Low Morphological Diversity in *Sturnus vulgaris* Across the United States

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Abstract:

European starlings (*Sturnus vulgaris*) were introduced to North America in the mid-19th century with a few dozen individuals in New York City's Central Park. Today, millions of starlings currently populate North America and range from the U.S.-Mexico border to Alaska, and every state in between. To test the impact of the Founder Effect in North American starling populations, the morphology of birds from the east and west coasts of the United States were compared. Mitochondrial DNA and morphological measurements were collected from each bird in this study. This study is the first to examine morphology and mtDNA data together from *Sturnus vulgaris* in these regions. No statistically significant differences were found between the morphological measurements from the East Coast birds to the West Coast birds. This conclusion is consistent with the original lack of diversity in the introduced population and the short period of time starlings have existed and been able to evolve morphologically in the United States.

Introduction:

European starlings (*Sturnus vulgaris*) were introduced to North America in the mid-19th century. They are endemic to Eurasia and North Africa, and have also been introduced to South Africa, Australia and New Zealand. These birds have been the subject of extensive ecological, physiological, and behavioral research (O'Hagan *et al*., 2015). Specifically, European starlings have been used to investigate the Founder effect, which describes the loss of genetic diversity as few individuals are taken from an original population and introduced to a new environment in North America (Cabe 1998) and Australia (Rollins *et al*., 2011). Mitochondrial DNA has shown to be an effective marker in analyzing the history of genetic diversity in starlings (Rollins *et al*., 2011).

Despite their short history in the US, European starlings have had a dramatic impact in North America by corrupting agricultural crops, intimidating native bird species, and flying into airplane engines. They have been highly adaptable to the varied North American environments they have colonized. This comes as a huge consequence to the indigenous species (Feare 1984). The root of the problem started with the American Acclimatization Society: a group founded in New York City in 1871 dedicated to introducing European flora and fauna into North America for both economic and cultural reasons. Eugene Schieffelin, a member of the society, released approximately 100 starlings in New York City's Central Park in 1890 and 1891 (Feare 1984). About the same date, the Portland Song Bird Club released 35 pairs of common starlings in Portland, Oregon. These birds became established but disappeared around 1902 (Feare 1984). Common starlings reappeared in the Pacific Northwest in the mid-1940s and these birds were probably descendants of the 1890 Central Park introduction. Expanding their range from New York rapidly, starlings reached the west coast of North America by the early 1950s, Alaska by the 1970s, and is now widely distributed throughout the continent.

A 2014 study examined the wing morphology of European starlings and how this allows them to colonize novel habitats (P-P. Bitton and B. A. Graham 2014). They hypothesized that the individuals leading the expansion wave had longer and pointier wings because these characteristics are associated with greater juvenile dispersal distances. Therefore, they predicted that successive generations of range expanding birds would display increasing wing length and pointedness. Contrary to their expectations, wing length and shape did not change during the colonization wave. However, the pointedness of European starling wings decreased across the continent by more than 3.8% over the last 120 years. This was caused by an increase in the length of the secondary feathers, not by a decrease in the length of primary feathers. So far, this is the only work that has done a comprehensive analysis of starling morphology across the US.

The objective of our research project specifically was to use morphological & genetic data to track the impact of the Founder effect on *Sturnus vulgaris* in the United States. Much of the data analyzed in this project was morphological, which is noteworthy since many explorations of the Founder effect focus solely on genetic data. The overall goal of this project was to examine patterns of diversity across the United States, to determine how this unique population history has impacted today's starlings. Illuminating the history of one of the world's worst invasive species will increase understanding of how a seemingly homogenous species can colonize such broad expanses of land, and adapt to highly varied environments. This work has implications for both understanding a fundamental evolutionary pattern, in addition to addressing ecological patterns and conservation concerns.

Materials and Methods:

Forty-eight birds were collected overall from various locations: Westchester Airport in Westchester, NY (1), LAX in Los Angeles County, CA (11), JFK Airport in Queens County, NY (7), and Albany Airport in Albany, NY (12).

The juvenile specimen from Westchester Airport was caught by Derek Colbert on September 12, 2014 in a V-top trap and euthanized via cervical dislocation. The 11 adult specimens from Los Angeles Airport were caught by Shannon Chandler on November 11, 2014 in a fly-in trap. The 7 specimens from JFK Airport were caught by Melissa Malloy on October 31, 2014 (2), and May 29, 2015 (5). They were caught via V-top traps, and euthanized via cervical dislocation. The 12 adult specimens from Albany Airport were caught by Allen Gosser on January 20, 2015 in a V-top trap and euthanized by cervical dislocation.

Morphological Methods:

Between two researchers, each fresh bird specimen was measured three times using Marathon digital calipers (sizes 8" and 12"), for a total of six measurements for each region, per bird. The anatomical regions measured included: beak length, beak depth, head length, wing length, tail length, tarsus length, and body length (Figure 1). For each region, 6 repeated measurements were averaged for the most accurate results possible.

Afterwards, the bird specimens were skinned by method of taxidermy in order to preserve the morphology of the bird. The taxidermy process began by slitting the bird down the sternal keel to reveal the organs (Figure 2); the sex of the bird was identified by looking at the

reproductive organs. Sawdust was used to absorb any excess blood (Figure 3). Next, the legs and wings were pulled up and through the skin, being careful not to puncture the skin. The skin was kept (Figure 4), and the organs were set aside. Next, the head was inverted so that the skin was flipped inside out. The brain was removed, along with the eyes and tongue. The anterior of the skull was truncated. Then the skin was flipped back to its original state; cotton was stuffed into the empty bird to fill the eye cavities and restore its original shape. The legs were tied, and the bird was wrapped in cotton to rest a few days until it was sufficiently dry and ready for research purposes (Figure 5).

The skinned birds were then measured with the same procedures described above, for the purpose of comparing pre-skinned and post-skinned measurements for each bird. The measurements from the skinned birds are then able to then be compared to other museum skins in ornithology collections.

FIGURE 1:

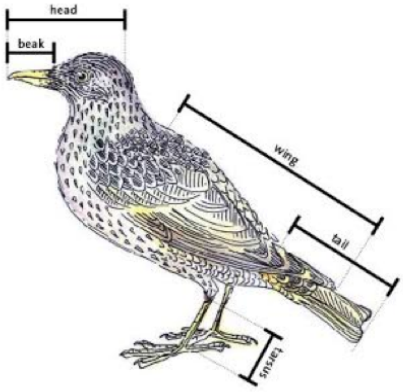


FIGURE 2:

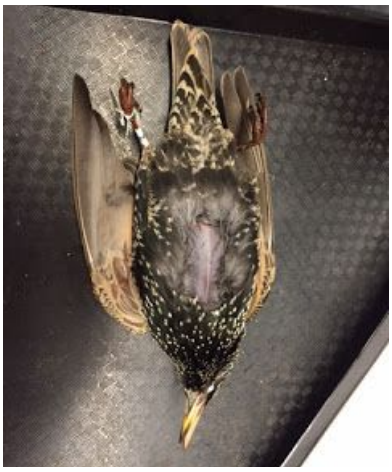


FIGURE 3:

FIGURE 4:

FIGURE 5:

Genetic Methods:

An ankle tag was created for each specimen, and a piece of the toe pad from each bird was cut off using scissors and scalpels. Each respective toe pad was placed into a collection tube, to be used for DNA extraction. Specimens were placed into bags and stored in the -20º freezer for later use.

The Qiagen DNeasy Blood & Tissue Kit was used for the DNA extraction process. Samples were first lysed using proteinase K. Next they were vortexed, 200µL of Buffer AL was added, and samples were placed into a water bath. The samples were then vortexed again, and 200µL of Ethanol was added. Samples were vortexed again, and then transferred into a DNeasy mini spin column and centrifuged. Next, the spin column was placed in a new collection tube with 500µL of Buffer AW1. This process was repeated with Buffer AW2. Samples were centrifuged again, and transferred to a new micro-centrifuge tube. 200µL of Buffer AE was added and the solution was incubated at room temperature. The next step was to isolate the mtDNA loci by performing a PCR.

Mitochondrial DNA was chosen to be examined because of its benefits in examining invasion history, and because it allows these sequences to be compared to previous mitochondrial analyses of starlings from other regions. Additionally, the likelihood of recovering mtDNA in small or degraded biological samples is greater than for nuclear DNA because mtDNA molecules are present in hundreds to thousands of copies per cell compared to the nuclear complement of two copies per cell. This is useful because some of the samples to be used in this project are museum specimens which degrade over time.

Certain regions of the mtDNA are more variable than others. The ND2 region and CO1 region are known to be particularly sensitive to genetic variation. These regions have also been sequenced in starling populations in the United Kingdom, Australia, New Zealand, and South Africa, which allows us to compare the sequences and relative levels of genetic diversity in each region (Rollins, 2011).

The primer sequences for the ND2 region are:
H6313, 5′CTCTTATTTAAGGCTTTGAAGGC-3′
L5216, 5′GCCCATACCCCRAMAATG-3′

The primer sequences for the CO1 region are:
vF1_t1, 5'-TCTCAACCAACCACAAAGACATTGG-3'
vR1d_t1, 5'-TAGACTTCTGGGTGGCCRAARAAYCA-3'.

We ran our extracted DNA samples through a polymerase chain reaction (PCR), a process used to isolate and amplify specific regions of the DNA. The total PCR volume for each sample was 25µL, this included 13.5µL of a mixture of distilled water, 5µL of each primer, and 1.5µL of DNA extraction sample into each of the collection tubes with the GE illustra PuReTaq Ready-To-Go PCR Bead.

Lastly, the PCR product was run through a gel electrophoresis. For each gel, 0.7 g of Agarose, 4mL of Sybr Safe, and 40mL of TAE (Buffer) were measured into an Erlenmeyer flask and heated in several short intervals until the solution became clear. Next, one 8-well comb and two gates were placed into each gel casting tray, and the solution was poured in. When the gel was solid, the comb was pulled out, TAE buffer was poured over the gel, and a pipette was used to insert a 7µL solution (5µL PCR and 2µL dye) into each well except for the last one

(reserved for the ladder). Next, the lid was placed on, and the electrode wires were connected to the power source. The power was turned to 100 volts, and was let run until blue dye approached the end of the gel. The final gel was examined using a UV light box; the samples which showed promising bands were sent to Genewiz for sequencing.

Morphological Analysis:

T-tests were conducted between the following groups: east coast/west coast, male/female, and pre-skinned/post-skinned (for each county).

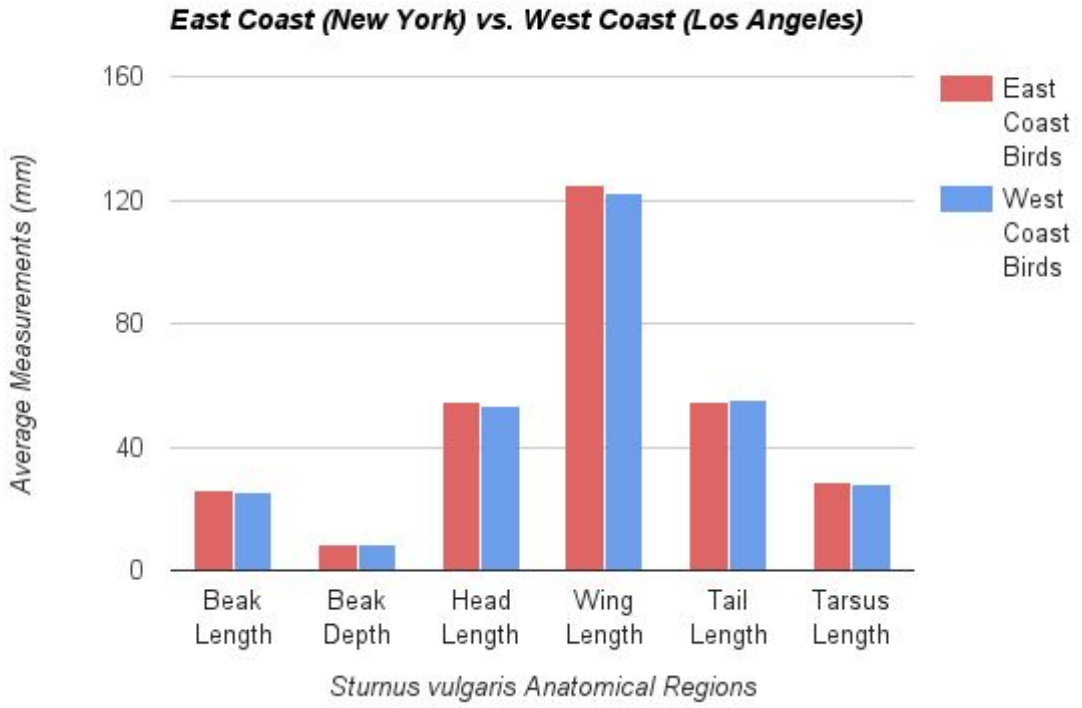
Results and Conclusions:

There was no statistically significant difference between the pre-skin and post-skin measurements of the birds, with the exception of the tail (Table 1). However, this may be due to other factors besides the actual morphology of the bird, since the tail measurements were subject to ambiguity. These results demonstrate that the skinning process does not notably distort the original morphology of the bird.

In conclusion, there were no statistically significant differences between any of the groups of birds we analyzed in our study: East Coast and West Coast birds (Figure 6), Male and Female birds (Figure 7), and the different counties - Westchester, Los Angeles, Albany, and Queens (Figure 8). These results are consistent with the lack of morphological diversity in this species. These findings should be expected since all current starling populations in the United States originated from a single introduction of some 60 individuals in 1890. Additionally, we found that West Coast birds had less standard deviation than East Coast birds, which could potentially mean that they are less variable; this would match predictions because, as a population grows and spreads across a land mass, it would make sense for the newest generations to be the least variable.

It is possible, however, that larger sample sizes could capture morphological variation that was not found in this study. In an upcoming analysis, genetic data will be looked at, which may uncover more diversity than is seen in the morphology.

FIGURE 6:



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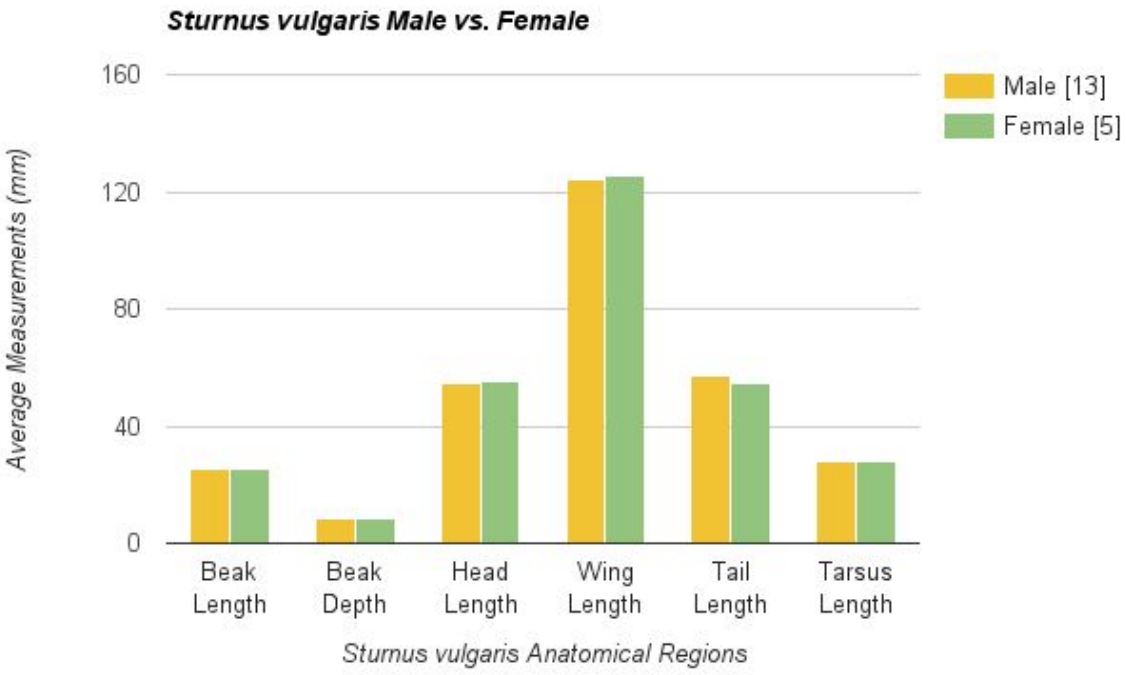

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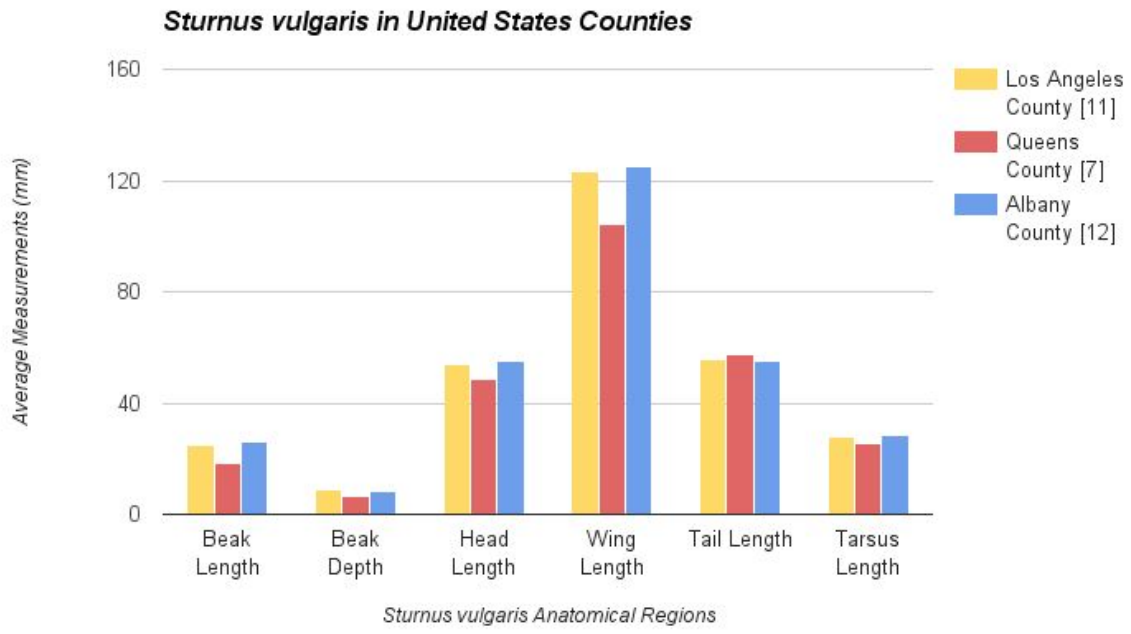

TABLE 1:

	East Coast [13]	West Coast [11]	P Value
Beak Length	26.1	25.1	0.89
Beak Depth	8.5	8.6	0.33
Head Length	54.9	53.5	0.88
Wing Length	125.1	122.6	0.98
Tail Length	54.7	55.4	0.36
Tarsus Length	28.5	27.8	0.85

	Male [13]	Female [5]	P Value
Beak Length	25.5	25.2	No Difference
Beak Depth	8.4	8.4	No Difference
Head Length	54.5	55.1	0.34
Wing Length	124.2	125.3	0.18
Tail Length	57	54.4	0.94
Tarsus Length	27.9	27.7	0.66

Comparisons between Pre-Skin and Post-Skin

	Los Angeles County [11]	P Value	Queens County [7]	P Value	Albany County [12]	P Value
Beak Length	25.1	No Difference	18.2	0.79	26.1	0.89
Beak Depth	8.7	0.82	6.6	0.83	8.5	1
Head Length	54	0.23	48.7	0.79	55	0.1417
Wing Length	123	0.22	104.3	0.58	125.2	0.14
Tail Length	55.7	0	57.4	0.02	55	0
Tarsus Length	27.9	1	25.4	0.93	28.4	1

Discussion:

In conclusion, these results are notable on multiple levels. First of all, it is important that there was no significant difference between pre-skinned and post-skinned measurements because this means that both types of specimens are suitable for research purposes, and that the taxidermy process does not distort the bird.

It is also important to note that there was a wider standard deviation range in the east coast compared to the west coast. This could be due to multiple reasons. Since the number of samples in the west coast was smaller than the number of samples in the east coast, it is possible that this was the source of a wider standard deviation variation in the east coast. However, it is also possible that the discrepancy exists because there is more morphological diversity in the east coast than on the west coast. Future studies can investigate this by

increasing the sample size as well as having more geographic diversity in the studied specimens so the samples are more representative of the places in which starlings live in the United States. Furthermore, future studies may ultimately compare samples from the United States to those from the United Kingdom in order to assess the true magnitude of the Founder effect.

This study holds value not only in understanding the invasive nature of starlings, but of all invasive species as well. One of the most puzzling aspects of *Sturnus vulgaris* is its ability to survive and adapt to almost any environment, but still retain such a low morphological (and likely genetic) diversity as well. Although organisms cannot plan for environmental change, it is widely understood that the more variation that exists in a population, the better prepared that population will be to adapt to change when it does occur; low diversity is often associated with reduced fitness. Thus, it seems that starlings are an outlier in this case, because despite being quite homogenous, they've been able to thrive in almost every part of the world,

Additionally, we know that the initial introduction in 1890 was around 60 birds; however, out of this small group, it is likely that not all of the birds survived to contribute to the next generation - so the initial population must've been even smaller than we think. This also asks the question - *why* and *how* were starlings so successful in such unfavorable conditions?

Starlings have had a huge impact on the United States in the short amount of time they have lived here - they are responsible for transferring diseases, consuming cultivated crops, and creating aircraft safety hazards. In turn, we end up paying for their damage - whether in the form of money being lost from crop loss, or the government paying specialists to kill starlings at airports. Thus, since starlings are affecting the US on an economic level, it only makes sense to try and better understand their invasive nature and reasons for success, in order to prohibit further damage. By understanding the biology of *Sturnus vulgaris*, we can solve this issue in a more effective way than just killing them individually.

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