

Determining the Role of Morphology in Reproductive Isolation between Two Related Species

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## **Abstract**

Very closely related species are ideal candidates for studying the finer mechanisms of evolution. This study aims to determine the role of ecologically valuable physical traits in maintaining reproductive isolation between two recently diverged species, the Red-naped Sapsucker (*Sphyrapicus nuchalis*) and the Red-breasted Sapsucker (*S. ruber*), by analyzing the morphological differences in a collection of specimens taken from an area in which they interbreed. I measured and recorded the mass, dimensions of the bill, length of the wing chord, and length of the tarsus for each of the specimens, then described how these traits vary by ancestry and sex. I found that the males of two species differed significantly in the length of the wing chord, the length of the tarsus, and in mass, but that any differences involving females were negligible. Of the traits that differed between the two parental species, we found evidence that only mass and wing chord are contributing to reproductive isolation between the subspecies. These findings show that traits that are not traditionally considered important to sexual selection still play a role in the early divergence of species.

## **Introduction**

It has been long established that subtle differences in morphology within a population can ultimately lead to the emergence of separate species, this being one of the foundational concepts of the Theory of Evolution by Natural Selection. Soon after the initial divergence of new species, these subtle differences, should they be strongly tied to genetics, are essential in driving the further evolution of those species. Hybridization occurs most often early into the process of speciation. Whether it results in the collapse or divergence of insipient lineages depends largely on the divergence of traits. When hybrids present the same phenotype as one parental species

despite their mixed genetics, it indicates that selection for that phenotype is strong enough to act as a form of reproductive isolation despite hybridization taking place because intermediate forms are disadvantageous (Delahaie et al. 2017). The traits most likely to become the targets of concentrated selection early on are those involved in sexual signaling, since they are easily seen and so easily selected upon (Harris et al. 2018, Delahaie et al. 2017). It is by virtue of this that species divergence can happen in the absence of physical barriers between populations (Semenov et al. 2017, Harris et al. 2018, Delahaie et al. 2017). Species divergence where, at some point, there is the absence of a physical barrier is the most common situation in which diverging species are found, and in these situations the diverging populations will continue to hybridize (Barton & Hewitt 1989). The area over which the ranges of the new species overlap is called a hybrid zone, and often manifests as a narrow channel that takes up a fraction of the full range of either species (Barton & Hewitt 1989). Speciation in these systems can be driven by strong assortative mating choice towards either species (Grossen et al. 2017, Delahaie et al. 2017) but may be helped along by characteristics of the hybrid zone, or hybrids themselves having low fitness compared to either parental, that make spatial and genetic convergence untenable. The hybrid zone may occupy a tenuous niche that risks disappearing through time (Billerman 2016), or the hybrids may be relatively rare or unfit compared to individuals of either parental species (Barton & Hewitt 1989). It is because of these factors that the existence of hybrid zones between newly diverged species does not prevent speciation from happening in these systems.

The Red-breasted Sapsucker (*S. ruber*) and the Red-naped Sapsucker (*S. nuchalis*) are relatively recently diverged species (Grossen et al. 2017). Historically, they have been recognized as different subspecies of Yellow-bellied Sapsucker (*Sphyrapicus varius*), and today share a hybrid zone and many physical and genetic traits (Grossen et al 2016). Their hybrid zone

is a well-known system that has been studied often over the last century. It is found in the northwestern portion of North America where the coastal *S. ruber* meets the inland species *S. nuchalis* (Billerman 2016). Due to how closely intertwined these species are, the exact ways in which they remain separate can be difficult to discern. Plumage coloration is already known to be one trait that reliably distinguishes and helps diverge the two (Billerman 2016) as it is known to do in many other birds undergoing speciation (Semenov et al. 2017, Harris et al. 2018, Delahaie et al. 2017). This study aims to determine which nonsexual, ecological morphological traits are promoting speciation in this system.

## **Methods**

### **Sampling**

The specimens used in this study were taken from the University of Wyoming Museum of Vertebrates. The specimens were taken from allopatric populations of both parental species as well as from the hybrid zone. Exact sampling methods for these specimens, as well as methods of determining their hybrid index scores, are explored in detail in (Billerman 2016). See Figure 1 for a depiction of sampling locations.

### **Measuring**

Measurements for the length of the wing chords, length of the tarsi, and length, width, and depth of the bill were taken for each specimen (66 males, 63 females, 2 unknown) using a pair of electronic calipers. Measurements were taken in millimeters to a precision of one hundredth of a unit. All bill measurements were standardized by the nares with the length of the bill defined as the distance from the proximal end of the nares to the tip of the bill, the width of the bill defined as the width at the proximal end of the nares, and the depth of the bill defined as

the depth at the proximal end of the nares. The length of the wing chord was defined as the length from the distal end of the radius to the tip of the last primary. The length of the tarsus was defined as the length from the hypotarsus to the second scale before the digits. In the case of the lengths of the wing chords and tarsi, the two measurements per specimen were averaged under as a way to support their accuracy. Mass was in grams and was copied from what was recorded when specimens were originally sampled, and hybrid index scores were calculated for each individual from genotyping by sequencing GBS data.

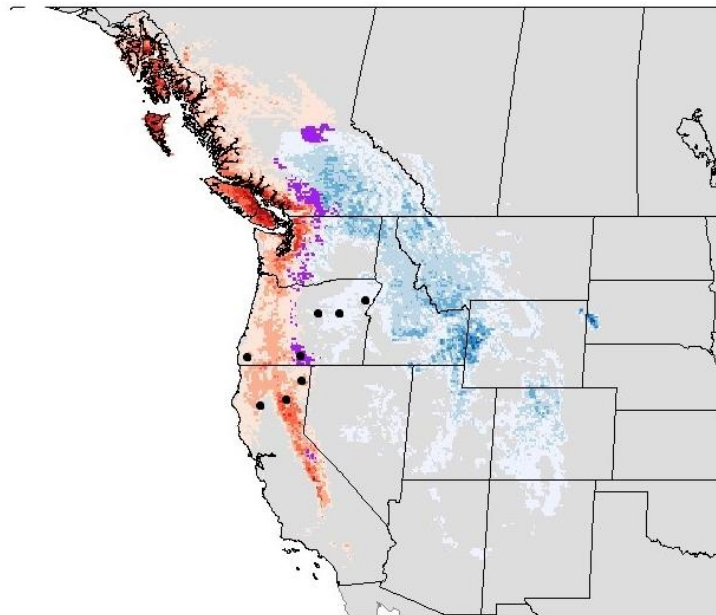


Figure 1: An abundance map generated using the ebirdst package showing the predicted abundances of *S. ruber* in red and *S. nuchalis* in blue shaded by the predicted relative abundance of each species, with the hybrid zone defined as the area where both species are predicted to occur and depicted in purple during a week in the breeding season of 2019. Each dot marks one of the locations at which sampling took place.

## **Analysis**

All analyses of the data were performed in R version 4.1.2. First, a t-test with an alpha level of 0.05 was used for all measured traits as they appeared in allopatric populations divided by sex (n=66 for males, n=63 for females). For the allopatric populations, any specimen with a hybrid index score of 0.2 or less was designated a Red-naped sapsucker and any with a score of 0.8 or more was designated a Red-breasted. This was done in order to determine if there were reasonable differences between *S. ruber* and *S. nuchalis* in terms of the measured traits.

Following this, linear regression models were made using the entire datasets for each trait divided by sex in order to visualize the general trends as well as how random the data was. Once the traits found to be significantly different between the parentals were determined, phenotypic clines were made of each using the R package hzar, which develops hierarchical Bayesian models to predict cline shape for both morphological traits and genetic data. For these clines, instead of using the physical distance across the sampling transect, I used the hybrid index score for each site as a proxy for the geographical range since the sampling transect moved through the hybrid zone between allopatric populations, and as a way to more definitively correlate the gradients of the traits with genetic information.

## **Results**

### **Bill Measurements**

All the data regarding bill measurements, regardless of sex, showed no significance in the initial t-test. For bill length in males the p-value was 0.493, in females it was 0.209. For bill width in males the p-value was 0.525, in females it was 0.073. For bill depth in males the p-value

was 0.9952, and in females it was 0.2844. There were also no visible trends showing differences in trait values across the datasets in the linear regressions (see Figure 2).

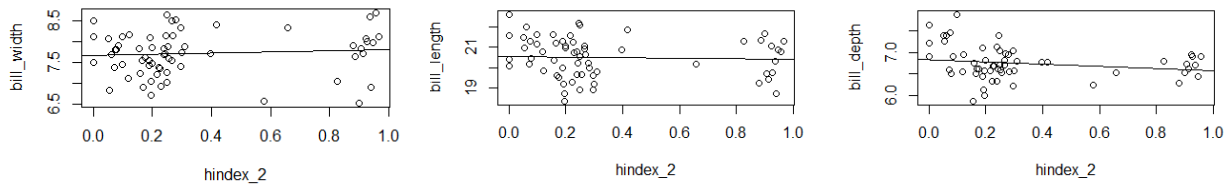


Figure 2: The linear regressions for bill depth (left), bill width (middle), and bill length (right) for males.

### Other Measurements in Females

Of the other traits measured, the only one to show a significant difference in the original t-test was the length of the tarsus which had a p-value of 0.005. The length of the wing chord had a p-value of 0.394 and mass had a p-value of 0.810. Despite tarsus length for females showing significance, as well as producing a linear regression model with an obvious trend, the cline for this trait did not show a gradient over the range, as was also true for the clines for the length of the wing chord and mass (see Figure 3).

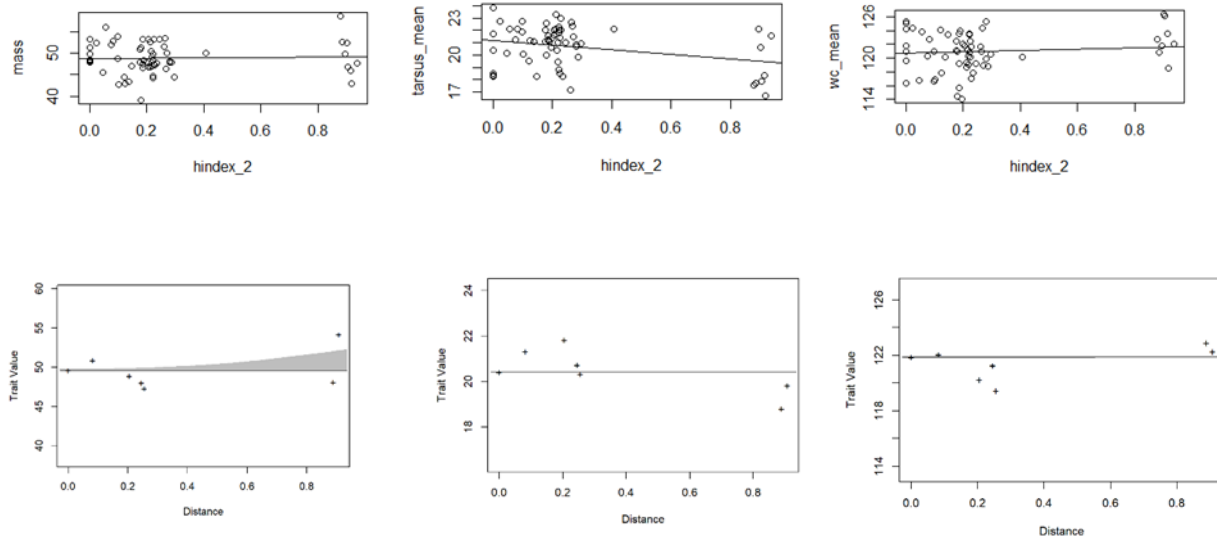


Figure 3: Top row: linear regressions for mass, the length of the tarsus, and the length of the wing chord in females. Bottom row: clines for the same traits in the same order.

### Other Measurements in Males

The length of the tarsus, as well as being the only significant trait measured in the females, had a pronounced difference between the males of both subspecies. The p-value for this trait was  $9.052 \times 10^{-9}$ , the most significant of all the measured traits. The linear regression showed the higher values belonging to *S. ruber*, the line starting at 22 mm and moving down towards *S. nuchalis* where the line ends near 19 mm. The cline produced from this trait did not have a curve, however, and strongly resembles the regression line (see Figure 4).

Aside from the bill measurements, the length of the wing chord in males showed the least differentiation between the two subspecies. The t-test resulted in a significant p-value of  $1.126 \times 10^{-4}$ . The linear regression showed this trait following a trend opposite to that of the tarsus length, with *S. ruber* tending to have a shorter wing chord than *S. nuchalis*, with the line moving



up from approximately 120 mm to 124 mm. This is also reflected in the cline, the curve of which happens between a hybrid index of 0.2 and 0.3 (see Figure 4).

The mass in males had a significant p-value of  $1.669 \times 10^{-3}$ . The trend seen in this trait moved in the same direction as that shown by the length of the tarsus, with the linear regression line starting near 50 g on the side of *S. ruber* and moving down to 45 g on the side of *S. nuchalis*. The cline produced for this trait had the steepest curve which exists around a hybrid index of 0.2. The cline for this trait also differed more from the regression line compared to the previous two traits, with the cline beginning near 55 g instead of remaining within the same values as the linear regression (see Figure 4).

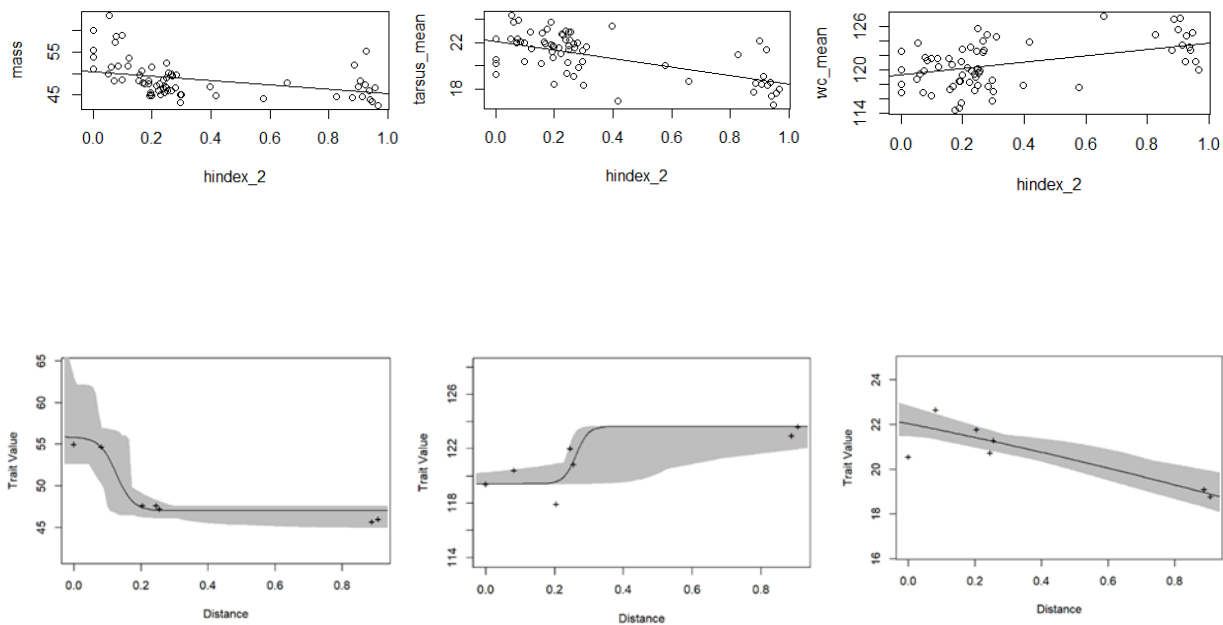


Figure 4: Top row: linear regressions for mass, the length of the tarsus, and the length of the wing chord in males. Bottom row: clines for the same traits in the same order.

## Differences between Males and Females

Once I obtained the results of the analysis of the female traits, I decided not to investigate the differences between males and females within each species further. The almost ubiquitous insignificance in differences between the females, as well as the same trends being exhibited in the measured traits in both males and females when the female data produced trends, indicated that any intraspecies differences, assumed to be smaller than interspecies differences, would not provide any significant data. It can be noted, however, that while the female data showed the same trends as the male data, there was a small exception when it came to mass which, in the cline at least, runs counter to the male data and rises slightly with *S. nuchalis* instead of the expected *S. ruber*.

## **Discussion**

### **Traits Not Contributing to Reproductive Isolation**

The length of the tarsus in males being as gradual a change across the cline as it was shows that despite there being a strong difference between the forms this trait takes in the parental populations, it likely is not contributing to differentiation between the two. The linear shape of the cline indicates that this trait exists as a gradient, so there is no clear separation between the subspecies in terms of this trait. This is also true for all the traits that failed to return even a significant difference between the subspecies, which are bill dimensions in both sexes and mass and the length of the wing chord in females, as well as for the length of the tarsus in females, which, despite a significant p-value, produced a completely flat cline showing no change in the trait across the hybrid zone. That more morphology did not differ between the species was slightly surprising, considering the differences in habitat between them is such that the hybrid zone is able to occupy its own niche (Billerman 2016), and natural selection is surely

acting on each independently in relation to those habitats. The lack of significant differentiation in females when compared to males could be a result of sexual selection acting in greater amounts on the males, allowing females to remain in a state more similar to that of the most recent common ancestor of both subspecies. This aligns with previous knowledge that many of the initial differences to appear in newly diverging systems are as a result of divergent sexual selection affecting the males. Since the sample sizes for males and females were almost equal, the idea that there are not enough females in the study to show potential differences can be reasonably disregarded. The lack of differences found could have been due a lack of sampling locations across more of the hybrid zone, though.

### **Traits Contributing to Reproductive Isolation**

The other two significant traits, mass and the length of the wing chord in males, did display a strong correlation with ancestry, depending on hybrid index, with individuals with a hybrid index greater than 0.2 being likely to take on the same trait values as *S. nuchalis*. It is obvious from the continued existence of hybrids in this system that the differences in these traits are not acting as a complete reproductive barrier, but they are still contributing to reproductive isolation nonetheless. Such a strong distinction based on hybrid index indicates that, over time, even as hybridization between the species continues to occur, the correlated traits will cease to exist in a gradient, but will only be found in the forms exhibited by either species. The trends found in this study show the majority of hybrids in this system appearing like *S. nuchalis*, even if they are genotypically more similar to *ruber*. It could be that the manifestations of the traits belonging to *S. ruber* are easily masked by those belonging to *S. nuchalis*, or that the collection being skewed somewhat in the direction of *ruber* is affecting the placement of the curves on the clines. Mass was the strongest contributor to reproductive isolation. The cline it produced had a

steep slope at a hybrid index of 0.2, marking that as a distinct point at which its manifestation changes from that of one subspecies to the next. This was also true for the length of the wing chord, though the softer slope on that cline indicates a less distinct point of divergence. Both these traits changing at the same place may indicate that they are inversely correlated with each other.

### **Next Steps**

Following this study could be investigation into more traits that are generally thought to be uninvolved in sexual selection. Several studies have already explored the role of sexual signals like plumage in driving speciation in this and similar systems (Harris et al. 2018, Grossen et al. 2017, Delahaie et al. 2017), given that those traits are the most likely to be affected by assortative mate choice in the early stages of speciation (Barton & Hewitt 1989). Alternatively, traits that are thought to be uninvolved in sexual selection could be investigated to see if that is truly the case, and if they are not, why they behave like those that are. Further investigation could also be given to the phenomenon exhibited here in which the majority of hybrids appeared like *S. nuchalis* despite the majority of specimens being more closely related to *S. ruber*, and what that indicates about the fitness of hybrids in the system.

### **Conclusion**

This study investigated the role of select morphologies in maintaining reproductive isolation in a system made up of newly diverged species that share a hybrid zone. The traits investigated are not traditionally associated with mate choice, and so provided a way to investigate less obvious mechanisms of early speciation. The results showed that only mass and the length of the wing chord in males have a role in maintaining reproductive isolation across

this system. The distributions of the values of these traits were not only different between members of both species, but failed to produce an even gradient across their respective clines. This is evidence of mate choice creating reproductive isolation through selecting against intermediate trait values in otherwise hybrid individuals.

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