

The Effects of Insularity on Beak Morphology in a Hybrid and a Non-Hybrid Species

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Master of Science Thesis



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Abstract

Despite an increased recognition that hybridization is an important evolutionary force, we are only starting to investigate the potential for adaptation in hybrid species. Historically, hybridization has been viewed as maladaptive, but transgression or increased genetic or phenotypic variation could contribute to hybrid species' potential for local adaptation. Investigating phenotypic variation in response to different environmental factors within a hybrid species is necessary to address whether hybrid species can use hybridization-derived variation to adapt locally. Previous studies comparing island and mainland populations of both Italian and Spanish sparrows have indicated that insularity is an important factor affecting the size and shape of the beak in both parallel and non-parallel ways. Here I investigate the potential to adapt to insularity in the homoploid hybrid Italian sparrow (*Passer italiae*) and one of its parent species, the Spanish sparrow (*Passer hispaniolensis*). I focus on differences in beak morphology between the two species and also between insular and mainland individuals. I found significant differences in beak size between insular and mainland individuals, with island individuals having smaller beaks than their mainland conspecifics. For beak shape, I found differences between Italian and Spanish sparrow, as well as between island and mainland individuals. I also present an alternative method for investigating beak and head dimensions, namely 3D scanning. I argue that this is a valuable method for future research as it captures more of the skull anatomy, which can reveal important connections between beak and other anatomical structures.

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1. Introduction

Speciation is traditionally thought of as a bifurcating process, where the two species originate from one common ancestor, but have diverged by natural selection and genetic drift (Coyne & Orr, 2004). Historically, a role for interspecific hybridization in generating biological diversity was thought unlikely in animals (Mayr, 1963), although it was considered to be of importance in generating plant diversity (Dowling & Secor, 1997; Stebbins, 1959). However, a role for hybridization in generating new animal diversity has been increasingly recognized and its consequences for adaptation and evolution is a rapidly developing topic of research (Abbott et al., 2013; Sætre, 2013). Hybridization is common in nature with 10% of animals and 25% of plants known to hybridize with at least one other species (Mallet, 2005). Hybridization can act as a creative force through enhancement of genetic variation, which can facilitate evolutionary change in a new direction (Grant & Grant, 1994). Further, hybrids possess larger additive genetic variation relative to their parent species, and hybrid genomes can sometimes produce extreme, transgressive phenotypes that go beyond the extent of both its parent species, as in the case of *Helianthus* sunflowers (Rieseberg et al., 2003). As a result, hybrids can sometimes occupy ecological niches or adaptive peaks that are inaccessible for its parent species (Mallet, 2007). How the potential for adaptive divergence within a hybrid species compares to that of its parents is, however, not well understood.

1.1 Hybrid speciation

One outcome of hybridization is hybrid speciation, where a species has originated through hybridization. A hybrid population is most likely to originate following secondary contact in new available habitats. Moreover, they might be most likely to evolve into a hybrid species when there is ecological space available that is not utilized by either parental species (Abbott et al., 2013). There are two main types of hybrid speciation. Polyploid hybrid speciation occurs when the resulting hybrid has a different number of chromosome sets relative to its parents, and is common in plants (Mallet 2007). Conversely, homoploid hybrid speciation produces a viable, fertile and reproductively isolated hybrid without a change in chromosome number (Gross & Rieseberg, 2005; Mallet, 2007). The homoploid hybrid Italian sparrow (*Passer italiae*) originated from hybridization between the Spanish sparrow (*Passer hispaniolensis*) and the house sparrow (*Passer domesticus*) (Elgvin et al., 2011; Hermansen et al., 2011; Trier et al., 2014).

For a hybrid species to be successfully established, reproductive isolation against the parent species must develop (Schumer et al., 2014). Polyploidy creates a strong, postzygotic, reproductive barrier between the hybrid and both parental species (Abbott et al., 2013). On the contrary, reproductive barriers are difficult to achieve for a homoploid hybrid species, since reproductive isolation must develop for hybrids to maintain genetic integrity in face of sympatry with a parent species. Different subsets of the incompatibilities which maintain the parent species separate can, however, form barriers against each of the parent species (Hermansen et al., 2014). In the Italian sparrow, mito-nuclear and sex-linked incompatibilities play a crucial role in forming reproductive barriers against both parents (Trier et al., 2014).

1.2 The effect of hybridization on potential for adaptation

The genome of an individual contains the potential to evolve novel functions. This potential is subject to a variety of selective constraints (Hall, 1999). Hybridization can enhance the genetic variance, which can affect the potential for adaptation. As closely related species tend to hybridize more frequently, hybridization is typically an especially important driver of speciation for rapidly radiating taxa (Abbott et al., 2013; Seehausen, 2004). There is likely to be a positive feedback between hybridization and speciation, as hybridization can increase the rate of speciation, and the resulting diversity of closely related species could then provide further opportunities for hybridization events (Seehausen, 2004).

Introgression, the gene flow across a reproductive barrier by hybridization and backcrossing, may result in introduction of selectively favoured alleles from one population into another and can hence provide novel material for adaptation (Abbott et al., 2013). Also, hybridization and backcrossing can lead to a burst of variation called a hybrid swarm in which variation ranges widely between the characteristics of the two parental species (Stebbins, 1959), generating novel variation that may enhance adaptive potential.

Variation derived from hybridization is different from that of mutation. Evolution of novel adaptations through mutation and natural selection is likely to be slow, since mutations are rare for each genetic locus (Abbott et al., 2013) and are often deleterious or neutral (Kimura, 1983). Grant & Grant (1994) found that new additive genetic variance introduced from hybridization in Darwin's finches was two to three orders of magnitude greater than that introduced by mutation.

Hybridization can transfer large coadapted complexes at the same time (Abbott et al., 2013). For instance, mimicry pattern in *Heliconius* butterflies has been shown to require introgression of large adaptive regions across species boundaries (Heliconius Genome Consortium, 2012). Moreover, genes introduced by hybridization has already been tested by selection in one of the parents and are thus less likely to be deleterious than mutations (Rieseberg et al., 2003).

How the parental species diverge may impact hybrid evolution. Stabilizing selection in parents increases the ability to evolve in hybrids, and can also produce hybrid transgressive phenotypes as the parents diverge (Bailey et al., 2013; Rieseberg et al., 1999). Directional selection causes intermediate hybrid phenotypes that are bound to evolve towards the differences between the parental taxa (Bailey et al., 2013). Novel adaptation mediated through transgressive phenotypes is considered to be the most likely cause of homoploid hybrid speciation, although it is much less frequent in animals than in plants (Rieseberg et al., 1999). However, a large part of introgressed variation is expected to be deleterious, and thus many hybridization events will leave no long-term impacts on evolution. Yet, hybridization events between closely related species are more likely to result in viable combinations (Abbott et al., 2013).

The level of divergence between the two parental species influences the outcome of hybridization (Abbott et al., 2013; Stelkens & Seehausen, 2009). If there is less divergence one can expect less chance of novelties being produced in hybrids, whereas for more divergent taxa novel phenotypes are more likely to form, but there is also an increased risk of intrinsic incompatibilities (Abbott et al., 2013).

Italian sparrows have been shown to be significantly more evolvable than both house and Spanish sparrows in a variety of plumage traits, and they also show transgressive phenotypes (Bache-Mathiesen, 2015). For beak morphology, beak size has been found to be the most transgressive phenotype, as both island and mainland Italian sparrows have larger beaks than either parental species (Helén, 2016). To address whether hybrid species differ from their parent species in potential to adapt to insularity, I investigate beak morphology differences between island and mainland populations of the homoploid hybrid Italian sparrow and of one of its parent species, the Spanish sparrow.

1.3 Adaptations to insularity

Observations of island biotas were of great importance to Darwin when he formulated his Theory of Evolution in 1859. Islands can be regarded as natural experiments (Whittaker, 1998), and groups of islands serve as replicates making it easier to distinguish between evolutionary patterns and unique outcomes (Losos & Ricklefs, 2009).

The same processes are important on islands as on continents. However, these processes operate in smaller and less complicated ecosystems on islands (Eliasson, 1995; Vitousek & Benning, 1995). Wallace (1881) identified two additional attributes of islands that make them suitable for evolutionary studies. The first attribute was their relative youth compared to continents, where the peak of evolutionary diversification has passed (Losos & Ricklefs 2009). The second attribute that Wallace identified as important was geographical isolation. Islands are often situated distantly from other landmasses and some have been isolated for

large periods of time. This in turn, can lead to island species diverging along new evolutionary trajectories.

Insular species have been shown to differ in comparison to mainland conspecifics in a range of traits. Foster (1964) described the pattern that small-bodied species develop larger body sizes, and larger species smaller body sizes, on islands. This pattern was later coined “the Island rule” (Van Valen, 1973), and has been shown to be related to ecological differences between island and mainland environments in some species (Runemark et al., 2015). Other traits, including life-history traits (Adler & Levins, 1994) and coloration (Hayashi & Chiba, 2004; Runemark et al., 2014) have also been shown to change on islands.

Interestingly, island populations of the Italian sparrow differ in genomic composition (Runemark et al., 2017) as well as in phenotypic traits (Bache-Mathiesen, 2015; Helén, 2016; Piñeiro, 2015). Further, island populations are more phenotypically diverged from each other with respect to beak morphology than mainland populations, and even more so in traits that are important for local adaptation (Helén, 2016).

Here, we take advantage of the expected differences in ecology between island and mainland populations, and use island and mainland populations of both study species to investigate whether the within species divergence differs between the hybrid and its non-hybrid parent.

1.4 Beak morphology

The diversity in morphology of bird beaks reflects the ecological importance of this trait (P. R. Grant, 1986). Beak size and shape are under strong selection to optimize foraging and food manipulation, facilitating the occupation of a large variety of niches. A well-known example of this is the adaptive radiation of Darwin's finches on the Galápagos Islands. The large variety of beak shapes that Darwin observed has later been shown to be a result of adaptation to different diets and food-handling (Bowman, 1961; Lack, 1947; Schluter et al., 1985).

Bite force has also been shown to be positively correlated with beak depth and width (Herrel et al., 2005). Selection for a harder diet would act directly on a beak dimension correlated with bite force and hence seed-crushing ability. Beak shapes and sizes often evolve to be adapted to local diet to increase foraging efficiency. Furthermore, the strong ecological selection during adaptation to different habitats and niches, can lead to acoustic divergence in song (Derryberry et al., 2012). Thus beak morphology can be a target of sexual selection (Huber & Podos, 2006). Hence, beak size and shape can have tremendous impacts on an individual's ability to survive and reproduce. Furthermore, beak shape seems to be a highly adaptable trait, correlated with local diet and climate in the Italian sparrow (Piñeiro, 2015). This makes it an ideal trait for investigating the effects of insularity in a hybrid and non-hybrid species.

1.5 Parallelism as a hallmark of adaptation

Parallel evolution is defined as the independent evolution of the same trait in different lineages (Futuyma, 1986). A variety of studies have shown that similar environmental conditions can result in independent populations acquiring similar phenotypic traits in parallel (Eroukhmanoff et al., 2009; Losos et al., 1998; Schluter et al., 2004). Genetic drift can also result in phenotypic evolution, however such changes would not be predictable, and hence not result in parallel changes (Schluter et al., 2004). Thus, consistent and shared phenotypic features in similar environments are unlikely to arise by genetic drift, and instead suggest a role of natural selection. Hence, parallel changes in phenotypic traits are widely regarded as hallmarks of adaptive evolution (Pelosi et al., 2006).

Comparing island populations and reference mainland populations of two independent sparrow species (Italian and Spanish sparrows) will allow me to assess the extent of parallelism in beak morphology on islands, and hence investigate potential adaptation to insularity.

2. Material and methods

2.1 Study species

To investigate beak shape variation between species, biogeographical settings and populations, I estimated beak size and shape of 207 Italian sparrows from four island and three mainland populations, and 145 Spanish sparrows from two island and three mainland populations (See supplementary table 1 for a break-down to populations and individuals). These are excellent species to address the question whether the extent of divergence and adaptation to insularity differ between a hybrid and a non-hybrid species, as both species are found on several islands, which provide replica populations that allows for studying divergence, and whether this correlates with local ecology. This will allow me to investigate whether divergence, and the selective forces causing this divergence and their relationship with divergence, differs between the species.

The study species differ in ecology. The Spanish sparrow is typically found in regions without human habitations and is a gregarious species, breeding in large colonies as well as foraging in flocks outside the breeding season (Summers-Smith, 1988). The Italian sparrow is also gregarious, but unlike the Spanish sparrow it is a human commensal, often found in cities and agricultural areas. Ecologically it resembles the house sparrow. However, when the Spanish sparrow is found in regions without the presence of the house sparrow, it can sometimes spread into inhabited regions and thrive as a human commensal, nesting in holes and crevices of man-made structures (Summers-Smith, 1988). While Spanish sparrow diet mainly consists of a variety of seeds, supplemented by leaves of young plants, Italian sparrow feeds mainly on seeds and insects.

2.2 Fieldwork, photographing and image protocol

Members of the Oslo Sparrow Group caught Italian sparrows from the Mediterranean islands of Corsica, Crete, Sicily and Malta during the springs in 2013 and 2014, and three localities in mainland Italy: Crotona, Guglionesi and Rimini during spring 2015 (Fig. 1). Spanish sparrows were caught from three mainland localities during springtime: Lago Salso, Italy (2011), Badajoz, Spain (2016) and Chokpak, Kazakhstan (2014), as well as from the islands Tenerife (2016) and Sardinia (2013). This study setup contains only two Spanish island populations. The original plan was to include Malta as the third island. However, the sparrows on Malta turned out to be Italian and not Spanish sparrows according to genetic analysis (Runemark et al., 2017). This unfortunately resulted in an unbalanced study design.

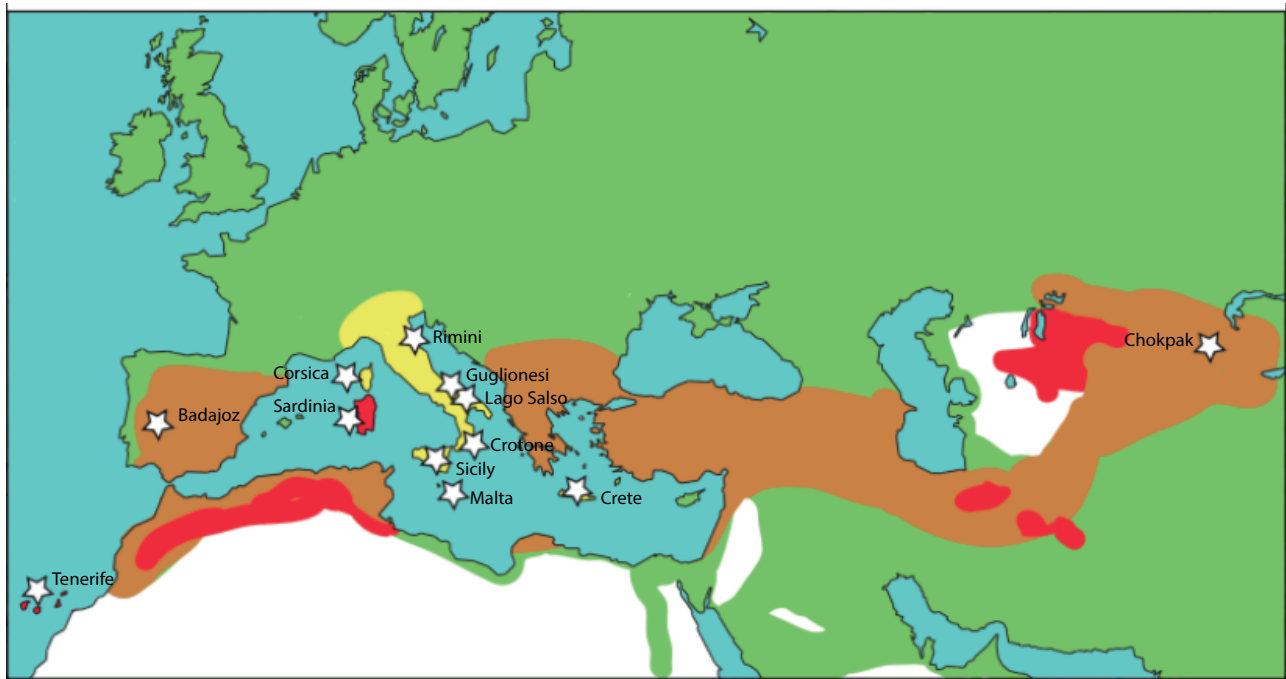


Figure 1: Distribution of species in the *Passer* hybrid complex. Areas where only Italian sparrows are found, are shown in yellow. Green symbolizes house sparrow, while areas in red are occupied by Spanish sparrows. Finally, areas where Spanish and house sparrows live in sympatry are shown in brown. Sampling locations are

indicated by star-symbols.

All birds were caught using mist nets and released immediately after data had been collected. In this way we minimized the amount of stress inflicted on the sparrows. The beak images used for digitalization were taken using a high resolution Nikon D500 with 16.2 megapixels. The birds were photographed in a standardized illumination environment, and a color checker (5.7 x 8.7 cm X-rite mini ColorChecker ®class) was placed next to them. The color checker also included a scale bar so that the scale of the landmark configuration could be computed. The background was covered in millimeter paper (the image setup is described in detail in Tesaker (2014)). The images were saved in RAW format (NEF-files), and later converted to JPG-files using the program ViewNX 2 (Nikon Corporation 2015). All the necessary permissions for sampling were obtained from respective national and local authorities.

2.3 Data acquisition: Geometric morphometrics

I used geometric morphometrics to get quantitative measures from images taken in the field. Studies using this approach are accomplished through the Procrustes paradigm (Adams et al., 2013). From each specimen, landmark coordinates are obtained. However, these raw coordinates do not only contain information about size, but also the location and orientation of the specimen. To overcome this issue and remove the less relevant non-shape variation, the Procrustes superimposition implements a series of operations (Adams et al., 2013; Rohlf & Slice, 1990). The geometric morphometric approach is able to discriminate substantially better between species than traditional methods and additional differences among species and small-

scale shape variation can also be detected more easily (D. J. Foster et al., 2008). Moreover, it allows for corrections for allometry (D. J. Foster et al., 2008).

I used the thin-plate-spline programs tpsUTIL (Rohlf, 2010b), tpsDIG2 (Rohlf, 2013) and tpsRELW (Rohlf, 2010a). TpsUTIL is a utility program that was used to convert pictures into tps files. TpsDIG2 was used for digitizing landmarks and capture outlines using semi-landmarks. TpsRELW was used to extract centroid sizes and relative warps from the landmark data.

Landmarks should be homologous anatomical loci that represent the same biological locations in every individual. Further, landmarks should provide adequate coverage of the morphology, be found repeatedly and reliably in every specimen and lie within the same plane (Zelditch et al., 2004). Following Moksnes (2014) and Helén (2016), I placed 5 homologous landmarks both on the upper and lower mandible, respectively, and then drew an outline of the beak with 7 equidistant semi-landmarks (See supplementary figure S1 and S2). The semi-landmarks are used to quantify morphology in areas where clear homologous landmarks are hard to define. To minimize noise in digitalization, I estimated the repeatability by digitizing the same set of beaks twice. I digitized the dataset included in the study when R-squared (R^2) for the training set was 0.898.

Lastly, tpsRELW was used to extract centroid sizes as well as relative warps from the landmark data. Centroid size is defined as the square root of the summed squared deviations of the coordinates from their centroid (Mitteroecker & Gunz, 2009). In other words, centroid size is based on the square root of the sum of all squared

distances from each landmark to the center of the beak. Relative warps (hereafter RWs) are commonly described as principal components of form and are used to represent shape (Zelditch et al., 2004). To correct the relative warps for allometric effects of size, I conducted a regression of the relative warps onto centroid size using Rstudio (RStudio Team, 2016). All further analyses were carried out in Rstudio unless otherwise stated.

2.4 Statistical analyses of 2D data

To be able to get a quick graphical overview of my dataset, and assess general trends in the data, I started my analyses by creating boxplots using the *ggplot2-package* (Wickham, 2009). Boxplots were made for both beak size grouped by species and insularity, as well as the three first RWs chosen based on the broken stick criteria (see supplementary figure S5). These three RWs are used in all further shape-analyses unless otherwise stated.

To investigate what factors affect beak size, as well as different components of shape in Spanish and Italian sparrows, I conducted a series of linear mixed models. This was done using the *lmer* function in the R-package *lme4* (Bates et al., 2015).

Centroid sizes, as well as the three first relative warps, were treated as response variables in four different models. The explanatory terms were insularity and species, and population was included as a random effect, nested within insularity. I used a backwards elimination approach to identify the best model. However, insularity could not be removed from the model as population was nested within as a random factor. The backwards elimination approach is conducted by removing interactions

with p-values above 0.2, starting with the interaction having the highest p-value. This is repeated until only interactions and factors with $p < 0.2$ remain in the final model.

To address the question of whether there is a significant difference between my groups (i.e. 1. mainland Spanish sparrows, 2. island Spanish sparrows, 3. mainland Italian sparrows and 4. island Italian sparrows) and what factors were of importance in describing these differences, I did a series of ANOVA tests. To test whether my data met the assumption of homogeneity of variances, I used the Levene test found in the *car*-package (Fox & Weisberg, 2011). After running the analyses I decided upon the best model using the Akaike Information Criterion (Akaike, 1974). The Akaike Information Criterion (AIC) is a relative measure of how good a model is among a candidate set of models given the data (Symonds & Moussalli, 2011), where the model with the lowest AIC score is preferred. AIC is often used when selecting models from analyses that explore a range of variables that may be associated with a particular trait, and thus are worthy for further investigation (Symonds & Moussalli, 2011). Since I used ANOVA to identify important predictors of differences between my groups, AIC was a suitable method for model selection. However, when there are relatively few data per estimated parameter, AICc (second-order Akaike's Information Criterion) is recommended (Burnham & Anderson, 2002). Models with a ΔAIC (difference in AICc value between two models) by less than two from the best model, is not considered as statistically worse in explaining variation. Thus, by looking at ΔAIC values, we can compare other models to the best one.

To investigate what variables significantly affect beak shape when the three most important components of shape are considered together, I decided to run a multivariate analysis of variance (MANOVA). While the ANOVA investigates differences in means between two or more groups, MANOVA tests for the difference in two or more vectors of means (French et al., 2002). I thus decided to run a MANOVA, as all three RWs are components of shape, and we might hypothesize that they could all be affected by changes in external factors. Model selection for the dependent variables RW1, RW2 and RW3 was conducted using the backwards elimination approach. The Pillai's Trace as well as p-values were used to assess the significance of each parameter in the selected model. The Pillai test is often considered to be the most robust and powerful test statistic, and it also results in the most conservative F-statistic (French et al., 2002). The Pillai's trace is a positive valued statistic that ranges from 0 to 1, where an increasing value of a variable corresponds to a stronger effect of the variable on multivariate variation. Further, parameter estimates were also extracted to assess to what extent each parameter in the model contributed to the responses in each separate response variable.

To investigate how changes in the major dimensions of divergence reflect changes in the beak, I ran a relative warp analysis (Rohlf, 1993) in MorphoJ (Klingenberg, 2011), which is essentially the same as a principal component analysis (PCA) on the procrustes coordinates. If we consider our multidimensional dataset projected onto several orthogonal axes, the first relative warp is the axis that lies in the direction of maximum variation. RW2 will be explaining the highest variance possible under the constraint that it is orthogonal relative to RW1, and so on for subsequent axes. By using this procedure, most of the variance in the dataset can be described using only a few RWs. I made a summary plot from the relative warp analysis, showing the

variances associated with the RWs. By looking at this plot, I decided to retain the three first RWs for further analysis, based on the broken stick criteria (See supplementary figure S5).

Whereas a relative warp analysis is useful to display the major features of shape variation in my dataset as well as to discover patterns in the relations among my observations (Klingenberg, 2011), canonical variates analysis (CVA) is generally helpful to describe whether groups differ in their mean tendency (Mitteroecker & Gunz, 2009). In this case it is especially useful to find the shape features that best distinguish between my groups of specimens. Thus, I performed a CVA on the procrustes coordinates in MorphoJ, and produced transformation grids for the three first canonical variates (CVs). CV1 describes the axis along which species are best discriminated. CV2 will be describing the axis in which species are best discriminated, under the constraint that it is uncorrelated to CV1, and so on for subsequent CVs. Insularity and species were used to create the groups of interest such that the differences in shape between island and mainland individuals of Spanish and Italian sparrows could be simplified and studied.

Finally, to investigate how correcting for the random factor population influenced my response variables, I performed a variance decomposition analysis (VC) using MCMC posterior sampling implemented in the R package *MCMCglmm* (Hadfield, 2010). This package fits generalized linear mixed models using Markov Chain Monte Carlo techniques. The method was suitable to investigate the effect of population, as it allows both fixed and random effects in the model, as well as multiple response variables. The model included the response variables RW1, RW2 and RW3, and their

distributions were set to Gaussian. The model also included the fixed effects insularity and species. Model selection was performed based on the Deviance Information Criterion (DIC) (Spiegelhalter et al., 2002). Calculation of DIC can easily be implemented in the code and is calculated as follows: $DIC = 2\bar{D} - D(\bar{\Omega})$, where \bar{D} is the mean deviance of all iterations and $D(\bar{\Omega})$ is the mean estimate of the parameters (Hadfield, 2010).

When the number of groups is low, the mixing of the MCMC chain becomes poor and gets stuck at values close to zero. This can be overcome using a technique called parameter expansion (Liu et al., 1998) that speeds up the rate of convergence in the MCMC chain. This was done by using information from a run with an uninformative prior on my data to choose proper values for the prior means and prior covariance matrix (alpha mean and variance), which then was used in the parameter expanded run afterwards. Also, I used a Cauchy prior in the parameter-expanded run as suggested in Hadfield (2010). The Cauchy prior has the alpha variance set to the square of the standard deviation (i.e. variance) of the posterior distribution gotten from the uninformative prior. The parameter-expanded model was run for 20 million iterations with a burn-in phase of 4 million and a thinning interval of 50.

2.5 3D scanning

In this study, I have performed geometric morphometric analyses of three-dimensional structures using two-dimensional images. 2D images are analytically advantageous, considering computational simplicity. Also, one can visualize shape changes in a very precise way using thin-plate spline (TPS) shape grids. Further, 2D data are generally easier to collect and also more cost-effective (Cardini, 2014).

To look further into the methodology and investigate whether 2D data are a good proxy for their 3D counterparts, or whether 3D methods can provide more insights, I decided to perform 3D scanning of sparrow skulls. This also makes it possible to study other parts of the skull and their relations to beak size and shape. Previous studies have found that beak dimensions, namely depth and width, are good predictors for bite force. Head width has also been found to be positively correlated to bite force, a wider head was interpreted as a probable consequence of the presence of larger jaw muscles (Herrel et al., 2005). Performing 3D imaging of the entire skull should make investigation of these interesting anatomical aspects possible.

To perform 3D scanning of sparrow skulls, a *Planmeca Planscan* device was used. The scanner is originally intended for intraoral scanning for restorative dentistry. It uses blue laser technology with small wavelengths of 450 nm. The scanner has removable heads with three different sizes to optimize the target field while scanning. I used the largest size as this managed to scan large parts of the skull and thus made each scanning quick, which is important to prevent the scanner from overheating. The device was operated through the *Planmeca Romexis software*, more specifically the *CAD/CAM module*. The skulls were placed securely upon a round homemade turntable using play dough. This made rotation of the skull possible while scanning and thus made the scanning process more efficient.

To investigate how 3D scanning performs compared to the 2D method and whether my findings from 2D data is related to skull shape, I scanned one individual of each sex for Italian sparrow and house sparrow (the other parent species), since Spanish

sparrow skulls were not available. The dataset is very small, however this is intended as a pilot study to test whether 3D scanning is a valuable method to include in future research. Once the 3D scanning was finished, the resulting 3D model was visualized using MeshLab (Cignoni et al., 2008). The models were rescaled to reflect real distance by entering the scale factor (i.e. ratio), which I found by dividing a selected distance on the skull (the widest two extremities at the transition between beak and eye socket) by the same distance in the 3D model. All distances were measured three times, and the means of these measurements were used in further calculations.

After rescaling the model, I measured three dimensions of the head and beak, namely length, width and depth. Total head length was measured from the back of the skull to the tip of the beak, head width was measured at the widest part of the skull posterior to the eye sockets and head depth was measured at the deepest part of the skull posterior to the eye sockets (Following Herrel et al. 2005). Beak length was measured from the base of the skull to the tip of the beak, which is suggested for passerines (De Beer et al., 2001). The beak width was measured at the widest part of the beak, and finally, depth was measured at the transition between skull and beak (See supplementary figure S3 for illustrations of measurements).

I also wanted to have a look at the hinges for the jaw muscles in the four individuals to see if there were connections between beak morphology and how well-developed the muscles were, and also to look for species differences in jaw muscles. The surface area of the muscle cavities along the jaw were approximated by using the standard formula for the surface area of an ellipse: $a \times b \times \pi$, where a is the major radius and b is the minor radius from the centre of the ellipse. The measurements

taken in MeshLab were the major and minor diameter (See supplementary figure S4). These distances were measured three times each and the average was divided by two to get the major and minor radius. I measured both the right and the left cavity for all individuals, as they were not consistent in size. However, in one individual, namely the Italian female sparrow, the left cavity was not suitable for scanning.

3. Results

3.1 Beak morphology differences between species and different geographical settings

3.1.1 Beak size differences

We tested which combination of factors best explained beak size differences to identify the forces driving this divergence. The best generalized linear mixed model explaining beak size includes insularity (whether the individual lives on an island or the mainland), species and population as a random factor nested within insularity. Δ AIC between this model and the second best where species is removed from the model was 5.51, which is a significantly worse model. Beak size differs significantly between insular populations and mainland populations ($P = 0.01$, Table 1), with island populations having smaller beaks than mainland counterparts (Fig. 2). Italian sparrows have slightly larger beaks than Spanish sparrows (Fig. 2), but this species effect is not significant ($P = 0.46$, Table 1).

Table 1: Summary from the generalized linear mixed model showing fixed factors included and their associated *p*-values

	Parameter Estimate	Standard Error	t-value	<i>P</i> -value
Insularity	37.54	14.37	2.61	0.01*
Species	-10.01	13.67	-0.73	0.46

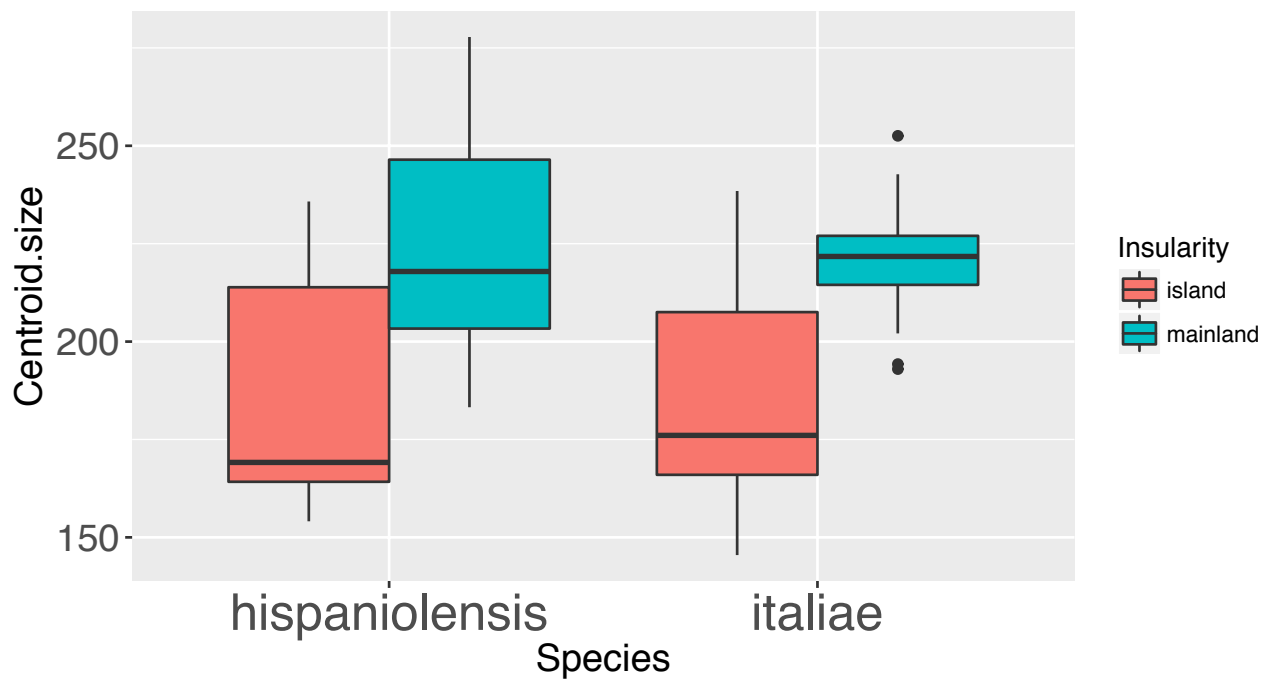


Figure 2: Boxplot showing differences in beak size between the two species. The species are also categorized by insularity, where blue represents mainland individuals and red represents island individuals. See supplementary figure S6 for histograms showing how proportion of different beak sizes vary within the two species, and between insular and mainland individuals.

To investigate if these results hold true for an analysis without population as a random factor, I decided to run an ANOVA. The best ANOVA model for beak size includes insularity, species and sex (AICc = 3217.26), with insularity and sex being most important in affecting the size of beaks. This is in correspondence with the linear model, also identifying insularity as being a significant factor. Beak size differs significantly between sexes ($P = 0.05$, Table 2), with males having bigger beaks than females (Fig. 3). The second best model includes the interaction between insularity and sex as well (AICc = 3219.187), this is not significantly worse model ($\Delta\text{AIC} = 1.932$), however, I selected the first one due to the preference for simpler models.

Table 2: Summary of ANOVA for centroid size, a measure reflecting overall beak size.

	Degrees of Freedom	Sum of Squares	Mean Squares	F-value	P-value
Insularity	1	128607	128607	239.83	<2e-16***
Species	1	663	663	1.236	0.27
Sex	1	2098	2098	3.913	0.05*
Residuals	348	186611	536		

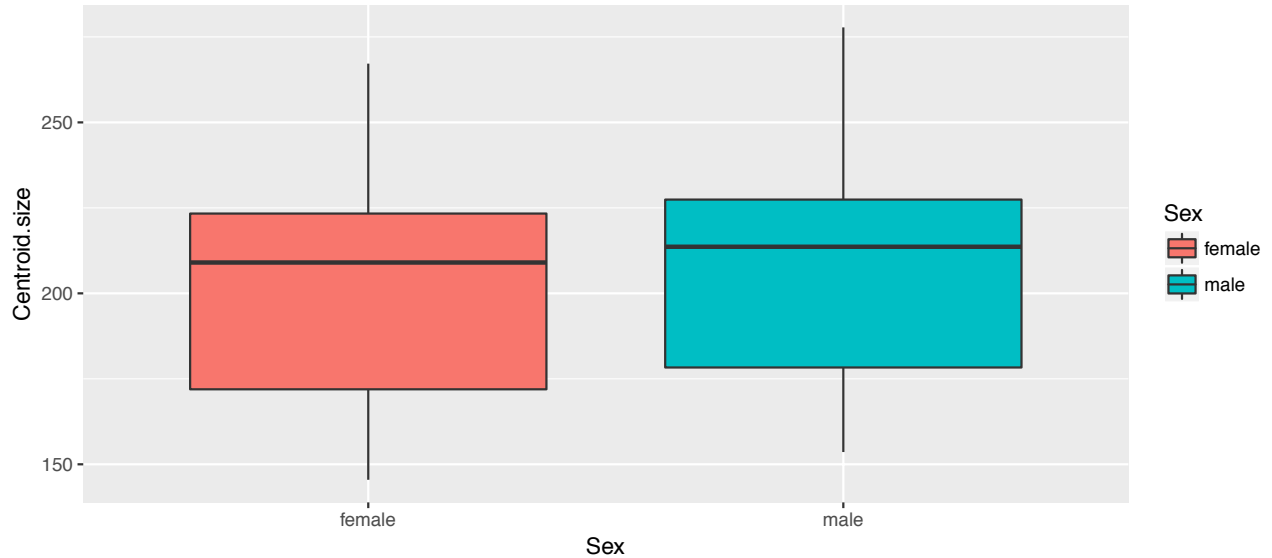


Figure 3: Boxplot showing differences in beak size between the two sexes. Blue boxes represent male individuals and red represents female individuals.

3.1.2 Beak shape differences

For beak shape, I found differences between Italian and Spanish sparrow, as well as between island and mainland individuals. The best linear model explaining the major axis of shape divergence, RW1, reflecting a change from a robust lower mandible towards a thinner one, and a bulkier upper mandible, includes insularity and species. This model has a ΔAIC of 6.13 to the second best model. The best model that describes the second major axis of divergence, RW2, reflecting a change towards a lower and longer beak, also includes insularity and species and has a ΔAIC of 9.16 to the second best model. Lastly, the model that best explains the third major axis of shape divergence, RW3, reflecting a shape change towards a pointier and less broad beak, is also including insularity and species and the ΔAIC is 8.12 between this

model and the second best one. The linear models show that species is the most important predictor for RW1 and RW2, however the species effect is only significant for RW2 ($P = 0.002$, Table 3). This is also evident in the boxplot of RW2 (Fig. 4c), where the mean for the boxes representing Spanish and Italian sparrows are further apart than in the other two plots, with Italian sparrows having higher values for RW2, which means that their beaks are lower and longer than for the Spanish sparrows. Interestingly, although not significant ($P = 0.192$, Table 3), RW3 shows contrasting results with the two previous RWs; insularity is more important than species. The boxplot (Fig. 4e) shows the effect of insularity with mainland individuals having lower values for RW3. This means that island individuals have a less broad and more pointy beak than mainland individuals.

Table 3: Summary of the three best linear models for the three first components of shape RW1, RW2 and RW3.

RW1	Parameter Estimate	Standard Error	t-value	P-value
Insularity	-0.004	0.014	-0.259	0.795
Species	-0.018	0.010	-1.698	0.089
RW2				
Insularity	-0.002	0.005	-0.441	0.660
Species	0.016	0.005	3.087	0.002*
RW3				
Insularity	-0.012	0.009	-1.305	0.192
Species	-0.007	0.009	-0.804	0.421

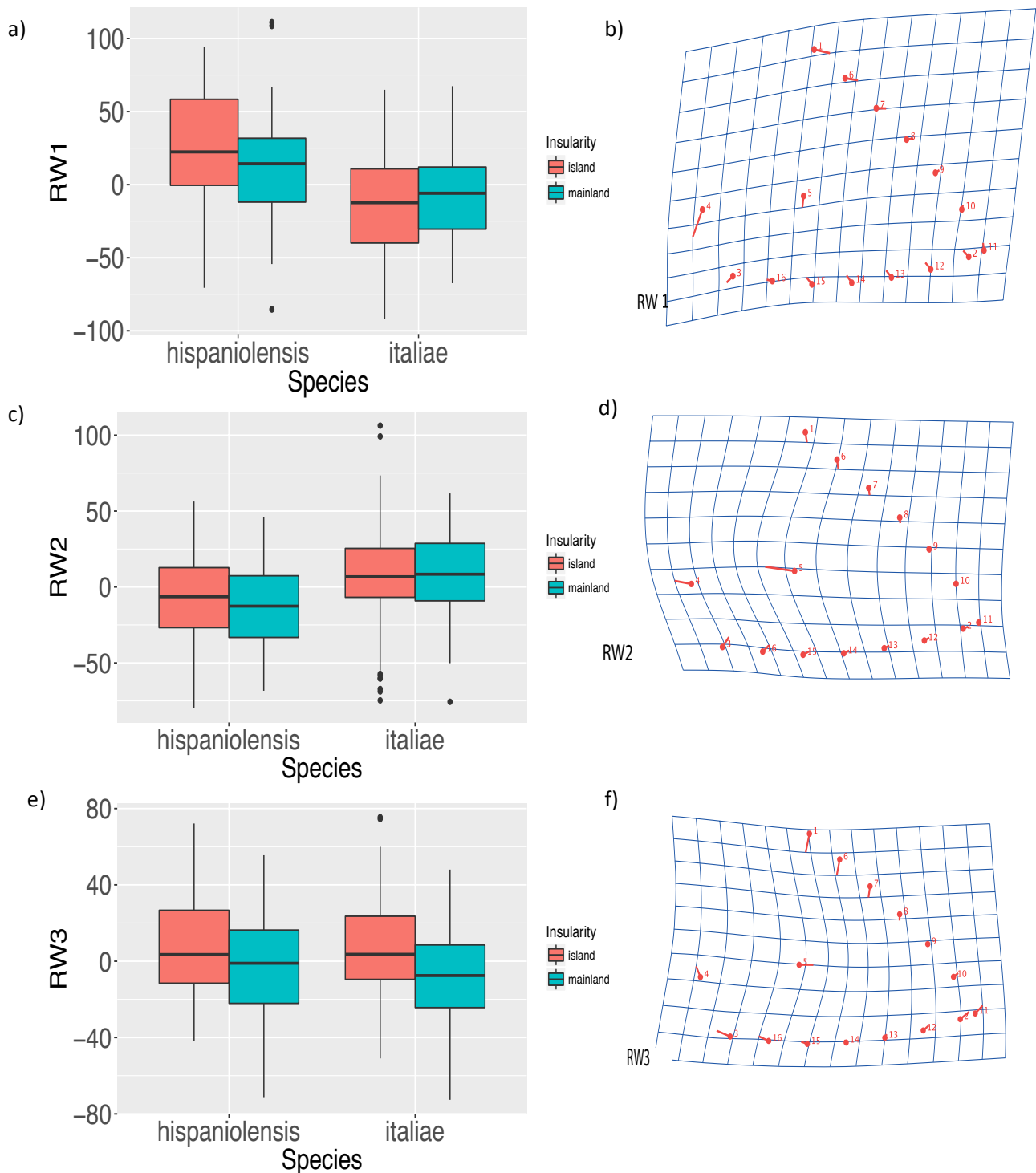


Figure 4: Boxplots and transformation grids for a-b) RW1, c-d) RW2, and e-f) RW3. The transformation grids show shape changes (as procrustes distance) associated with each RW and have a scale factor of 1.0, which corresponds to a change of the RW score by 0.1 units in the positive direction. Note that the grids were produced in MorphoJ and have not been corrected for allometric effects on size, thus these

transformation grids are only intended to give an idea of directions of change. See supplementary figure S7 for a visual representation of the variation within each RW.

Looking further into the various components of shape, we find that the best ANOVA model for RW1 includes insularity, species and sex as well as the interactions between insularity and species and the interactions between insularity and sex (AICc = -1380.77), this model and the next best model has a Δ AIC of 1.903, and we keep the model with the lowest number of factors (i.e. the most parsimonious). The model for RW2 includes insularity, species and sex as well as the interaction between insularity and sex (AICc = -1478.20) with a Δ AIC of 1.062 separating it from the second best model, still we prefer the simpler model since including more factors does not make the model better. For RW3, the best model includes insularity, species, sex, and the interaction between insularity and sex (AICc = -1589.06) with a Δ AIC of 1.608 between this model and the next best one, still the simplest model is used since this difference is not significant. Species seems to be the most important factor shaping beaks as it is the most significant factor for both RW1 ($P = 5.69e-11$, Table 4) and RW2 ($P = 2.77e-07$, Table 4). However, insularity is also significant for RW2 ($P = 0.016$, Table 4) and nearly so for RW1 ($P = 0.07$, Table 4). For RW3, the ANOVA indicates that insularity ($P = 6.6e-06$, Table 4) is more important than species ($P = 0.140$, Table 4), this is also what I found in the linear model (Table 3). For RW3 there is also a significant effect of sex ($P = 0.007$, Table 4). There are also certain interactions that are identified as significant predictors, namely the interaction between insularity and species ($P = 0.007$, Table 4) for RW1, with Insular Spanish sparrows having a robust upper mandible, and insular Italian sparrows having robust lower mandible. The interaction between insularity and sex is significant ($P = 0.011$, Table 4)

for RW2, with insular female sparrows having a lower and longer beak, and males not being significantly affected by insularity.

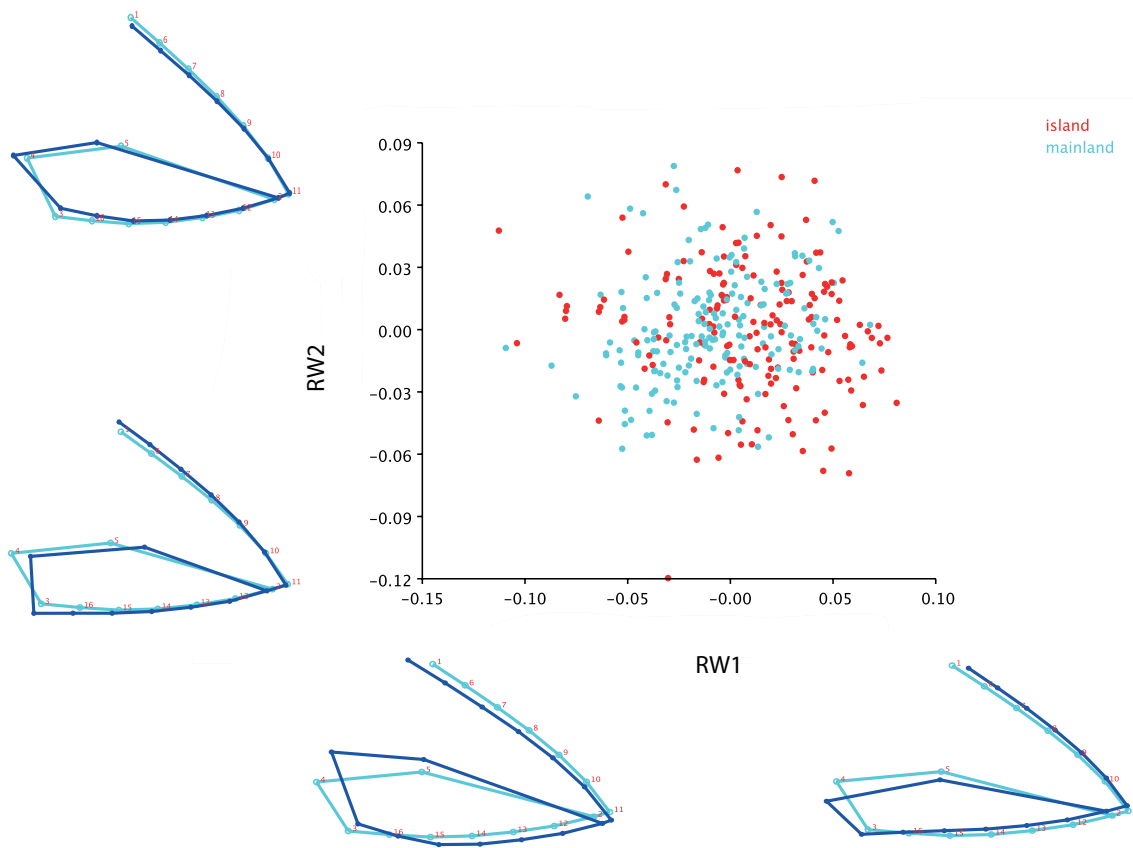
Table 4: Summary of ANOVA for shape; RW1, RW2, RW3

RW1	Degrees of Freedom	Sum of Squares	F-value	P-value
Insularity	1	0.004	3.297	0.070
Species	1	0.052	45.772	5.69e-11***
Sex	1	0.000	0.021	0.885
Insularity:Species	1	0.008	7.396	0.007**
Insularity:Sex	1	0.002	1.691	0.194
Residuals	346	0.392		
RW2				
Insularity	1	0.005	5.914	0.016*
Species	1	0.024	27.478	2.77e-07***
Sex	1	0.0002	0.180	0.672
Insularity:Sex	1	0.006	0.594	0.011*
Residuals	347	0.299		
RW3				
Insularity	1	0.013	20.944	6.6e-06***
Species	1	0.001	2.190	0.140
Sex	1	0.005	7.454	0.007**
Insularity:Sex	1	0.001	2.270	0.133
Residuals	347	0.218		

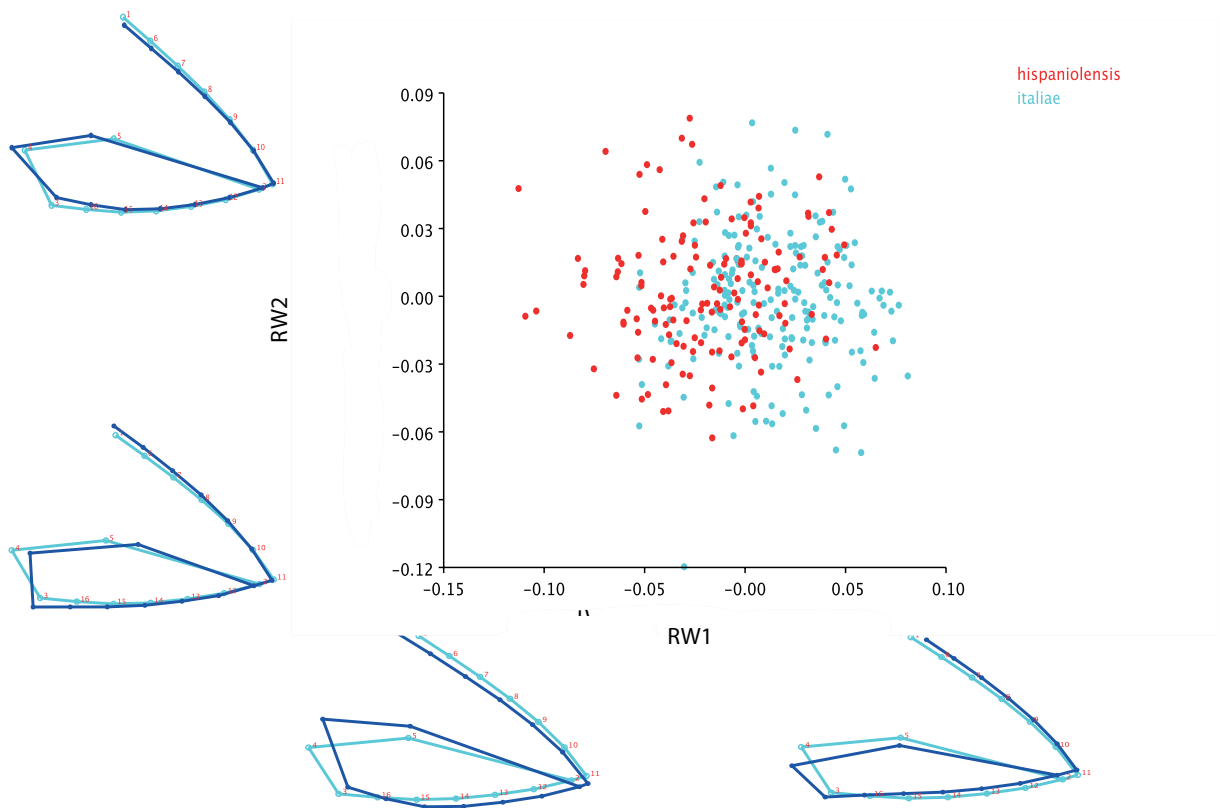
From the plots constructed from the relative warp analysis (Fig. 5), we can see that Spanish sparrows seem to have a more robust and thick lower mandible, whereas Italian sparrows have a larger upper mandible, represented by a clear separation of Spanish and Italian individuals along RW1, with Spanish sparrows having lower RW1 values than Italian sparrows (Fig. 5b and 5d). The species effect on RW1 is also apparent in the boxplot (Fig. 4a). Insular individuals (Fig. 5a and 5c), tend to have more elongated and narrow lower mandibles and large bulky upper mandibles, as represented by higher RW1 values. Conversely, mainland individuals seem to

have bulkier lower mandibles and smaller upper mandibles, as shown by their lower RW1 values (Fig. 5a and 5c). RW2 and RW3 do not show any clear tendencies in figure 4. However, one can see that island individuals have somewhat higher RW3 values than mainland individuals (Fig. 5c). Once again indicating the effect of insularity found in the previous boxplot, namely that island individuals have a less broad and more pointy beak than mainland individuals.

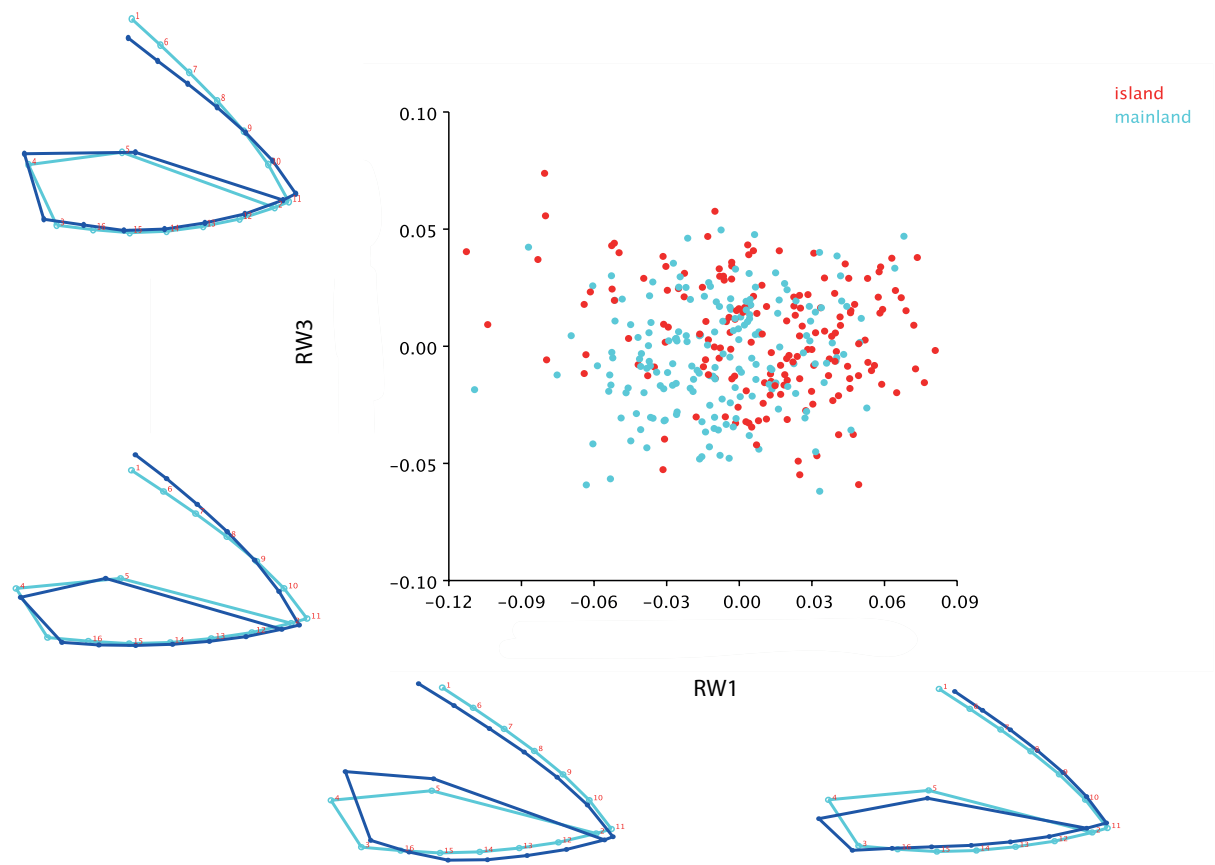
a)



b)



c)



d)

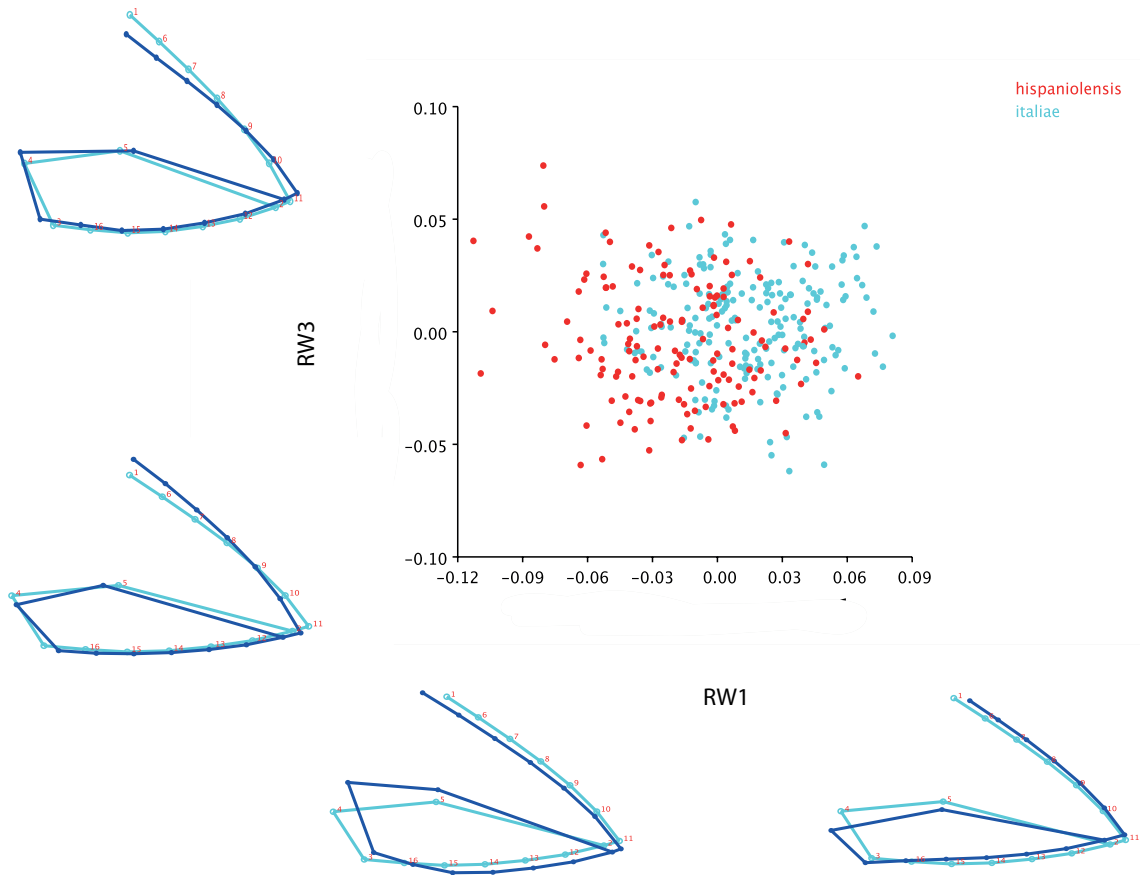


Figure 5: Illustration of the shape changes represented by Relative warp 1, Relative warp 2 and Relative warp 3; a) RW1 vs. RW2 categorized by insularity, b) RW1 vs. RW2 categorized by species, c) RW1 vs. RW3 categorized by insularity and d) RW1 vs. RW3 categorized by species.

To assess the degree of separation in the mean tendencies of my groups I performed a CVA. I found that my four groups were well separated when I made histograms of the CVscores (Fig. 6). The CV shape changes that best separate my groups are presented in figure 7. CV1 represents a change similar to that of RW1, with the lower mandible becoming smaller and the upper mandible becoming larger. Thus, this is the change that best separates the groups from one another. The shape changes represented in CV2 and CV3 are less easy to interpret as a whole, however one can see the movements of each individual landmark in figure 7. It is important to note that these grids have been made in MorphoJ and have not been corrected for allometric effects on size, thus these transformation grids are only intended to give an idea of the directions of phenotypic change associated with the CVs. This change is the same as can be obtained by regression of shape onto the scores for the respective CV (Rohlf et al., 1996). The scale factor for CV shape changes is in units of Mahalanobis distance (in this case the scale factor is 10.0) — the shape change per unit of within-group shape variation.

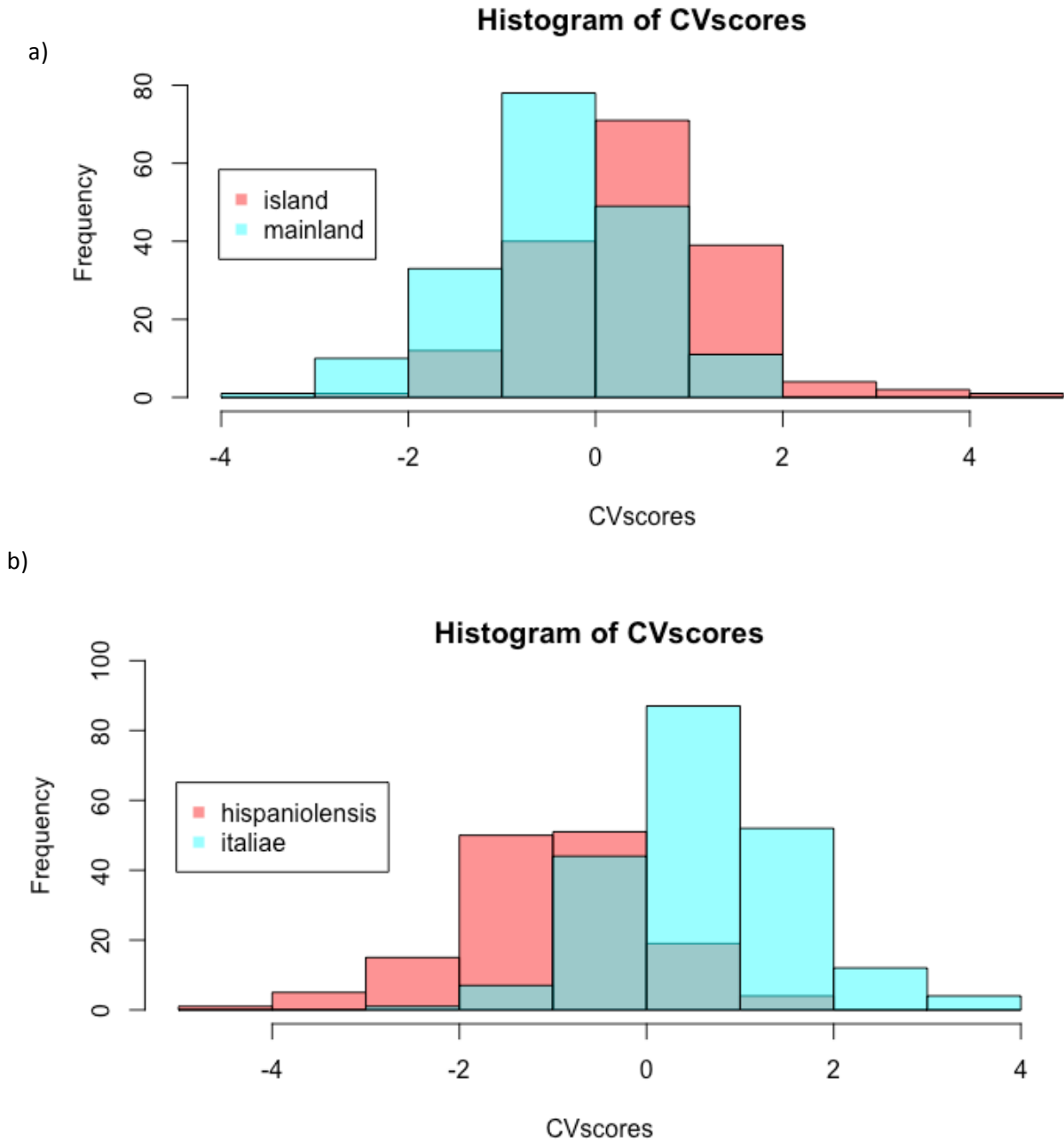


Figure 6: CVA for shape. The above histograms show the CVscores for specimens grouped by a) insularity (mean = $-2.35e-17$, SD = 1.09), and b) species (mean = $-6.15e-17$, SD = 1.29). The response variables are all 28 of the relative warps.

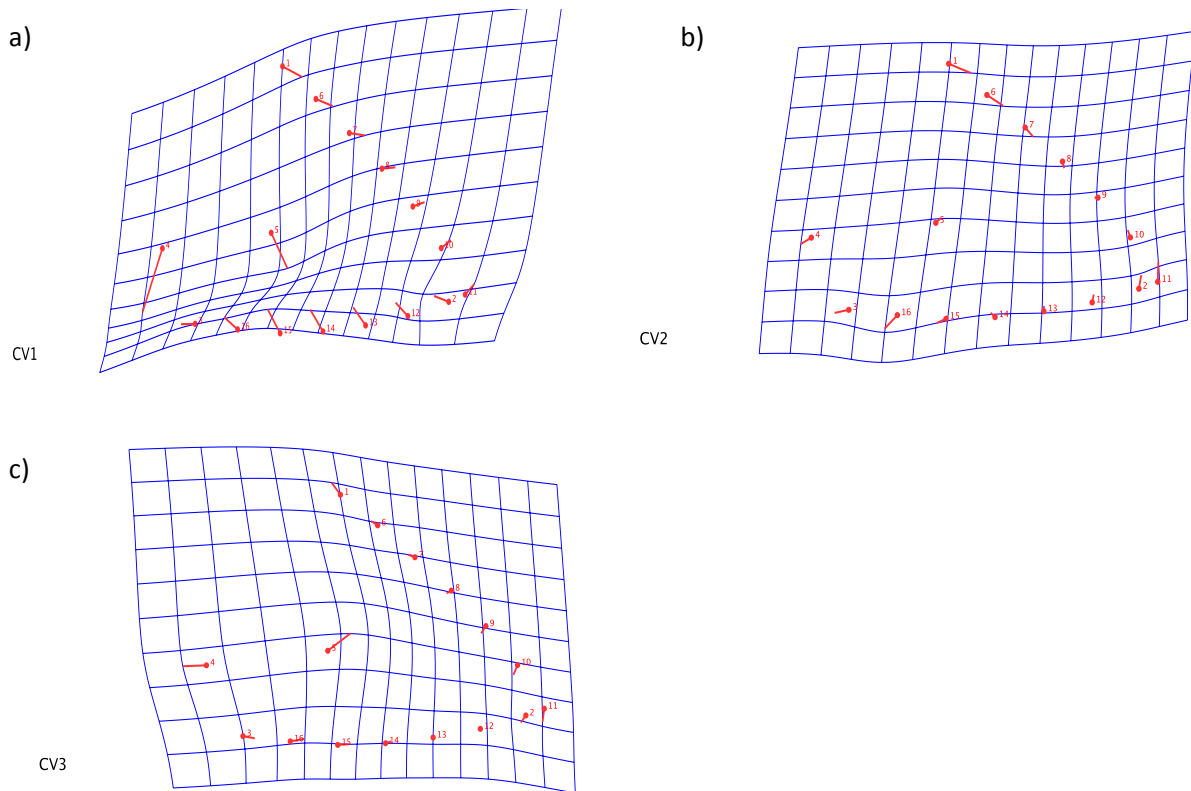


Figure 7: Shape changes associated with the canonical variates from the CVA, CV1 (a), CV2 (b) and CV3 (c).

To estimate the variables explaining overall shape differences along all significant shape dimensions we used a multivariate model. The best multivariate MANOVA model, including all significant shape variables (RW1, RW2 and RW3) and hence providing information on overall shape divergence along several dimensions, includes species, insularity, sex and the interactions between species and insularity, and insularity and sex. The results mirror the results from the ANOVA in that species is the most significant factor in explaining beak shape variation ($P = 2.2e-16$, Table 5), with Spanish sparrows having bigger lower mandibles, and the Italian sparrow having an overall lower and longer beak. Insularity is also a significant predictor ($P = 3.765e-05$, Table 5), with insular individuals having a less broad and pointier beak than mainland individuals. Further, island individuals have a bulkier upper mandible, and mainland individuals have bulkier lower mandibles.

Other less important but still significant variables includes the interactions between species and insularity and insularity and sex, which correspond to the interactions identified as important in the ANOVA. The interaction between species and insularity results in higher RW1 values for Spanish sparrows on island, and lower for Italian sparrows on islands. Insular Spanish sparrows will have a robust upper mandible, and insular Italian sparrows have a robust lower mandible. Spanish sparrows have higher values of RW2 on islands and will have a lower and longer beak, meanwhile Italian sparrows are not affected by insularity for RW2. RW3 changes in parallel in the two species, with both having higher values on islands and hence have a pointier and less broad beak. The interaction between sex and insularity shows a dramatic drop in RW1 values for male sparrows on islands, indicating a robust low mandible, whereas the females are hardly affected by insularity when it comes to the robustness of their mandibles. Female sparrows have a large increase in RW2 values on islands, representing a lower and longer beak, and males are not affected significantly. For RW3, both sexes are affected in the same way, having higher values on islands, indicating a pointier and less broad beak.

Table 5: Summary from MANOVA including Pillai's trace value, associated F-statistic and p-values.

	Degrees of Freedom	Pillai trace	Approximate F-value	Degrees of Freedom	P-value
Species	1	0.204	29.357	344	<2.2e-16***
Insularity	1	0.065	7.969	344	3.765e-05***
Sex	1	0.022	2.521	344	0.058
Species:Insularity	1	0.024	2.799	344	0.040*
Insularity:Sex	1	0.027	3.198	344	0.024*
Residuals	346				

Table 6: Associated parameter estimates from MANOVA model

	Parameter Estimate	Standard Error	t-value	P-value
Response RW1				
Species	-0.037	0.006	-6.417	4.572e-10
Insularity	-0.017	0.007	-2.518	1.224e-02
Sex	-0.005	0.005	-0.977	3.291e-01
Species:Insularity	0.020	0.008	2.674	7.861e-03
Insularity:sex	0.009	0.007	1.300	1.943e-01
Response RW2				
Species	0.014	0.005	2.725	0.007
Insularity	-0.015	0.006	-2.528	0.012
Sex	-0.009	0.005	-2.094	0.037
Species:Insularity	0.007	0.007	1.002	0.317
Insularity:sex	0.016	0.006	2.532	0.012
Response RW3				
Species	-0.003	0.004	-0.590	0.556
Insularity	-0.007	0.005	-1.389	0.166
Sex	0.011	0.004	2.946	0.003
Species:Insularity	-0.003	0.006	-0.603	0.547
Insularity:sex	-0.008	0.005	-1.484	0.139

To be able to include random effects, it is necessary to run a mixed model. As I had both a multivariate response variable and a random factor, I used MCMCglmm (Hadfield, 2010). In the MCMCglmm I included population as a random factor. The null model without factors has a DIC = 9950.33. A model including species and insularity (DIC = 9950.57) is a better fit than when species is removed from the model (DIC = 9950.64). Adding the interaction between species and insularity did not improve the model (DIC = 9951.0). Since the simplest model is preferred, we would keep the null model in this instance since the Δ DIC values are so low. This implies that population of origin best explains shape, and thus more populations would be needed to refute the null model of drift between populations as driving the shape differences.

Table 7: The table shows the results from an expanded parameter run ran for 20 million iterations with a burn-in phase of 4 million and a thinning interval of 50. The model includes population as a random effect, and insularity + species as fixed effects. The model has a DIC = 9950.57.

Trait	Posterior mean	l - 95% CI	u - 95% CI	Effective sample size	P-value
RW1	0.680	-19.371	20.353	59600	0.942
RW2	0.463	-11.567	13.634	58615	0.999
RW3	1.823	-13.125	16.365	57774	0.801
Insularity	-5.221	-15.612	4.927	59600	0.299
Species	4.840	-9.221	17.820	55519	0.471

3.2 Beak morphology differences between populations

From what we saw in the MCMCglmm, population effects might be responsible for much of the variation observed in beak shape. When the data points are coloured by population (Fig. 8), we can see that it looks like the variation may be explained by population differences to some extent, consistent with the findings of population of origin best explaining variation in the MCMCglmm. However, there are no clear trends, except that interestingly, individuals from Tenerife seem to have quite differently shaped beaks compared to the other populations (Fig. 8).

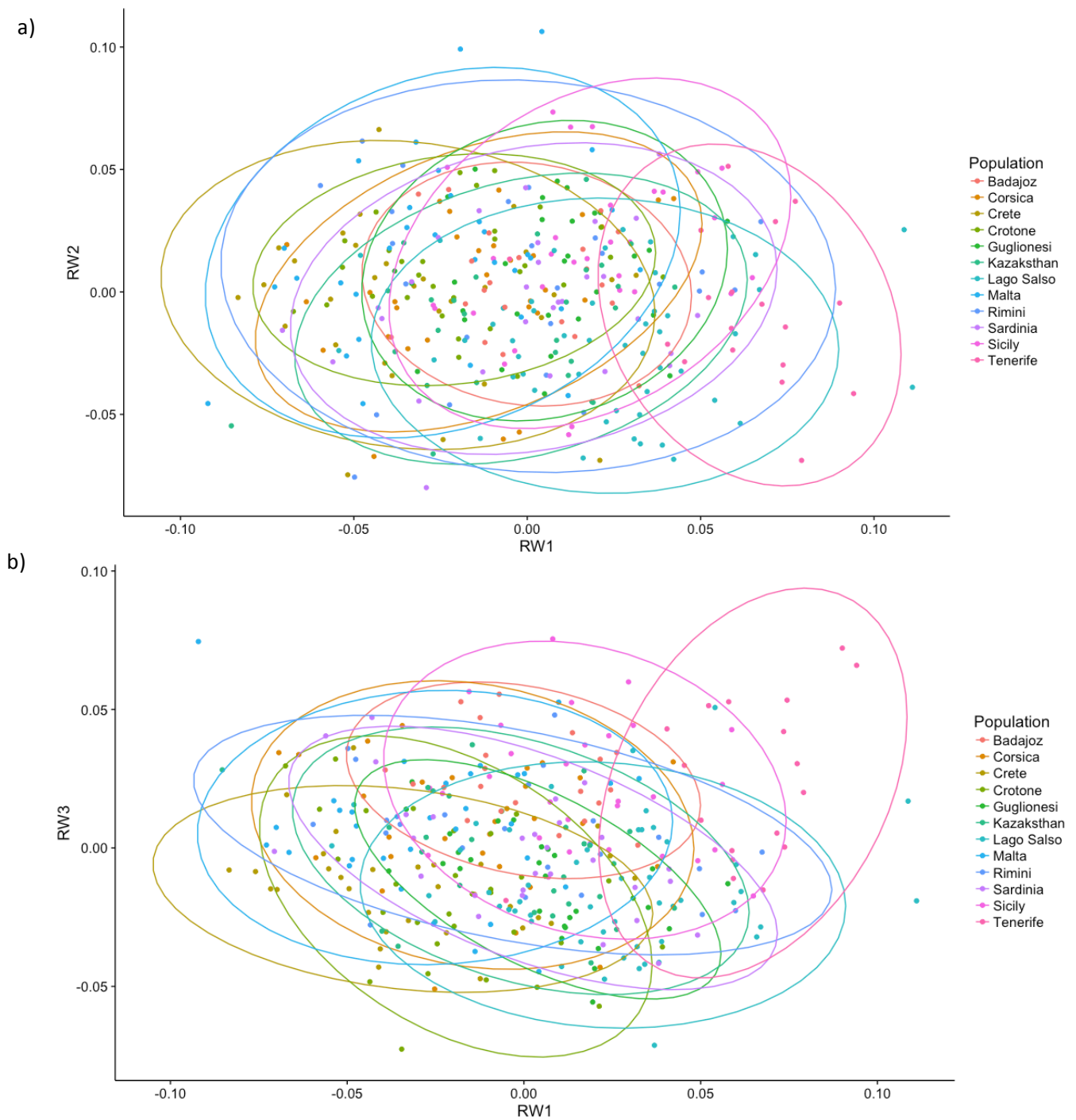


Figure 8: Relative warp 1 plotted against relative warp 2 (a). Relative warp 1 plotted against relative warp 3 (b). The data points are coloured by populations and the plots include 95% confidence ellipses.

3.3 Beak morphology differences investigated using 3D scanning

3.3.1 Differences in head and beak dimensions

I found that the two species differed in some aspects of beak and head morphology. Italian sparrows have longer heads (mean = 30.27 mm, Table 8) than house sparrows (mean = 28.37 mm, Table 8); this is especially true for the females having a head length difference of 2.65 mm, compared to the males having a difference of 1.16 mm. However the width and depth of the head are similar between the two species. Also, I found that the beaks were longer in Italian sparrows (mean = 14.6 mm) than in house sparrows (mean = 12.74 mm, Table 8); once again this difference is larger between the females that have a beak length difference of 2.14 mm, whereas the two males have a difference of 1.57 mm. The beak depth and width does not show any large contrasts, although there seems to be a slight trend towards house sparrows having deeper bills than Italian sparrows.

Table 8: Measurements of head and beak dimensions from 3D models of skulls for Italian and house sparrows. The numbers are the mean value of three measurements, and the standard deviations of these measurements are given in parentheses. Average standard deviations of each dimension across individuals are also reported.

	Total head length	Head width	Head depth	Beak length	Beak width	Beak depth
House sparrow female	27.64 mm (SD=0.162)	15.02 mm (SD=0.052)	15.18 mm (SD=0.041)	12.40 mm (SD=0.192)	7.68 mm (SD=0.005)	8.00 mm (SD=0.046)
House sparrow male	29.09 mm (SD=0.327)	16.66 mm (SD=0.122)	16.71 mm (SD=0.137)	13.08 mm (SD=0.125)	7.53 mm (SD=0.046)	8.85 mm (SD=0.170)
Italian sparrow female	30.29 mm (SD=0.302)	15.64 mm (SD=0.063)	15.95 mm (SD=0.115)	14.54 mm (SD=0.083)	7.58 mm (SD=0.004)	7.53 mm (SD=0.057)
Italian sparrow male	30.25 mm (SD=0.102)	15.41 mm (SD=0.123)	15.78 mm (SD=0.191)	14.65 mm (SD=0.113)	7.34 mm (SD=0.060)	7.97 mm (SD=0.037)
	$\overline{SD} = 0,223$	$\overline{SD} = 0.090$	$\overline{SD} = 0.121$	$\overline{SD} = 0.128$	$\overline{SD} = 0.029$	$\overline{SD} = 0.078$

3.3.2 Differences in jaw muscle cavities

When estimating cavities in the jaw where the jaw muscles have been situated, I first had to make several measurements of the cavities to be able to calculate the surface area of them. Table 9 shows the average major and minor diameters that were used to do these calculations (See supplementary figure S4 for more information about these measurements). The average standard deviation for average major diameter and average minor diameter was the same ($\overline{SD} = 0.033$), which means that overall the variability in measurements was low to moderate.

Table 9: Average for the measurements of major and minor diameter, as well as standard deviations for these measurements. Average standard deviations of all measurements for the major and minor diameter, are shown at the end of the table. The measures are shown for both L = left and R = right cavity for all four specimens. NA = not applicable.

	Cavity	Average major diameter	Average minor diameter
House sparrow female	R	8.311 mm (SD=0.006)	1.600 mm (SD=0.057)
	L	7.146 mm (SD=0.020)	1.614 mm (SD=0.027)
House sparrow male	R	7.883 mm (SD=0.075)	2.568 mm (SD=0.012)
	L	8.983 mm (SD=0.023)	1.908 mm (SD=0.013)
Italian sparrow female	R	8.276 mm (SD=0.041)	1.734 mm (SD=0.042)
	L	NA	NA
Italian sparrow male	R	8.849 mm (SD=0.024)	2.438 mm (SD=0.039)
	L	9.039 mm (SD=0.043)	2.806 mm (SD=0.044)
		$\overline{SD}=0.033$	$\overline{SD}=0.033$

I found that the male house and Italian sparrow had much larger cavities than the females, and this was consistent for both left and right cavity (Table 10). The average surface for a female jaw cavity is 10.25 mm², whereas for males the average surface is 16.56 mm². Further, Italian sparrows seem to have somewhat larger cavity surfaces compared to house sparrows, with an average of 16.04 mm² in Italian sparrows compared to an average of 12.21 mm² in house sparrows, although this result may be influenced by the missing information for the female Italian sparrow. I found no pattern suggesting that either left or right cavity always tends to be the bigger one, however each skull had one cavity bigger than the other, except for the female Italian sparrow missing information about the left cavity. Also, there was a large variation in cavity surface area, ranging all the way from 9.058 mm² to 19.919 mm².

Table 10: *Calculated surface area of right and left cavity for all four specimens. NA = not applicable.*

	Surface area of right cavity	Surface area of left cavity
House sparrow female	10.433 mm ²	9.058 mm ²
House sparrow male	15.897 mm ²	13,464 mm ²
Italian sparrow female	11.270 mm ²	NA
Italian sparrow male	16.941 mm ²	19.919 mm ²

4. Discussion

4.1 Insularity - an important predictor of beak size that favors small beaks

I found parallelism in beak size across species, with insular Italian and Spanish sparrows both having smaller beaks than their mainland counterparts. This means that insularity may cause beaks to diverge along the same axis in both species, hence that selective pressure may be equivalent with respect to insularity. Interestingly, the Italian sparrow has been reported to have smaller beaks in islands than on mainland in earlier studies (Helén, 2016). Smaller beaks in insular individuals is unexpected as the island rule states that small-bodied species develop larger body sizes, and larger species smaller body sizes on islands (J. B. Foster, 1964; Van Valen, 1973). However, it is possible that a single trait, such as beak size, does not necessarily evolve in a way that reflects body size evolution as described by Van Valen. Further, Meiri et al. (2008) found no evidence for the island rule when conducting phylogenetic comparative analyses on a large dataset of different clades of mammals. Instead, they found that size evolution on islands is likely to be affected by biotic and abiotic characteristics of different islands.

The convergence in beak size of insular Italian and Spanish sparrows may be linked directly to islands. Islands have a different ecology than adjacent mainland localities, and biodiversity on islands is often reduced and hence there is lower competition (Runemark et al., 2014). This can result in more open niches, and thus it might be possible that a broad beak with strong bite force for cracking hard seeds is not necessarily adaptive, as the abundance of other smaller seeds and other food sources is adequate. The importance of diet for beak size in insular and mainland individuals

has been addressed before. The carbon composition of the diet (amount of $\delta^{13}\text{C}$), annual precipitation and sex explains the beak size variation in individuals on islands better than in mainland populations (Helén, 2016). These factors might hence be responsible for the parallel reduction in beak size on islands. On the other hand, the Spanish sparrow can become a human commensal on islands due to competitive release in the absence of house sparrows. The Italian sparrow is a human commensal and resembles its other parent, the house sparrow (Summers-Smith, 1988). Thus, signs of convergence between insular Spanish and Italian sparrow could be linked directly to islands, but also be reinforced by selective pressures related to human commensalism. On the other side, adaptation to food sources originating from human societies has been found to favor a more robust beak (Riyahi et al., 2013). I found that insular individuals had smaller beaks, and thus human commensalism is not likely to be the driver behind this pattern. However, the parallelism found in beak size on islands indicates that islands must exert specific selective pressures that have molded the beak in the same way. It is possible that this is facilitated by genetic variation shared between the hybrid and its parent species, which in turn has made it easier to evolve the same beak size under similar selective pressures.

I found no support for species differences in beak size. Eroukhmanoff et al. (2013) found beak size, namely beak height and length, to be strongly correlated with environmental factors. Environmental factors can contribute by affecting local agriculture and hence the availability of types of seeds, and in this manner affects the local optima for beak size. This is consistent with the findings of Herrel et al. (2005), who found beak depth and width, to be good predictors for bite force. Also, considering that Spanish sparrows can take on a role as human commensals in islands, the potential role for environmental factors affecting local agriculture can be of

importance for both Spanish and Italian sparrows, and thus result in the two species becoming more similar.

I found significant sex differences in beak size as well, with males having a bigger beak than the females for both species, however the effect of sex was much smaller than insularity. Interestingly, Grant & Grant (2002) conducted a 30-year study of evolution of size and shape traits in two populations of Darwin's finches, where they found that beak size traits were subject to selection more often than was beak shape. This can be an important factor contributing to the strong insular and environmental effects upon beak size. Further, size traits are significantly less likely than shape traits to experience stasis (Hunt, 2007), and it has also been suggested that body size may be more evolvable than aspects of shape according to the fossil record (Stanley & Yang, 1987). Thus, considering all the aspects above, it is not surprising to find signs of selection on beak size.

4.2 Beak shape – an effect of insularity, species and sex

I found effects of both species and insularity on beak shape. The Italian sparrow has an overall lower and longer beak than the Spanish sparrow, having a more robust lower mandible. I also found that shape changes in parallel in the two species, resulting in island individuals having a bulkier upper mandible, and mainland individuals have bulkier lower mandibles. This is the same as Piñeiro (2015) reported in the Italian sparrow as well. Further, I found that insularity favours a less broad and more pointy beak in both species, this convergence may be caused by adaptation to diet. Interestingly, Herrel et al. (2005) did not only identify beak width and depth to be important predictors of bite force, but also beak shape. As Piñeiro (2015) found

beak shape to be affected by the carbon composition of the diet in the Italian sparrow, it is plausible that ecological factors contribute to the parallel changes observed here. As mentioned earlier, shape seems to be less evolvable than size, and thus a strong directional selection pressure such as diet may be needed to achieve stronger changes.

I also found sex to be an important predictor for beak shape (especially for RW2 and RW3), i.e. sexual dimorphism. This is consistent with the fact that in birds, the sexes usually differ in size and also in body proportions, including the morphological features used for foraging (Amadon, 1959). Further, the traditional explanation for varying degrees of sexual dimorphism between species is variation in social mating systems and the pattern of parental care (Darwin, 1871). In sparrows, the male feeding rates are high until the nestlings reach 10 days of age, and in this period the nestlings are fed with insects. The male feeding rate then declines significantly as the nestlings get older and the female takes on the role of feeding the young, mostly with plants (Hegner & Wingfield, 1987). These differences in participation in parental care between the male and female sparrow might explain some of the sex differences in beak shape. Invertebrate foraging may also require different morphological features than plant foraging and thus this may also explain the sexual dimorphism.

As now seen, beak shape is affected by insularity, species and sex. Interestingly there were also significant effects of interactions between these factors. The interaction between species and insularity results in insular Spanish sparrows having a robust upper mandible, and insular Italian sparrows have a robust lower mandible. Spanish sparrows on islands will have a lower and longer beak than insular Italian sparrows,

and both species have a pointier and less broad beak on islands. This means that insularity is affecting my two study species differently for the two most important components of shape, however both have a pointier and thinner beak on islands, and this convergence may be indicative of adaptation to insularity. However, in order to be sure that this indeed is an adaptation to insularity, these changes must be shown to be genetic or have a genetic basis, and that selection in islands is indeed responsible for these changes. However, as Pelosi et al. (2008) discuss, fitness itself is a complex trait that results from all interactions among the molecular components of the whole organism. Therefore it is important to assess the extent of parallel evolution for as many traits as possible in a given system. Thus identifying traits that are subject to parallelism in the *Passer* hybrid complex is important to accompany genetic analyses to get a fuller picture of fitness and adaptation.

The second interaction I identified was between sex and insularity. This interaction results in males having robust lower mandibles on islands. On the other hand, female sparrows have a lower and longer beak on islands. We also see both sexes having a pointier and overall less broad beak when living on islands. This means that although the lower mandible of the males on islands is bulkier, the beak in itself is less broad.

Population of origin seemed to be the best explanation for variation in beak shape according to the mixed model, and also individuals from Tenerife differed more from the other populations. The fact that Spanish sparrows from Tenerife differ from other populations of Spanish sparrow is an interesting observation. Since not only beak size (Helén, 2016), but also beak shape (Piñeiro, 2015) in the Italian sparrow is

strongly correlated with the amount of carbon in the diet, the diverged beak morphology on Tenerife might be a result of a slightly different climate that in turn results in different food sources being present there compared to other populations investigated. Another important aspect is that Tenerife is of volcanic origin (Ancochea et al., 1990), and is thus a much younger island than Sardinia. Young islands are typically species-poor and hence have lower competition (Losos & Ricklefs, 2009). The first species establishing themselves on a young island will often diversify in novel directions as they will find untapped resources, and lack the constraints of a resident biota already being present (Losos & Ricklefs, 2009). It is a plausible explanation that Tenerife is relatively species-poor in comparison to other islands and this might have caused the Spanish sparrows there to diversify in a novel direction. The size of the islands themselves differ a lot as well, with Tenerife being approximately 2058 km² (Ancochea et al., 1990) and Sardinia 24,090 km² (Pisanu et al., 2009). Small islands often have less species than larger islands (Losos & Ricklefs, 2009). Thus, both the size and age of Tenerife may contribute to a more species-poor environment. On the other hand, Tenerife is part of an archipelago: The Canary Islands. Although birds do not diverge into multiple species on small islands, they do so readily on archipelagos composed of small islands (Mayr & Diamond, 2001). The radiation of Darwin's finches is an example of this phenomenon with 13 species having been produced in the Galápagos archipelago. I propose that Tenerife's relative youth and small size, may result in less competition and niche differentiation that may have spurred specific evolution of beak morphology among the Spanish sparrows residing there.

4.3 3D scanning – what can we learn from it?

The 3D scanning revealed that there is much to learn about features of the bird skull that have not been studied extensively with classical geometric morphometric measurements, and that 3D scanning indeed works to capture these features. Not only did I find that the beak was longer in Italian sparrows than in house sparrows, but also head length differed in the same manner. A possible explanation for these beak length differences, is the role of the beak as a thermoregulatory organ (Hagan & Heath, 1980; Tattersall et al., 2017). Bird species living in colder climates have been found to have significantly shorter beaks than species in warm climates, as a means of minimizing heat loss from the highly vascularized surface of the beak (Symonds & Tattersall, 2010). This is also in correspondence with Allen's Rule, which states that the appendages of endotherms are smaller relative to body size in colder climates, in order to reduce heat loss (Allen, 1877). Thus, Italian sparrows may have longer beaks to be able to cope with a warmer Mediterranean climate. I also found that jaw muscles seemed to differ between the sexes, with males having the largest cavities. This is consistent with the fact that among birds, the male is larger than the female for most species (Amadon, 1959).

Regarding the method itself, transforming the 3D model into real life distances was easily achieved in MeshLab, where one could just add the calculated ratio directly into the settings. Further, doing measurements in MeshLab proved to be quite accurate as variability in measurements was low to moderate across all measurements. I discovered there were two aspects that one needs to keep in mind when performing a 3D scan. First you have to pay close attention when selecting skulls for scanning. Some skulls may prove hard to scan due to the thickness of the bones and cartilage

that composes it. This was the case for one of my individuals, namely the Italian female, where the wavelengths from the scanner penetrated right through the skull, resulting in double scanning of certain parts. This in turn led to the left jaw cavity not being possible to measure because it was completely closed up due to the double scanning of this area. Second, when using the Planmeca Planscan device like I did here, it is important to note that it was intended for scanning in restorative dentistry. This means the models created in MeshLab will have a base, just like the gum would be the base of a tooth. The issue was overcome by starting the scanning from the tip of the beak, as we discovered that the scanning device always creates a base at the opposite side of the starting point of scanning. However, keeping these two obstacles in mind, I argue that 3D scanning combined with the software MeshLab is something future researchers could benefit from implementing in comparative, developmental, functional, and quantitative studies of morphology in birds and especially sparrows. The method allows for accessing more accurate measures of the entire skull and jaws, and might make it possible to derive more robust conclusions about the biological reasons why beaks may vary in shape and size. Moreover, combining these measures with measures related to anatomy, such as jaw cavities, makes it possible to derive correlations between dimensions and anatomy. This might in turn be useful for further studies on for instance bite force, where there has been found a link between beak/head dimensions and bite force, with this connection being a probable result of the presence of larger muscles (Herrel et al., 2005). I suggest that future studies implement a 3D approach to investigate the muscles that are important in generating bite force.

In conclusion, I did not find support for the hybrid species being more variable in size/shape than the non-hybrid. Instead, I found strong insular effects on beak size, and that insular environments favored smaller beaks in both species. I also found effects of both insularity and species on beak shape, with Spanish sparrows having larger lower mandibles, and the Italian sparrow having an overall longer and lower beak than Spanish sparrows. Insular individuals had a less broad and pointier beak than mainland individuals. Further, island individuals have a bulkier upper mandible, as mainland individuals have bulkier lower mandibles. I hence find evidence consistent with adaptation to the species- and setting specific ecology in both size and shape both for the parent and hybrid species. To further investigate this phenomenon, I suggest that future research both add further populations to the analyses to improve the power and accompany the original 2D measurements with 3D scanning to capture the full multidimensional variation in shape. More accurate multidimensional measures are likely to spur new insights in studies of morphology and evolution.

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Appendix I

Supplementary figures



Figure S1: Example of digitization of beak shape in 2D. This picture shows the placement of the five homologous landmarks, as well as the millimeter paper where one square enclosed by bold lines equals 1 cm². Best-fit curves containing 7 equidistant semi-landmarks were drawn between landmark 1-2 and 2-3 (not shown in picture).

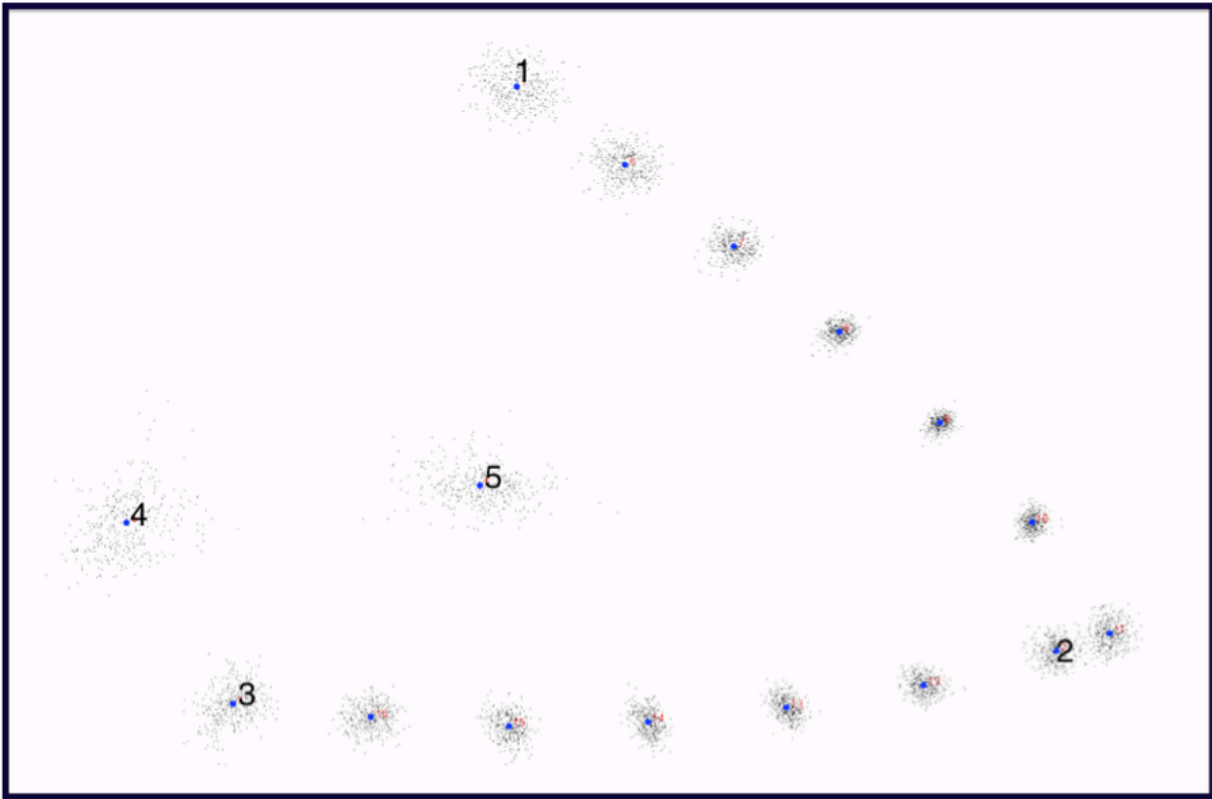


Figure S2: Consensus plot showing the variation of landmark and semi-landmark placement for all 352 individuals (black dots). The blue dots represent the mean configuration of the landmark

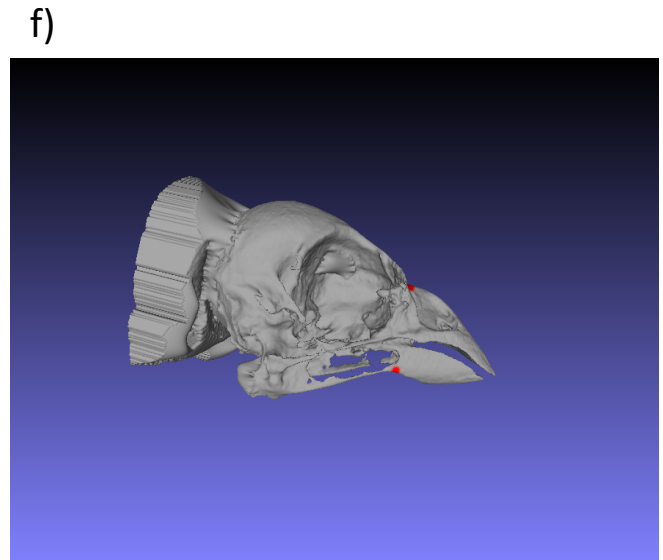
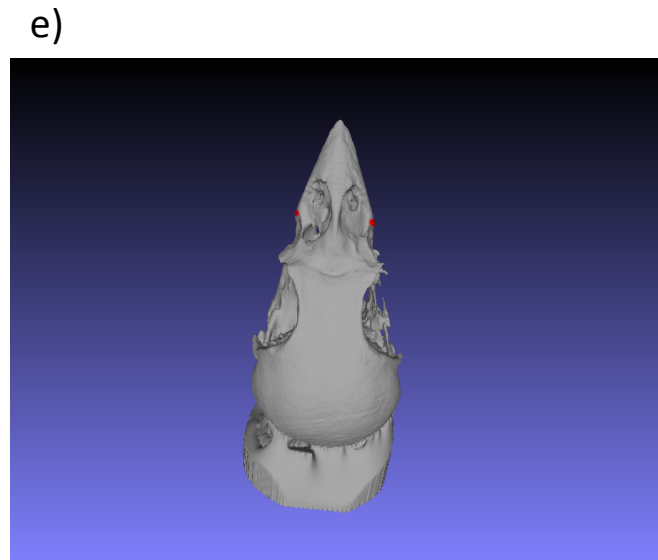
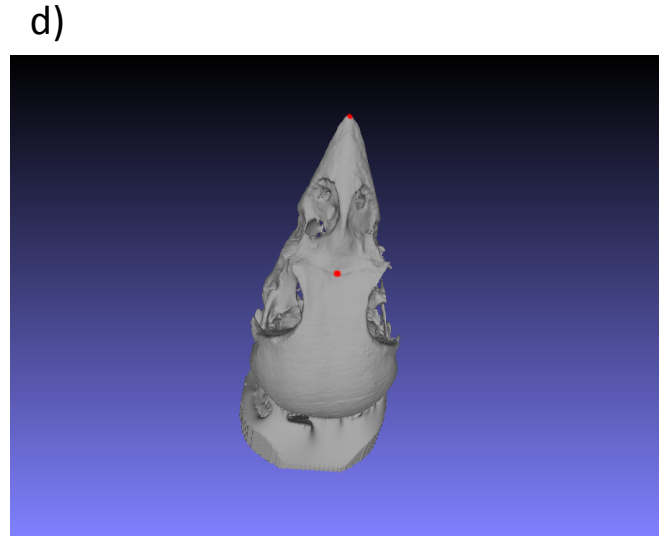
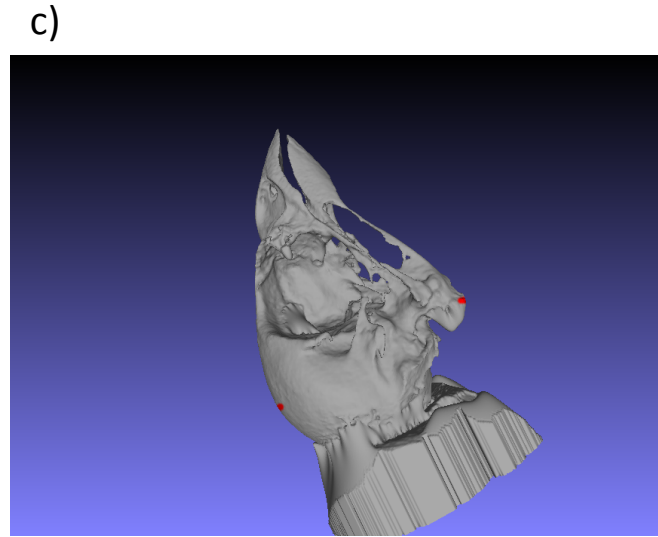
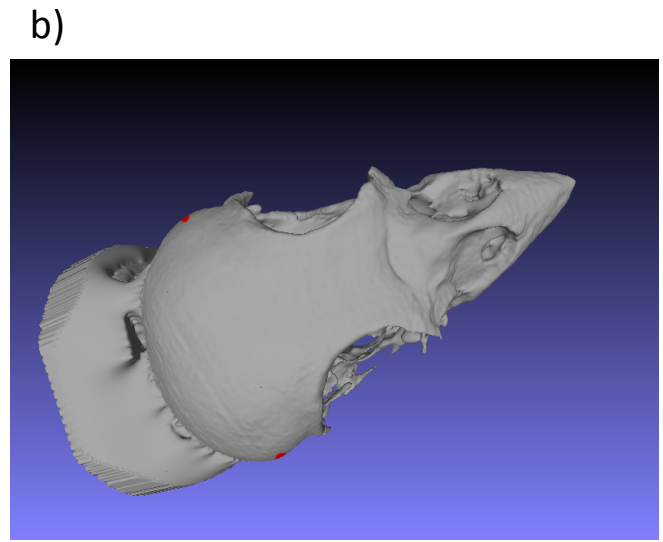
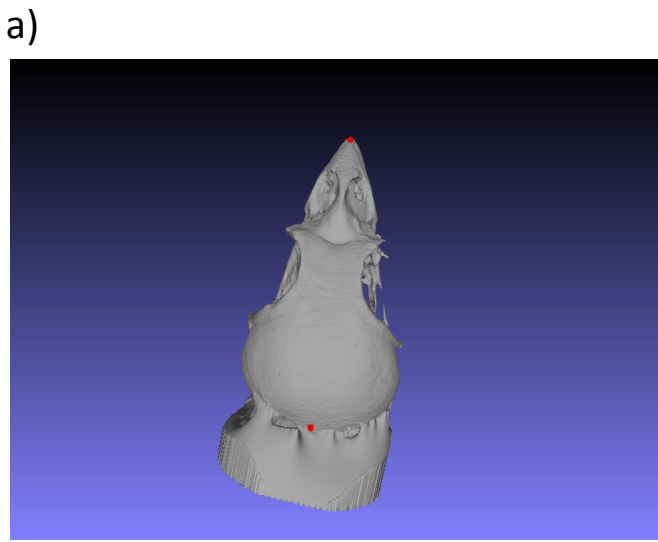
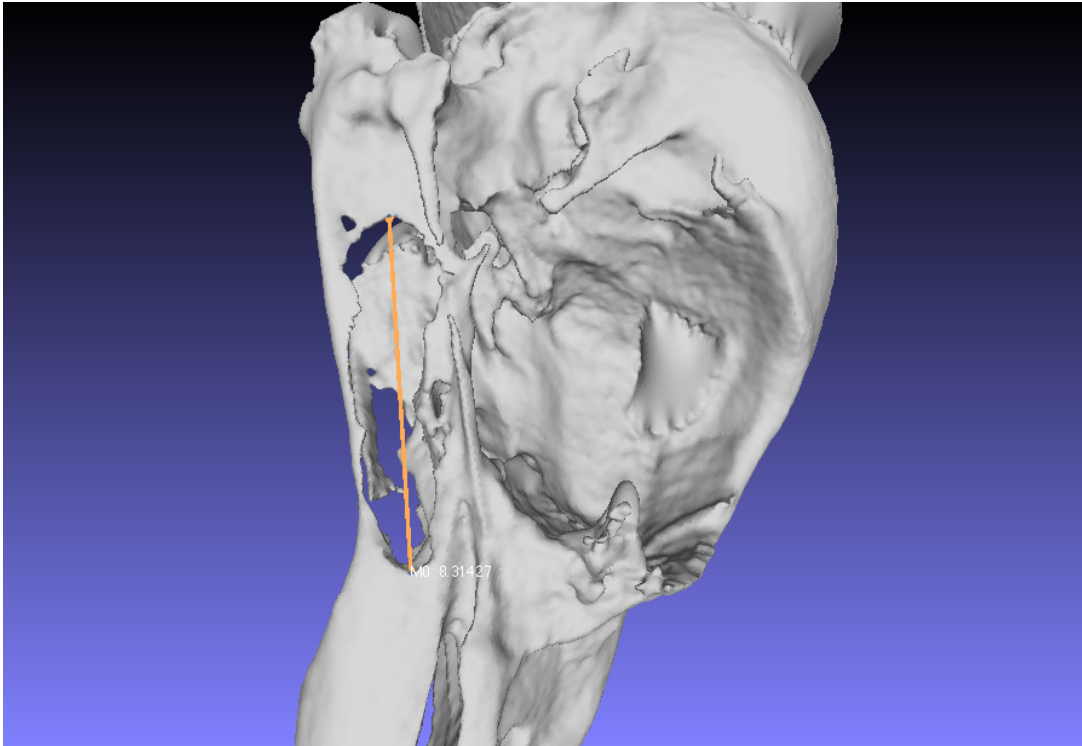


Figure S3: Pictures showing measurements of beak and head dimensions on 3D models. a) Total head length, b) head width, c) head depth, d) beak length, e) beak width and f) beak depth.

a)



b)

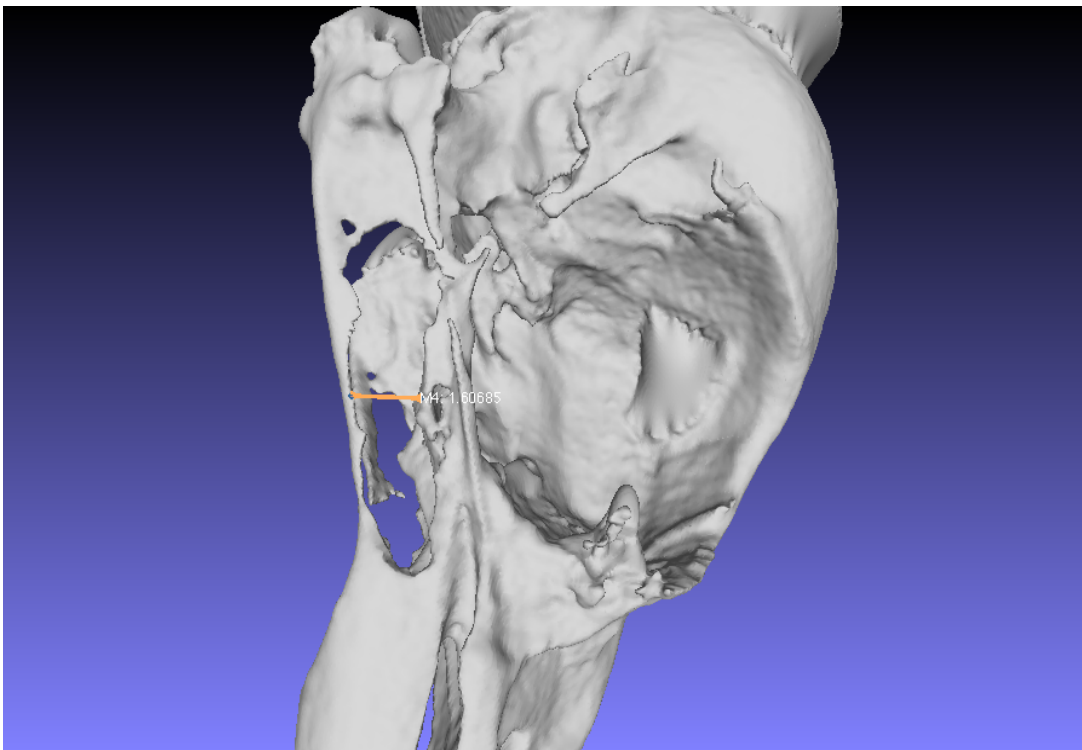


Figure S4: Measurement of muscle cavities. A) measuring major diameter, B) measuring minor diameter.

PCA

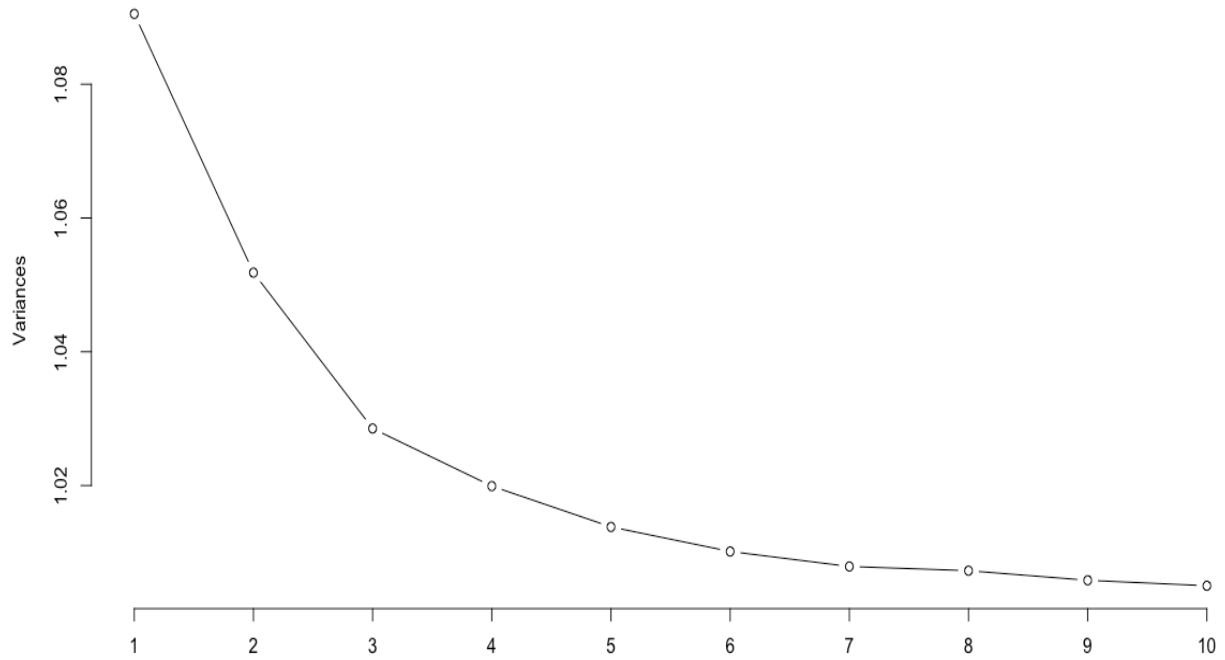
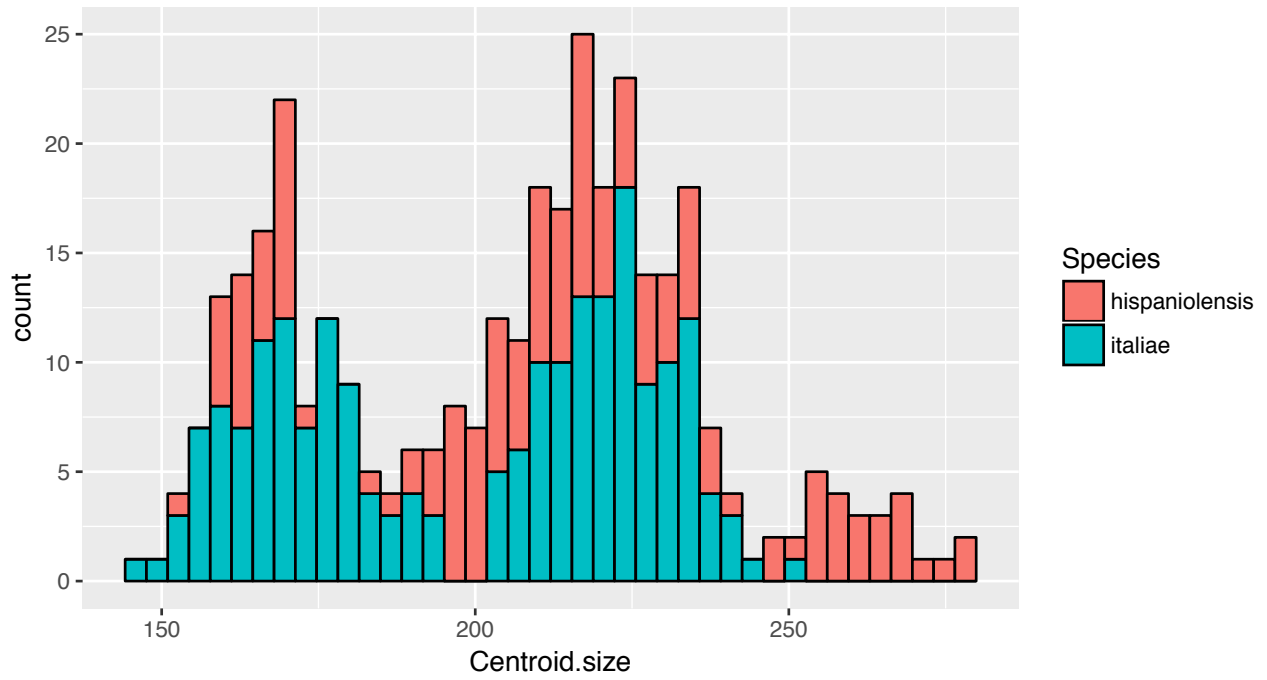


Figure S5: The following plot is a summary from a PCA / relative warp analysis, showing the variances (y-axis) associated with the RWs (x-axis). From the broken stick criteria I decided to retain three RWs for further analysis.

a)



b)

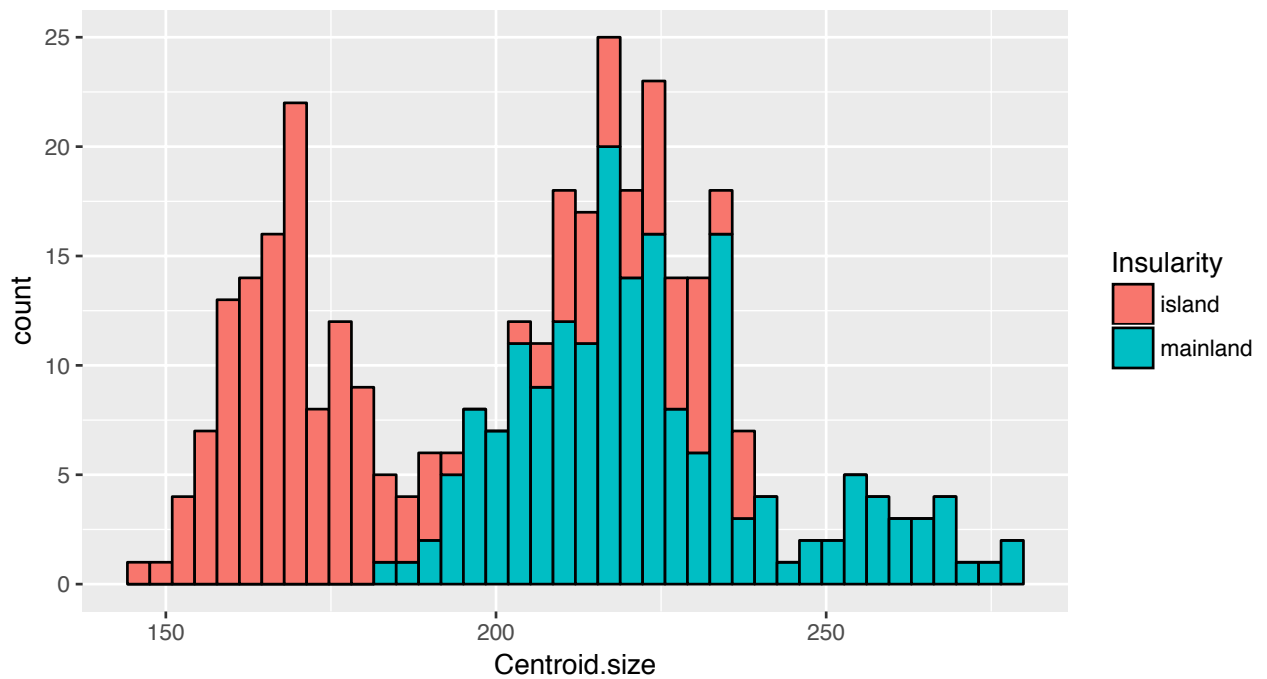


Figure S6: histograms coloured by a) species and b) insularity, showing how the proportion of different beak sizes vary within these two groups.

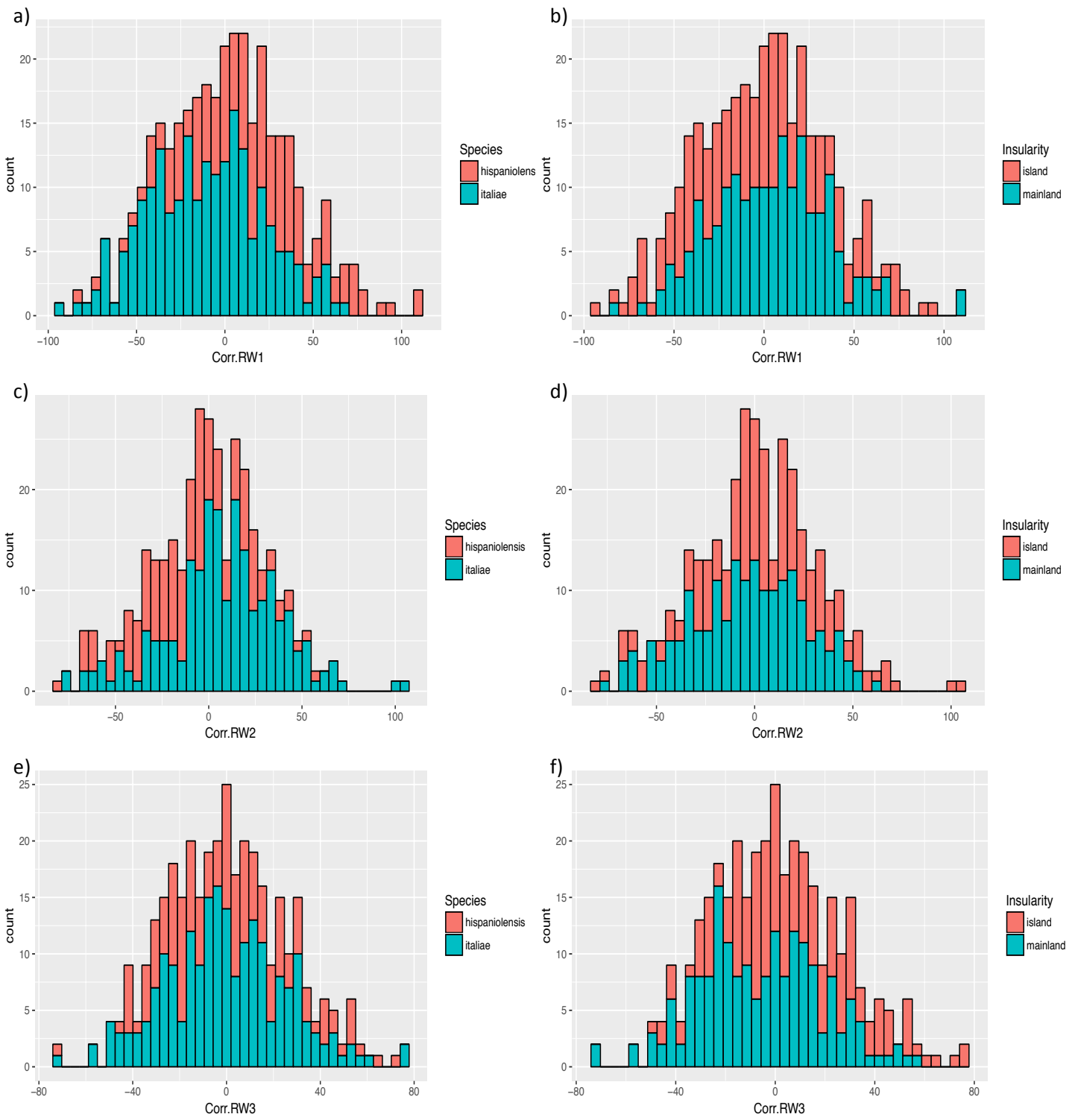


Figure S7: These histograms are intended to be a visual representation of variation in the components of shape *RW1* (a&b), *RW2* (c&d) and *RW3* (e&f) among the individuals coloured by species and insularity.

Appendix II

Supplementary tables

Table S1: Overview of all populations of individuals included in the analyses.

Location	Species	Group	Males	Females
Corsica	<i>P. italiae</i>	Island	15	15
Crete	<i>P. italiae</i>	Island	15	15
Sicily	<i>P. italiae</i>	Island	15	15
Malta	<i>P. italiae</i>	Island	21	12
Crotone	<i>P. italiae</i>	Mainland	15	15
Guglionesi	<i>P. italiae</i>	Mainland	15	15
Rimini	<i>P. italiae</i>	Mainland	13	11
Lago Salso	<i>P. hispaniolensis</i>	Mainland	25	25
Badajoz	<i>P. hispaniolensis</i>	Mainland	12	9
Kazakhstan	<i>P. hispaniolensis</i>	Mainland	13	14
Sardinia	<i>P. hispaniolensis</i>	Island	15	14
Tenerife	<i>P. hispaniolensis</i>	Island	7	11

