous, hybrids, and migratory Allen's were present across a continuum within the principal component space, although one individual from the edge of the hybrid zone and the range of migratory Allen's (Mendocino County, CA) grouped with Rufous along PC1 (Figure 3.4A). This bird had 75% of its genome introgressed from Rufous Hummingbird (Figure 3.3B).

Genome-wide F_{ST} between migratory Allen's and Rufous was 0.09 ($P < 0.05$), while F_{ST} between migratory and non-migratory Allen's was 0.06 ($P < 0.05$). Within the parental groups, I found 322 fixed SNPs between migratory Allen's and Rufous Hummingbird, with 169 of them on the Z chromosome. I found 26 fixed SNPs between non-migratory and migratory Allen's: only one was located on the Z chromosome (Figure 3.5). F_{ST} increased with geographic distance, and all pairwise comparisons between the admixed + parental groups were significantly different, while individuals within each group had a similar F_{ST} (Figure 3.6, Table 3.1, $P < 0.05$). The coefficient of correlation of genetic and geographic distance across groups suggested that groups located further apart tended to be less genetically similar ($R^2 = 0.21$, $P < 0.05$, Figure 3.6).

Gene flow Estimates

I found no evidence that founder effects from the colonizing mainland population of non-migratory Allen's Hummingbird preceded their rapid population expansion (Tajima's D=0.37 ± 0.86).

Among the three non-migratory groups of Allen's Hummingbird, Santa Cruz Island was the most isolated. F_{ST} values from the mainland (F_{ST}=0.10, $P < 0.05$) and the southern Channel Islands (F_{ST}=0.21, $P < 0.05$, Table 3.1) were both high. Other group comparisons showed less differentiation than Santa Cruz Island, although they were still significantly different. The southern Channel Islands and the mainland (F_{ST}=0.05, $P < 0.05$), the southern migratory Allen's and Rufous Hummingbird (F_{ST}=0.08, $P < 0.05$), and northern migratory Allen's and Rufous (F_{ST}=0.05, $P < 0.05$, Table 3.1) were all significantly differentiated.

Among non-migratory Allen's groups, gene flow from Santa Cruz Island to both the southern Channel Islands and the mainland was the lowest of all groups; these *M* estimates corresponded with the relative isolation shown by F_{ST} values (Figure 3.7, Table 3.1). *M* to Santa Cruz Island from the southern islands and the mainland was significantly higher, indicating asymmetric gene flow (Figure 3.7). Gene flow from the mainland to the southern islands was not significantly more frequent than gene flow to the mainland from the southern islands (Figure 3.7).

Migratory Allen's Hummingbird in northern California was influenced by a high *M* from the Allen's × Rufous hybrid zone*,* while Rufous Hummingbird had a significantly higher *M* into the hybrid zone than from the hybrid zone to Rufous (Figure

3.7). Migratory Allen's in central California had a significantly lower *M* from non-migratory Allen's in mainland southern California than non-migratory mainland Allen's did from migratory Allen's (Figure 3.7).

Speciation History

Of the three speciation scenarios I tested, Scenario 1 was best-supported (0.43 probability, 95% confidence interval=0.39-0.44) compared to Scenario 2 (0.27 probability, 95% CI=0.22-0.34) and Scenario 3 (0.30 probability, 95% CI=0.27-0.34). In Scenario 1, the ancestral *Selasphorus* diverged from Anna's Hummingbird 1.30 million years ago (MYA), Allen's and Rufous diverged 0.65 MYA, and non-migratory and migratory Allen's split from each other 0.43 MYA (Figure 3.8). Thus, the data reject the hypothesis that migratory Allen's Hummingbird is a hybrid taxon of Rufous and non-migratory Allen's Hummingbird and that Rufous and migratory Allen's are sister taxa.

DISCUSSION

Speciation Modeling and Population Selection

The diversification of hummingbirds in North America has been rapid, considering the group is estimated to have colonized North America about 5.0 MYA (McGuire et al. 2014). Here, I find that the ancestral *Selasphorus* diverged from Anna's Hummingbird about 1.30 MYA, Allen's and Rufous diverged 650,000 years ago, and migratory and non-migratory Allen's are each other's closest relative and diverged 430,000 years ago (Figure 3.8). Support for this scenario (Scenario 1) was significantly higher than for scenarios 2 and 3; the relatively narrow margin of significance given by the 95% CI is likely due to the small amount of differences in the genomes of migratory and non-migratory Allen's and Rufous Hummingbird identified here. Previous work hypothesized secondary contact between migratory Allen's and Rufous following the late Pleistocene 10,000-12,000 years ago (Myers et al. 2019). Other work has estimated divergence times of ancestral *Selasphorus* from Anna's Hummingbird about 1.6 MYA and 5.1 MYA (McGuire et al. 2014, Licona-Vera and Ornelas 2017), divergence of Allen's from Rufous from 5,000-100,000 years ago (Battey 2019), and divergence of the Allen's and Calliope Hummingbird ancestor from Rufous at about 300,000 years ago (McGuire et al. 2014). Finally, previous work failed to resolve the relationship between Allen's and Rufous Hummingbird (Licona-Vera and Ornelas 2017).

Some of the discrepancies between my results and previous studies are likely explained by hybridization between Allen's and Rufous Hummingbird. Accounting for gene flow is a critical part of estimating divergence, as recent and ancient hybridization obscures phylogeny (McDade 1992, McVay et al. 2017). Ensuring that samples used for divergence dating are not heavily introgressed will also prevent estimates of divergence that are too recent due to gene flow (Leaché et al. 2014). Previous work primarily utilized Allen's Hummingbird specimens from northern California (McGuire et al. 2014, Licona-Vera and Ornelas 2017, Battey 2019). Strikingly, nearly all migratory Allen's samples acquired in this study were introgressed with Rufous Hummingbird, and all Allen's Hummingbirds in northern California were introgressed with Rufous Hummingbird. Thus, it is likely that gene flow led to previous recent divergence estimates (Figure 3.3, McGuire et al. 2014, Licona-Vera and Ornelas 2017, Battey 2019).

Analyses rejected the hypothesis of a hybrid origin, and further, genomic variation demonstrated a clinal pattern (Figure 3.3B). Samples from northern California showed introgression from Rufous, while samples from Monterey showed less introgression from Rufous, and samples from San Luis Obispo showed no introgression from Rufous, with minimal admixture from non-migratory Allen's (Figure 3.3B). Thus, my data suggest that introgression is responsible for the variable genomic architecture observed across the range of migratory Allen's Hummingbird.

Migratory Allen's sampled from southern California mostly did not receive introgression from non-migratory Allen's north of Santa Barbara County. This may be an artifact of group designation. Because birds sampled in the Santa Barbara area were phenotypically similar to non-migratory Allen's and populations in the area are observed year-round, I considered these individuals as non-migratory Allen's for purposes of analysis. However, migratory Allen's was reported to breed as far south as Ventura County before the colonization of non-migratory Allen's (Grinnell and Miller 1944) and may still breed in the area. Further, all of the individuals in the area were genomically hybrid. If, instead, I considered birds in Santa Barbara and Ventura County as migratory Allen's *a priori*, gene flow estimates from non-migratory Allen's into the range of migratory Allen's might have been different.

Patterns of Introgression

Introgression is a well-documented threat to several species with limited ranges (Levin et al. 1996, Roberts et al. 2010, Witte et al. 2013). Extensive gene flow from Rufous Hummingbird into the range of migratory Allen's might be indicative of swamping, although the Z chromosome of migratory Allen's appears to be more resistant to introgression in comparison to the rest of the genome (Figure 3.3). Strikingly, 168 of the 322 fixed SNPs between Allen's and Rufous Hummingbird reside on the Z, indicating this sex chromosome is an important contributor to reproductive isolation between these two species (Figure 3.5). When interspecific hybridization occurs, loci associated with reproductive isolation do not tend to pass beyond regions of hybridization, as demonstrated across a howler monkey (*Alouatta palliata* × *A. pigra*) hybrid zone (Cortés-Ortiz et al. 2019). I observed a similar pattern here; thus, within migratory Allen's and Rufous Hummingbird, the observed patterns suggest that the Z chromosome makes a disproportionate contribution to reproductive isolation between these species, making complete genomic swamping an unlikely quandary (Figure 3.3).

Genetic rescue is another possible product of gene flow (Whiteley et al. 2015). The rapid expansion of non-migratory Allen's Hummingbird is likely connected to food resources from hummingbird feeders, ornamentals, and exotic plants (for example, Cape Honeysuckle, *Tecoma capensis* and Tree Tobacco, *Nicotiana glauca*, Clark and Mitchell 2013, Clark 2017). Thus, future work should evaluate whether introgression via hybridization from non-migratory Allen's and Rufous into the range of migratory Allen's Hummingbird benefits this subspecies, especially from non-migratory Allen's, which is able to exploit habitat that migratory Allen's is not.

Within non-migratory Allen's, the finding that M is lower from Santa Cruz Island to the mainland than M from the mainland to Santa Cruz Island is perplexing, as island populations are often at least as likely to colonize the mainland as they are to colonize an island from the mainland (Sofaer et al. 2012, Mason et al. 2014, Hanna et al. 2019). I also found that Santa Cruz Island is the most genetically isolated from all other non-migratory Allen's groups, in congruence with several other bird studies on the Channel Islands (Figure 3.4, Table 3.1, Ashley and Willis 1987, Caballero and Ashley 2011, Sofaer et al. 2012, Walsh 2015, Wilson et al. 2015, Hanna et al. 2019). In contrast to the asymmetric gene flow present between Santa Cruz Island and the mainland, I found high, symmetric gene flow between the southern Channel Islands and the mainland, even though the southern islands are further away than Santa Cruz Island (Figure 3.7). This finding may be an artifact of the founding mainland population 60 years ago, which likely originated from the southern Channel Islands (the Palos Verdes Peninsula, the site of colonization, is geographically closest to Santa Catalina Island, Bradley 1980, Allen et al. 2016). Thus, because gene flow from Santa Cruz Island to the southern Channel Islands is low, and because the mainland population was recently colonized by a population from the southern Channel Islands, the genomic signature of the southern islands on the mainland remains.

Although non-migratory Allen's Hummingbird is estimated to have colonized mainland southern California from the southern Channel Islands ~60 years ago, I found no evidence of a founder effect (Bradley 1980, Allen et al. 2016). However, in systems with high gene flow, analyses sometimes fail to identify a founder effect scenario (Busch et al. 2007, Reynolds and Fitzpatrick 2013). Two sources of gene flow, one from migratory Allen's Hummingbird, and the other from continued gene exchange between the mainland and the Channel Islands since the initial colonization of the mainland, might be masking the signature of any founder effects that occurred.

Intergradation of Non-migratory and Migratory Allen's Hummingbird

Clark (2017) hypothesized that the non-migratory Allen's Hummingbird came into contact with the migratory Allen's Hummingbird in the vicinity of the border between Santa Barbara and Ventura County area. The data presented here support the hypothesis of Clark (2017), where intergradation likely initiated in the area of overlap in Santa Barbara and Ventura County, with gene flow into Los Angeles and Riverside counties (Figure 3.3). The two subspecies likely co-occur at least in Santa Barbara County, and possibly further south. There are admixture levels of individuals of 40-50% as far south as Los Angeles County, indicating gene flow. I also find evidence of gene flow on Santa Cruz Island, in San Diego County, and in Riverside County, consistent with the asymmetric estimates of M, where gene flow is higher in the direction of non-migratory Allen's than in the direction of migratory Allen's (Figure 3.7). I observed low F_{ST} between southern migratory Allen's and mainland non-migratory Allen's (Table 3.1).

The underlying ecological differences between migratory and non-migratory Allen's, and analyses of the mechanisms that make non-migratory Allen's seemingly more plastic than migratory Allen's Hummingbird, are unknown. Island populations are often outcompeted by mainland populations. Non-migratory Allen's, originally an island subspecies, may be outcompeting migratory Allen's, especially when considering that non-migratory Allen's has higher fecundity and a longer breeding season (Wells and Baptista 1979, Clark and Mitchell 2013, Clark 2017). As hypothesized by Clark (2017), non-migratory Allen’s appears to be better at exploiting disturbed habitat. All of the above factors may affect southern populations of migratory Allen’s, which was historically described to breed as far south as Ventura County (Grinnell and Miller 1944). Thus, given the high levels of admixture and gene flow in Santa Barbara County, our data suggest that since colonization of the mainland, non-migratory Allen’s has advanced into the previously described range of migratory Allen’s.

CONCLUSIONS

In Chapter 3, I analyzed the extent of gene flow and the evolutionary history of non-migratory Allen’s, migratory Allen’s, and Rufous Hummingbird. Using whole-genome data, I found evidence of extensive gene flow from Rufous Hummingbird into the range of migratory Allen’s, recent intergradation between non-migratory and migratory Allen’s in southern California, and high gene flow across the entire non-migratory Allen’s, migratory Allen’s, and Rufous Hummingbird species complex. I also found evidence of selection on the Z chromosome between Allen’s and Rufous Hummingbird, which resists introgression from Rufous. I also found a lack of gene flow from the northern Santa Cruz Island to the mainland, with high gene flow to Santa Cruz Island from the mainland. Finally, I estimated divergence dates of Allen’s and Rufous to be further back in time than previously reported and rejected the hypotheses that migratory Allen’s is a hybrid taxon of non-migratory Allen’s and Rufous Hummingbird and that Rufous and migratory Allen’s are sister taxa.

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FIGURES AND TABLES

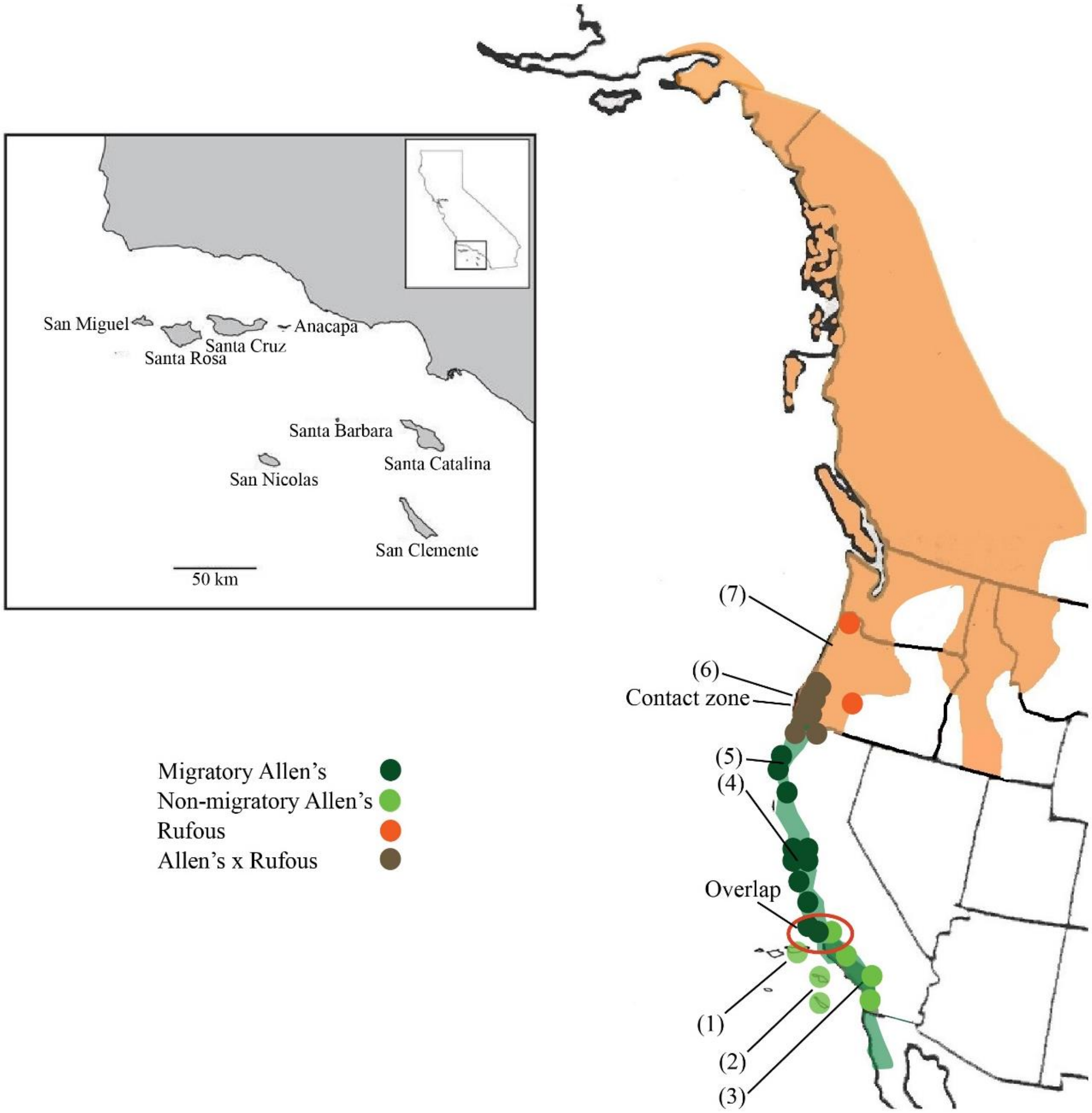


Figure 3.1 – **Approximate breeding ranges of migratory Allen's, non-migratory Allen's, and Rufous Hummingbird, and sampling areas** (Myers et al. 2019). Numbers in parentheses denote sampling group number. Inset map shows the locations of the Channel Islands.

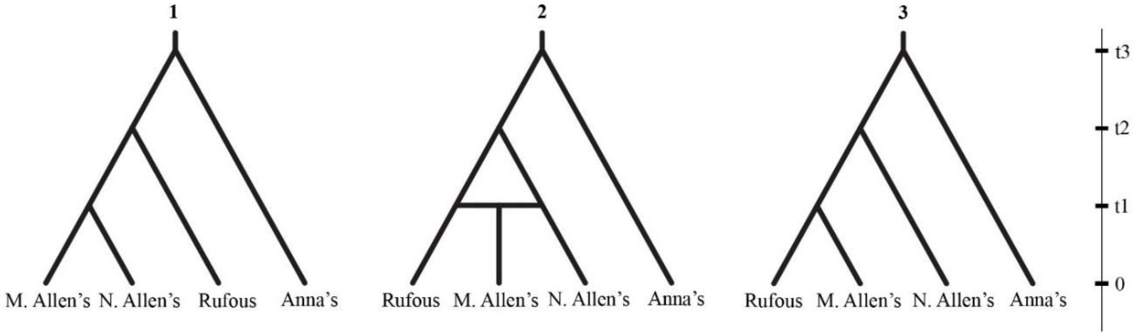


Figure 3.2 – **Three hypotheses of the speciation history of migratory Allen's, non-migratory Allen's, and Rufous Hummingbird. 1)** a split between Allen's and Rufous, followed by a split between migratory and non-migratory Allen's, **2)**, a split between non-migratory Allen's and Rufous, followed by hybridization between them to form migratory Allen's, a hybrid species, and **3)** a split between non-migratory Allen's and the ancestor of migratory Allen's and Rufous, followed by a split between Rufous and migratory Allen's Hummingbird.

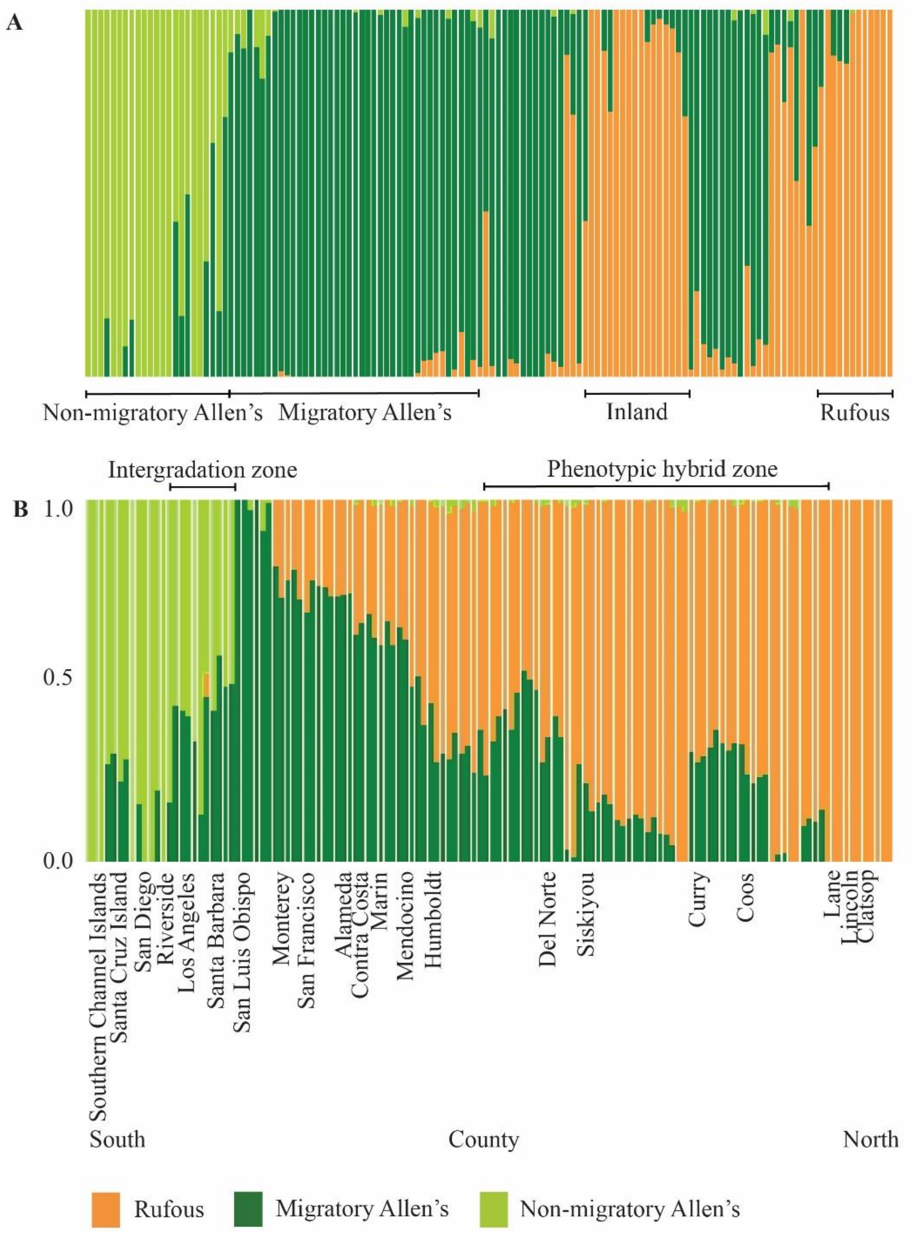


Figure 3.3 – **ADMIXTURE plot for K=3 groups for A) the Z chromosome and B) across the whole genome.** “Inland” indicates individuals sampled from an inland transect of the hybrid zone in Siskiyou County, which is south of Curry and east of Del Norte County. There was introgression by Rufous Hummingbird into the range of migratory Allen’s Hummingbird, beyond the phenotypic hybrid zone described in Myers et al. (2019) for the whole genome, although introgression was limited on the Z. There was also evidence of intergradation between non-migratory and migratory Allen’s, with more limited introgression on the Z.

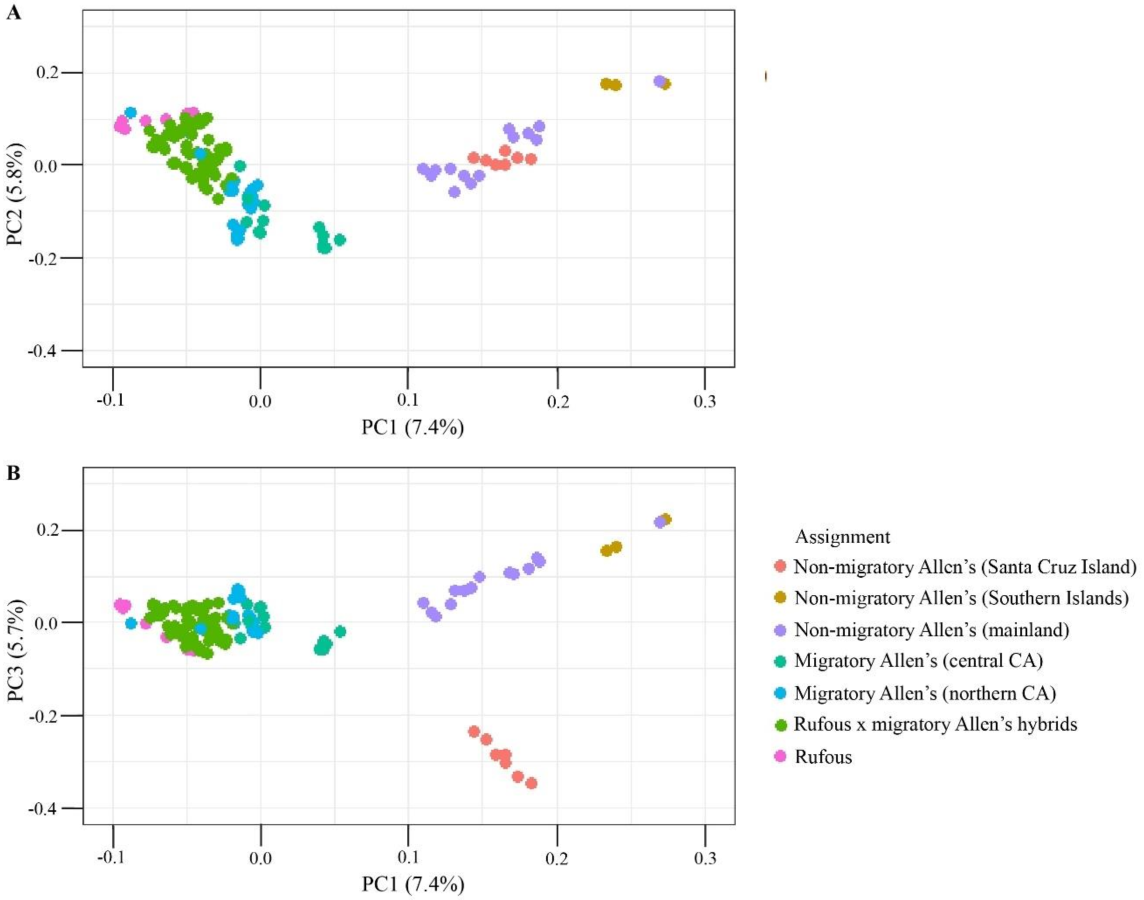


Figure 3.4 – **Principal component analysis (PCA) of the seven admixed + parental groups across the first three principal components.** Based on 1,770,572 single nucleotide polymorphisms (SNPs). **A**) Three main clusters, which were mostly separated by PC1 and PC2 (7.4% and 5.8% of the variation respectively). One mainland bird (purple) from San Diego County, CA, clustered with the southern islands (gold). **B**) Four main clusters, which were mostly separated by PC1 and PC3 (7.4% and 5.7% of the variation respectively).

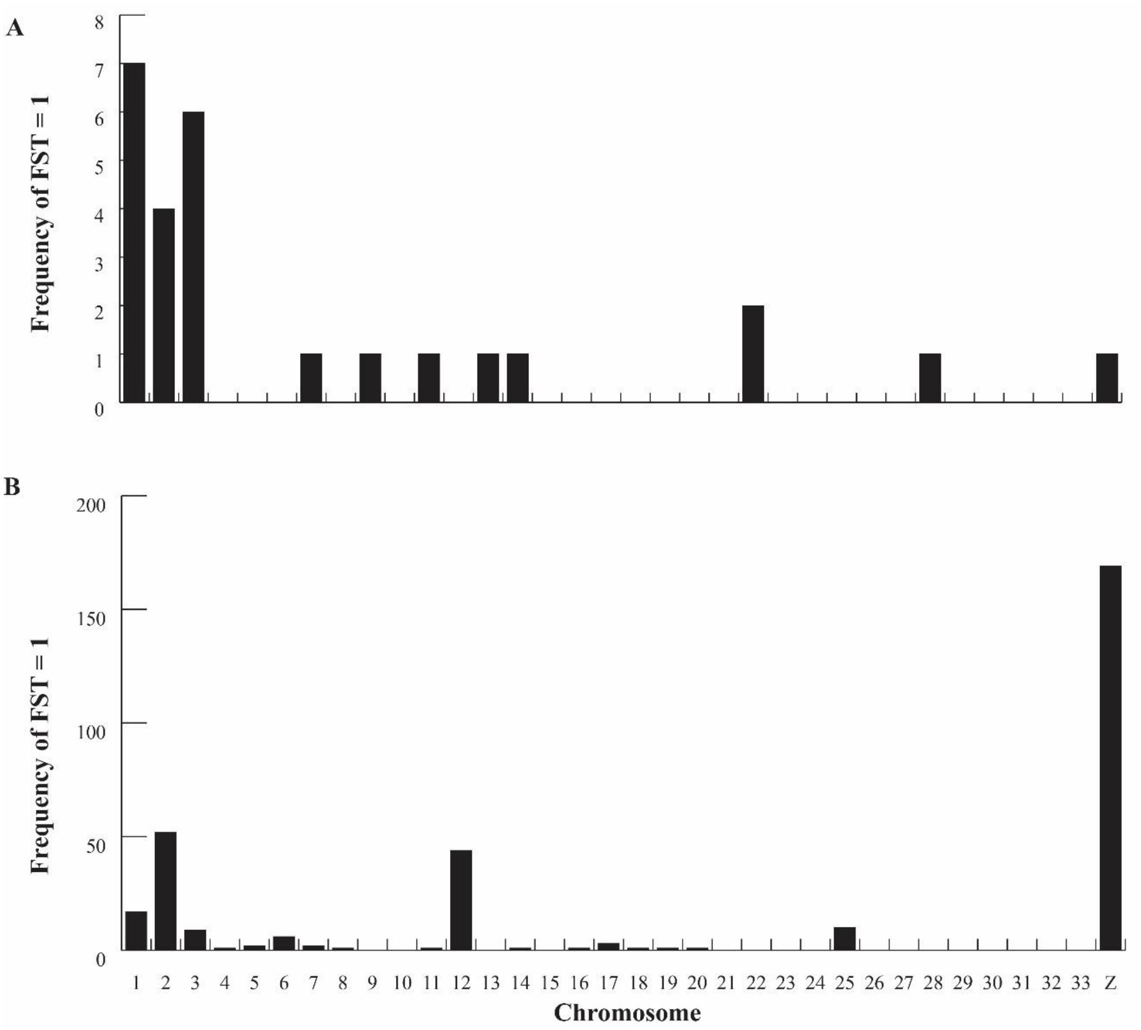


Figure 3.5 – **Fixed differences (SNPs) between A) migratory and non-migratory Allen's and B) migratory Allen's and Rufous Hummingbird.** There are 26 fixed SNPs between migratory and non-migratory Allen's and 322 fixed SNPs between migratory Allen's and Rufous, with 169 differences on the Z chromosome.

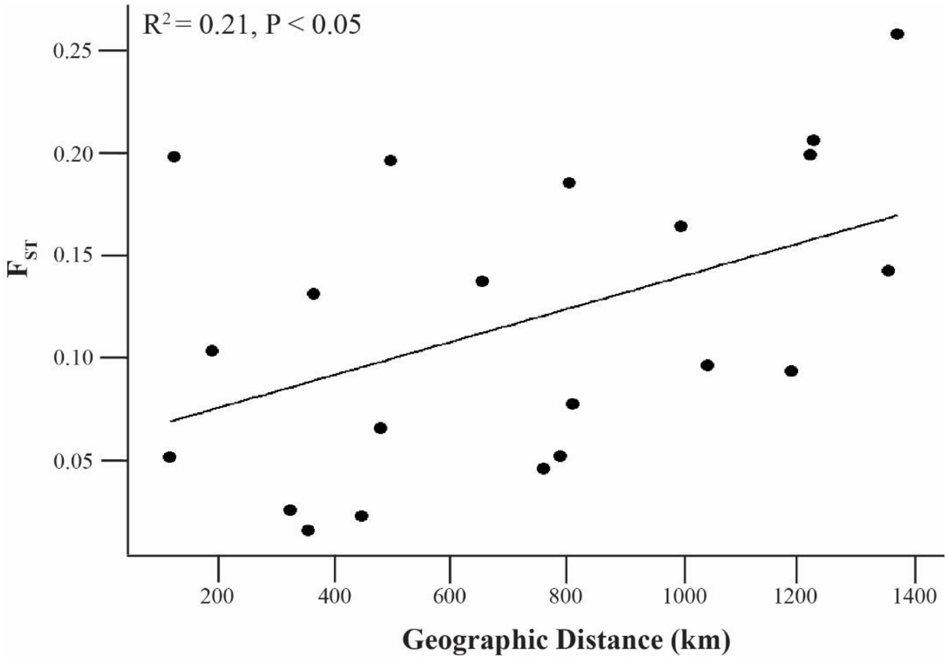


Figure 3.6 – **Isolation by distance scatterplot illustrating the pairwise relationship between genetic distance (F_{ST}) between the seven admixed + parental groups and geographic distance.** Pairwise distances of the seven admixed + parental groups were plotted on the x-axis based on average distances of the sampled localities within one group from the average distances of the sampled localities in the other. There was a significant, positive association of increased genetic distance with increased geographic distance, showing clinal genetic variation in the dataset.

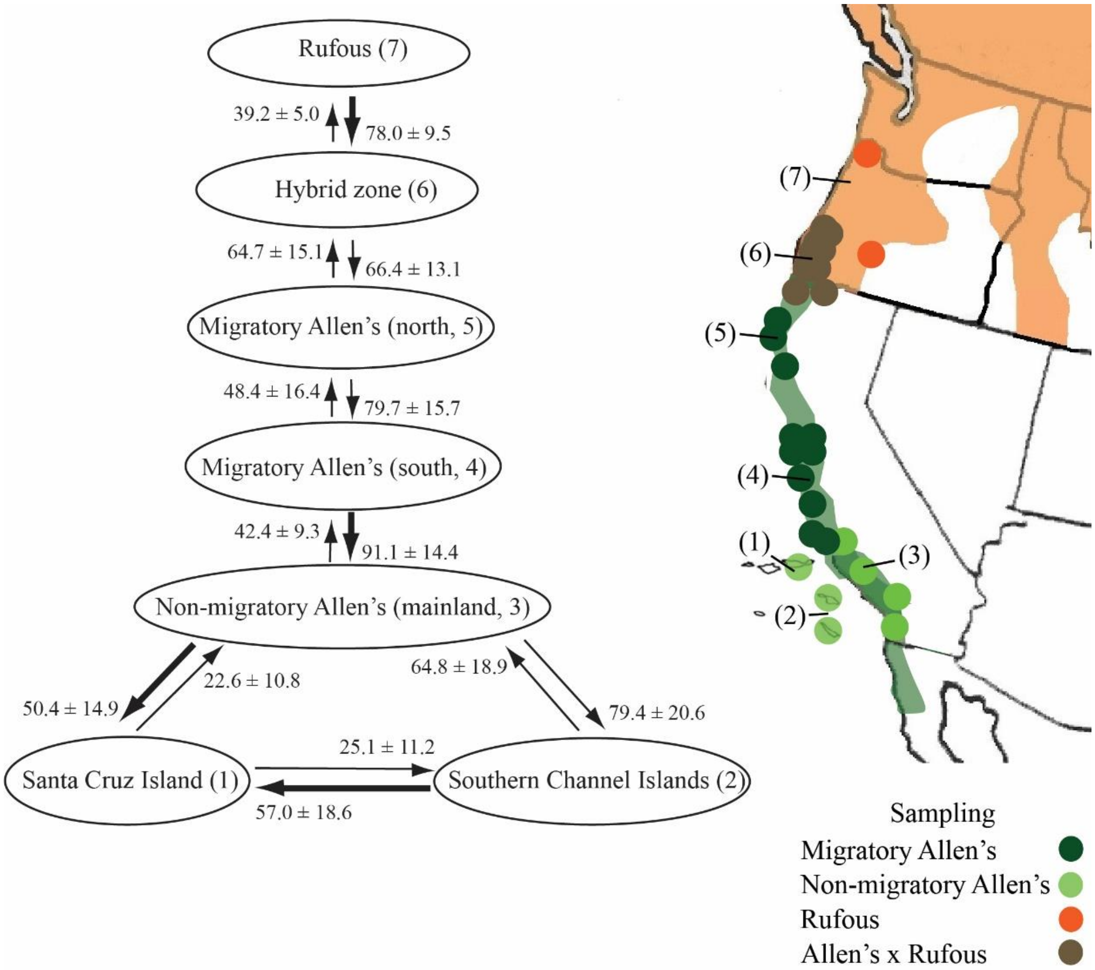


Figure 3.7 – **Bayesian estimates of A) the long-term migration rates (*M*) across the admixed + parental groups, implemented in LAMARC (Kuhner 2006).** Thickness of the arrows are proportional to *M*. Numbers in parentheses denote the geographic vicinity of each group.

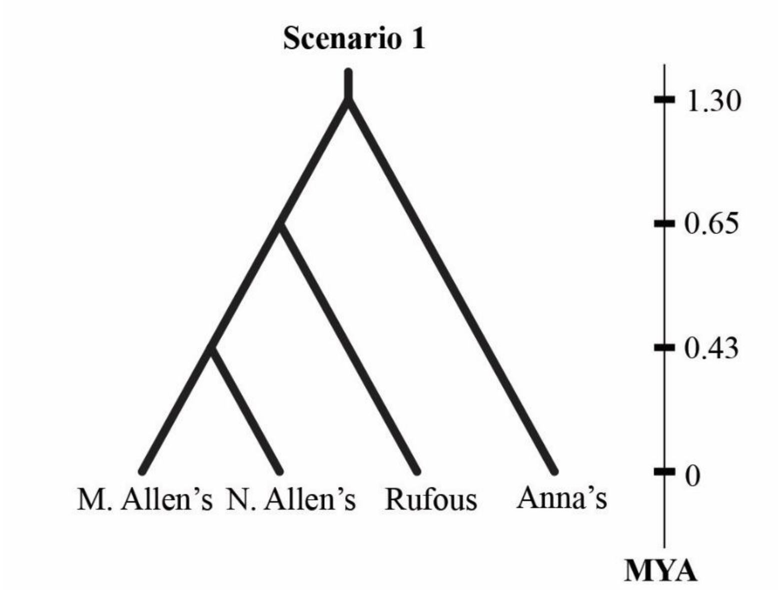


Figure 3.8 – **The best-supported scenario (0.43 probability, 95% confidence interval=0.39-0.44) representing the demographic history of migratory Allen's, non-migratory Allen's, and Rufous Hummingbird.**

Table 3.1. **Below diagonal, F_{ST} values, above diagonal, average geographic distance (km), between groups in the admixed + parental group.** Average distance was calculated based on the average distance of the sampled localities between two groups. (1) northern Santa Cruz Island non-migratory Allen's, (2) Southern Channel Island non-migratory Allen's, (3) mainland non-migratory Allen's, (4) southern migratory Allen's, (5) northern migratory Allen's, (6) individuals in the migratory Allen's × Rufous hybrid zone, and (7) Rufous Hummingbird. All F_{ST} values were significant ($P < 0.05$).

	1	2	3	4	5	6	7
1		122	187	364	656	1000	1225
2	0.200		114	497	807	1231	1376
3	0.103	0.051		480	813	1192	1360
4	0.131	0.196	0.065		323	762	1046
5	0.137	0.185	0.077	0.025		446	792
6	0.154	0.206	0.093	0.045	0.022		354
7	0.195	0.258	0.122	0.076	0.051	0.015	

CONCLUSIONS OF THE DISSERTATION

Allen’s Hummingbird, which resides along the coast in California and southern Oregon, is comprised of two subspecies: one migratory and one sedentary. Non-migratory Allen’s breeds from Baja California to Santa Barbara County, California, and on the Channel Islands off the coast of southern California. Migratory Allen’s breeds from Santa Barbara County to southern Oregon, where it forms a hybrid zone with Rufous Hummingbird. My dissertation has focused on the discovery and evolutionary maintenance of the hybrid zone between migratory Allen’s and Rufous Hummingbird, centered in northern California and southern Oregon. By examining the morphological, genetic, and behavioral composition of birds across this hybrid zone, I examined how natural selection, sexual selection, and biogeographic processes have affected the diversification and interactions of Allen’s and Rufous.

In Chapter 1, I described a discovered hybrid zone between Allen’s and Rufous Hummingbird using courtship behavior and morphology, where I used Linear Discriminant Function Analysis, cline analysis, and a hybrid index to diagnose hybrids and map the geographic extent of the zone. I also assessed how selection acts on the 20 behavioral and morphological characters I studied for male individuals. When species form a hybrid zone, there are prezygotic barriers, for example, sexual selection, that drive isolation, and postzygotic barriers, such as hybrid incompatibility or inviability. I found evidence for a prezygotic barrier across this hybrid zone: as narrow as 3 km, clines for sexually selected courtship behaviors were far narrower than others (as wide as 300 km), showing the strength of sexual selection in driving separation of Allen’s and Rufous.

Building on the behavioral data accumulated in Chapter 1, the second chapter of my dissertation is based on display behavior across the hybrid zone and parental species ranges, where I assigned individual Allen's, Rufous, and hybrid behavioral phenotypes based on the sequences of their courtship displays, using k-mer analysis. The courtship display is a fixed action pattern comprised of underlying stereotyped elements in Allen's and Rufous Hummingbird. Thus, I discovered numerous novel and transgressive courtship displays across the contact zone based on a reorganization of underlying elements (novel traits) or extreme expressions of the underlying elements, beyond the range of either parental species (transgressive traits). A goal of future work is to use the findings of this chapter to map courtship traits to their underlying genes, with the assumption that these courtship displays are innate.

For Chapter 3, in consideration of the extent of hybridization between migratory Allen's and Rufous, I evaluated the effects of gene flow from the hybrid zone on the genomes of populations across the range of migratory Allen's Hummingbird, and found evidence of extensive gene flow from Rufous Hummingbird into the range of migratory Allen's. I also found evidence of selection on the Z chromosome in migratory Allen's and Rufous Hummingbird, which resists introgression from Rufous, showing that the Z chromosome, similar to courtship behavior, apparently has a role as a species barrier between migratory Allen's and Rufous Hummingbird. I also presented evidence of recent intergradation between non-migratory and migratory Allen's in Santa Barbara County, southern California, and high gene flow across the entire non-migratory Allen's, migratory Allen's, and Rufous Hummingbird species complex.

Given the extent of hybridization between non-migratory Allen's, migratory Allen's, and Rufous Hummingbird, I evaluated their evolutionary relationships, and tested three scenarios: 1) a split between Allen's and Rufous, followed by a split between migratory and non-migratory Allen's, 2) a split between non-migratory Allen's and Rufous, followed by a hybridization event between them to form migratory Allen's, a hybrid species, and 3) a split between non-migratory Allen's and Rufous, followed by a split between Rufous and migratory Allen's Hummingbird. I found that Scenario 1, a split between Allen's and Rufous, followed by divergence of non-migratory and migratory Allen's, is most likely. I also estimated divergence dates of Allen's and Rufous Hummingbird that were further back in time than reported by previous work.

Future work that stems from my dissertation will address whether human activity perpetuates hybridization in Allen's and Rufous Hummingbird. Future investigations will also address the underlying ecological differences between migratory and non-migratory Allen's and evaluate the hypothesis raised in Clark (2017) that non-migratory Allen's is more plastic than migratory Allen's Hummingbird and better at exploiting disturbed habitat.

APPENDICES

Appendix A: Supplementary Tables/Figures for Chapter 1

Table S1.1 – **Coordinates, locality, and county for Rufous Hummingbird (*S. rufus*, RUHU), Allen's Hummingbird (*S. s. sasin*, ALHU), and hybrid (HYHU) samples gathered in the field.** Classifications based on LDA results. "NB" indicates a bird that was not collected or banded. Individuals with a band number or "NB" were released after blood sample collection; all other samples are from collected individuals.

Band/ID	Species	Sex	Locality	County	Latitude (N)	Longitude (W)
SDSU 2983	ALHU	M	Pismo State Beach, CA	San Luis Obispo	35°6'38.15"	120°37'39.18"
SDSU 2984	ALHU	M	Montana de Oro State Park, CA	San Luis Obispo	35°6'35.98"	120°37'39.30”
E05847	ALHU	M	Pismo State Beach, CA	San Luis Obispo	35°6'38.15"	120°37'39.18"
E05846	ALHU	M	Pismo State Beach, CA	San Luis Obispo	35°6'38.15"	120°37'39.18"
E05908	ALHU	M	Pismo State Beach, CA	San Luis Obispo	35°6'38.15"	120°37'39.18"
E05909	ALHU	M	Pismo State Beach, CA	San Luis Obispo	35°6'35.98"	120°37'39.30"
SDSU 2985	ALHU	M	Carmel River State Beach, CA	Monterey	36°31'45.14"	121°55'27.52"
SDSU 2986	ALHU	M	Carmel River State Beach, CA	Monterey	36°31'45.14"	121°55'27.52"
E05998	ALHU	M	Carmel River State Beach, CA	Monterey	36°31'45.14”	121°55'27.52"
E05907	ALHU	M	Carmel River State Beach, CA	Monterey	36°31'45.14”	121°55'27.52"
E05844	ALHU	M	Carmel River State Beach, CA	Monterey	36°31'45.14”	121°55'27.52"
SDSU 2987	ALHU	M	Mendocino Headlands State Park, CA	Mendocino	39°18'10.53"	123°47'16.98"
SDSU 2988	ALHU	M	Mendocino Headlands State Park, CA	Mendocino	39°18'10.53"	123°47'16.98”
E05996	ALHU	M	Mendocino Headlands State Park, CA	Mendocino	39°18'10.53"	123°47'16.98"
E05905	ALHU	M	Van Damme State Park, CA	Mendocino	39°16'29.18"	123°47'24.35"
E05997	ALHU	F	MacKerricher State Park, CA	Mendocino	39°29'13.11"	123°47'45.76"
SDSU 2989	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'3.26"	124° 0'41.75"
SDSU 2990	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'3.26"	124° 0'41.75"
E05902	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'3.26"	124° 0'41.75"
E05920	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'9.77"	124°0'28.43"
E05918	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'9.77"	124°0'28.43"
SDNHM 56129	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'11.72"	124°0'30.39"

SDNHM 56130	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'11.72"	124°0'30.39"
SDNHM 56131	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'6.29"	124° 0'31.88"
E05917	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'11.72"	124°0'30.39"
E05916	ALHU	M	Humboldt Redwoods State Park, CA	Humboldt	40°21'6.29"	124°0'31.88"
E05901	HYHU	M	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'27.78"	124°8'28.43"
E05949	ALHU	M	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'27.78"	124°8'28.43"
SDSU 2992	ALHU	M	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.55"	124°8'29.30"
SDSU 2991	ALHU	M	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.55"	124°8'29.30"
E05915	ALHU	M	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'22.73"	124°8'28.43"
E05992	ALHU	F	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'22.73"	124°8'28.43"
E05940	ALHU	F	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.13"	124°8'30.93"
E05950	HYHU	F	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.13"	124°8'30.93"
E05959	ALHU	F	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.13"	124°8'30.93"
E05841	HYHU	F	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.13"	124°8'30.93"
SDNHM 56127	ALHU	M	Patrick's Point State Park, CA	Humboldt	41°8'21.16"	124°9'1.06"
SDSU 2995	ALHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05937	ALHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05947	HYHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05936	ALHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
SDSU 2996	ALHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05960	ALHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05938	ALHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05970	ALHU	M	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05958	ALHU	F	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05919	ALHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"

E05903	ALHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05995	ALHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05842	ALHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDNHM 56128	ALHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDSU 2993	ALHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDSU 2994	ALHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05843	HYHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05994	HYHU	M	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDSU 3007	ALHU	F	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05904	HYHU	F	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDSU 2997	ALHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124° 5'51.62"
E05935	HYHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124° 5'51.62"
E05969	ALHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124° 5'51.62"
E05946	HYHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124° 5'51.62"
SDSU 2998	ALHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124° 5'51.62"
E05957	HYHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124° 5'51.62"
E05955	HYHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'56.79"	124°6'18.30"

K53386	HYHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'56.79"	124°6'18.30"
E05933	HYHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'54.77"	124°6'16.36"
E05956	HYHU	M	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'54.77"	124°6'16.36"
E05968	ALHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 3029	HYHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 3030	ALHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 3031	HYHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
K53373	ALHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
E05932	ALHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
E05913	ALHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
K53374	HYHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
K53375	HYHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
E05914	HYHU	M	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 3032	ALHU	F	Highway 197, Crescent City, CA	Del Norte	41°50'14.75"	124°6'49.76"
NB24	ALHU	F	Highway 197, Crescent City, CA	Del Norte	41°50'14.75"	124°6'49.76"
NB25	HYHU	F	Highway 197, Crescent City, CA	Del Norte	41°50'14.75"	124°6'49.76"
NB26	HYHU	F	Highway 197, Crescent City, CA	Del Norte	41°50'14.75"	124°6'49.76"
NB51	HYHU	F	Highway 197, Crescent City, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDNHM 56055	HYHU	M	Happy Camp, CA	Siskiyou	41°46'38.09"	123°23'48.75"
SDNHM 56065	HYHU	M	Happy Camp, CA	Siskiyou	41°46'38.09"	123°23'48.75"

SDNHM 56057	RUHU	F	Happy Camp, CA	Siskiyou	41°46'45.83"	123°22'56.94"
SDNHM 56053	RUHU	F	Happy Camp, CA	Siskiyou	41°46'45.83"	123°22'56.94"
SDNHM 56062	HYHU	M	Seiad Valley, CA	Siskiyou	41°50'10.66"	123°10'24.85"
SDNHM 56059	RUHU	M	Seiad Valley, CA	Siskiyou	41°51'28.80"	123°14'22.80"
SDNHM 56060	RUHU	F	Seiad Valley, CA	Siskiyou	41°51'28.80"	123°14'22.80"
SDNHM 56061	RUHU	F	Seiad Valley, CA	Siskiyou	41°51'28.80"	123°14'22.80"
SDSU 2999	HYHU	M	Alfred Loeb State Park, CA	Curry	42°6'45.34"	124°11'9.98"
SDSU 3000	ALHU	M	Alfred Loeb State Park, CA	Curry	42°6'45.34"	124°11'9.98"
E05944	HYHU	M	Alfred Loeb State Park, CA	Curry	42°6'45.34"	124°11'9.98"
E05954	HYHU	M	Whaleshead Beach, OR	Curry	42°8'43.67"	124°21'17.37"
K53383	HYHU	M	Alfred Loeb State Park, CA	Curry	42°6'43.99"	124°11'12.30"
K53376	HYHU	M	Alfred Loeb State Park, CA	Curry	42°6'43.99"	124°11'12.30"
K53377	HYHU	M	Harris Beach State Park, OR	Curry	42°4'3.93"	124°18'29.80"
SDSU 3001	HYHU	M	Whaleshead Beach, OR	Curry	42°8'43.67"	124°21'17.37"
E05942	HYHU	F	Harris Beach State Park, OR	Curry	42°8'42.67"	124°21'17.37"
K53378	HYHU	F	Harris Beach State Park, OR	Curry	42°4'3.93"	124°18'29.80"
E05945	HYHU	F	Harris Beach State Park, OR	Curry	42°4'3.93"	124°18'29.80"
SDNHM 56113	HYHU	M	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDNHM 56114	RUHU	M	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDNHM 56115	HYHU	M	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDNHM 56116	RUHU	M	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDNHM 56117	RUHU	M	Selma, OR	Josephine	42°16'49"	123°37'6.65"
K53385	ALHU	M	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
E05952	HYHU	M	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
SDSU 3002	HYHU	M	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
E05943	ALHU	M	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"

SDSU 3003	HYHU	M	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
K53381	HYHU	M	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
K53382	HYHU	M	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
SDSU 3004	HYHU	M	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
K53386	HYHU	M	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
NB50	HYHU	F	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
SDNHM 56124	HYHU	M	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3033	HYHU	M	Cape Blanco State Park, OR	Curry	42°50'3.15"	124°33'21.31"
SDSU 3034	HYHU	M	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3035	HYHU	M	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3036	HYHU	M	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3037	HYHU	M	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
NB12	HYHU	M	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3038	HYHU	M	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
SDSU 3039	HYHU	M	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
SDSU 3060	HYHU	M	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
SDSU 3062	HYHU	M	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
E05951	HYHU	M	Arizona State Beach, OR	Curry	42°36'54.99"	124°23'49.29"
E05952	HYHU	M	Arizona State Beach, OR	Curry	42°36'54.99"	124°23'49.29"
E05964	HYHU	M	Arizona State Beach, OR	Curry	42°36'54.99"	124°23'49.29"
E05966	HYHU	M	Arizona State Beach, OR	Curry	42°36'56.97"	124°23'44.57"
SDSU 3063	HYHU	F	Arizona State Beach, OR	Curry	42°36'56.97"	124°23'44.57"
NB39	ALHU	F	Humbug Mountain State Park, OR	Curry	41°13'20.33"	124°26'36.68"
NB40	ALHU	F	Humbug Mountain State Park, OR	Curry	41°13'20.33"	124°26'36.68"
NB41	HYHU	F	Humbug Mountain State Park, OR	Curry	41°13'20.33"	124°26'36.68"
E05963	HYHU	F	Arizona State Beach, OR	Curry	42°36'56.97"	124°23'44.57"

E05961	HYHU	F	Arizona State Beach, OR	Curry	42°36'56.97"	124°23'44.57"
SDNHM 56122	HYHU	F	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
NB51	HYHU	F	Humbug Mountain State Park, OR	Curry	41°13'20.33"	124°26'36.68"
E05989	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'56.78"	124°24'7.80"
E05988	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'56.78"	124°24'7.80"
E05987	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'53.97"	124°24'13.62"
E05986	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'59.76"	124°24'7.00"
E05985	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'56.78"	124°24'7.80"
SDNHM 56077	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'59.76"	124°24'7.00"
SDNHM 56106	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'53.97"	124°24'13.62"
SDNHM 56107	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'56.78"	124°24'7.80"
SDNHM 56078	HYHU	M	Bullard's Beach State Park, OR	Coos	43° 8'59.76"	124°24'7.00"
SDSU 3064	HYHU	M	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56112	HYHU	M	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56111	HYHU	M	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56080	HYHU	M	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SSDNHM 56075	RUHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56105	RUHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56072	HYHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56108	HYHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56079	HYHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56076	ALHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
SDNHM 56075	HYHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°24'7.08"
*	ALHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
*	RUHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
SDNHM 56071	HYHU	F	Bullard's Beach State Park, OR	Coos	43°8'56.81"	124°26'2.05"

SDSU 3065	HYHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
SDNHM 56125	HYHU	F	Bradley Lake County Park, OR	Coos	43°4'0.72"	124°25'44.10"
*	ALHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
*	HYHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
*	HYHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
*	ALHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
*	ALHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
*	HYHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
*	HYHU	F	Bandon State Park, OR	Coos	43°8'56.81"	124°26'2.05"
SDSU 3066	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'53.69"	124°22'8.56"
SDSU 3067	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'53.69"	124°22'8.56"
SDSU 3061	HYHU	M	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3068	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3069	HYHU	M	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3070	HYHU	M	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3071	HYHU	M	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3072	HYHU	M	Sunset Bay State Park, OR	Coos	43°19'52.17"	124°22'14.54"
SDSU 3073	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'52.17"	124°22'14.54"
SDSU 3074	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'52.17"	124°22'14.54"
SDSU 3075	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'52.17"	124°22'14.54"
SDSU 3076	HYHU	M	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB1	HYHU	M	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB2	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB3	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB4	RUHU	M	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
SDNHM 56113	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"

NB52	HYHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB53	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB54	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB55	HYHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB56	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB57	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB58	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB59	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
SDSU 3077	RUHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB60	HYHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB61`	HYHU	F	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
SDNHM 56067	HYHU	F	Powers, OR	Coos	42°53'0.39"	124°4'23.35"
SDNHM 56093	RUHU	M	Apple Creek, Umpqua National Forest, OR	Douglas	43°18'10.24"	122°40'18.43"
SDNHM 56092	RUHU	M	Apple Creek, Umpqua National Forest, OR	Douglas	43°18'10.24"	122°40'18.43"
SDNHM 56091	RUHU	M	Apple Creek, Umpqua National Forest, OR	Douglas	43°18'10.24"	122°40'18.43"
SDNHM 56089	RUHU	M	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56088	RUHU	M	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56087	RUHU	M	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56086	RUHU	M	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56090	RUHU	F	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56104	RUHU	F	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDSU 3078	RUHU	F	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDSU 3079	RUHU	M	Spinreel Campground, OR	Coos	43°34'9.98"	124°12'11.93"

SDSU 3080	RUHU	M	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
SDSU 3081	RUHU	M	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
NB10	RUHU	M	Tugman State Park, OR	Coos	43°34'13.45"	124°12'10.19"
SDSU 3082	RUHU	M	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"
SDSU 3083	RUHU	M	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"
SDSU 3084	HYHU	M	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"
SDSU 3085	HYHU	M	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
NB11	RUHU	M	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
SDSU 3086	RUHU	F	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"
SDSU 3087	RUHU	M	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
SDNHM 56122	RUHU	M	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
NB5	RUHU	M	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
SDSU 3088	RUHU	M	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
SDNHM 56123	HYHU	M	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
NB6	RUHU	M	Umpqua Lighthouse State Park, OR	Douglas	43°39'47.58"	124°11'56.49"
SDSU 3089	RUHU	F	Umpqua Lighthouse State Park, OR	Douglas	43°39'47.58"	124°11'56.49"
NB36	HYHU	F	Umpqua Lighthouse State Park, OR	Douglas	43°39'47.58"	124°11'56.49"
NB37	RUHU	F	Umpqua Lighthouse State Park, OR	Douglas	43°39'47.58"	124°11'56.49"
NB38	RUHU	F	Umpqua Lighthouse State Park, OR	Douglas	43°39'47.58"	124°11'56.49"
SDNHM 56119	RUHU	M	Siltcoos Campground, OR	Lane	43°52'32.70"	124°8'35.55"
SDSU 3090	RUHU	M	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
SDSU 3091	RUHU	M	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
SDSU 3092	RUHU	M	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
SDNHM 56120	RUHU	M	Siltcoos Campground, OR	Lane	43°52'36.13"	124°8'36.09"
SDSU 3093	HYHU	M	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
SDSU 3094	RUHU	F	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"

NB23	RUHU	F	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
E05978	RUHU	M	Honeyman State Park, OR	Lane	43°55'53.82"	124° 6'39.33"
SDSU 3006	RUHU	M	Honeyman State Park, OR	Lane	43°55'53.82"	124° 6'39.33"
E05973	RUHU	M	Honeyman State Park, OR	Lane	43°55'53.82"	124° 6'39.33"
E05976	RUHU	M	Honeyman State Park, OR	Lane	43°55'53.82"	124° 6'39.33"
E05972	RUHU	M	Honeyman State Park, OR	Lane	43°55'59.46"	124° 6'43.25"
E05990	RUHU	M	Honeyman State Park, OR	Lane	43°55'56.38"	124° 6'42.71"
E05977	RUHU	M	Honeyman State Park, OR	Lane	43°55'56.21"	124° 6'41.02"
SDSU 3005	RUHU	M	Honeyman State Park, OR	Lane	43°55'56.21"	124° 6'41.02"
E05971	HYHU	M	Honeyman State Park, OR	Lane	43°55'59.46"	124°6'43.25"
E05979	HYHU	F	Honeyman State Park, OR	Lane	43°55'56.21"	124°6'41.02"
E05974	RUHU	F	Honeyman State Park, OR	Lane	43°55'56.21"	124°6'41.02"
SDNHM 56094	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'36.22"	123°54'1.71"
SDNHM 56096	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'36.22"	123°54'1.71"
SDNHM 56097	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'36.22"	123°54'1.71"
SDNHM 56098	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'41.12"	123°53'35.48"
SDNHM 56099	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'41.12"	123°53'35.48"
SDNHM 56101	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'27.09"	123°53'45.82"
SDNHM 56102	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'27.09"	123°53'45.82"
SDNHM 56103	RUHU	M	Clatsop State Forest, OR	Clatsop	45°43'27.09"	123°53'45.82"

Table S1.2 – **Morphological characters of adult males from references in the literature; mean and standard deviation in parentheses when available.** The top range for each character listed is for Allen's Hummingbird (*S. s. sasin*), the bottom for Rufous Hummingbird (*S. rufus*). Measurements are in mm.

Character	Pyle (1997)	Colwell (2005)	Stiles (1972)
Tail length	21.9-25.9	N/A	22.6-25.8, (24.37 ± 0.74)
	22.5-28.3	N/A	25.3-28.9, (27.36 ± 0.91)
Folded wing chord	36.2-43.3	38.33 ± 0.99	36.8-39.6, (38.08 ± 0.84)
	38.1-46.6	40.88 ± 0.88	38.7-41.6, (40.32 ± 0.87)
Exposed culmen	14.0-17.0	N/A	14.9-17.0, (15.92 ± 0.49)
	14.4-18.0	N/A	15.1-17.6, (16.07 ± 0.60)
*r2 outer distance	N/A	0.0, (0.0 ± 0.0)	N/A
	N/A	3.0-4.7, (3.75 ± 0.39)	N/A
*r2 outer depth	N/A	0.0, (0.0 ± 0.0)	N/A
	N/A	0.2-0.9, (0.57 ± 0.16)	N/A
*r2 inner distance	N/A	0.0, (0.0 ± 0.0)	N/A
	N/A	4.0-6.0, (4.98 ± 0.46)	N/A
*r2 inner depth	N/A	0.0, (0.0 ± 0.0)	N/A
	N/A	0.6-2.0, (1.27 ± 0.32)	N/A
*r5 width	1.2-1.9	0.9-1.9, (1.44 ± 0.22)	N/A
	1.8-2.6	2.0-2.7, (2.39 ± 0.18)	N/A
Percent green back (%)	60-100	80-100	N/A
	0-50	Variable	N/A

Table S1.3 – **Morphological characters of adult females from references in the literature; mean and standard deviation in parentheses when available.** The top range for each character listed is for Allen's Hummingbird (*S. s. sasin*), the bottom for Rufous Hummingbird (*S. rufus*). All measurements are in mm.

Character	Pyle (1997)	Colwell (2005)	Stiles (1972)
Tail length	21.9-25.9	N/A	22.3-25.8, (23.89 ± 0.96)
	23.9-28.3	N/A	16.3-18.8, (17.63 ± 0.64)
Folded wing chord	39.5-43.3	N/A	39.8-42.8, (41.46 ± 0.80)
	42.6-46.6	N/A	42.6-45.8, (44.40 ± 0.81)
Exposed culmen	15.8-18.8	N/A	16.0-18.6, (17.32 ± 0.64)
	16.4-19.0	N/A	16.3-18.8, (17.63 ± 0.64)
*r2 notch length	Absent	N/A	Absent/Present
	Present	N/A	N/A
*r1 width	N/A	N/A	6.0-7.8, (6.98 ± 0.42)
	N/A	N/A	7.5-8.7, (7.96 ± 0.29)
*r5 width	2.0-2.8	N/A	2.0-2.7, (2.39 ± 0.21)
	2.7-4.0	N/A	2.8-4.0, (3.34 ± 0.31)

*r1-r5 designate tail rectrix measurements.

Table S1.4 – **Average Linear Discriminant Function scores (LDA) for each phenotypic character for males and females; scores furthest away from zero indicate the highest contributions to group membership for k=3 for Allen's Hummingbird (*S. s. sasin*), Rufous Hummingbird (*S. rufus*), and hybrids.**

Character	Male LDF	Female LDF
Tail length	17.8	20.3
Folded wing chord	48.7	33.4
Exposed culmen	47.6	22.1
*r1 width	27.9	17.1
*r2 outer distance	4.3	-5.1
*r2 outer depth	-21.4	N/A
*r2 inner distance	-6.9	N/A
*r2 inner depth	-11.5	N/A
*r2 width	-5.4	-2.4
*r3 width	24.7	16.5
*r4 width	-20.1	44.1
*r5 width	47.8	4.1
Percent green back (%)	0.6	N/A
Post-shuttle chirrups	2.9	N/A
Post-dive chirrups	14.20	N/A
Fundamental frequency of dive (kHz)	0.1	N/A
Maximum consecutive dives	-5.4	N/A
Maximum consecutive pendulums	3.0	N/A
Pendulums to dives (ratio)	1.7	N/A

*r1-r5 designate tail rectrix measurements.

Table S1.5 – **Linear Discriminant Function Analysis classifications for male Allen's Hummingbird (*S. s. sasin*), Rufous Hummingbird (*S. rufus*), and hybrid hummingbirds sampled in the field; k=3 groups were used: Allen's Hummingbird, Rufous Hummingbird, and hybrid.**

Nearest city, County, State	N	Allen's	Hybrid	Rufous
San Luis Obispo, San Luis Obispo, CA	6	6	0	0
Monterey, Monterey, CA	5	5	0	0
Mendocino, Monterey, CA	4	4	0	0
Weott, Humboldt, CA	10	10	0	0
Arcata, Humboldt, CA	5	3	2	0
Trinidad, Humboldt, CA	9	8	1	0
Orick, Humboldt, CA	9	7	2	0
Crescent City, Del Norte, CA	20	7	13	0
Happy Camp, Siskiyou, CA	2	0	2	0
Seiad Valley, Siskiyou, CA	2	0	1	1
Brookings, Curry, OR	8	1	7	0
Selma, Josephine, OR	5	0	3	2
Gold Beach, Curry, OR	9	2	7	0
Port Orford, Curry, OR	15	0	15	0
Bandon, Coos, OR	13	0	13	0
Clearwater, Douglas, OR	7	0	0	7
Charleston, Coos, OR	16	0	7	9
Lakeside, Coos, OR	9	0	2	7
Winchester Bay, Douglas, OR	6	0	1	5
Dunes City, Lane, OR	6	0	1	5
Florence, Lane, OR	9	0	1	8
Astoria, Clatsop, OR	8	0	0	8

Table S1.6 – **Linear Discriminant Function Analysis classifications for female Allen's Hummingbird (*S. s. sasin*), Rufous Hummingbird (*S. rufus*), and hybrid hummingbirds sampled in the field; k=3 groups were used: Allen's Hummingbird, Rufous Hummingbird, and hybrid.**

Nearest city, County, State	N	Allen's	Hybrid	Rufous
Mendocino, Mendocino, CA	1	1	0	0
Arcata, CA (Humboldt)	5	3	2	0
Trinidad, CA (Humboldt)	1	1	0	0
Orick, CA (Humboldt)	2	1	1	0
Crescent City, CA (Del Norte)	5	2	3	0
Happy Camp, CA (Siskiyou)	2	0	0	2
Seiad Valley, CA (Siskiyou)	2	0	0	2
Brookings, OR (Curry)	3	0	3	0
Gold Beach, OR (Curry)	1	0	1	0
Port Orford, OR (Curry)	8	2	6	0
Bandon, OR (Coos)	19	5	11	3
Charleston, OR (Coos)	12	0	4	8
Powers, OR (Coos)	1	0	1	0
Clearwater, OR (Douglas)	3	0	0	3
Lakeside, OR (Coos)	1	0	0	1
Winchester Bay, OR (Douglas)	4	0	1	3
Dunes City, OR (Lane)	2	0	0	2
Florence, OR (Lane)	2	0	1	1

*64 Rufous Hummingbird and Allen's Hummingbird females were also utilized from museum collections to supplement my sample size (not included in table).

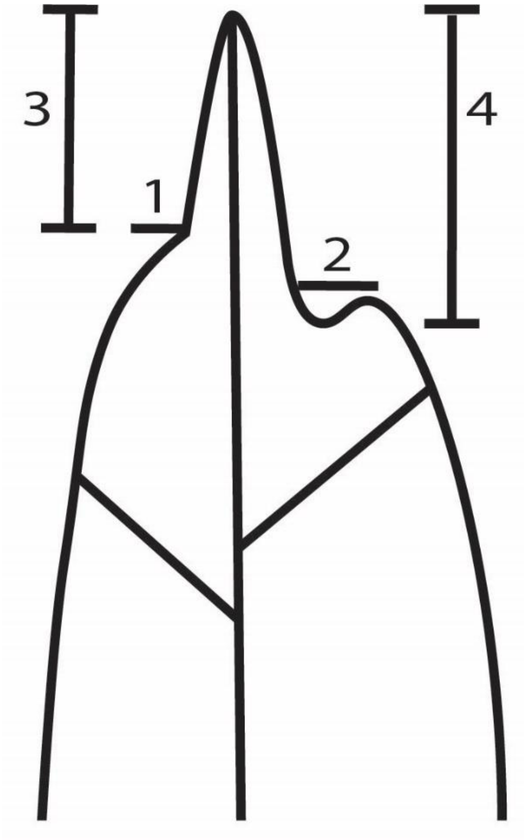


Figure S1.1 – **Shape of the second rectrix of adult male Rufous Hummingbird (*S. rufus*).** Measurements from Colwell (2005): (1) depth of emargination of the outer web, (2) depth of the notch of the inner web, (3) length of emargination of the outer web, and (4) length of the notch from the tip on the inner web.

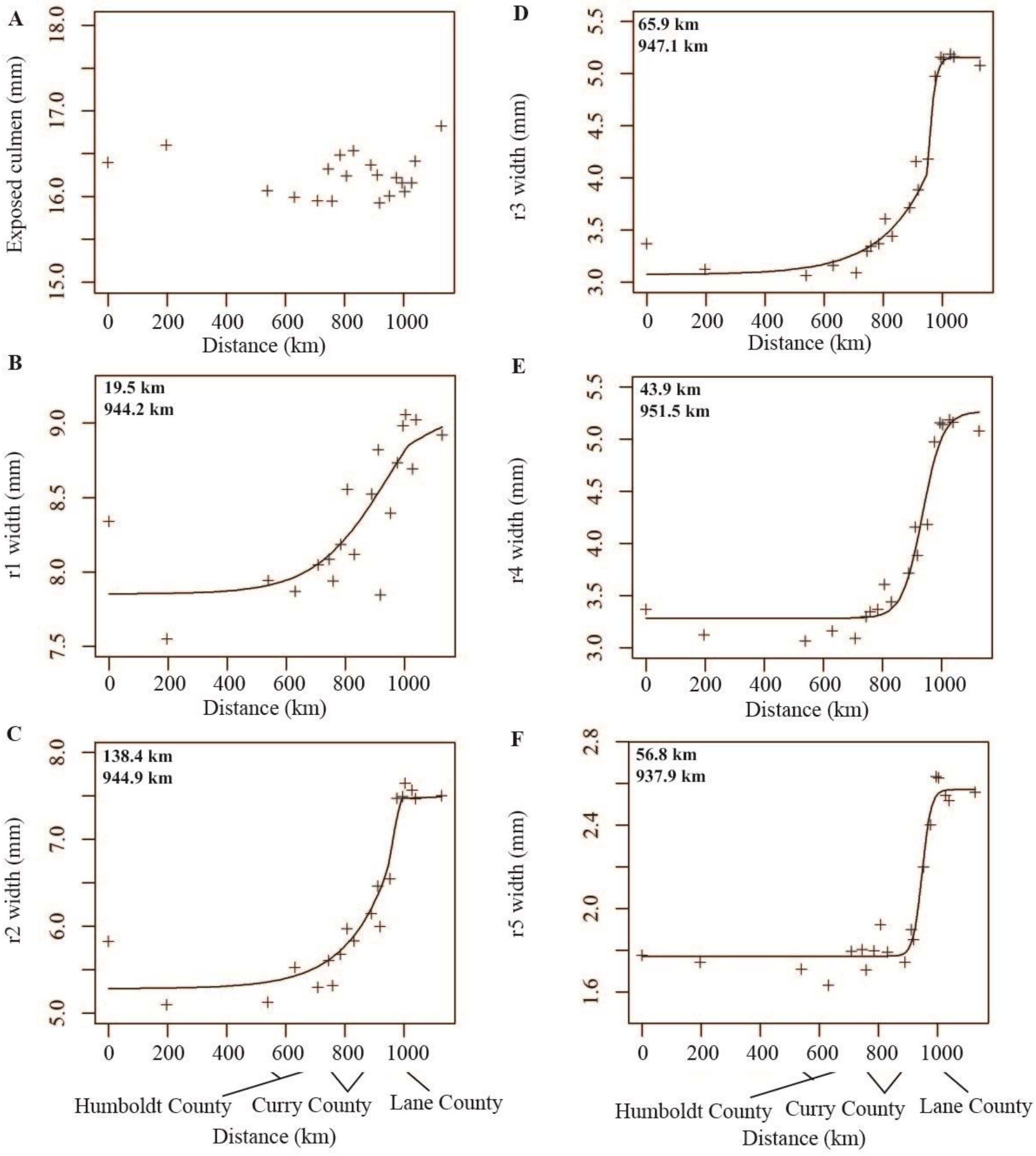


Figure S1.2 – **Cline analysis for males across the coastal, north-south transect for the following characters: (A) exposed culmen (mm), (B) rectrix one width (mm), (C) rectrix two width (mm), (D) rectrix three width (mm), (E) rectrix four width (mm), and (F) rectrix five width (mm).** The southernmost Allen's Hummingbird (*S. s. sasin*) population (San Luis Obispo County, CA) was considered the starting point for distance, and given a distance of zero km, with the northernmost Rufous Hummingbird (*S. rufus*) population (Clatsop County, OR) 1200 km away from the southernmost Allen's Hummingbird population. Cline and associated data for exposed culmen were removed due to wide confidence intervals that spanned the extent of the transect. Within each plot, top distance is cline width (km), bottom distance designates cline center (km).

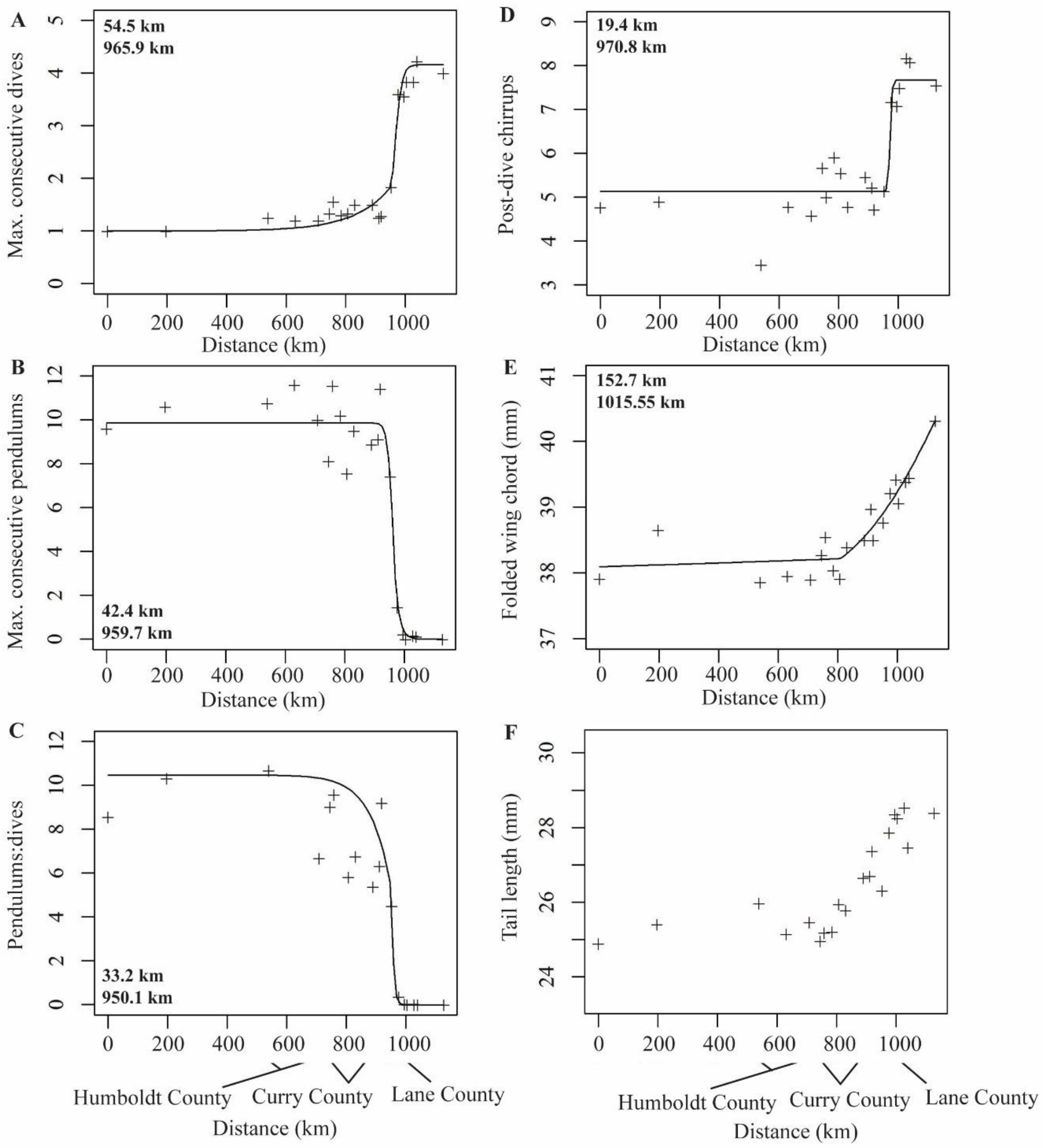


Figure S1.3 – **Cline analysis for males across the coastal, north-south transect for the following characters: (A) maximum consecutive dives, (B) maximum consecutive pendulums, (C) the ratio of pendulums to dives performed by each individual, (D) the number of post-dive chirrups, (E) folded wing chord (mm), and (F) tail length (mm).** Cline and associated data for tail length were removed due to wide confidence intervals that spanned the extent of the transect. The southernmost Allen's Hummingbird population (San Luis Obispo County, CA) was considered the starting point for distance, and given a distance of zero km, with the northernmost Rufous Hummingbird population (Clatsop County, OR) 1200 km away from the southernmost Allen's Hummingbird population. Within each plot, top distance is cline width (km), bottom distance designates cline center (km).

Appendix B: Supplementary Tables/Figures for Chapter 2

Table S2.1 – **Locality data for *Selasphorus* males.** "NB" indicates a bird that was not collected or banded. Individuals with a band number or "NB" were released after blood sample collection; all other samples are from collected individuals.

Band/ID	Locality	County	Latitude	Longitude
SDSU 2983	Pismo State Beach, CA	San Luis Obispo	35°6'38.15"	120°37'39.18"
SDSU 2984	Montana de Oro State Park, CA	San Luis Obispo	35°6'35.98"	120°37'39.30"
E05846	Pismo State Beach, CA	San Luis Obispo	35°6'38.15"	120°37'39.18"
E05908	Pismo State Beach, CA	San Luis Obispo	35°6'38.15"	120°37'39.18"
E05909	Pismo State Beach, CA	San Luis Obispo	35°6'35.98"	120°37'39.30"
SDSU 2985	Carmel River State Beach, CA	Monterey	36°31'45.14"	121°55'27.52"
SDSU 2986	Carmel River State Beach, CA	Monterey	36°31'45.14"	121°55'27.52"
E05998	Carmel River State Beach, CA	Monterey	36°31'45.14"	121°55'27.52"
E05907	Carmel River State Beach, CA	Monterey	36°31'45.14"	121°55'27.52"
SDSU 2987	Mendocino Headlands State Park, CA	Mendocino	39°18'10.53"	123°47'16.98"
SDSU 2988	Mendocino Headlands State Park, CA	Mendocino	39°18'10.53"	123°47'16.98"
E05996	Mendocino Headlands State Park, CA	Mendocino	39°18'10.53"	123°47'16.98"
E05905	Van Damme State Park, CA	Mendocino	39°16'29.18"	123°47'24.35"
SDSU 2989	Humboldt Redwoods State Park, CA	Humboldt	40°21'3.26"	124°0'41.75"
SDSU 2990	Humboldt Redwoods State Park, CA	Humboldt	40°21'3.26"	124°0'41.75"
E05902	Humboldt Redwoods State Park, CA	Humboldt	40°21'3.26"	124°0'41.75"
E05920	Humboldt Redwoods State Park, CA	Humboldt	40°21'9.77"	124°0'28.43"
E05918	Humboldt Redwoods State Park, CA	Humboldt	40°21'9.77"	124°0'28.43"

SDNHM 56129	Humboldt Redwoods State Park, CA	Humboldt	40°21'9.77"	124°0'28.43"
SDNHM 56130	Humboldt Redwoods State Park, CA	Humboldt	40°21'6.29"	124°0'31.88"
SDNHM 56131	Humboldt Redwoods State Park, CA	Humboldt	40°21'6.29"	124°0'31.88"
E05917	Humboldt Redwoods State Park, CA	Humboldt	40°21'11.72"	124°0'30.39"
E05916	Humboldt Redwoods State Park, CA	Humboldt	40°21'11.72"	124°0'30.39"
E05901	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'27.78"	124°8'28.43"
E05949	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'27.78"	124°8'28.43"
SDSU 2992	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.55"	124°8'29.30"
SDSU 2991	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'24.55"	124°8'29.30"
E05915	Humboldt Bay Bird Observatory, CA	Humboldt	40°53'22.78"	124°8'28.43"
SDNHM 56127	Patrick's Point State Park, CA	Humboldt	41°8'21.16"	124°9'1.06"
SDSU 2995	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05937	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05947	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05936	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
SDSU 2996	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05960	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05938	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"

E05970	Humboldt Lagoons State Park, CA	Humboldt	41°13'20.33"	124°5'46.76"
E05919	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05903	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05995	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05842	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDNHM 56128	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDSU 2993	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDSU 2994	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05843	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
E05994	Prairie Creek Redwoods State Park, CA	Humboldt	41°21'49.68"	124°1'6.54"
SDSU 2997	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124°5'51.62"
E05935	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124°5'51.62"
E05969	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124°5'51.62"
E05946	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124°5'51.62"
SDSU 2998	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124°5'51.62"
E05957	Del Norte Coast Redwoods State Park, CA	Del Norte	41°42'10.03"	124°5'51.62"
E05955	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'56.79"	124°6'18.30"

K53386	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'56.79"	124°6'18.30"
E05933	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'54.77"	124°6'16.36"
E05956	Del Norte Coast Redwoods State Park, CA	Del Norte	41°41'54.77"	124°6'16.36"
E05968	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 3029	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 3030	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 3031	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
E05932	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
E05913	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
K53374	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
K53375	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
E05914	Jedediah Smith Redwoods State Park, CA	Del Norte	41°50'14.75"	124°6'49.76"
SDSU 2999	Alfred Loeb State Park, CA	Curry	42°6'45.34"	124°11'9.98"
SDSU 3000	Alfred Loeb State Park, CA	Curry	42°6'45.34"	124°11'9.98"
E05944	Alfred Loeb State Park, CA	Curry	42°6'45.34"	124°11'9.98"
E05954	Whaleshead Beach, OR	Curry	42°8'43.67"	124°21'17.37"
K53383	Alfred Loeb State Park, CA	Curry	42°6'43.99"	124°11'12.30"
K53376	Alfred Loeb State Park, CA	Curry	42°6'43.99"	124°11'12.30"
K53377	Harris Beach State Park, OR	Curry	42°4'3.93"	124°18'29.80"
SDSU 3001	Whaleshead Beach, OR	Curry	42°8'43.67"	124°21'17.37"

K53385	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
E05952	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
SDSU 3002	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
E05943	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
SDSU 3003	Lobster Creek Campground, OR	Curry	42°30'4.03"	124°17'51.67"
K53381	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
K53382	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
SDSU 3004	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
K53386	Quosatana Campground, OR	Curry	42°29'53.91"	124°14'5.12"
SDNHM 56124	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3033	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3034	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3035	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3036	Cape Blanco State Park, OR	Curry	42°49'50.96"	124°32'51.25"
SDSU 3037	Cape Blanco State Park, OR	Curry	42°49'49.07"	124°32'49.43"
NB12	Cape Blanco State Park, OR	Curry	42°49'49.07"	124°32'49.43"
SDSU 3038	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
SDSU 3039	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
SDSU 3060	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
SDSU 3062	Humbug Mountain State Park, OR	Curry	42°41'17.96"	124°26'36.68"
E05951	Arizona State Beach, OR	Curry	42°36'54.99"	124°23'49.29"
E05952	Arizona State Beach, OR	Curry	42°36'54.99"	124°23'49.29"
E05964	Arizona State Beach, OR	Curry	42°36'54.99"	124°23'49.29"
E05966	Arizona State Beach, OR	Curry	42°36'54.99"	124°23'49.29"
E05812	New River, OR	Coos	42°59'50.05"	124°26'59.47"

E08527	New River, OR	Coos	42°59'50.05"	124°26'59.47"
E08528	New River, OR	Coos	42°59'50.05"	124°26'59.47"
E08526	New River, OR	Coos	42°59'50.05"	124°26'59.47"
E08525	New River, OR	Coos	42°59'50.05"	124°26'59.47"
E08523	New River, OR	Coos	42°59'50.05"	124°26'59.47"
E05989	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
E05988	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
E05987	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
E05986	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
E05985	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56077	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56106	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56107	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56078	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDSU 3064	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56112	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56111	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56080	Bullard's Beach State Park, OR	Coos	43°8'53.97"	124°24'13.62"
SDNHM 56139	Coos Forest, OR	Coos	43°11'39.25"	124°18'10.8"
SDNHM 56140	Coos Forest, OR	Coos	43°11'39.25"	124°18'10.8"
SDNHM 56141	Coos Forest, OR	Coos	43°11'39.25"	124°18'10.8"
NB63	Coos Forest, OR	Coos	43°11'39.25"	124°18'10.8"
NB64	Coos Forest, OR	Coos	43°11'39.25"	124°18'10.8"
E05813	South Slough, OR	Coos	43°17'44.86"	124°20'6.73"
E05818	South Slough, OR	Coos	43°17'44.86"	124°20'6.73"
E05817	South Slough, OR	Coos	43°17'44.86"	124°20'6.73"
E05815	South Slough, OR	Coos	43°17'44.86"	124°20'6.73"
E05983	South Slough, OR	Coos	43°17'44.86"	124°20'6.73"
E05982	South Slough, OR	Coos	43°17'44.86"	124°20'6.73"
E085821	South Slough, OR	Coos	42°59'44.49"	124°27'4.02"
E08522	South Slough, OR	Coos	42°59'44.49"	124°27'4.02"
SDSU 3066	Sunset Bay State Park, OR	Coos	43°19'53.69"	124°22'8.56"

SDSU 3067	Sunset Bay State Park, OR	Coos	43°19'53.69"	124°22'8.56"
SDSU 3061	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
SDSU 3068	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3069	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3070	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3071	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3072	Sunset Bay State Park, OR	Coos	43°19'51.96"	124°22'13.72"
SDSU 3073	Sunset Bay State Park, OR	Coos	43°19'52.17"	124°22'14.54"
SDSU 3074	Sunset Bay State Park, OR	Coos	43°19'52.17"	124°22'14.54"
SDSU 3075	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
SDSU 3076	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB1	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB2	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB3	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
NB4	Sunset Bay State Park, OR	Coos	43°19'53.24"	124°22'8.69"
SDNHM 56093	Apple Creek, Umpqua National Forest, OR	Douglas	43°18'10.24"	122°40'18.43"
SDNHM 56092	Apple Creek, Umpqua National Forest, OR	Douglas	43°18'10.24"	122°40'18.43"
SDNHM 56091	Apple Creek, Umpqua National Forest, OR	Douglas	43°18'10.24"	122°40'18.43"
SDNHM 56089	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56088	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56087	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDNHM 56086	Toketee Lake, Umpqua National Forest, OR	Douglas	43°16'20.47"	122°24'38.62"
SDSU 3079	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"

SDSU 3080	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
SDSU 3081	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
NB10	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
SDSU 3082	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"
SDSU 3083	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"
SDSU 3084	Spinreel Campground, OR	Coos	43°34'12.16"	124°12'20.90"
SDSU 3085	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
NB11	Tugman State Park, OR	Coos	43°34'9.98"	124°12'11.93"
SDSU 3087	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
SDNHM 56122	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
NB5	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
SDSU 3088	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
SDNHM 56123	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
NB6	Umpqua Lighthouse State Park, OR	Douglas	43°39'45.11"	124°11'57.18"
SDNHM 56119	Siltcoos Campground, OR	Lane	43°52'32.70"	124°8'35.55"
SDSU 3090	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
SDSU 3091	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
SDSU 3092	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
SDNHM 56120	Siltcoos Campground, OR	Lane	43°52'32.70"	124°8'35.55"
SDSU 3093	Siltcoos Campground, OR	Lane	43°34'12.16"	124°12'20.90"
E05855	Siltcoos Campground, OR	Lane	43°52'33.98"	124°8'41.88"
E05857	Siltcoos Campground, OR	Lane	43°52'33.98"	124°8'41.88"
E05856	Siltcoos Campground, OR	Lane	43°52'33.98"	124°8'41.88"

E05978	Honeyman State Park, OR	Lane	43°55'53.82"	124°6'39.33"
SDSU 3006	Honeyman State Park, OR	Lane	43°55'53.82"	124°6'39.33"
E05973	Honeyman State Park, OR	Lane	43°55'53.82"	124°6'39.33"
E05976	Honeyman State Park, OR	Lane	43°55'59.46"	124°6'43.25"
E05972	Honeyman State Park, OR	Lane	43°55'59.46"	124°6'43.25"
E05990	Honeyman State Park, OR	Lane	43°55'56.38"	124°6'42.71"
E05977	Honeyman State Park, OR	Lane	43°55'56.21"	124°6'41.02"
SDSU 3005	Honeyman State Park, OR	Lane	43°55'56.21"	124°6'41.02"
E05971	Honeyman State Park, OR	Lane	43°55'59.46"	124°6'43.25"
SDNHM 56094	Clatsop State Forest, OR	Clatsop	45°43'36.22"	123°54'1.71"
SDNHM 56096	Clatsop State Forest, OR	Clatsop	45°43'36.22"	123°54'1.71"
SDNHM 56097	Clatsop State Forest, OR	Clatsop	45°43'36.22"	123°54'1.71"
SDNHM 56098	Clatsop State Forest, OR	Clatsop	45°43'41.12"	123°53'35.48"
SDNHM 56099	Clatsop State Forest, OR	Clatsop	45°43'41.12"	123°53'35.48"
SDNHM 56101	Clatsop State Forest, OR	Clatsop	45°43'27.09"	123°53'45.82"
SDNHM 56102	Clatsop State Forest, OR	Clatsop	45°43'27.09"	123°53'45.82"
SDNHM 56103	Clatsop State Forest, OR	Clatsop	45°43'27.09"	123°53'45.82"
E05858	Beaver Creek, OR	Lincoln	44°30'53.14"	124°2'56.35"
SDSU 3027	Beaver Creek, OR	Lincoln	44°30'53.14"	124°2'56.35"
E05860	Beaver Creek, OR	Lincoln	44°30'53.14"	124°2'56.35"
E05831	Beaver Creek, OR	Lincoln	44°30'53.14"	124°2'56.35"
SDSU 3028	Beaver Creek, OR	Lincoln	44°30'53.14"	124°2'56.35"
E05834	Beaver Creek, OR	Lincoln	44°30'53.14"	124°2'56.35"
E05833	Beaver Creek, OR	Lincoln	44°30'53.14"	124°2'56.35"
SDNHM 56150	Juneau, AK	-	58°20'13.2"	134°33'36.65"
SDNHM 56151	Juneau, AK	-	58°20'13.2"	134°33'36.65"
SDNHM 56152	Juneau, AK	-	58°20'13.2"	134°33'36.65"
SDNHM 56153	Juneau, AK	-	58°20'13.2"	134°33'36.65"
SDNHM 56154	Juneau, AK	-	58°20'13.2"	134°33'36.65"
SDNHM 56149	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"

SDNHM 56142	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"
SDNHM 56144	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"
SDNHM 56145	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"
SDNHM 56146	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"
SDNHM 56147	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"
SDNHM 56148	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"
SDNHM 6138	Ketchikan, AK	-	55°26'3.92"	131°36'57.52"
E05900	Aiken's Creek, CA	Humboldt	41°14'24.16"	123°39'20.78"
E05899	Aiken's Creek, CA	Humboldt	41°14'24.16"	123°39'20.78"
SDSU 3008	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
SDSU 3009	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
SDSU 3010	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
K53310	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
E08529	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
K53294	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
SDNHM 56137	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
E08530	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
K53300	Aiken's Creek, CA	Humboldt	41°13'41.26"	123°39'25.15"
E05825	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
E05830	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
SDSU 3023	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
SDSU 3024	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
SDSU 3025	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
SDSU 3026	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
E05829	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
E05827	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
E05826	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"

E05824	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
E05828	Ullathorne, CA	Humboldt	41°17'15.96"	123°34'16.38"
E05896	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
E05829	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
E05897	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
E05898	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
SDSU 3013	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
SDSU 3012	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
SDSU 3011	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
E05930	Green Riffle, CA	Humboldt	41°26'25.91"	123°30'20.67"
SDSU 3017	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
E05925	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
E05895	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
SDSU 3014	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
SDSU 3015	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
SDSU 3016	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
E05928	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
E05926	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
E05927	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
E05894	Ti Creek, CA	Humboldt	41°31'30.52"	123°31'42.86"
E05861	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
E05870	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
E05862	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
E05865	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
E05891	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
SDSU 3020	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"

SDSU 3019	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
SDSU 3018	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
SDSU 3021	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
SDSU 3022	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
E05864	Wingate Bar, CA	Siskiyou	41°43'22.31"	123°26'14.94"
E05893	Happy Camp, CA	Siskiyou	41°48'28.21"	123°21'26.94"
E05921	Happy Camp, CA	Siskiyou	41°48'28.21"	123°21'26.94"
E05869	Happy Camp, CA	Siskiyou	41°48'28.21"	123°21'26.94"
E05867	Happy Camp, CA	Siskiyou	41°48'28.21"	123°21'26.94"
E05892	Happy Camp, CA	Siskiyou	41°47'20.96"	123°22'51.12"
E05868	Happy Camp, CA	Siskiyou	41°47'20.96"	123°22'51.12"
E05866	Happy Camp, CA	Siskiyou	41°47'20.96"	123°22'51.12"
E05922	Happy Camp, CA	Siskiyou	41°47'20.96"	123°22'51.12"
E05923	Happy Camp, CA	Siskiyou	41°47'20.96"	123°22'51.12"
SDNHM 56055	Happy Camp, CA	Siskiyou	41°46'38.09"	123°23'48.75"
SDNHM 56065	Happy Camp, CA	Siskiyou	41°46'38.09"	123°23'48.75"
SDNHM 56062	Seiad Valley, CA	Siskiyou	41°50'10.66"	123°10'24.85"
SDNHM 56059	Seiad Valley, CA	Siskiyou	41°50'10.66"	123°10'24.85"
E05806	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05821	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05810	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05808	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05822	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05807	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05805	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05809	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05823	Seiad, CA	Siskiyou	41°50'25.04"	123°11'49.55"
E05803	Horse Creek, CA	Siskiyou	41°49'58.14"	123°0'20.53"
E05840	Horse Creek, CA	Siskiyou	41°49'58.14"	123°0'20.53"
E05839	Horse Creek, CA	Siskiyou	41°49'58.14"	123°0'20.53"
E05838	Horse Creek, CA	Siskiyou	41°49'58.14"	123°0'20.53"
E05837	Horse Creek, CA	Siskiyou	41°49'58.14"	123°0'20.53"
E05835	Horse Creek, CA	Siskiyou	41°44'23.40"	123°0'15.50"
E05804	Horse Creek, CA	Siskiyou	41°44'23.40"	123°0'15.50"
E05802	Horse Creek, CA	Siskiyou	41°44'23.40"	123°0'15.50"
SDNHM 56114	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDNHM 56115	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDNHM 56116	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDNHM 56117	Selma, OR	Josephine	42°16'49"	123°37'6.65"

SDNHM 56118	Selma, OR	Josephine	42°16'49"	123°37'6.65"
SDSU 3096	Oregon Mountain Road, CA	Del Norte	41°58'32.65"	123°44'34.74"
SDNHM 56069	Remote, OR	Coos	43°0'44.66"	123°53'3.12"
SDNHM 56070	Remote, OR	Coos	43°0'36.51"	123°53'23.44"

Table S2.2 – **First-order Markov Chain transition probability matrices for each phenotype for *Selasphorus* male courtship behavior, including A) Rufous Hummingbird, B) Allen's Hummingbird, and C) hybrids.**

A) Rufous 1

	X	S	H	R	Z
X	0.00	0.32	0.00	0.68	0.00
S	0.00	0.00	0.73	0.04	0.23
H	0.00	0.20	0.00	0.46	0.32
R	0.00	0.05	0.00	0.60	0.35
Z	0.00	0.00	0.00	0.00	1.00

Rufous 2

	X	S	H	R	R_X	R_A	Z
X	0.00	0.40	0.00	0.60	0.00	0.00	0.00
S	0	0	0.75	0.25	0	0	0
H	0.00	0.00	0.00	0.89	0.00	0.11	0.00
R	0.00	0.00	0.00	0.42	0.08	0.08	0.42
R_X	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R_A	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Z	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Rufous 3

	X	S	H	R	R_X	R_A	Z
X	0.00	0.38	0.00	0.62	0.01	0.00	0.00
S	0.00	0.00	0.35	0.04	0.02	0.00	0.59
H	0.00	0.06	0.00	0.24	0.00	0.06	0.66
R	0.00	0.06	0.00	0.63	0.00	0.04	0.27
R_X	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R_A	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Z	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Rufous 4

	X	S	H	R	R_X	Z
X	0.00	0.29	0.00	0.71	0.00	0.00
S	0.00	0.00	0.40	0.00	0.00	0.60
H	0.00	0.00	0.00	0.50	0.00	0.50
R	0.00	0.00	0.00	0.56	0.11	0.33
R_X	0.00	0.00	0.00	0.00	0.00	1.00
Z	0.00	0.00	0.00	0.00	0.00	1.00

Rufous 5

	X	S	H	R	R_X	R_L	Z
X	0.00	0.20	0.00	0.80	0.00	0.00	0.00
S	0.00	0.00	0.50	0.50	0.00	0.00	0.00
H	0.00	0.00	0.00	1.00	0.00	0.00	0.00
R	0.00	0.00	0.00	0.50	0.04	0.08	0.38
R_X	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R_L	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Z	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Rufous 6

	X	S	H	H_X	R	Z
X	0.00	0.55	0.00	0.00	0.45	0.00
S	0.00	0.00	0.20	0.10	0.05	0.65
H	0.00	0.17	0.00	0.00	0.33	0.50
H_X	0.00	0.00	0.00	0.00	0.00	1.00
R	0.00	0.26	0.00	0.00	0.52	0.22
Z	0.00	0.00	0.00	0.00	0.00	1.00

Rufous 7

	X	S	H_E	R	Z
X	0.00	0.70	0.00	0.30	0.00
S	0.00	0.27	0.00	0.73	0.00
H_E	0.00	0.00	0.00	1.00	0.00
R	0.00	0.05	0.00	0.55	0.40
Z	0.00	0.00	0.00	0.00	1.00

Rufous 8

	X	S	H	R_E	Z	∑
X	0.00	4.00	0.00	2.00	0.00	6.00
S	0.00	0.00	1.00	0.00	3.00	4.00
H	0.00	0.00	0.00	0.00	1.00	1.00
R_E	0.00	0.00	0.00	5.00	2.00	7.00
Z	0.00	0.00	0.00	0.00	0.00	0.00
∑	0.00	4.00	1.00	7.00	6.00	18.00

B) Allen's 1

	X	S	H	A	P	Z
X	0	.22	0	0	.78	0
S	0	0	.71	0	0	.29
H	0	.06	0	0	.63	.31
A	0	.04	0	0	.14	.82

P	0	.04	0	.08	.83	.05
Z	0	0	0	0	0	1

Allen's 2

	X	S	H	A	A_X	A_A	P	Z
X	0	.42	0	0	0	0	.58	0
S	0	0	.73	0	0	0	0	.27
H	0	.16	0	0	0	0	.74	.1
A	0	0	0	0	0	0	.1	.9
A_X	0	0	0	0	0	0	0	1
A_A	0	0	0	0	0	0	0	1
P	0	.08	0	.08	.03	.01	.75	.05
Z	0	0	0	0	0	0	0	1

Allen's 3

	X	S	H	R	A	A_X	A_A	P	Z
X	0	.62	0	0	0	0	0	.38	0
S	0	0	.46	0	0	0	0	0	.54
H	0	.04	0	0	0	0	0	.52	.44
R	0	0	0	0	0	0	0	.11	.89
A	0	0	0	0	.04	0	.05	.09	.82
A_X	0	0	0	0	0	0	0	0	1
A_A	0	0	0	0	0	0	0	0	1
P	0	.01	0	.04	.1	.04	0	.72	.08
Z	0	0	0	0	0	0	0	0	1

Allen's 4

	X	S	H	A	A_A	P	Z
X	0	.11	0	0	0	.89	0
S	0	0	.80	0	0	0	.2
H	0	.08	0	.08	0	.5	.34
A	0	.03	0	0	0	.22	.75
A_A	0	0	0	0	0	0	1
P	0	.02	0	.11	0.04	.79	.04
Z	0	0	0	0	0	0	1

Allen's 5

	X	S	H	R	A	P	Z
X	0	.08	0	0	0	.92	0
S	0	0	.67	0	0	0	.33
H	0	0	0	0	0	.57	.43

R	0	0	0	0	0	.33	.67
A	0	.07	0	0	0	.14	.79
P	0	.05	0	0.02	.05	.78	.1
Z	0	0	0	0	0	0	1

Allen's 6

	X	S	H	A	P	Z
X	0	.13	0	0	.87	0
S	0	0	.33	0	0	.67
H	0	0	0	0	0	1
A	0	0	0	.27	.17	.56
P	0	.01	0	.14	.78	.07
Z	0	0	0	0	0	1

Allen's 7

	X	S	H	A	A_X	P	Z
X	0	.24	0	0	0	.76	0
S	0	0	.25	0	0	0	.75
H	0	0	0	0	0	0	1
A	0	0	0	0	0	0	1
A_X	0	0	0	0	0	0	1
P	0	0	0	.08	.05	.85	.02
Z	0	0	0	0	0	0	1

Allen's 8

	X	S	H	R_X	A	P	Z
X	0	.46	0	0	0	.54	0
S	0	0	.66	0	0	0	.34
H	0	.48	0	0	0	.26	.26
R_X	0	0	0	0	0	0	1
A	0	0	0	0	0	.08	.92
P	0	.01	0	.01	.09	.8	.09
Z	0	0	0	0	0	0	1

C) Hybrids

Hybrid 1

	X	S	H	R	A	P	Z
X	0	.83	0	0	0	.17	0
S	0	0	.75	0	0	0	.25
H	0	.11	0	0	0	.11	.78
R	0	0	0	0	0	1	0
A	0	0	0	0	.66	0	.34

P	0	.17	0	0	0	0	.83
Z	0	0	0	0	0	0	1

Hybrid 2

	X	S	H	R	A	P	Z
X	0	.05	0	.68	0	.27	0
S	0	0	.88	.12	0	0	0
H	0	.24	0	.08	.08	.3	.3
R	0	0	.16	0	.47	.23	.14
A	0	0	0	0	0	0	1
P	0	.04	0	.68	0	.18	.1
Z	0	0	0	0	0	0	1

Hybrid 3

	X	S	H	R	A	P	Z
X	0	.43	0	.29	.24	.04	0
S	0	0	.33	0	0	0	.67
H	0	0	0	.5	0	0	.5
R	0	.13	0	.26	.13	.13	.35
A	0	.07	0	0	.5	.07	.36
P	0	0	0	.75	0	0	.25
Z	0	0	0	0	0	0	1

Hybrid 4

	X	S	H	R	A	P	Z
X	0	.25	0	.63	0	.12	0
S	0	0	.82	0	0	0	.18
H	0	0	0	.38	0	.12	.5
R	0	.02	0	.67	.01	.09	.21
A	0	0	0	0	0	0	1
P	0	0	0	.41	0	.36	.23
Z	0	0	0	0	0	0	1

Hybrid 5

	X	S	H	R	R_X	R_A	A	P	Z
X	0	.10	0	.86	.04	0	0	0	0
S	0	0	.66	.07	0	0	0	0	.27
H	0	0	0	.1	0	.18	0	.36	.36
R	0	.2	0	.53	0	0	0	.08	.19
R_X	0	0	0	0	.34	0	0	.33	.33
R_A	0	0	0	0	0	0	0	0	1
A	0	0	0	0	0	0	0	0	1

P	0	0	0	0	0	0	.14	0	.86
Z	0	0	0	0	0	0	0	0	1

Hybrid 6

	X	S	H	R	R_X	R_A	P	Z
X	0	.15	0	.79	.03	0	.03	0
S	0	0	.14	.28	0	.14	0	.44
H	0	.33	0	0	0	0	0	.67
R	0	.02	0	.55	0	.02	.05	.36
R_X	0	0	0	0	.67	0	0	.33
R_A	0	0	0	0	0	0	0	1
P	0	0	0	0	0	0	0	1
Z	0	0	0	0	0	0	0	1

Hybrid 7

	X	S	H	R	R_A	P	Z
X	0	.33	0	.60	0	.07	0
S	0	0	.82	0	0	0	.18
H	0	.26	0	.35	.09	.18	.15
R	0	0	0	.61	.02	.03	.34
R_A	0	0	0	0	0	0	1
P	0	0	0	.9	0	0	.1
Z	0	0	0	0	0	0	1

Hybrid 8

	X	S	H	R	P	Z
X	0	.34	0	.59	.07	0
S	0	0	.69	.06	0	.25
H	0	.16	0	.72	0	.12
R	0	.17	0	.53	.03	.27
P	0	0	0	.83	0	.17
Z	0	0	0	0	0	1

Hybrid 9

	X	S	H	R	R_A	P	Z
X	0	.19	0	.79	0	.02	0
S	0	0	.88	.03	0	0	.09
H	0	.25	0	.21	.17	.04	.33
R	0	.16	0	.55	.04	0	.25
R_A	0	0	0	0	0	0	1
P	0	0	0	.33	0	0	.67

Z	0	0	0	0	0	0	1

Hybrid 10

	X	S	H	R	R_X	P	Z
X	0	.31	0	.53	0	.16	0
S	0	0	.33	.07	0	0	.6
H	0	.2	0	.3	0	0	.5
R	0	.02	0	.51	.02	.11	.35
R_X	0	0	0	.5	0	0	.5
P	0	0	0	.17	0	0	.83
Z	0	0	0	0	0	0	0

Hybrid 11

	X	S	H	R	R_A	A	Z
X	0	.45	0	.55	0	0	0
S	0	0	.6	0	0	0	.4
H	0	0	0	.67	0	0	.33
R	0	0	0	.59	.05	.05	.31
R_A	0	0	0	0	0	0	1
A	0	0	0	0	0	0	1
Z	0	0	0	0	0	0	1

Hybrid 12

	X	S	H	R	P	Z
X	0	.57	0	.43	0	0
S	0	0	.71	.12	0	.17
H	0	.08	0	.66	.26	0
R	0	.12	0	.44	0	.44
P	0	0	0	0	0	1
Z	0	0	0	0	0	1

Hybrid 13

	X	S	H	R	A	P	Z
X	0	.05	0	.1	0	.85	0
S	0	0	.88	0	0	0	.12
H	0	0	0	.29	0	.58	.13
R	0	.08	0	.23	.15	.19	.35
A	0	.11	0	.06	.28	.22	.39
P	0	.04	0	.19	.12	.60	.05
Z	0	0	0	0	0	0	1

Hybrid 14

	X	S	H	R	A	A_A	A_M	P	P_D	Z
X	0.00	0.79	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.00
S	0.00	0.00	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.06
H	0.00	0.28	0.00	0.05	0.05	0.05	0.05	0.42	0.05	0.05
R	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.33
A	0.00	0.00	0.00	0.00	0.15	0.15	0.00	0.28	0.00	0.42
A_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
A_M	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.67
P	0.00	0.04	0.00	0.00	0.10	0.02	0.02	0.71	0.02	0.09
P_D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 15

	X	S	H	R	A	A_A	P	Z
X	0	.25	0	.33	.04	0	.38	0
S	0	0	.83	.09	0	0	0	.08
H	0	0	0	.64	0	0	.36	0
R	0	.16	0	.5	0	.03	.03	.38
A	0	.04	0	0	.31	.04	.15	.46
A_A	0	0	0	0	0	0	0	1
P	0	.01	0	0	.24	0	.74	.01
Z	0	0	0	0	0	0	0	1

Hybrid 16

	X	S	H	R	R_X	A	P	Z
X	0	.24	0	.21	.01	0	.54	0
S	0	0	.81	.03	0	0	0	.16
H	0	.23	0	.08	0	0	.42	.27
R	0	.09	0	.19	0	0.02	.34	.36
R_X	0	0	0	0	0	0	0	1
A	0	0	0	0	0	0.14	0	.86
P	0	.06	0	.34	0	0.02	.47	.11
Z	0	0	0	0	0	0	0	1

Hybrid 17

	X	S	H	R	A	A_X	A_A	P	Z
X	0	.79	0	0	.04	0.04	0	.13	0
S	0	0	.81	0	0	0	0	0	.19
H	0	0	0	.12	.35	0	0	.35	.18
R	0	0	0	0	.5	0	0	0	.5
A	0	0	0	0	0	0	0	.09	.91

A_X	0	0	0	0	0	0.33	0	0	0.67
A_A	0	0	0	0	0	0	0	0	1
P	0	.03	0	0	.09	0.03	.06	.7	.09
Z	0	0	0	0	0	0	0	0	1

Hybrid 18

	X	S	H	R	A	A_A	P	Z
X	0	.54	0	.08	0	.04	.34	0
S	0	0	.91	0	0	0	.09	.09
H	0	.04	0	.04	0	.04	.44	.44
R	0	0	0	.14	0	0	.43	.43
A	0	.05	0	0	.27	0	.14	.54
A_A	0	0	0	0	0	0	0	1
P	0	.01	0	.03	.12	.01	.73	.1
Z	0	0	0	0	0	0	0	1

Hybrid 19

	X	S	H	R	R_X	A	A_A	P	Z
X	0	0	0	0	0	0	0	1	0
S	0	0	1	0	0	0	0	0	0
H	0	.45	0	0	0	0	0	0	.55
R	0	0	0	0	0	0	0	0	1
R_X	0	0	0	0	0	0	0	0	1
A	0	0	0	0	0	.36	0	.36	.28
A_A	0	0	0	0	0	0	0	0	1
P	0	.08	0	.01	.01	.09	.03	.74	.04
Z	0	0	0	0	0	0	0	0	1

Hybrid 20

	X	S	H	R	R_X	A	P	Z
X	0	.53	0	0	.07	.07	.33	0
S	0	0	.25	0	0	0	0	.75
H	0	0	0	0	0	0	0	1
R	0	0	0	0	0	0	0	1
R_X	0	0	0	0	0	0	0	1
A	0	0	0	0	0	.34	.33	.33
P	0	0	0	.05	0	.15	.65	.15
Z	0	0	0	0	0	0	0	1

Hybrid 21

	X	S	H	R	A	P	Z
X	0	.37	0	0	0	.63	0
S	0	0	.77	0	0	0	.23
H	0	.25	0	.05	0	.4	.3
R	0	.56	0	.11	.11	0	.22
A	0	.04	0	0	.17	.21	.58
P	0	.01	0	.06	.15	.70	.08
Z	0	0	0	0	0	0	1

Hybrid 22

	X	S	H	R	R_X	R_A	A	P	Z
X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
S	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
H	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.64	0.18
R	0.00	0.00	0.00	0.16	0.00	0.05	0.16	0.10	0.53
R_X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.92
P	0.00	0.04	0.00	0.06	0.01	0.00	0.04	0.79	0.06
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 23

	X	S	H	R	A	A_A	P	Z
X	0.00	0.16	0.00	0.02	0.02	0.00	0.80	0.00
S	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
H	0.00	9.00	0.00	0.00	0.00	0.00	0.74	0.17
R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
A	0.00	0.06	0.00	0.04	0.35	0.04	0.08	0.43
A_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.05	0.00	0.00	0.14	0.02	0.73	0.06
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 24

	X	S	H	R	A	A_X	A_L	P	Z
X	0	.5	0	0	0	0	0	.5	0
S	0	0	.17	0	0	0	0	0	.83
H	0	0	0	0	0	0	0	1	0
R	0	0	0	0	.5	0	.5	0	0

A	0	0	0	0	0	.2	.2	.2	.4
A_X	0	0	0	0	0	0	0	0	1
A_L	0	0	0	0	0	0	0	0	1
P	0	.02	0	.02	.06	0	0	.86	.04
Z	0	0	0	0	0	0	0	0	1

Hybrid 25

	X	S	H	R	A	A_A	P	Z
X	0.00	0.14	0.00	0.02	0.00	0.00	0.84	0.00
S	0.00	0.00	0.94	0.03	0.00	0.00	0.00	0.03
H	0.00	0.00	0.00	0.00	0.00	0.02	0.86	0.12
R	0.00	0.00	0.00	0.06	0.06	0.00	0.29	0.59
A	0.00	0.06	0.00	0.03	0.08	0.00	0.16	0.67
A_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.05	0.00	0.02	0.10	0.02	0.79	0.02
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 26

	X	S	H	R	A	P	Z
X	0	.24	0	.31	0	.45	0
S	0	0	.76	.05	0	0	.19
H	0	.13	0	0	.13	.06	.68
R	0	.29	0	.58	0	0	.13
A	0	0	0	0	.21	0	.79
P	0	.05	0	.03	.15	.77	0
Z	0	0	0	0	0	0	1

Hybrid 27

	X	S	H	R	R_X	A	P	Z
X	0.00	0.47	0.00	0.00	0.00	0.00	0.53	0.00
S	0.00	0.00	0.74	0.00	0.00	0.00	0.00	0.26
H	0.00	0.10	0.00	0.00	0.00	0.00	0.60	0.30
R	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.75
R_X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
A	0.00	0.00	0.00	0.00	0.00	0.34	0.14	0.52
P	0.00	0.05	0.00	0.02	0.00	0.14	0.70	0.09
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 28

	X	S	H	R	A	P	Z
X	0.00	0.23	0.00	0.02	0.00	0.75	0.00
S	0.00	0.00	0.71	0.00	0.00	0.00	0.29
H	0.00	0.06	0.00	0.00	0.00	0.91	0.03
R	0.00	0.00	0.00	0.00	0.11	0.11	0.78
A	0.00	0.03	0.00	0.07	0.12	0.08	0.70
P	0.00	0.04	0.00	0.01	0.15	0.75	0.05
Z	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 29

	X	S	H	R	A	A_A	P	Z
X	0.00	0.33	0.00	0.00	0.00	0.00	0.67	0.00
S	0.00	0.00	0.85	0.00	0.00	0.00	0.01	0.14
H	0.00	0.21	0.00	0.00	0.03	0.00	0.49	0.27
R	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.67
A	0.00	0.05	0.00	0.00	0.00	0.03	0.07	0.85
A_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.07	0.00	0.01	0.06	0.01	0.78	0.07
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 30

	X	S	H	R	R_L	A	P	Z
X	0.00	0.09	0.00	0.00	0.00	0.00	0.91	0.00
S	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.50
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
R_L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
A	0.00	0.00	0.00	0.00	0.00	0.22	0.22	0.56
P	0.00	0.00	0.00	0.03	0.03	0.18	0.66	0.10
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 31

	X	S	H	R	R_X	R_A	A	A_A	P	P_D	Z
X	0.00	0.30	0.00	0.02	0.00	0.00	0.00	0.00	0.68	0.00	0.00
S	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30
H	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.00	0.30
R	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.74
R_X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.92
A_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.06	0.00	0.05	0.01	0.01	0.04	0.01	0.71	0.07	0.04
P_D	0.00	0.00	0.00	0.37	0.00	0.00	0.13	0.00	0.00	0.00	0.50
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 32

	X	S	H	R	A	A_A	P	P_X	Z
X	0.00	0.15	0.00	0.03	0.01	0.00	0.80	0.01	0.00
S	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.20
H	0.00	0.16	0.00	0.03	0.00	0.00	0.53	0.06	0.22
R	0.00	0.14	0.00	0.05	0.02	0.00	0.51	0.02	0.26
A	0.00	0.00	0.00	0.00	0.09	0.00	0.13	0.00	0.78
A_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.06	0.00	0.10	0.06	0.02	0.70	0.02	0.04
P_X	0.00	0.09	0.00	0.16	0.00	0.00	0.16	0.09	0.50
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 33

	X	S	H	R	A	P	P_X	P_D	Z
X	0.00	0.08	0.00	0.75	0.00	0.17	0.00	0.00	0.00
S	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R	0.00	0.00	0.00	0.76	0.02	0.07	0.00	0.00	0.15
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.00	0.00	0.18	0.00	0.36	0.09	0.09	0.28
P_X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P_D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 34

	X	S	H	R	R_X	R_M	A	A_A	P	Z
X	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.00
S	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.14
R	0.00	0.00	0.00	0.50	0.00	0.50	0.00	0.00	0.00	0.00
R_X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R_M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

A	0.00	0.06	0.00	0.00	0.00	0.00	0.29	0.06	0.18	0.41
A_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.04	0.00	0.02	0.02	0.02	0.24	0.00	0.68	0.00
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 35

	X	S	H	H_W	R	R_A	A	P	Z
X	0.00	0.27	0.00	0.00	0.19	0.00	0.00	0.54	0.00
S	0.00	0.00	0.93	0.00	0.00	0.00	0.00	0.00	0.07
H	0.00	0.20	0.00	0.10	0.04	0.00	0.00	0.43	0.23
H_W	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.33
R	0.00	0.04	0.00	0.00	0.50	0.04	0.00	0.00	0.42
R_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
A	0.00	0.07	0.00	0.00	0.00	0.00	0.07	0.07	0.79
P	0.00	0.08	0.00	0.00	0.04	0.01	0.10	0.74	0.03
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 36

	X	S	H	R	R_X	A	P	P_W	Z
X	0.00	0.51	0.00	0.00	0.00	0.00	0.49	0.00	0.00
S	0.00	0.00	0.91	0.00	0.00	0.00	0.00	0.00	0.09
H	0.00	0.43	0.00	0.00	0.00	0.03	0.48	0.00	0.06
R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
R_X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
A	0.00	0.00	0.00	0.14	0.00	0.05	0.05	0.00	0.76
P	0.00	0.01	0.00	0.00	0.01	0.19	0.63	0.03	0.13
P_W	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 37

	X	S	H	R	R_M	R_A	P_X	Z
X	0.00	0.24	0.00	0.47	0.24	0.00	0.05	0.00
S	0.00	0.00	0.71	0.00	0.00	0.00	0.00	0.29
H	0.00	0.00	0.00	0.20	0.00	0.40	0.00	0.40
R	0.00	0.06	0.00	0.38	0.12	0.00	0.00	0.44
R_M	0.00	0.29	0.00	0.00	0.00	0.14	0.00	0.57
R_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P_X	0.00	0.00	0.00	0.00	0.50	0.00	0.50	0.00

Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 38

	X	S	H	R_E	R_A	P	Z
X	0.00	0.68	0.00	0.26	0.00	0.06	0.00
S	0.00	0.00	1.00	0.00	0.00	0.00	0.00
H	0.00	0.06	0.00	0.60	0.06	0.19	0.06
R_E	0.00	0.03	0.00	0.22	0.06	0.28	0.41
R_A	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.00	0.00	0.44	0.00	0.48	0.08
Z	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Hybrid 39

	X	S	H_E	R	A	P	Z
X	0.00	0.15	0.00	0.79	0.00	0.06	0.00
S	0.00	0.00	0.89	0.11	0.00	0.00	0.00
H_E	0.00	0.00	0.00	0.56	0.00	0.00	0.44
R	0.00	0.04	0.00	0.70	0.01	0.02	0.23
A	0.00	0.00	0.00	0.00	0.00	0.00	1.00
P	0.00	0.00	0.00	0.22	0.00	0.44	0.34
Z	0.00	0.00	0.00	0.00	0.00	0.00	1.00

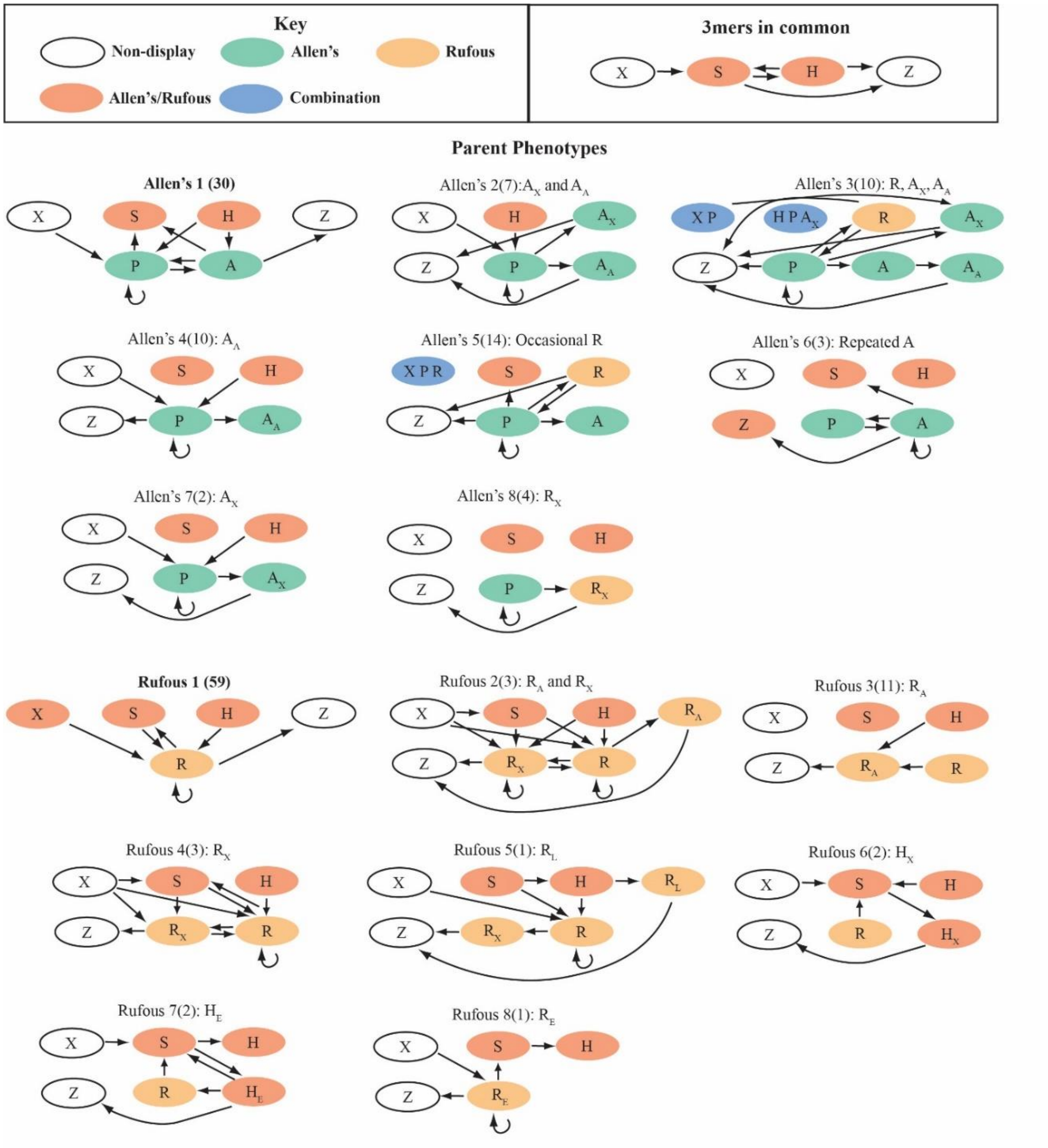
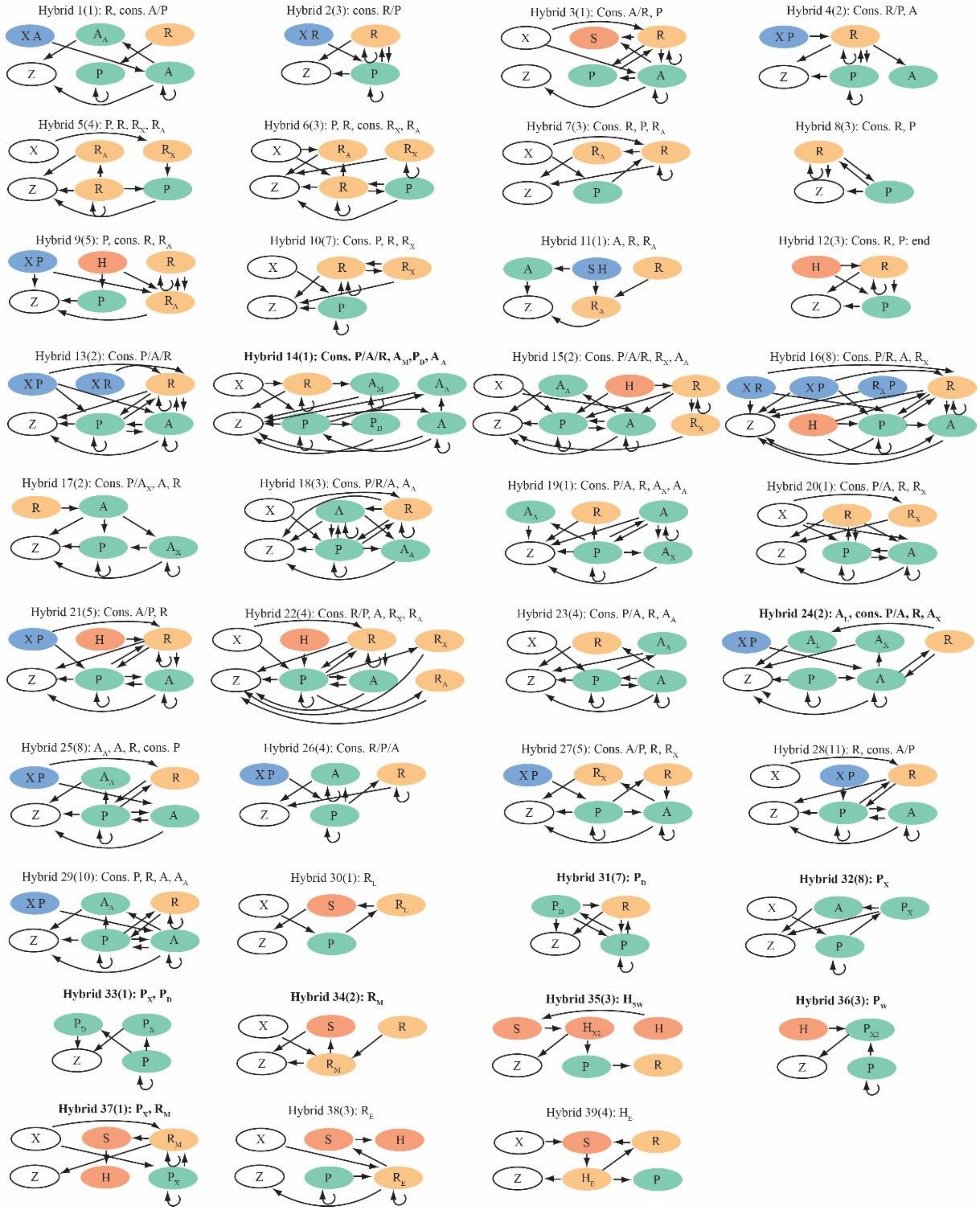

Key
Non-display
Allen's
Rufous
Allen's/Rufous
Combination
3mers in common
X
S
H
Z
Parent Phenotypes
Allen's 1 (30)
Allen's 2(7): A_X and A_A
Allen's 3(10): R, A_X, A_A
X P
H P A_X
Allen's 4(10): A_A
Allen's 5(14): Occasional R
X P R
Allen's 6(3): Repeated A
Allen's 7(2): A_X
Allen's 8(4): R_X
Rufous 1 (59)
Rufous 2(3): R_A and R_X
Rufous 3(11): R_A
Rufous 4(3): R_X
Rufous 5(1): R_L
Rufous 6(2): H_X
Rufous 7(2): H_E
Rufous 8(1): R_E

Figure S2.1 – **Courtship behavior phenotypes for 3-mers of display sequences in Allen's and Rufous Hummingbird.** 3-mers are embedded within each phenotype: starting at any oval, follow any arrow from the focal oval to the next oval, then follow any arrow attached to that oval to another oval to complete the 3-mer. Typical phenotypes that characterized most individuals of each species are in bold: all 3-mers observed were included here. Besides phenotype 1 for Allen's and Rufous, which was the most common, 3-mers for phenotypes only include 3-mers that were unique to each phenotype, i.e., these are the 3-mers that made each phenotype different from the rest. See Figure 2.3 for line drawings and Table 2.2 for definitions of displays. For simplification, uninformative transitions (**3-mers in common)** between shuttles (S) and half pendulums (H) that occur within both species were omitted from this figure. Red ovals indicate displays shared by Allen's and Rufous (S, H), green ovals indicate displays that occur in parental Allen's and not Rufous (pendulum, P, Allen's dive, A), orange ovals indicate displays that occur in parental Rufous but not Allen's (Rufous dive, R), "non-display" ovals indicate either a start (X) or end (Z) state in the sequence, and blue ovals indicate multiple displays combined into the same oval to provide an accurate depiction of the 3-mer sequence (i.e., in Allen's 3, X P was only followed by A_X, whereas if X and P were included in different ovals, the transition of X to P to the next display could have been X-P-R, X-P-A, X-P-P, or X-P-Z). Phenotype number, sample size (N) in parentheses, and distinguishing displays of that phenotype are displayed above each phenotype.

Hybrid Phenotypes



Hybrid 1(1): R, cons. A/P

Hybrid 2(3): cons. R/P

Hybrid 3(1): Cons. A/R, P

Hybrid 4(2): Cons. R/P, A

Hybrid 5(4): P, R, R_X, R_A

Hybrid 6(3): P, R, cons. R_X, R_A

Hybrid 7(3): Cons. R, P, R_A

Hybrid 8(3): Cons. R, P

Hybrid 9(5): P, cons. R, R_A

Hybrid 10(7): Cons. P, R, R_X

Hybrid 11(1): A, R, R_A

Hybrid 12(3): Cons. R, P: end

Hybrid 13(2): Cons. P/A/R

Hybrid 14(1): Cons. P/A/R, A_M,P_D, A_A

Hybrid 15(2): Cons. P/A/R, R_X, A_A

Hybrid 16(8): Cons. P/R, A, R_X

Hybrid 17(2): Cons. P/A_X, A, R

Hybrid 18(3): Cons. P/R/A, A_A

Hybrid 19(1): Cons. P/A, R, A_X, A_A

Hybrid 20(1): Cons. P/A, R, R_X

Hybrid 21(5): Cons. A/P, R

Hybrid 22(4): Cons. R/P, A, R_X, R_A

Hybrid 23(4): Cons. P/A, R, A_A

Hybrid 24(2): A_L, cons. P/A, R, A_X

Hybrid 25(8): A_A, A, R, cons. P

Hybrid 26(4): Cons. R/P/A

Hybrid 27(5): Cons. A/P, R, R_X

Hybrid 28(11): R, cons. A/P

Hybrid 29(10): Cons. P, R, A, A_A

Hybrid 30(1): R_L

Hybrid 31(7): P_D

Hybrid 32(8): P_X

Hybrid 33(1): P_X, P_D

Hybrid 34(2): R_M

Hybrid 35(3): H_{SW}

Hybrid 36(3): P_W

Hybrid 37(1): P_X, R_M

Hybrid 38(3): R_E

Hybrid 39(4): H_E

Figure S2.2 – **Courtship behavior phenotypes for 3-mers of display sequences in hybrids.** 3-mers are embedded within each phenotype: starting at any oval, follow any arrow from the focal oval to the next oval, then follow any arrow attached to that oval to another oval to complete the 3-mer. Phenotypes that include transgressive (14, 34, 37) and novel (14, 24, 31, 32, 33, 35, 36, 37) displays are bold. 3-mers for hybrid phenotypes only include 3-mers that were unique to each phenotype, i.e., these are the 3-mers that made each phenotype different from the rest. See Figure Figure 2.3 for line drawings and Table 2.2 for definitions of displays. I only included intermediate transitions between S and H or transitions that were part of a 3-mer to a transgressive or novel behavior within a phenotype. Blue ovals indicate multiple displays combined into the same oval to provide an accurate depiction of the 3-mer sequence (i.e., in Hybrid 1, X-A was only followed by A, whereas if X and A were included in different ovals, the transition of X to A to the next display could have been X-A-R, X-A-A, X-A-P, or X-A-Z). Phenotype number, sample size (N) in parentheses, and distinguishing displays of that phenotype are displayed above each phenotype.

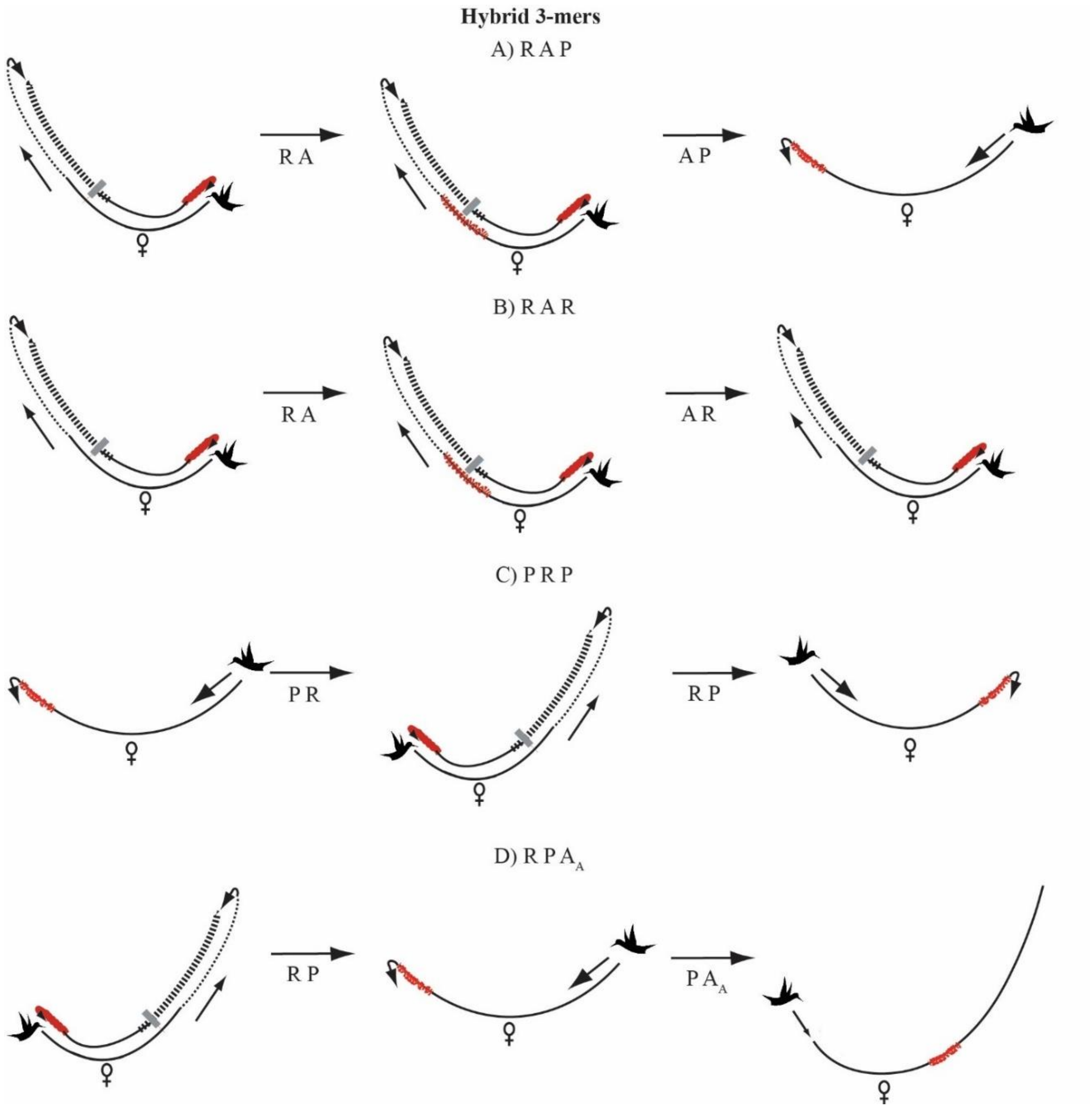


Figure S2.3 – **Line drawings of 3-mers absent from Allen's and Rufous Hummingbird but common in some hybrids.** Some common 3-mers that were represented in many hybrid display bouts were **A)** Rufous dive, Allen's dive, pendulum (R-A-P), **B)**, R-A-R, **C)** P-R-P, and **D)** R-P-A_A (aborted Allen's dive).

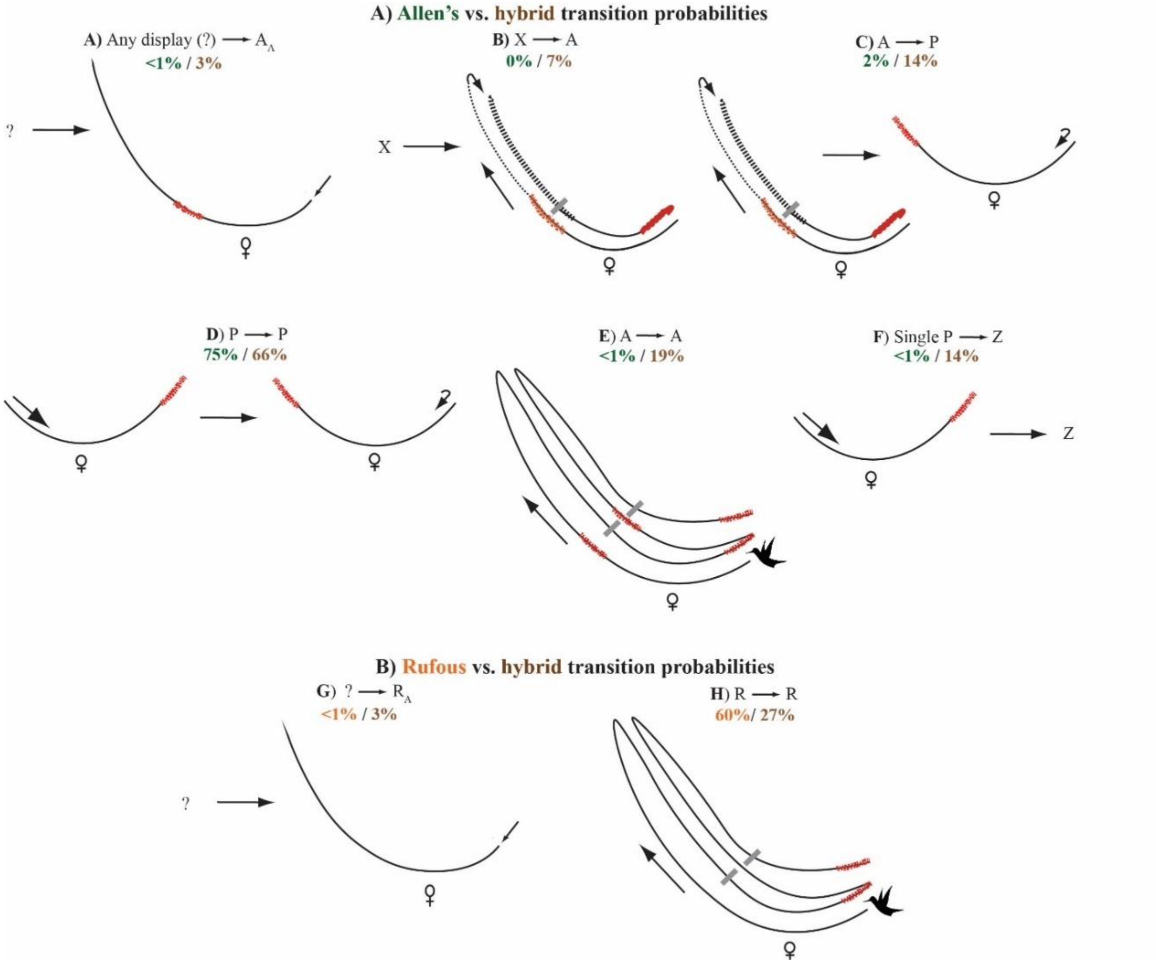


Figure S2.4 – **Display transitions within Allen's and Rufous phenotypes that are more or less common in hybrids than in either parental species and their associated probabilities under a first-order Markov Chain.** Allen's transition probabilities are in green, Rufous in orange, hybrids in brown. "?" indicates that any display can be substituted in the space of the sequence occupied by the "?", dive sound is designated by a gray hashmark, while red squiggles indicate writhing. Comparing Allen's to hybrid phenotypes, **(A)** aborted Allen's dives (A_A) occurred <1% of the time within all Allen's, and 3% of the time across hybrid phenotypes, **(B)** bouts never began with an Allen's dive (A) in Allen's Hummingbird, while bouts began with an A 7% of the time in hybrids, **(C)** a pendulum (P) followed an Allen's dive 2% of the time in Allen's and 14% of the time in hybrids, **(D)** a current P was followed by an additional P 75% of the time in Allen's and 66% of the time in hybrids, **(E)** consecutive A occurred <1% of the time in Allen's, and 19% of the time in hybrids, **(F)** bouts of display ended (Z) with a single P display <1% of the time in Allen's and 14% of the time in hybrids. Comparing Rufous to hybrid phenotypes, **(G)** aborted Rufous dives (R_A) occurred <1% of the time within all Rufous, and 3% of the time across hybrid phenotypes, **(H)** a current R was followed by a subsequent R 61% of the time in Rufous and 27% of the time in hybrids.

Appendix C: Supplementary Tables/Figures for Chapter 3

Table S3.1 – **Coordinates, locality, and county for all samples (N=133).** Vouchered tissue samples are indicated with an associated museum: San Diego State University Museum of Biodiversity (SDSU), California Academy of Sciences (CAS), Museum of Southwestern Biology (MSB), San Diego Natural History Museum (SDNHM), Burke Museum (UWBM), and Museum of Vertebrate Zoology (MVZ). “NB” indicates a bird that was not collected or banded and “CJC” indicates the collector number for tissues not yet housed at a museum. Individuals with a band number or “NB” were released after blood sample collection; all other samples are from collected individuals.

Band or museum ID	Approx. breeding range	Collection date	Locality	County, State	Latitude (N)	Longitude (W)
SDSU2980	Non-migratory Allen’s	3/8/2017	Point Mugu State Park	Los Angeles, CA	34°4'21.00"	119°0'49.80”
E05849	Non-migratory Allen’s	3/17/2017	University of California, Santa Barbara	Santa Barbara, CA	34°24'32.61"	119°52'41.28”
SDSU2983	Migratory Allen’s	3/22/2017	Pismo State Beach	San Luis Obispo, CA	35° 6'38.15"	120°37'39.18"
SDSU2984	Migratory Allen’s	3/23/2017	Pismo State Beach	San Luis Obispo, CA	35° 6'35.98"	120°37'39.30”
SDSU2986	Migratory Allen’s	3/27/2017	Carmel River State Beach	Monterey, CA	36°31'45.14"	121°55'27.52"
SDSU2987	Migratory Allen’s	3/27/2017	Mendocino Headlands State Park	Mendocino, CA	39°18'10.53"	123°47'16.98"
SDSU2988	Migratory Allen’s	3/27/2017	Mendocino Headlands State Park	Mendocino, CA	39°18'10.53"	123°47'16.98”
SDNHM51665	Non-migratory Allen’s	4/5/2007	San Clemente Island	Los Angeles, CA	32°55'01.14”	118°30'38.34”
SDSU2996	Allen’s × Rufous	5/2/2017	Humboldt Lagoons State Park	Humboldt, CA	41°13'20.33”	124°5'46.76”
K53374	Allen’s × Rufous	4/27/2017	Tugman State Park	Coos, OR	41°50'14.75”	124°6'49.76”
E05965	Allen’s × Rufous	5/6/2017	Humbug Mountain State Park	Curry, OR	42°36'54.99”	124°23'49.29”
E05962	Allen’s × Rufous	5/6/2017	Humbug Mountain State Park	Curry, OR	42°36'54.99”	124°23'49.29”
SDSU3008	Allen’s × Rufous	3/31/2018	Aikens Creek Campground	Humboldt, CA	41°13'41.26"	123°39'25.15"
E08529	Allen’s × Rufous	3/31/2018	Aikens Creek Campground	Humboldt, CA	41°13'41.26"	123°39'25.15"
E08530	Allen’s × Rufous	4/1/2018	Aikens Creek Campground	Humboldt, CA	41°13'41.26"	123°39'25.15"

K53300	Allen’s × Rufous	4/1/2018	Aikens Creek Campground	Humboldt, CA	41°13'41.26"	123°39'25.15"
K53310	Allen’s × Rufous	4/1/2018	Aikens Creek Campground	Humboldt, CA	41°13'41.26"	123°39'25.15"
K53377	Allen’s × Rufous	5/2/2017	Harris Beach State Park	Curry, OR	42°4'3.93”	124°18'29.80”
SDSU3064	Allen’s × Rufous	4/27/2016	Bullard's Beach State Park	Coos, OR	43°8'56.81”	124°24'7.08”
CJC399	Allen’s × Rufous	4/18/2016	Bullard's Beach State Park	Coos, OR	43°8'56.81”	124°24'7.08”
E05987	Allen’s × Rufous	5/15/2017	Bullard's Beach State Park	Coos, OR	43°8'53.97”	124°24'13.62”
E05985	Allen’s × Rufous	5/17/2017	Bullard's Beach State Park	Coos, OR	43°8'56.78”	124°24'7.80”
NB28	Allen’s × Rufous	4/27/2016	Bullard's Beach State Park	Coos, OR	43° 8'56.78”	124°24'7.80”
E05831	Rufous	4/28/2018	Beaver Creek State Natural Area	Lincoln, OR	44°30'53.14”	124°2'56.35”
E05858	Rufous	4/28/2018	Beaver Creek State Natural Area	Lincoln, OR	44°30'53.14”	124°2'56.35”
E05860	Rufous	4/28/2018	Beaver Creek State Natural Area	Lincoln, OR	44°30'53.14”	124°2'56.35”
SDSU3031	Allen’s × Rufous	5/18/2016	Jedediah Smith Redwoods State Park	Del Norte, CA	41°50'14.75”	124°6'49.76”
SDSU3012	Allen’s × Rufous	4/3/2018	Green Riffle River Access	Humboldt, CA	41°26'25.91"	123°30'20.67"
SDSU3013	Allen’s × Rufous	4/3/2018	Green Riffle River Access	Humboldt, CA	41°26'25.91"	123°30'20.67"
SDSU3014	Allen’s × Rufous	4/3/2018	Ti Creek Campground	Humboldt, CA	41°31'30.52"	123°31'42.86"
SDSU3015	Allen’s × Rufous	4/9/2018	Ti Creek Campground	Humboldt, CA	41°31'30.52"	123°31'42.86"
SDSU3017	Allen’s × Rufous	4/9/2018	Wingate Bar River Access	Siskiyou, CA	41°31'30.52"	123°31'42.86"
SDSU3018	Allen’s × Rufous	4/10/2018	Wingate Bar River Access	Siskiyou, CA	41°43'22.31"	123°26'14.94"
SDSU3098	Non-migratory Allen’s	2/2/2016	Santa Catalina Island	Los Angeles, CA	33°21'23.17"	118°26'22.81”
SDSU3097	Non-migratory Allen’s	2/2/2016	Santa Catalina Island	Los Angeles, CA	33°21'23.17”	118°26'22.81”

CAS90566	Migratory Allen’s	3/8/1996	Cambria	San Luis Obispo, CA	35°34'8.28"	121° 4'32.71"
CAS95622	Migratory Allen’s	3/23/2003	Lucas Valley	Marin, CA	38°5'23.67"	122°46'58.17"
CAS95623	Migratory Allen’s	5/12/2003	Olema	Marin, CA	38° 5'17.66"	122°46'25.63"
CAS96922	Migratory Allen’s	4/6/2009	Golden Gate Park	San Francisco, CA	37°46'15.09”	122°27'58.42"
CAS96923	Migratory Allen’s	4/6/2009	Golden Gate Park	San Francisco, CA	37°46'13.04"	122°27'55.18"
CAS96943	Migratory Allen’s	5/8/2009	Cambria	San Luis Obispo, CA	35°34'8.28"	121° 4'32.71"
CAS96992	Migratory Allen’s	6/3/2009	Golden Gate Park	San Francisco, CA	37°46'13.04”	122°27'55.18”
CAS97003	Migratory Allen’s	6/4/2009	Golden Gate Park	San Francisco, CA	37°46'13.04”	122°27'55.18”
CAS97088	Migratory Allen’s	3/13/2008	San Francisco Zoo	San Francisco, CA	37°43'55.74”	122°30'05.68”
CAS97717	Migratory Allen’s	5/27/2013	Golden Gate Park	San Francisco, CA	37°46'10.05”	122°27'56.48”
CAS97720	Migratory Allen’s	7/30/2013	Golden Gate Park	San Francisco, CA	37°46'10.05”	122°27'56.48”
CAS97760	Migratory Allen’s	6/19/2011	Golden Gate Park	San Francisco, CA	37°46'10.05”	122°27'56.48”
CAS97778	Migratory Allen’s	6/28/2012	Golden Gate Park	San Francisco, CA	37°46'10.05”	122°27'56.48”
E05892	Allen’s × Rufous	5/11/2018	Chambers Flat	Siskiyou, CA	41°47'20.96"	123°22'51.12"
E05868	Allen’s × Rufous	4/13/2018	Chambers Flat	Siskiyou, CA	41°47'20.96"	123°22'51.12"
E05866	Allen’s × Rufous	4/14/2018	Chambers Flat	Siskiyou, CA	41°47'20.96"	123°22'51.12"
E05867	Allen’s × Rufous	4/14/2018	Chambers Flat	Siskiyou, CA	41°47'20.96"	123°22'51.12"
CJC378	Rufous	5/25/2015	Clatsop State Forest	Clatsop, OR	45°43'36.22"	123°54'1.71”
CJC380	Rufous	5/25/2015	Clatsop State Forest	Clatsop, OR	45°43'36.22”	123°54'1.71”
CJC382	Rufous	5/26/2015	Clatsop State Forest	Clatsop, OR	45°43'36.22”	123°54'1.71”
CJC383	Rufous	5/26/2015	Clatsop State Forest	Clatsop, OR	45°43'41.12”	123°53'35.48”

CJC386	Rufous	5/27/2015	Clatsop State Forest	Clatsop, OR	45°43'41.12”	123°53'35.48”
CJC387	Rufous	5/27/2015	Clatsop State Forest	Clatsop, OR	45°43'27.09”	123°53'45.82”
E05998	Migratory Allen’s	3/29/2017	Carmel River State Beach	Monterey, CA	36°31'16.87"	121°55'36.99”
E05907	Migratory Allen’s	4/1/2017	Carmel River State Beach	Monterey, CA	36°31'16.87"	121°55'36.99"
E05844	Migratory Allen’s	3/29/2017	Carmel River State Beach	Monterey, CA	36°31'16.87"	121°55'36.99"
E05898	Allen’s × Rufous	4/3/2018	Green Riffle River Access	Humboldt, CA	41°26'25.91"	123°30'20.67"
E05897	Allen’s × Rufous	4/4/2018	Green Riffle River Access	Humboldt, CA	41°26'25.91"	123°30'20.67"
E05804	Allen’s × Rufous	4/22/2018	Horse Creek	Siskiyou, CA	41°49'58.14"	123°0'20.53"
E05802	Allen’s × Rufous	4/22/2018	Horse Creek	Siskiyou, CA	41°49'58.14"	123°0'20.53"
E05803	Allen’s × Rufous	4/22/2018	Scott's Bar	Siskiyou, CA	41°44'23.40"	123° 0'15.50"
E05838	Allen’s × Rufous	4/23/2018	Scott's Bar	Siskiyou, CA	41°44'23.40"	123° 0'15.50"
E05835	Allen’s × Rufous	4/22/2018	Horse Creek	Siskiyou, CA	41°49'58.14"	123°0'20.53"
E05960	Allen’s × Rufous	4/21/2017	Humboldt Lagoons State Park	Humboldt, CA	41°13'20.33"	124°5'46.76"
E05970	Allen’s × Rufous	4/23/2017	Humboldt Lagoons State Park	Humboldt, CA	41°13'20.33"	124°5'46.76"
E05936	Allen’s × Rufous	4/24/2017	Humboldt Lagoons State Park	Humboldt, CA	41°13'20.33”	124°5'46.76”
E05902	Allen’s × Rufous	4/15/2017	Humboldt Redwoods State Park-Albee Creek	Humboldt, CA	40°21'3.26”	124°0'41.75”
E05920	Allen’s × Rufous	4/16/2017	Humboldt Redwoods State Park-Albee Creek	Humboldt, CA	40°21'9.77"	124° 0'28.43"
MSB25142	Migratory Allen’s	8/1/2001	Marin	Marin, CA	38° 5'17.66"	122°46'25.63"
E05982	Allen’s × Rufous	3/22/2018	South Slough Estuarine Research Center	Coos, OR	43°17'44.86”	124°20'6.73”
E05983	Allen’s × Rufous	3/22/2018	South Slough Estuarine Research Center	Coos, OR	43°17'44.86”	124°20'6.73”
E05944	Allen’s × Rufous	5/2/2017	Harris Beach State Park	Curry, OR	42°6'43.99"	124°11'12.30"

E05918	Allen’s × Rufous	4/18/2017	Humboldt Redwoods State Park-Albee Creek	Humboldt, CA	40°21'9.77”	124°0'28.43”
E05980	Non-migratory Allen’s	3/13/2017	Point Mugu State Park	Los Angeles, CA	34°4'21.00”	119°0'49.80”
MVZ183713	Migratory Allen’s	3/13/2006	Eastshore State Park	Alameda, CA	37°53'22.58”	122°19'07.74”
MVZ182025	Migratory Allen’s	3/30/2005	Livermore	Alameda, CA	37°40'52.60”	121°45'21.46”
MVZ180045	Migratory Allen’s	3/17/2002	Orinda	Contra Costa, CA	37°51'56.04”	122°09'07.26”
MVZ183549	Non-migratory Allen’s	3/27/2006	Santa Cruz Island Reserve	Santa Barbara, CA	33°59'45.80”	119°43'30.46”
MVZ183551	Non-migratory Allen’s	3/27/2006	Santa Cruz Island Reserve	Santa Barbara, CA	33°59'45.80”	119°43'30.46”
MVZ183552	Non-migratory Allen’s	3/27/2006	Santa Cruz Island Reserve	Santa Barbara, CA	33°59'45.80”	119°43'30.46”
MVZ183553	Non-migratory Allen’s	3/27/2006	Santa Cruz Island Reserve	Santa Barbara, CA	33°59'45.80”	119°43'30.46”
MVZ183554	Non-migratory Allen’s	3/28/2006	Santa Cruz Island Reserve	Santa Barbara, CA	33°59'45.80”	119°43'30.46”
MVZ183555	Non-migratory Allen’s	3/29/2006	Santa Cruz Island Reserve	Santa Barbara, CA	33°59'45.80”	119°43'30.46”
MVZ183556	Non-migratory Allen’s	3/29/2006	Santa Cruz Island Reserve	Santa Barbara, CA	33°59'45.80”	119°43'30.46”
MVZ182072	Migratory Allen’s	4/29/2006	Tilden Regional Park	Contra Costa, CA	37°53'49.27”	122°13'53.15”
MVZ180487	Migratory Allen’s	4/30/2001	Concord	Contra Costa, CA	37°58'18.54”	122°00'44.29”
MVZ180488	Migratory Allen’s	3/19/2001	Contra Costa	Contra Costa, CA	37°46'09.43”	121°59'14.58”
E05847	Migratory Allen’s	3/22/2017	Pismo State Beach	San Luis Obispo, CA	35°6'38.15”	120°37'39.18”
E05908	Migratory Allen’s	3/24/2017	Pismo State Beach	San Luis Obispo, CA	35°6'38.15”	120°37'39.18”
E05856	Allen’s × Rufous	4/29/2018	Siltcoos Campground	Lane, OR	43°52'33.98"	124° 8'41.88"
E05857	Allen’s × Rufous	4/29/2018	Siltcoos Campground	Lane, OR	43°52'33.98"	124° 8'41.88"
E05855	Allen’s × Rufous	4/29/2018	Siltcoos Campground	Lane, OR	43°52'33.98"	124° 8'41.88"

SDNHM50767	Non-migratory Allen’s	11/25/2002	Tarzana	Los Angeles, CA	34°10'28.65”	118°33'11.62”
E05905	Migratory Allen’s	4/8/2017	Mendocino Headlands State Park	Mendocino, CA	39°16'29.18”	123°47'24.35”
SDNHM51608	Non-migratory Allen’s	12/26/2006	Ocean Beach	San Diego, CA	32°44'41.41”	117°14'34.81”
SDNHM51657	Non-migratory Allen’s	3/13/2007	Hancock Park	Los Angeles, CA	34°04'04.04”	118°19'57.27”
SDSU2997	Allen’s × Rufous	4/26/2017	Humboldt Lagoons State Park	Humboldt, CA	41°13'20.33”	124° 5'46.76”
SDNHM53030	Non-migratory Allen’s	5/4/2011	Encinitas	San Diego, CA	33°02'20.04”	117°17'17.88”
E05818	Allen’s × Rufous	3/20/2018	South Slough Estuarine Research Center	Coos, OR	43°17'44.86”	124°20'6.73”
E05817	Allen’s × Rufous	3/20/2018	South Slough Estuarine Research Center	Coos, OR	43°17'44.86”	124°20'6.73”
E05815	Allen’s × Rufous	3/20/2018	South Slough Estuarine Research Center	Coos, OR	43°17'44.86”	124°20'6.73”
E05813	Allen’s × Rufous	3/25/2018	South Slough Estuarine Research Center	Coos, OR	43°17'44.86”	124°20'6.73”
E05951	Allen’s × Rufous	5/6/2017	Humbug Mountain State Park	Curry, OR	42°36'54.99”	124°23'49.29”
K53373	Allen’s × Rufous	4/27/2017	Tugman State Park	Coos, OR	41°50'14.75"	124° 6'49.76"
K53375	Allen’s × Rufous	4/29/2017	Tugman State Park	Coos, OR	41°50'14.75"	124° 6'49.76"
CJC375	Allen’s × Rufous	5/22/2015	Seiad	Siskiyou, CA	43°16'20.47"	122°24'38.62"
CJC374	Allen’s × Rufous	5/22/2015	Seiad	Siskiyou, CA	41°50'10.66”	123°10'24.85”
E05808	Allen’s × Rufous	4/20/2018	Seiad	Siskiyou, CA	41°50'25.04”	123°11'49.55”
E05822	Allen’s × Rufous	4/20/2018	Seiad	Siskiyou, CA	41°50'25.04”	123°11'49.55”
E05821	Allen’s × Rufous	4/20/2018	Seiad	Siskiyou, CA	41°50'25.04”	123°11'49.55”
E05810	Allen’s × Rufous	4/20/2018	Seiad	Siskiyou, CA	41°50'25.04”	123°11'49.55”

E05806	Allen’s × Rufous	4/21/2018	Seiad	Siskiyou, CA	41°50'25.04”	123°11'49.55”
E05807	Allen’s × Rufous	4/21/2018	Seiad	Siskiyou, CA	41°50'25.04”	123°11'49.55”
E05805	Allen’s × Rufous	4/21/2018	Seiad	Siskiyou, CA	41°50'25.04”	123°11'49.55”
SDSU3101	Non-migratory Allen’s	1/19/2016	University of California, Riverside	Riverside, CA	33°58'23.44"	117°19'21.80"
SDSU3099	Non-migratory Allen’s	12/21/2015	University of California, Riverside	Riverside, CA	33°58'23.44"	117°19'21.80"
E05850	Non-migratory Allen’s	3/16/2017	University of California, Santa Barbara	Santa Barbara, CA	34°24'32.61”	119°52'41.28”
SDSU2981	Non-migratory Allen’s	3/9/2017	Point Mugu State Park	Los Angeles, CA	34°4'21.00”	119°0'49.80”
E05999	Non-migratory Allen’s	3/17/2017	University of California, Santa Barbara	Santa Barbara, CA	34°24'32.61”	119°52'41.28”
E05848	Non-migratory Allen’s	3/16/2017	University of California, Santa Barbara	Santa Barbara, CA	34°24'32.61”	119°52'41.28”
UWBM80061	Migratory Allen’s	5/2/2002	Bolinas Lagoon	Marin, CA	38°5'17.66"	122°46'25.63"
SDNHM51760	Migratory Allen’s	5/4/2006	Cambria	San Luis Obispo, CA	35°34'8.28"	121° 4'32.71"
K53294	Allen’s × Rufous	3/31/2018	Aikens Creek Campground	Humboldt, CA	41°13'41.26"	123°39'25.15"
E05996	Migratory Allen’s	4/7/2017	Mendocino Headlands State Park	Mendocino, CA	39°18'10.53”	123°47'16.98”
MSB25414	Migratory Allen’s	5/29/2001	Marin	Marin, CA	38° 5'17.66"	122°46'25.63"
K53382	Allen’s × Rufous	5/3/2017	Quosatana Campground	Curry, OR	42°29'53.91"	124°14'5.12"
SDSU3071	Allen’s × Rufous	5/2/2016	Sunset Bay State Park	Coos, OR	43°19'51.96"	124°22'13.72"
E05818	Allen’s × Rufous	3/20/2018	South Slough Estuarine Research Center	Coos, OR	43°17'44.86”	124°20'6.73”

Table S3.2 – **Library prep information for all samples (N=133).** Vouchered tissue samples are indicated with an associated museum: San Diego State University Museum of Biodiversity (SDSU), California Academy of Sciences (CAS), Museum of Southwestern Biology (MSB), San Diego Natural History Museum (SDNHM), Burke Museum (UWBM), and Museum of Vertebrate Zoology (MVZ). “NB” indicates a bird that was not collected or banded and “CJC” indicates the collector number for tissues not yet housed at a museum. Individuals with a band number or “NB” were released after blood sample collection; all other samples are from collected individuals.

Band or museum ID	Approx. breeding range	County, State	Sex	Sequencer	Read length	Depth
SDSU2980	Non-migratory Allen’s	Los Angeles, CA	M	HiSeq X, Novogene	150bp PE	4.17
E05849	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq X, Novogene	150bp PE	2.55
SDSU2983	Migratory Allen’s	San Luis Obispo, CA	M	HiSeq X, Novogene	150bp PE	5.25
SDSU2984	Migratory Allen’s	San Luis Obispo, CA	M	HiSeq X, Novogene	150bp PE	4.79
SDSU2986	Migratory Allen’s	Monterey, CA	M	HiSeq X, Novogene	150bp PE	6.14
SDSU2987	Migratory Allen’s	Mendocino, CA	M	HiSeq X, Novogene	150bp PE	2.63
SDSU2988	Migratory Allen’s	Mendocino, CA	M	HiSeq X, Novogene	150bp PE	3.67
SDNHM51665	Non-migratory Allen’s	Los Angeles, CA	M	HiSeq X, Novogene	150bp PE	2.27
SDSU2996	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	3.20
K53374	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	2.79
E05965	Allen’s × Rufous	Curry, OR	M	HiSeq X, Novogene	150bp PE	2.75
E05962	Allen’s × Rufous	Curry, OR	M	HiSeq X, Novogene	150bp PE	5.63
SDSU3008	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	3.35
E08529	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	1.41
E08530	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	1.54
K53300	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	1.34
K53310	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	2.13
K53377	Allen’s × Rufous	Curry, OR	M	HiSeq X, Novogene	150bp PE	3.41
SDSU3064	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	1.66

CJC399	Allen's × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	2.22
E05987	Allen's × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	3.43
E05985	Allen's × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	3.56
NB28	Allen's × Rufous	Coos, OR	F	HiSeq X, Novogene	150bp PE	5.82
E05831	Rufous	Lincoln, OR	M	HiSeq X, Novogene	150bp PE	1.01
E05858	Rufous	Lincoln, OR	M	HiSeq X, Novogene	150bp PE	0.88
E05860	Rufous	Lincoln, OR	M	HiSeq X, Novogene	150bp PE	2.17
SDSU3031	Allen's × Rufous	Del Norte, CA	M	HiSeq X, Novogene	150bp PE	1.44
SDSU3012	Allen's × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	2.50
SDSU3013	Allen's × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	1.24
SDSU3014	Allen's × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	3.29
SDSU3015	Allen's × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	2.58
SDSU3017	Allen's × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	2.97
SDSU3018	Allen's × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	2.01
SDSU3098	Non-migratory Allen's	Los Angeles, CA	M	HiSeq X, Novogene	150bp PE	6.01
SDSU3097	Non-migratory Allen's	Los Angeles, CA	M	HiSeq X, Novogene	150bp PE	2.73
CAS90566	Migratory Allen's	San Luis Obispo, CA	M	HiSeq X, Novogene	150bp PE	5.12
CAS95622	Migratory Allen's	Marin, CA	M	HiSeq X, Novogene	150bp PE	2.43
CAS95623	Migratory Allen's	Marin, CA	F	HiSeq X, Novogene	150bp PE	3.09
CAS96922	Migratory Allen's	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	6.98
CAS96923	Migratory Allen's	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	6.90
CAS96943	Migratory Allen's	San Luis Obispo, CA	F	HiSeq X, Novogene	150bp PE	2.99
CAS96992	Migratory Allen's	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	8.35
CAS97003	Migratory Allen's	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	7.59
CAS97088	Migratory Allen's	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	9.75
CAS97717	Migratory Allen's	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	8.98

CAS97720	Migratory Allen’s	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	6.67
CAS97760	Migratory Allen’s	San Francisco, CA	F	NextSeq 500, UC Riverside	150bp PE	7.30
CAS97778	Migratory Allen’s	San Francisco, CA	M	NextSeq 500, UC Riverside	150bp PE	7.73
E05892	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	5.86
E05868	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	7.81
E05866	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	2.79
E05867	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	5.23
CJC378	Rufous	Clatsop, OR	M	NextSeq 500, UC Riverside	150bp PE	10.77
CJC380	Rufous	Clatsop, OR	M	NextSeq 500, UC Riverside	150bp PE	6.37
CJC382	Rufous	Clatsop, OR	M	NextSeq 500, UC Riverside	150bp PE	3.32
CJC383	Rufous	Clatsop, OR	M	NextSeq 500, UC Riverside	150bp PE	9.47
CJC386	Rufous	Clatsop, OR	M	NextSeq 500, UC Riverside	150bp PE	7.91
CJC387	Rufous	Clatsop, OR	M	NextSeq 500, UC Riverside	150bp PE	10.45
E05998	Migratory Allen’s	Monterey, CA	M	HiSeq X, Novogene	150bp PE	5.65
E05907	Migratory Allen’s	Monterey, CA	M	HiSeq X, Novogene	150bp PE	2.76
E05844	Migratory Allen’s	Monterey, CA	F	HiSeq X, Novogene	150bp PE	6.29
E05898	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	2.25
E05897	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	4.94
E05804	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	5.82
E05802	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	5.41
E05803	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	5.20
E05838	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	7.38
E05835	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	8.17
E05960	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	5.50
E05970	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	3.36
E05936	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	6.18

E05902	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	6.26
E05920	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	3.54
MSB25142	Migratory Allen’s	Marin, CA	F	HiSeq X, Novogene	150bp PE	2.00
E05982	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	1.49
E05983	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	2.94
E05944	Allen’s × Rufous	Curry, OR	M	HiSeq X, Novogene	150bp PE	6.22
E05918	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	2.68
E05980	Non-migratory Allen’s	Los Angeles, CA	M	HiSeq X, Novogene	150bp PE	5.40
MVZ183713	Migratory Allen’s	Alameda, CA	M	HiSeq 4000, UC Berkeley	150bp PE	2.18
MVZ182025	Migratory Allen’s	Alameda, CA	F	HiSeq 4000, UC Berkeley	150bp PE	1.94
MVZ180045	Migratory Allen’s	Contra Costa, CA	F	HiSeq 4000, UC Berkeley	150bp PE	2.49
MVZ183549	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq 4000, UC Berkeley	150bp PE	2.00
MVZ183551	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq 4000, UC Berkeley	150bp PE	3.36
MVZ183552	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq 4000, UC Berkeley	150bp PE	2.73
MVZ183553	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq 4000, UC Berkeley	150bp PE	2.88
MVZ183554	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq 4000, UC Berkeley	150bp PE	3.09
MVZ183555	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq 4000, UC Berkeley	150bp PE	2/42
MVZ183556	Non-migratory Allen’s	Santa Barbara, CA	M	HiSeq 4000, UC Berkeley	150bp PE	3.54
MVZ182072	Migratory Allen’s	Contra Costa, CA	M	HiSeq 4000, UC Berkeley	150bp PE	3.18
MVZ180487	Migratory Allen’s	Contra Costa, CA	M	HiSeq 4000, UC Berkeley	150bp PE	2.70
MVZ180488	Migratory Allen’s	Contra Costa, CA	M	HiSeq 4000, UC Berkeley	150bp PE	1.62
E05847	Migratory Allen’s	San Luis Obispo, CA	M	HiSeq X, Novogene	150bp PE	5.78

E05908	Migratory Allen’s	San Luis Obispo, CA	M	HiSeq X, Novogene	150bp PE	5.29
E05856	Allen’s × Rufous	Lane, OR	M	HiSeq X, Novogene	150bp PE	3.96
E05857	Allen’s × Rufous	Lane, OR	M	HiSeq X, Novogene	150bp PE	2.23
E05855	Allen’s × Rufous	Lane, OR	M	HiSeq X, Novogene	150bp PE	1.22
SDNHM50767	Non-migratory Allen’s	Los Angeles, CA	M	HiSeq X, Novogene	150bp PE	2.88
E05905	Migratory Allen’s	Mendocino, CA	M	HiSeq X, Novogene	150bp PE	1.56
SDNHM51608	Non-migratory Allen’s	San Diego, CA	M	HiSeq X, Novogene	150bp PE	5.49
SDNHM51657	Non-migratory Allen’s	Los Angeles, CA	F	HiSeq X, Novogene	150bp PE	2.94
SDSU2997	Allen’s × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	2.77
SDNHM53030	Non-migratory Allen’s	San Diego, CA	F	HiSeq X, Novogene	150bp PE	2.60
E05818	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	0.92
E05817	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	1.74
E05815	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	2.38
E05813	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	2.90
E05951	Allen’s × Rufous	Curry, OR	M	HiSeq X, Novogene	150bp PE	5.00
K53373	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	4.29
K53375	Allen’s × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	2.19
CJC375	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	1.21
CJC374	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	0.78
E05808	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	1.87
E05822	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	2.50
E05821	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	2.37
E05810	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	2.05
E05806	Allen’s × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	1.32

E05807	Allen's × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	0.93
E05805	Allen's × Rufous	Siskiyou, CA	M	HiSeq X, Novogene	150bp PE	0.57
SDSU3101	Non-migratory Allen's	Riverside, CA	M	HiSeq X, Novogene	150bp PE	6.22
SDSU3099	Non-migratory Allen's	Riverside, CA	M	HiSeq X, Novogene	150bp PE	4.71
E05850	Non-migratory Allen's	Santa Barbara, CA	M	HiSeq X, Novogene	150bp PE	6.22
SDSU2981	Non-migratory Allen's	Los Angeles, CA	M	HiSeq X, Novogene	150bp PE	4.17
E05999	Non-migratory Allen's	Santa Barbara, CA	M	HiSeq X, Novogene	150bp PE	6.64
E05848	Non-migratory Allen's	Santa Barbara, CA	F	HiSeq X, Novogene	150bp PE	2.80
UWBM80061	Migratory Allen's	Marin, CA	F	HiSeq X, Novogene	150bp PE	2.49
SDNHM51760	Migratory Allen's	San Luis Obispo, CA	M	HiSeq X, Novogene	150bp PE	5.07
K53294	Allen's × Rufous	Humboldt, CA	M	HiSeq X, Novogene	150bp PE	1.16
E05996	Migratory Allen's	Mendocino, CA	M	HiSeq X, Novogene	150bp PE	3.23
MSB25414	Migratory Allen's	Marin, CA	M	HiSeq X, Novogene	150bp PE	2.60
K53382	Allen's × Rufous	Curry, OR	M	HiSeq X, Novogene	150bp PE	6.20
SDSU3071	Allen's × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	0.90
E05818	Allen's × Rufous	Coos, OR	M	HiSeq X, Novogene	150bp PE	0.91

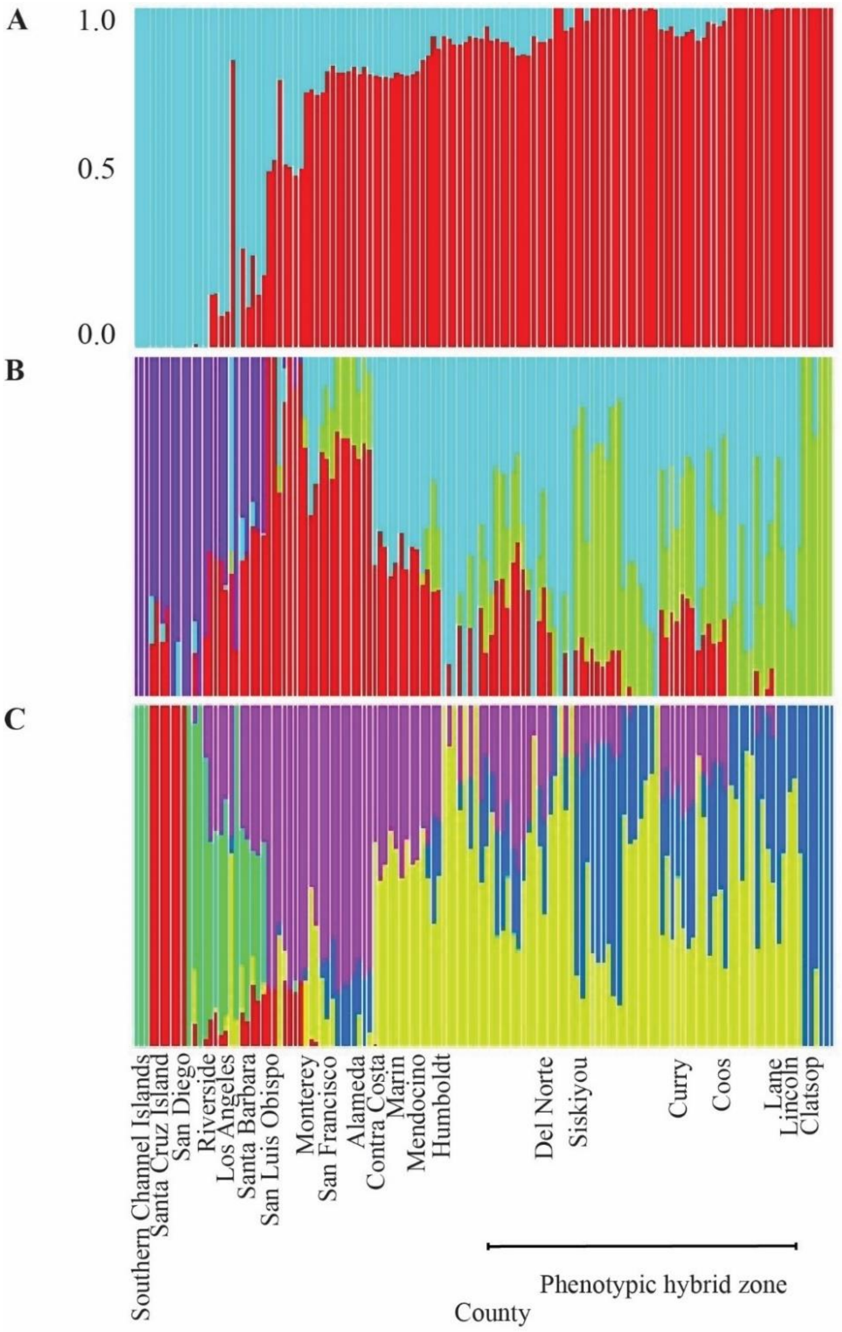


Figure S3.1 – **ADMIXTURE plots showing mixed ancestry between populations and individuals for A) K=2, B) K=4, and C) K=5.** Individuals were sampled across the historic ranges of non-migratory Allen's, migratory Allen's, and Rufous Hummingbird. **A)** K=2 clustered migratory Allen's and Rufous together, separate from non-migratory Allen's, **B)** K=4 separated migratory Allen's into two separate, highly admixed clusters, and separate clusters for non-migratory Allen's and Rufous, while **C)** K=5 split non-migratory Allen's into a Santa Cruz Island cluster and mainland + southern Channel Island cluster, followed by two separate (highly admixed) clusters of migratory Allen's Hummingbird, and a Rufous Hummingbird cluster.