



RESEARCH ARTICLE

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

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ABSTRACT

Allen's (*Selasphorus sasin*) and Rufous (*S. rufus*) hummingbird have long been suspected to hybridize, and potentially form a hybrid zone where their ranges overlap in southern Oregon. Migratory Allen's Hummingbird (*S. s. sasin*) breeds along a narrow strip of the California coast up to the Oregon border, while Rufous Hummingbird breeds from southern Oregon to Alaska. Analysis of behavioral and morphological data for 183 males and morphological data from 138 females showed that Allen's and Rufous hummingbird form a hybrid zone in southern Oregon and northern California. Linear discriminant function analysis and cline analysis of 20 phenotypic characters for males and 9 phenotypic characters for females suggested the center of the coastal transect of this north–south hybrid zone spanned from Bandon, Oregon (Coos County), to Port Orford, Oregon (Curry County). The contact zone extended north into the breeding range of Rufous (into Florence, Lane County, Oregon) and south into the range of Allen's (into Arcata, Humboldt County, California). Sporadic inland sampling suggested the hybrid zone extended at least 94 km inland from the coast. Behavioral data included courtship displays, which were composed of discrete, modular, behavioral elements. Sexual selection acted on these courtship displays, as behavioral clines related to courtship behaviors were more narrow than morphological clines. Some of the courtship behaviors analyzed included previously undescribed diagnostic behavioral characters for Allen's and Rufous hummingbird.

Keywords: admixture, cline, contact zones, environment, introgression, phenotype, selection, sexual selection

Evidencia de comportamiento y morfológica de una zona híbrida de *Selasphorus sasin* × *S. rufus* en el sur de Oregón y norte de California

RESUMEN

Durante mucho tiempo se ha sospechado que *Selasphorus sasin* y *S. rufus* se hibridan y que forman potencialmente una zona híbrida en el sur de Oregón donde sus rangos se superponen. La subespecie migratoria *S. s. sasin* cría a lo largo de una estrecha franja de la costa de California hasta el límite con Oregón, mientras que *S. rufus* cría desde el sur de Oregón hasta Alaska. El análisis de datos de comportamiento y morfológicos de 183 machos y datos morfológicos de 138 hembras muestran que *S. sasin* y *S. rufus* forman una zona híbrida en el sur de Oregón y el norte de California. Un análisis de función discriminante lineal y un análisis de clina de 20 caracteres fenotípicos para los machos y nueve caracteres fenotípicos para las hembras sugirieron que el centro de la transecta costera de esta zona híbrida norte-sur abarcó desde Bandon, Oregón (Condado de Coos), hasta Port Orford, Oregón (Condado de Curry). La zona de contacto se extendió al norte hacia el rango reproductivo de *S. rufus* (en Florence, Condado de Lane, Oregón), y al sur hacia el rango de *S. sasin* (en Arcata, Condado de Humboldt, CA). El muestreo esporádico tierra adentro sugirió que la zona híbrida se extendió al menos 94 km tierra adentro desde la costa. Los datos de comportamiento incluyeron despliegues de cortejo que estuvieron compuestos por elementos conductuales discretos y modulares. La selección sexual actuó en estos despliegues de cortejo, ya que las clinas de comportamiento relacionadas a las conductas de cortejo fueron más estrechas que las clinas morfológicas. Algunas de las conductas de cortejo analizadas incluyeron caracteres diagnósticos de comportamiento anteriormente no descriptos para *S. sasin* y *S. rufus*.

Palabras clave: ambiente, clina, fenotipo, introgresión, mezcla, selección, selección sexual, zonas de contacto

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 (*S. rufus*) × 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, 2 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 (*S. sasin*) × 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, we describe the phenotypic data that indicate these 2 species form a hybrid zone in southern Oregon and northern California.

Speculation of hybridization between Allen's and Rufous 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 1972). 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 1972, 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. Further, 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. As these birds were sampled on migration, outside the breeding range of either species, 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 another instance of "one-off" (sporadic) hybridization for which hummingbirds are well known, 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 to 0.8 kHz while Allen's Hummingbird produce sound with r3 with a fundamental frequency from 1.8 to 1.9 kHz (Clark et al. 2011, Clark 2014). Within Rufous Hummingbird, dozens of harmonics give the dive sound a buzzy 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). Allen's Hummingbird display sequences consist of multiple

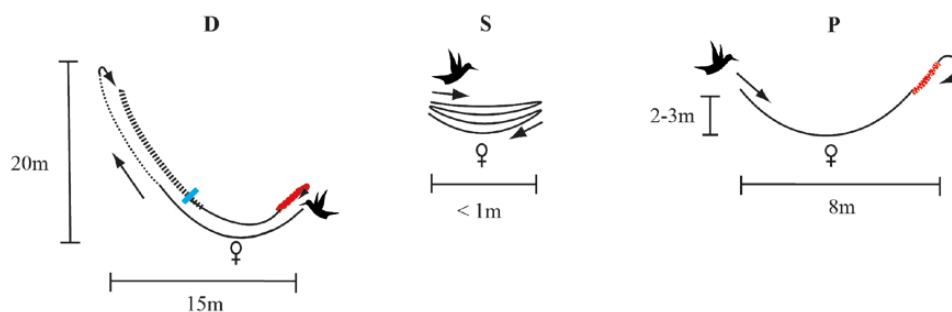


FIGURE 1. Courtship displays of male Allen's Hummingbird (*S. s. sasin*) and male Rufous Hummingbird (*S. rufus*). Bird silhouette is not to scale. ♀: approximate position of the female during the male's courtship dive. During the dive in both species (**D**), the bird ascends for the dive, turns around, 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 4.

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 “chirrup,” which are short, loud pulses of wing trill produced during a behavior we 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).

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; 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, we define a “bout” of display as the sequence of courtship behaviors a male performs toward his stimulus within a single courtship episode.

Males also 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 Allen’s and Rufous hummingbird and any putative hybrids.

Tail feather morphology produces the different dive sounds of Allen’s and Rufous hummingbird during courtship, doing 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 transitioned from r2 to r3 (Clark 2014). This “harmonic hopping” hypothesis makes 2 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, several possibilities exist for hybrids to produce sounds: they could produce Rufous-like buzzy dive sounds (+r2, -r3), Allen’s-like pure tone dive sounds (-r2, +r3), as well as 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, we describe 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. We also investigate the underlying behavioral elements that form courtship behaviors within each species.

METHODS

Life History

Allen’s and Rufous hummingbird breed within forest edges and disturbed areas along the Pacific coast (Healy and Calder 2006, Clark and Mitchell 2013; Figure 2). Allen’s Hummingbird breeds in riparian habitats adjacent to scrub and forest edge habitat along the California coast (Jewett 1929, Gilligan et al. 1994, Healy and Calder 2006; Figure 2). Allen’s Hummingbird has 2 subspecies: *Selasphorus sasin sasin* (migratory) and the slightly larger *S. s. sedentarius* (nonmigratory), which has a breeding range restricted to southern California (Stiles 1972). In this paper, 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 (Healy and Calder 2006). This species breeds in open areas and riparian habitat, usually in general association with fir, spruce, and hemlock-dominated Pacific rain forests.

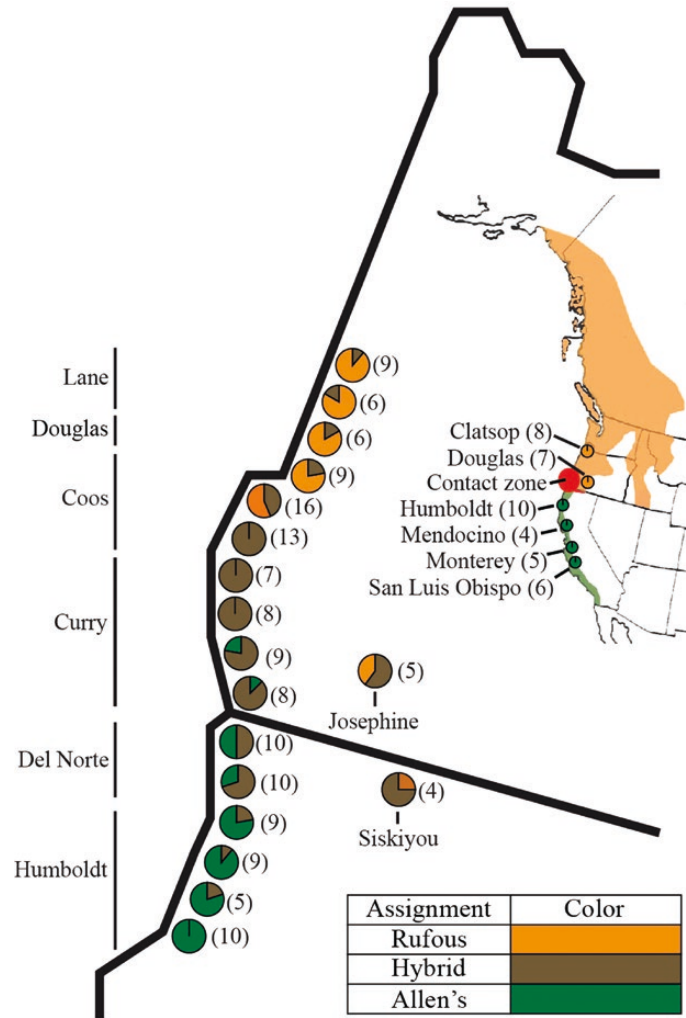


FIGURE 2. Approximate breeding ranges of Allen's (*S. s. sasin*) and Rufous (*S. rufus*) hummingbird (inset map) and linear discriminant function analysis (LDA) of phenotypic characters along a coastal transect and other localities for Rufous Hummingbird, Allen's Hummingbird, and putative hybrid males. Note: only migratory Allen's Hummingbird (*S. s. sasin*) is addressed here. For breeding ranges and sampling of reference parent species outside the hybrid zone (inset map): orange: Rufous Hummingbird breeding range; green: Allen's Hummingbird breeding range; red: hybrid zone sampling area, from Lane County (north) to Humboldt County (south; Healy and Calder 2006, Clark and Mitchell 2013). Each pie represents the proportion of the designated phenotype in that population, as determined by LDA.

Sampling

We 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 (Supplemental Material Table S1). We sampled Allen's Hummingbird parent 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 our coastal transect in Curry County, Oregon. We 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 our coastal transect in Curry County, Oregon. 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 nonbreeding (i.e. migrating) birds in our dataset. Female Allen's and Rufous hummingbird were identified based on range (only those collected outside of the area of overlap between the 2 species, described above, and away from the range of nonmigratory Allen's Hummingbird during the breeding season were measured) because of the difficulty in discriminating females based on morphology (Stiles 1972, 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. We 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, we did not diagnose females 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, we recorded males displaying to natural stimuli, such as wild females, other males, or (rarely) unknown stimuli. After a minimum of 10 bouts of displays were recorded, target males were then captured. We considered an individual bout as concluded when a male ceased displaying for 0.5 s or longer. We recorded 183 territorial males using a shotgun microphone (Sennheiser MKH 70, Wedemark, Germany) and a 24-bit digital recorder (Sound Devices 702, 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. Measurements of r2 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 (Supplemental Material Figure S1). 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 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 1972, Newfield 1983, Hurly et al. 2001, Colwell 2005, Healy and Calder 2006). For females, measurements were the same for males, except there are no back color measurements, and we only measured the length of the outer notch of r2, which is the most prominent portion of the less distinct r2 in females.

Behavior

Courtship behaviors can be broken apart into their underlying elements, which we define as small behavioral units that are below the level of a fixed action pattern (i.e. elements are the “building blocks” of displays). Carola's Parotia (*Parotia carolae*; Scholes 2006, 2008) and *Habronattus* jumping spiders (Elias et al. 2012) provide analyses of courtship display sequences made of behavioral elements that correspond to every movement contained within the display. Here, we describe the courtship displays of Allen's and Rufous hummingbirds by naming display elements in order to identify display differences between 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 considered 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, could be quantified (Figure 4, Table 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), we analyzed the high end of the dive sound.

The behavioral characters we analyzed included average fundamental frequency of the dive, which was 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. We 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. We also analyzed courtship behaviors based on underlying behavioral elements that we 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 2 species and tests for differences among groups that are defined a priori (Whitmore 1983, Heaney and Timm 1985, James and McCulloch 1990, Poulsen and French 1996). We used

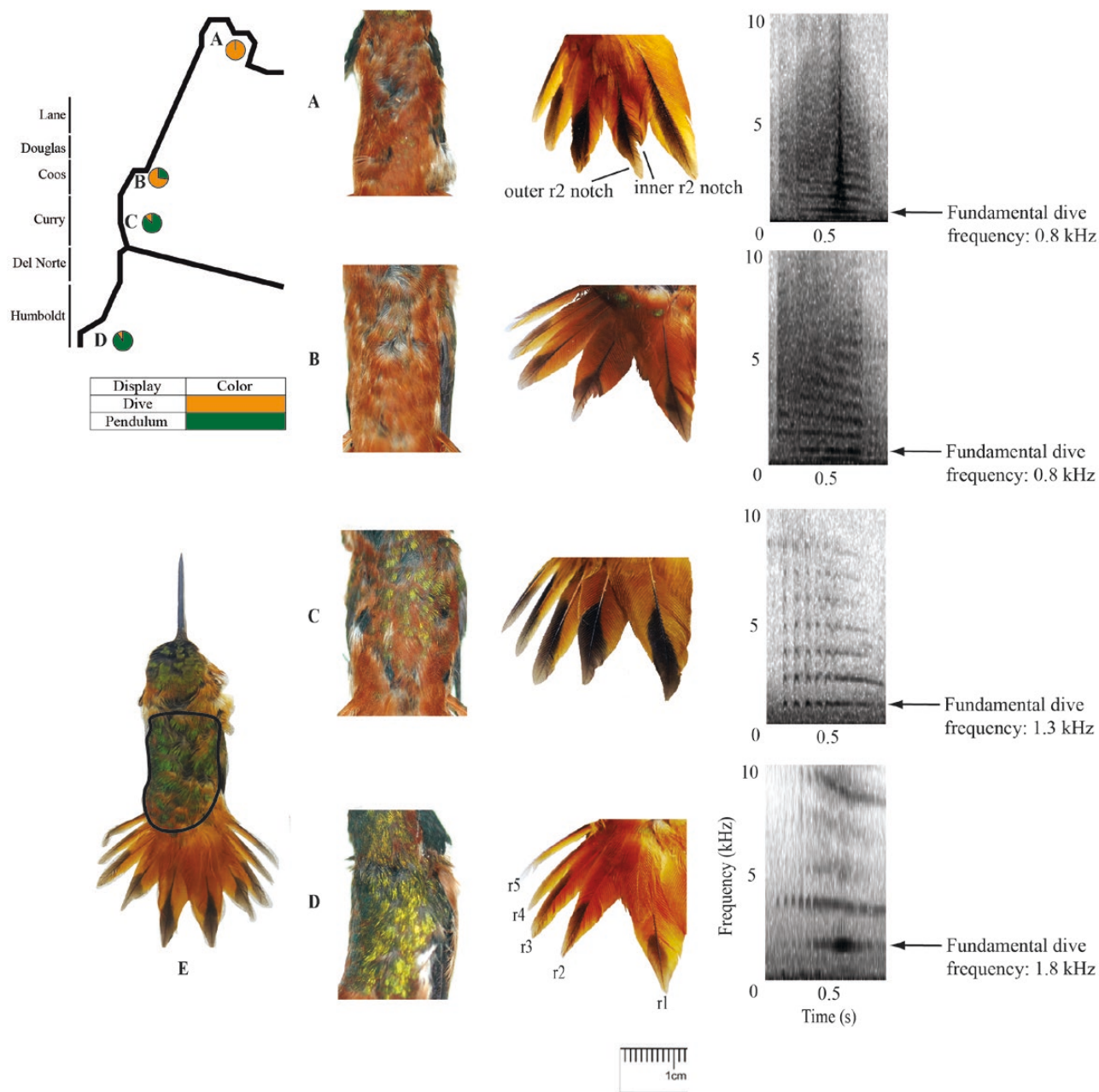


FIGURE 3. Back, rump, tail, fundamental frequency of the dive sound, and the proportion of pendulums to dives along the coastal gradient. (A) Rufous Hummingbird with a 10% green back (Clatsop State Forest, Clatsop County, Oregon; CJC 382). (B) A Rufous-like hybrid with a 0% green back; (Sunset Bay State Park in Coos County, Oregon; SDSU 3074). (C) An Allen's-like hybrid with a 50% green back (Alfred A. Loeb State Park, Curry County, Oregon; SDSU 2999). (D) An Allen's Hummingbird with a 100% green back, (Humboldt Redwoods State Park, Humboldt County, California; SDSU 2989). Along the north to south gradient, the rectrix 2 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 (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.

LDA to assign individuals to groups defined a priori, evaluate the extent of hybrid individuals across the transect, and assess how populations differ across our sampling transect (James and McCulloch 1990).

We implemented several definitions and characters to diagnose hybrids. Here, we defined a hybrid character as one that falls outside the 95% confidence interval (CI) of the variation present in the population of each parent

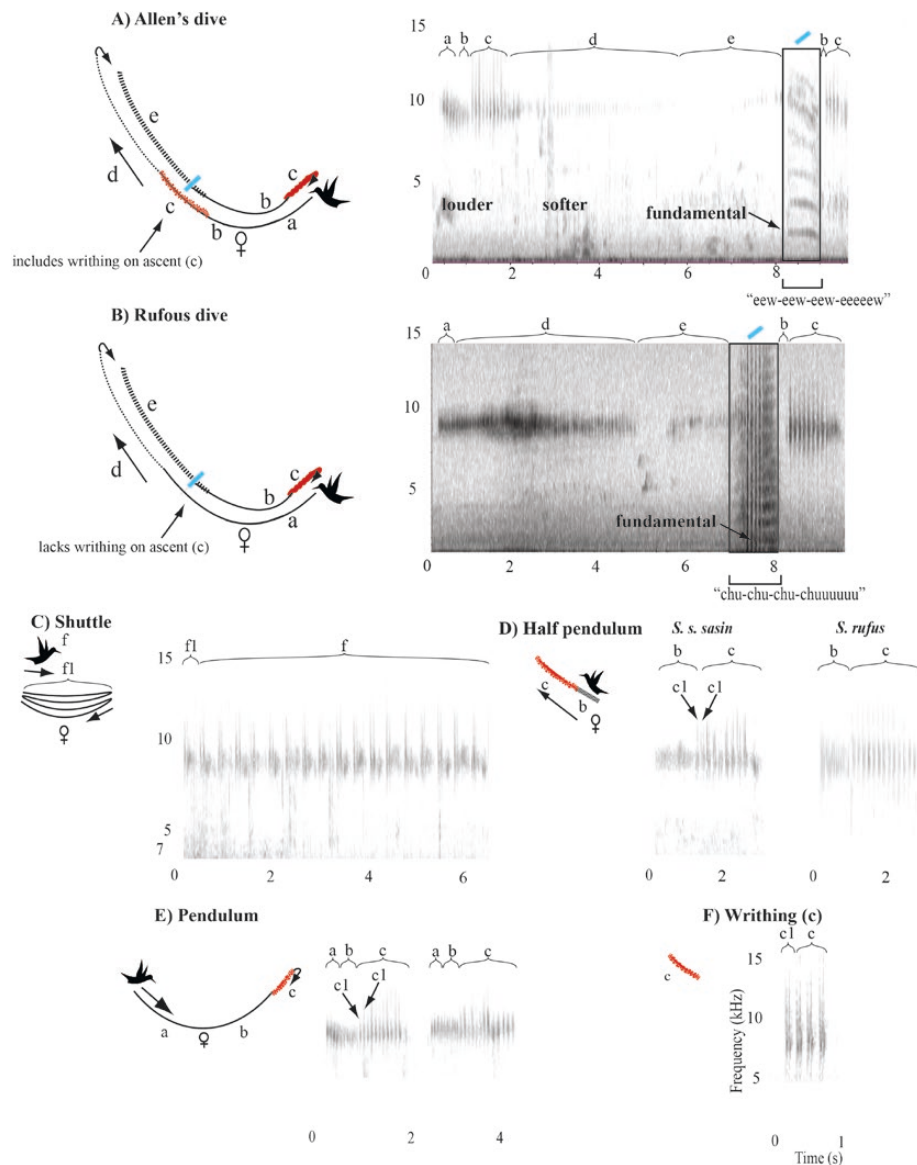


FIGURE 4. Courtship display kinematic sequences and sounds of male Allen's (*S. s. sasin*) and Rufous (*S. rufus*) hummingbird. Lowercase letters refer to display elements, as defined in Table 1 and as follows: a = short descent, b = short ascent, c = bout of writhing (c1 = a single chirrup, "c" denotes 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 *eeew-eeew-eeew-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 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.

TABLE 1. Definitions of display behaviors and elements; ID refers to display elements illustrated in Figure 4.

Display element	ID	Description of element
Short descent	a	A descent of about 2–3 m in first half of a shallow half-U shape.
Short ascent	b	An ascent of about 2–3 m 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 ~20 m. 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 ~20 m high, 15 m 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 ~20 m high, 15 m wide.
Shuttle	S	Male flashes gorget, flies with variable vertical trajectory in overall left/right motion; within 1–2 m of female, 25 m high; consists of individual shuttle segments.
Half pendulum	H	Male performs short ascent, concluding with writhing; 2–3 m high, 2–4 m 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; 2–3 m high, 8 m wide.

species using the same method as Pyle (1997), where we estimated the 95% CI as $\pm$ twice the standard deviation (SD) from the mean for each character. An intermediate character is one that is in between 2 parental phenotypes (e.g., a dive sound of 1.1 kHz). We diagnosed hybrids using the phenotypic characters that are used in identification of each parent species, where intermediate individuals with characters representing a mosaic of each parent species' phenotypes (e.g., an Allen's-like bird with a Rufous-like character) were used to identify putative hybrids, following Graves (1990). We 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 our measurements to other work (Table 2, Supplemental Material Tables S2 and S3). We defined a diagnostic character as one that falls 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. We used the 95% confidence interval to assign hybrid and parent characters to individuals. Characters falling outside of the 95% CI for a given species were considered hybrid characters. A character outside of the 95% CI might not actually be hybrid, since, assuming a normal distribution, parental samples will fall outside of the 95% CI, 5% of the time. 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 parent 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 $\pm$ SD.

To classify hybrid vs. parent individuals, we performed an LDA. Before performing this analysis, we used a cluster analysis of observations to determine the best number of groups to use for males and females, so that individuals could be assigned 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, we standardized our data to have a mean of zero. For clustering, we 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 our 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 farthest away from each other. Based

TABLE 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 mm.

Character	Allen's (n = 27)		Rufous (n = 34)	
	95% CI	Mean ± SD	95% CI	Mean ± SD
Morphology				
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 chirrup	6.0–10.0	8.0 ± 1.0	7.1–17.5	12.3 ± 2.6
Post-dive chirrup	1.8–7.4	4.6 ± 1.4	5.7–10.1	7.9 ± 1.1
Fundamental frequency of dive (kHz)	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 3. Morphological characters of adult female Allen's (*S. s. sasin*) and Rufous (*S. rufus*) hummingbird. 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.

on similarity and distance levels, $K = 3$ groups also showed the strongest support for female classification. Thus, for LDA, we used $K = 3$ groups for male clusters, and $K = 3$ groups for female clusters. We assigned each individual to a group 1–3 for males, and 1–3 for females. Using 3 groups, we designated each male into 1 of 3 categories: Rufous Hummingbird, hybrid, or Allen's Hummingbird. For females, morphological data for 9 characters were incorporated in all analyses (Table 3). The 3 clusters designate each female into 1 of 3 categories: Rufous Hummingbird, hybrid, or Allen's Hummingbird.

We 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 parent group to quantify populations across the hybrid zone (Rao 1948, James and McCulloch 1990, Poulsen and French 1996). We used LDA to evaluate classifications of individuals within each population estimated a priori, based on the presence of intermediate characters that partition individual samples into different clusters, and yield which variables maximize 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 2 or more characters to be scored as hybrid. For example, a Rufous-like bird performing a pendulum display was scored as hybrid, as we never observed a pendulum display in Rufous Hummingbird ($n = 34$). For females, individuals with one or more hybrid characters were also considered to be hybrid *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, we performed a geometric morphometric analysis on 29 landmarks for each tail feather (r1 through r5) for each individual (Figure 5A). We 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, we implemented a principal components analysis in MorphoJ v1.06d (Klingenberg 2011). PC1 represented a continuum of Allen's-like to Rufous-like tail shape. We 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, we 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 to 1, with the most intermediate individuals scoring in the middle (Hatheway 1962, Hubbard 1969, Anderson and Daugherty 1974, Mettler and Spellman 2009, Abbott and Brennan 2014). We incorporated our 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 in R 3.4.3, which uses the Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm to estimate cline parameters (Derryberry et al. 2014, R Core Team 2015). We 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 parent 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, we fit all characters to clines for Model 3. HZAR calculated cline center and width for each character using the MCMC. We ran 100,000 iterations for each cline parameter with a burn-in of 10,000 generations. We used 2-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 could be split into 3 types: dives, shuttles, and pendulums (Table 1). Within these displays, there were multiple types of pendulum displays: regular pendulums and a previously undescribed variant that we call "half pendulums." We found that dive displays could be split into 2 types typified by each parent species: "Allen's dive" and "Rufous dive" (described in Table 1, Figure 4A, 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 2 kinds of dives, we 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). Next, we described detailed elements of courtship displays in male Allen's and Rufous hummingbird that we sampled (Table 1, Figures 4 and 6).

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 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 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 4). During a long ascent, a bird ascended

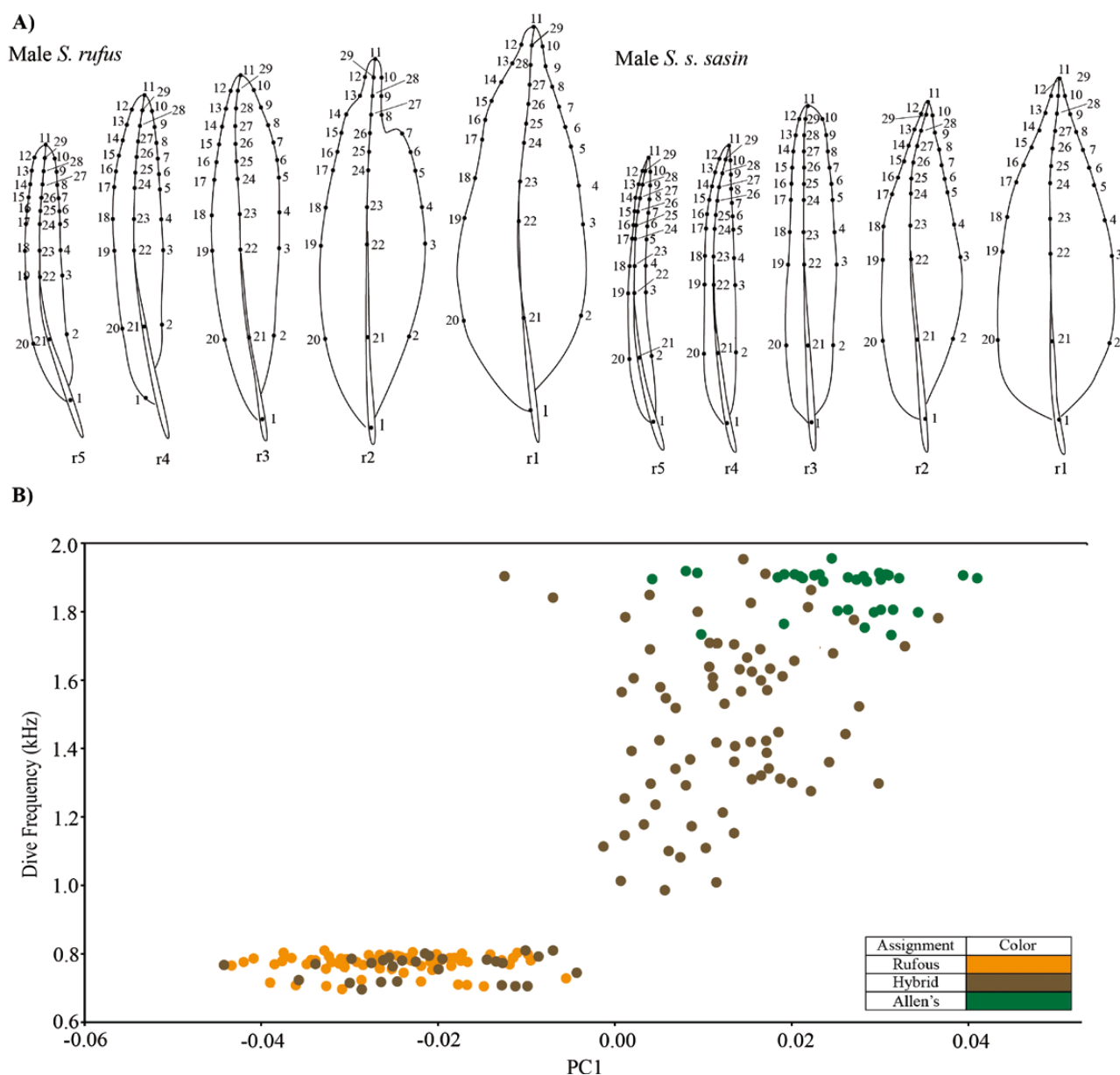


FIGURE 5. Geometric morphometric landmarks (**A**) and analysis (**B**) of one set of 5 tail feathers for individuals spanning the hybrid zone. (**A**) For one set of tail feather rectrices (r1, r2, r3, r4, and r5) for each individual, 29 landmarks per tail feather were taken. (**B**) Morphometric data suggest differences in tail shape drive differences in the fundamental frequency (kHz) of the sound made toward the end of a dive. Allen's Hummingbird generates sound with rectrix 3, while Rufous Hummingbird makes sound with rectrix 2, which has a notched tip that Allen's Hummingbird lacks. Putative hybrids produce intermediate sounds based on mixed tail feather morphology.

~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, spreading his tail feathers, and producing a dive sound (Figure 4A, 4B). Finally, shuttle segments are individual left-to-right or right-to-left motions; multiple shuttle segments form shuttle displays (Figure 4C; all display elements are further described in Table 1 and Figure 4).

The difference between the 2 species is that behavioral elements are assembled in a different order to form species-diagnostic displays (Table 1, Figure 6). For example, the elements of the pendulum display, which is found in Allen's and absent in Rufous, are not unique per se to the pendulum display or Allen's Hummingbird. Rather, the pendulum display assembles the elements in an *order* not observed in Rufous Hummingbird (short descent, short ascent with writhing; Figure 4).

Dive display. The dives performed by Allen's Hummingbird (hereafter, "Allen's dive") were (usually) composed of the following display elements (see also Figure 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 (Table 1 and Figure 4A, 4E).

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 composed of (e) a long descent, then another short ascent (b), and a bout of writhing (c; Figure 4B). Individuals of both species reached ~20 m in height during a dive display (Table 1 and Figure 4A, 4B). Kinematically, the 2 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 ($n = 34$; Table 1 and Figure 4B). Before writhing after the dive was performed, Rufous Hummingbird made a sound with the tail feathers toward 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 toward 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 ($n = 27$; Table 1 and Figure 4A). Allen's Hummingbird performed writhing on the ascent for the dive 90.5% of the time (Tables 1 and 2). Although a few male Allen's Hummingbird occasionally performed individual dives without writhing on the ascent, we sampled at least 10 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 composed of individual "shuttle segments," which occurred as individual right-to-left or left-to-right motions, with sound made by the wings (Figure 4C). We did not detect any differences between Allen's Hummingbird (either subspecies), Rufous Hummingbird, or any hybrid populations in how males performed this display. Thus, we did not focus any of our 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 (Tables 1 and 2; Figure 4E). 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 2 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 2 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 variant, 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 (Table 1, Figure 4D). 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 behavior and a regular pendulum display is that the male began the display next to the female, and thus, did not do the short descent that comprises 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 ($n = 34$; Tables 1 and 2, Figure 4D) and Allen's Hummingbird averaged 8.0 ± 0.5 individual chirrup sounds ($n = 27$; Tables 1 and 2, Figure 4D).

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 (Table 2, Figure 3A, Supplementary Material Table S1).

Evidence of Hybridization

Of the 183 breeding males sampled from the hybrid zone, most behavioral and morphological characters were clinal (Table 2). There was no sharp boundary that would demarcate the limit between 2 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 parent species (Figure 2).

LDA revealed a gradual north–south shift from a Rufous-like to an Allen's-like phenotype (Figure 2). Across the hybrid zone, there was a shift in tail feather morphology and the resultant fundamental frequency of the dive sound (Figures 3 and 4A–D) with some putative hybrids producing intermediate dive sounds (Figure 3). Sequences

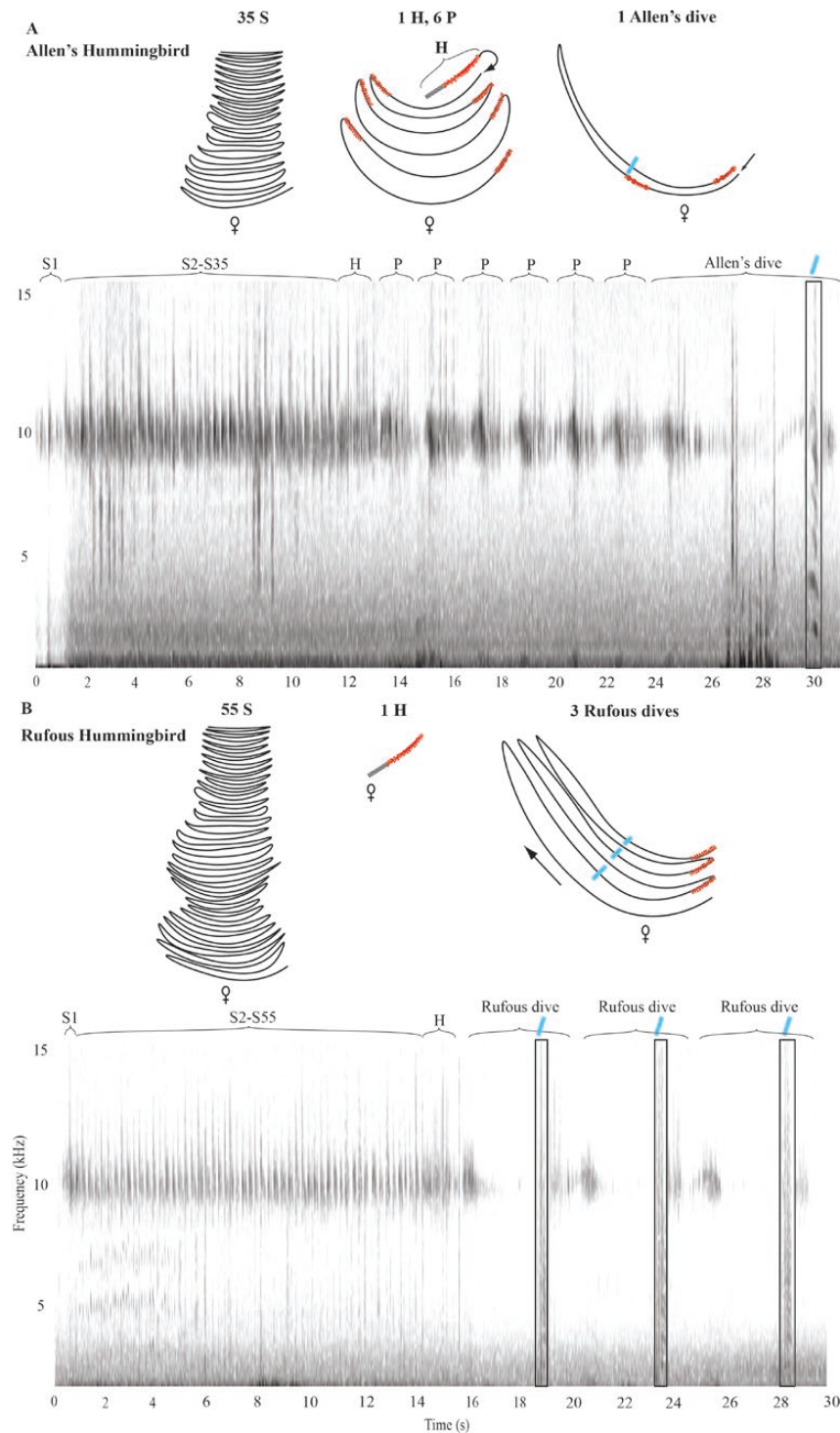


FIGURE 6. Typical courtship display sequences in male Allen's (*S. sasin*; **A**) and Rufous (*S. rufus*; **B**) hummingbird, from populations on the coastal transect. Display elements for kinematics (top) and spectrograms for each display (bottom), as in Figure 4: a = short descent, b = short ascent, 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 3 consecutive dives with no writhing on the ascent (Rufous dives). The red line indicates a bout of writhing.

of courtship behavior also differed along the transect, with a shift from a Rufous-like to Allen's-like behavioral phenotype (Figure 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 5B). PC1 was uncorrelated with fundamental frequency for Rufous Hummingbird, then fundamental frequency showed a sudden, discontinuous break between 0.8 and 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:

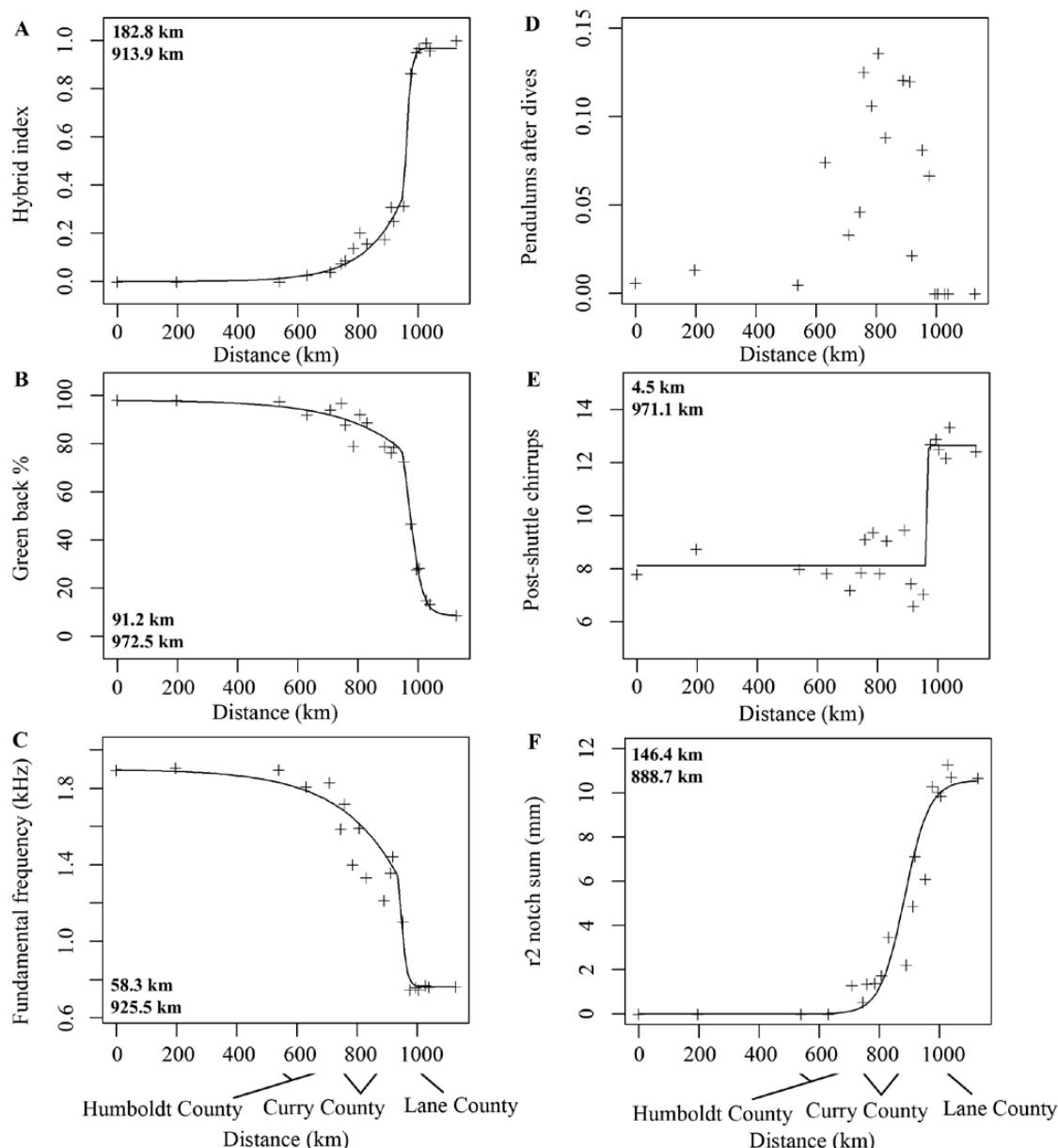


FIGURE 7. Cline analysis for males across the coastal, north-south transect for the following characters based on population means: (A) the hybrid index based on all characters, (B) percent green back, (C) fundamental dive frequency (kHz), (D) proportion of pendulums after dives, (E) post-shuttle chirrups, 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) 1,200 km away from the southernmost Allen's Hummingbird population. Within each plot, top distance is cline width (km), bottom distance designates cline center (km).

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 (although not as high-pitched as Allen’s), and tonal, without the buzzy quality of Rufous Hummingbird. We 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. We 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 parent species to which they were geographically closest. The northernmost transect locality, in Lane County, Oregon, was almost fully phenotypically Rufous Hummingbird. Our southernmost hybrid population, in Humboldt County, California, 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 3 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 2, Supplementary Material Tables S4 and S5).

Similar morphological characters best separated females as well (Supplementary Material Tables S4 and S6). The characters with the highest LDA scores, indicating the characters that best diagnosed across our 3 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, Oregon, as far south as Humboldt County, California (Supplementary Material Table S6). Most females identified as hybrid were found in Coos and Curry counties, Oregon. 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 our character suite (Table 4). Most clines centered between Port Orford, Oregon (Curry County), and Bandon, Oregon (Coos County; Figure 8, Supplemental Material Figures S2 and S3). For 8 of the remaining 15 characters, the position of the cline centers were within 20 km of Bandon, Oregon (km 950 on our transect, relative to km 0–1127, which spans 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 8E, Supplemental Material Figure S3E).

Based on the interval bound by 2 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 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 5 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 4). Generally, decay was lower on the left tail of the cline center (toward Allen’s range) than on the right side of the cline center (toward Rufous Hummingbird range). Generally, decay was lower for courtship behaviors than for morphological characters across both sides of the cline center.

DISCUSSION

Our data indicate 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, Oregon, in the north into Humboldt County in northern California to the south (Figure 2). The center of our 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,

TABLE 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)	1,152.4 (1,140.4–1,164.4)	1,309.1 (1,301.4–1,316.7)
Rectrix 1 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)	1,093.7 (0.0–1,429.4)	681.8 (2.8–1,424.7)
Rectrix 2 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 (36.0–1,384.1)
Rectrix 3 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 (36.0–1,384.1)
Rectrix 4 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 5 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 (0.0–1.423.8)	325.9 (2.4–1,336.9)
Rectrix 2 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 (1.1–1,329.8)
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)	1,311.3 (3.1–1,429.9)
Folded wing chord (mm)	1,015.55 (975.7–1,065.1)	152.7 (101.5–274.8)	0.3 (0.0–1.0)	0.1 (0.0–1.0)	674.9 (24.2–1,381.8)	16.5 (2.1–1,228.4)
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)	1,129.9 (193.1–1,424.5)
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 (0.9–1,429.2)	191.3 (0.2–1,429.1)
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 (2.2–1,426.3)	168.5 (1.1–1,425.4)
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 (3.7–1,406.1)
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)

California, implying that the hybrid zone also has an east-west component along the Klamath River, for which we did not have samples to include in the cline analyses presented here (Siskiyou County; [Figure 2](#)). Although geographic clines for phenotypic characters are often concordant in spatial position and width, there are documented exceptions ([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, can 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 we sampled had 10–20% green on the back, while most Allen’s Hummingbird had 90–100% green on the back ([Figure 3A](#), 3D). One hypothesis for these green-backed Rufous was that they were 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, personal communication; CJC personal observation) 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 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 about 0.8 and 1.1 kHz. We 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. We did not detect any polymorphic birds that produced separate sound with both r2 and r3 separately. Because these 2 feathers are adjacent and aerodynamically coupled, it may be physically impossible for r2 and r3 to flutter at different frequencies. We also did not detect any cases of birds that produced no

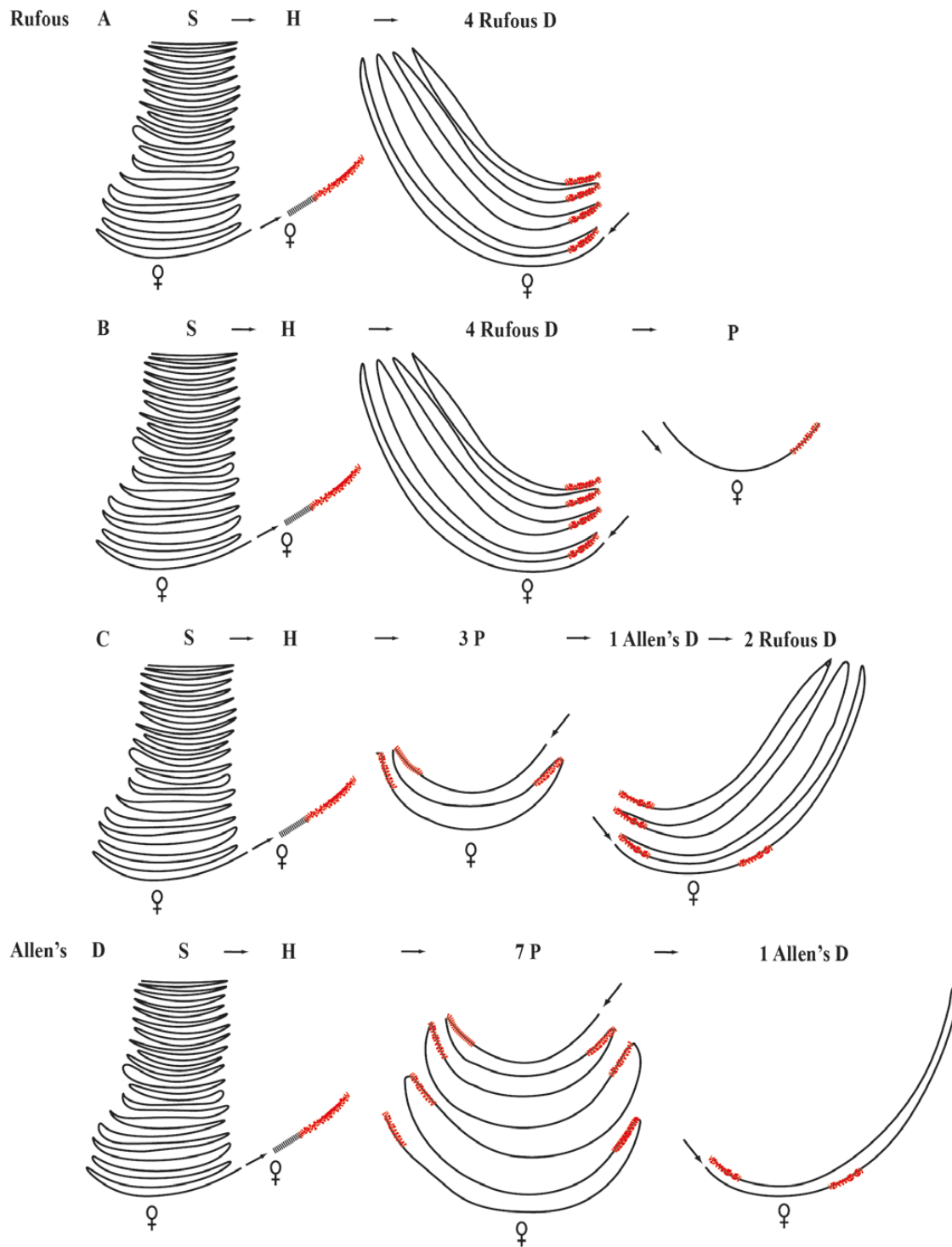


FIGURE 8. Typical courtship display sequences in males across the hybrid zone, from (A) north in Rufous Hummingbird, and (B) south into a Rufous-like hybrid population, (C) an Allen's-like hybrid population, and (D) an Allen's Hummingbird population. From north to south, there is a transition from Rufous-like 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.

dive sound (i.e. neither feather as a source), although qualitatively, some hybrids seemed to produce rather faint dive sounds. We 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

We did not find evidence of any populations of Rufous Hummingbird that perform O-shaped dives (Figure 4B), contrary to Johnsgard's (1983) description. Birds we sampled in Clatsop County, Oregon, and the Umpqua National Forest, Oregon, 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, we described the presence of a previously undescribed display, the "half pendulum" display, which is present in both Rufous and Allen's hummingbird (Figure 4D). Hurly et al. (2001) provided a brief, ambiguous description of Rufous Hummingbird performing an undescribed display, 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" we describe here. Half pendulums always followed the shuttle display in all Rufous Hummingbird and Allen's Hummingbird populations we studied, contra the Hurly et al. (2001) description of it as a "separate" display. We interpret the Hurly et al. (2001) account as referring to the half pendulum display (Table 1), as it is parsimonious to assume that they saw the same display we found to be widespread within all populations of Rufous Hummingbird we studied, rather than a display we observed only in Allen's Hummingbird.

All of the courtship behaviors described here comprise a set of discrete behavioral elements, common to both Rufous and Allen's hummingbird (Table 1, Figure 4; Barlow 1968, 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 differs (Figure 4). An example of courtship behaviors differing due to a rearrangement of common elements found within Rufous and Allen's hummingbird can be found within the half pendulum display of both species. The half pendulum

is composed 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 (Table 1, Figure 4D, 4E). 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 6A, 6B). Taken further, the element sequence of the pendulum display can be found within the first 3 elements of the sequence of an Allen's dive (Table 1, Figure 4A, 4E).

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 8 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, we 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. We will 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 parent species.

The displays often occur in a typical order (Figures 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 7A, 7D). The orders in which these displays occurred was homogeneous 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 (Supplementary Material Figure S3).

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 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 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 $\times$ 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 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 served 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 1992, 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 1992, Lipshutz et al. 2017). Alternatively, across a Black-capped (*Parus atricapillus*) $\times$ Carolina (*P. carolinensis*) chickadee 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.

We detected a substantial portion of individuals with an intermediate phenotype in the center of the hybrid zone. We will address the actual frequency of hybridization across the Allen's and Rufous hummingbird hybrid zone with genetic data in a subsequent study. In conjunction with the phenotypic data presented here, genetic data will tease apart whether these individuals are the result of high rates of interbreeding, or whether they are late-generation backcrosses with either few or no recent-generation hybrids. The latter case would suggest substantial reproductive isolation over a long period of time, while the former would not. 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 (Supplemental Material Tables S5 and S6). While this could be the product of sex bias in dispersal, we find it more likely that the LDA has misclassified 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 $\times$ Rufous hummingbird hybrid zone (Figure 8E, Supplemental Material Figure S3E).

Our results indicate clinal variation across our suite of phenotypic characters for males, especially between 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 2 traits, making it unclear why the LDA strongly loaded culmen length to discriminate between groups (Supplementary Material Table S4, Figure S2A). 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 parent species (Figure 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 (Table 4, Figure 8, Supplementary Material Figure S2, S3). Furthermore, shorter cline decay for courtship behaviors compared to morphology on both sides of our clines implies stronger selection on courtship behaviors (Szymura and Barton 1986). Selection is stronger (decay is lower) on the left side of the cline center (toward Allen's Hummingbird range) than on the right (toward Rufous Hummingbird 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 could help 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 1992, Shaw 1996, Curry et al. 2007). Here, geographic cline analysis suggests stronger selection on courtship behaviors relative to morphological traits. We also found evidence of stronger selection of behavior toward the range of Allen's Hummingbird than toward Rufous Hummingbird.

Knowledge of the mechanisms of reproductive isolation can provide 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 et al. 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 et al. 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 parent species within the area of contact. Incorporation of genetic cline analysis in future work will corroborate our 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 yr, Sitka spruce (*Picea sitchensis*) and Douglas fir (*Pseudotsuga menziesii*), 2 coniferous trees within the temperate 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 can be 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 bracteosum*, *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 we did not observe such concordance in our data, we 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, can 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, we expect elevated linkage disequilibrium and genetic diversity in the center of the zone, which is to be expected when 2 divergent populations make contact (Durrett et al. 2000, Chavez-Galarza et al. 2015).

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Conflict of interest statement: The authors of this manuscript certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Data depository: Our specimens have been deposited at the San Diego State University Museum of Biodiversity and the San Diego Natural History Museum. We are currently working on additional manuscripts with our dataset; thus, although we plan to make our data available on Dryad and the Macaulay Library, we do not wish to do so until we conclude writing of our other manuscripts.

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