

# Genomic contingencies and beak shape variation in a hybrid species

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## 25 Abstract

26 Hybridization is increasingly recognized as a potent evolutionary force. Though  
 27 additive genetic variation and novel combinations of parental genes theoretically  
 28 increase the potential for hybrid species to adapt, few empirical studies have  
 29 investigated the adaptive potential within a hybrid species. Here, we investigate  
 30 factors promoting phenotypic divergence using genomically diverged island  
 31 populations of the homoploid hybrid Italian sparrow *Passer italiae* from Crete,  
 32 Corsica, and Sicily. We address whether genomic contingencies, adaptation to climate  
 33 or diet best explain divergence in beak morphology. Populations vary significantly in  
 34 beak morphology, both between and within islands of origin. Temperature seasonality  
 35 best explains population divergence in beak size. Interestingly, beak shape along all  
 36 significant dimensions of variation was best explained by annual precipitation,  
 37 genomic composition and their interaction, suggesting a role for contingencies.  
 38 Moreover, beak shape similarity to a parent species correlates with proportion of the  
 39 genome inherited from that species, consistent with the presence of contingencies. In  
 40 conclusion, adaptation to local conditions and genomic contingencies arising from  
 41 putatively independent hybridization events jointly explain beak morphology in the  
 42 Italian sparrow. Hence, hybridization may induce contingencies and restrict evolution  
 43 in certain directions dependent on the genetic background.

## 44 Introduction

45 Adaptation to divergent ecological niches is a major factor in population divergence  
 46 and speciation (Schluter 2000; Grant and Grant 2008; Schluter 2009). Adaptation in  
 47 key traits where novel morphologies can allow for the invasion of new niches  
 48 (Dumont et al. 2012), are of particular interest since divergence in these can drive  
 49 speciation (Hunter 1998). Key traits can also enable co-existence with closely related  
 50 species (Miraldo and Hanski 2014) and hence spur adaptive radiations (Schluter  
 51 2000), and can generate specious groups, such as birds (Jarvis et al. 2014). The beak  
 52 is such a key trait, since beak shape adaptations have significantly contributed to the  
 53 niche diversity in birds (Mallarino et al. 2012). Variation in beak size- and shape is  
 54 important both for feeding efficiency (Benkman 2002; 2016) and thermoregulation  
 55 (Symonds and Tattersall 2010). It is also affects song (Derryberry et al. 2012), and  
 56 can hence be a target of sexual selection (Huber and Podos 2006). A classical  
 57 example of beak morphology adaptation is the radiation of Darwin's finches on the  
 58 Galapagos Islands, where divergent selection between groups of birds with different  
 59 dietary preferences have caused a dramatic beak shape diversity (Grant and Grant  
 60 2006). Interestingly, hybridization can also generate new beak shapes that allow more  
 61 efficient use of specific dietary resources (Grant and Grant 1996; Lamichhaney et al.  
 62 2015; 2016).

63  
 64 Hybridization is increasingly recognized as an important source of novel genetic  
 65 variation (Mallet 2005; 2007; Abbott et al. 2013). It can spur novel adaptations by  
 66 increasing genomic diversity, and through changing the constraints on the direction of  
 67 evolution. Hybrids are expected to have more additive genetic variation than the  
 68 parental species' genomes, and this increase is highest when the parent species are

69 fixed for different alleles at each locus (Bailey et al. 2013; Seehausen 2013;  
70 Eroukhmanoff et al. 2013b). Furthermore, the mosaic genome from the combination  
71 of the two parental genome complexes (Rieseberg 2003) can give rise either to  
72 phenotypes that are intermediate or mosaic versions of the parents, or transgressive  
73 phenotypes, which are beyond the range of the parental species (Rieseberg et al. 1999;  
74 Dittrich-Reed and Fitzpatrick 2012). The increase in additive genetic variation and the  
75 novel combinations of parental genes may increase the potential for hybrids to adapt  
76 (Rieseberg 2003; Eroukhmanoff et al. 2013b). Interestingly, different hybrid  
77 populations can attain strongly divergent genomic composition (Runemark et al. n.d.).  
78 However, hybrid species can also be subjected to constraints or contingencies  
79 resulting from mosaic patterns of parental inheritance or conditions during initial  
80 hybridization and genome stabilization (Eroukhmanoff et al. 2013b). Moreover,  
81 depending on the type of selection acting on the parent species' phenotypes, hybrid  
82 morphology is expected to be more or less restricted. For traits under stabilizing  
83 selection in parents, hybrids are expected to be free to evolve towards a variety of  
84 different potential fitness optima, even those extending beyond those of the parents  
85 (Bailey et al. 2013). However, when directional selection has contributed to parent  
86 species differences, hybrid phenotypes are predicted to be intermediate of the parent  
87 taxa, and restricted to evolve along the axis of divergence between them (Bailey et al.  
88 2013). This could facilitate convergence towards parental phenotypes (Bailey et al.  
89 2013). In the latter situation, populations of hybrid species could be restricted to trait  
90 values reflecting the relative proportion of the genome inherited from the parent  
91 species. Hence, hybrid populations differing in genomic composition could either be  
92 divergent due to genomic contingencies or to adaptation in response to local selection

93 pressures. Tests for presence of such genomic contingencies in hybrid species have,  
 94 however, rarely been made.

95

96 To address the relative importance of genomic contingencies and ecology for hybrid  
 97 phenotypes, we investigated how diet, climate and genomic composition affect beak  
 98 shape and size in a hybrid species. Our study species, the Italian sparrow, is a  
 99 homoploid hybrid resulting from the interbreeding between the Spanish sparrow  
 100 (*Passer hispaniolensis*) and the house sparrow (*Passer domesticus*) (Hermansen et al.  
 101 2011; ELGVIN et al. 2011; Trier et al. 2014). To be able to address the effect of  
 102 genomic background, we use three island populations of Italian sparrow from each of  
 103 the islands Crete, Corsica and Sicily that show strong differences in genomic  
 104 composition and appear to represent independent hybridization events (Runemark et  
 105 al. n.d.). In the absence of contingencies, populations experiencing the same selection  
 106 pressures are expected to develop similar phenotypes (Ravinet et al. 2012; Runemark  
 107 et al. 2014; 2015). Therefore, if there is strong ecological selection on the beak we  
 108 expect beak size and shape to correlate with diet or climate measures despite  
 109 individuals having different genomic compositions as long as contingencies are not  
 110 important in the system. On the other hand, if contingencies are important we expect  
 111 that island origin (reflecting genomic composition) better explains beak morphology.  
 112 Diet (Grant and Grant 1996; Neto et al. 2016) and climate (Eroukhmanoff et al.  
 113 2013a; Gardner et al. 2016) have previously been found to affect beak morphology,  
 114 but these factors have not been studied in genomically divergent populations.  
 115 Investigating these factors jointly will shed light on whether population differences  
 116 within hybrid species can be adaptive or may be restricted to values along the axis of  
 117 parental divergence. We used stable isotopes as a proxy for diet, a set of climatic

variables previously shown to influence beak size in the Italian sparrow  
(Eroukhmanoff et al. 2013a), and whole genome estimates of relative parental  
proportions from an earlier study on the island populations (Runemark et al. n.d.) to  
address which factors shape phenotypic variation in a hybrid species.

122

## 123 Materials and Methods

124 We sampled three populations of Italian sparrows from each island of Crete, Corsica  
125 and Sicily during the spring 2013 (Fig. 1a). We caught 10-38 birds in each population  
126 (see Supplementary Table 1 for sample sizes and sex) using mist netting, and took  
127 digital pictures of the right side of each birds' head with a Nikon D-500 16.2  
128 megapixel camera. The background was millimeter squared paper, and we ensured  
129 that the head of the bird was not tilted. Geometric morphometrics was used to analyze  
130 beak shape. We used the thin-plate spline based programs developed by (Rohlf 1998)  
131 for file conversion (tpsUTIL) and digitization of landmarks (tpsDIG2). Five  
132 homologous landmarks were placed on the beak, and we drew an outline with 7  
133 equidistant points, i.e. semi-landmarks to further capture beak shape (Supplementary  
134 Figure 1). PAST (Hammer et al. 2001) was used to estimate Relative Warps (RWs)  
135 and centroid size. Relative warps are principal components of shape (Zelditch et al.  
136 2004), and were extracted (n=32) and imported to R for further analysis. All further  
137 statistical analyses were performed in R (team n.d.). As feathers for female stable  
138 isotope analysis were only sampled for one population on each island, we performed  
139 all tests on two additional datasets to ensure that this did not bias our findings. The  
140 two data sets included only set with one population from each island where both  
141 males and females were sampled, and set with all nine populations where only males  
142 were sampled. .

143

144 First, we established whether there were significant differences in beak size and shape  
145 using centroid size and the four RWs deviating from the noise floor (Supplementary  
146 Table 2) as response variables in ANOVA and MANOVA, respectively. We tested  
147 both for the presence of overall population variation and for variation among  
148 populations within islands using models with population nested within island.

149

150 Next, we investigated which factors best explain size and shape variation. We used  
151 stable isotopes as a proxy for dietary differentiation. The combination of  $\delta^{15}\text{N}$  and  
152  $\delta^{13}\text{C}$  isotope ratios provide a comprehensive picture of diet;  $\delta^{15}\text{N}$  differentiation  
153 increases with each trophic level and is indicative of the trophic position in the food  
154 web (reviewed in (Caut et al. 2009).  $\delta^{13}\text{C}$  varies between  $\text{C}^3$  and  $\text{C}^4$ -plants (Fry 2006)  
155 and  $\delta^{13}\text{C}$  ratios in plants decrease with rainfall (Stewart et al. 1995; Ferrio and Voltas  
156 2005); therefore  $\delta^{13}\text{C}$  values are a proxy for dietary source. To obtain stable isotope  
157 values, we sent great covert feathers sampled during spring (March-June; 1 mg +/- 0.2  
158 mg finely cut samples in tin capsules, article no. D1008, Elemental Microanalysis,  
159 Devon, UK) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis at UC Davis Stable Isotope Facility. As the  
160 aim was to examine population differences, and since sparrows feed on a wide variety  
161 of resources, we did not attempt to examine isotopic contents of potential diet items,  
162 but rather whether diet differed. As baseline climatic differences could affect isotopic  
163 contents, we examined whether values clustered within islands. This was not the case  
164 (data not shown), and dietary differences were therefore not overrun by baseline  
165 signatures. We also used climatic factors previously shown to correlate with a beak  
166 size measurements in Italian sparrows (Eroukhmanoff et al. 2013a) as proxies for  
167 local climate. We extracted climate variables; annual temperature, annual

precipitation, temperature seasonality and precipitation seasonality from the Worldclim database (Hijmans et al. 2005) using the R-packages raster (Hijmans and van Etten 2016), rgdal (Bivand et al. 2016) and foreach (Calaway et al. 2015). Population hybrid index estimates were retrieved from (Runemark et al. n.d.). They were based on a whole genome ADMIXTURE analysis (Alexander et al. 2009), and the mean population probability of house sparrow ancestry was used as an index. The genomic hybrid index differs between all islands, and if beak shape similarity to the parent species corresponds to genomic resemblance, this would be an important factor in the models. Thus, these variables were used as explanatory factors in our models.

Centroid size and shape were used as dependent variables. Two models were run for shape: One with only the main axis of divergence, RW1, explaining > 60% of the variation in shape, and another including all four relative warps that deviate from the noise floor. As climate is identical for all individuals within a population whereas diet may vary between individuals within a population, one population level dataset was created to address the effects of both diet and climate, and one individual level dataset solely with individual diet estimates. To test which models best explain size and shape we used a model selection framework based on applicable information criteria.

### *Population level analyses*

For the population level analyses, we first tested which ecological factors best explain population divergence in beak size and shape. For the models with centroid size as dependent variable, AICc and importance were estimated using the R-package MuMIn (Barton 2016). AICc is a version of Akaike's Information Criterion, (Akaike 1974) which is especially suited for small datasets, and importance is the sum of



193 Akaike weights (Wagenmakers and Farell 2004) over all models including the  
194 explanatory variable. The variables with highest importance were used in subsequent  
195 models. We then tested which of all possible models best explained data based on  
196 AICc with sex, hybrid index and their interactions as explanatory variables. The same  
197 model was repeated for size, except RW1, reflecting a change from wide to a narrow  
198 basal part of the beak (Supplementary Figure 2) was used as a response variable. For  
199 the shape analysis including the four main RWs (Supplementary Figure 2), selection  
200 was based on AIC on MANOVA, first using models including only one climate or  
201 diet variable, and then testing if adding sex, hybrid index and/or their interactions  
202 improved the model.

203

#### 204 *Individual level analyses*

205 For the individual level dataset, model selection was performed as in the population  
206 level analyses, but on mixed models with population as a random factor with centroid  
207 size and RW1 as response variables, respectively. We used the lmer command from  
208 the R-package lme4 (Bates et al. 2016) for these analyses. We first tested which of the  
209 ecological variables best explained the model, and then explored whether adding  
210 hybrid index, sex and/or the interactions improved the model in the same manner as  
211 the population level analyses. To retrieve *F*- and *P*-values for the mixed models, we  
212 used the mixed function supplied in the R package afex (Singman et al. 2016).

213

214 For the shape analyses including all four main RWs, the R package MCMCglmm  
215 (Hadfield 2010) was used. When the number of groups is low the posterior  
216 distribution of the variance becomes increasingly tail-heavy, causing poor mixing of  
217 the MCMC chain. To mitigate this, we used parameter expansion (Hadfield 2010), on

218 the MCMCglmm algorithm to speed up the rate of convergence in the MCMC chain.  
 219 This entails using information from a run with an uninformative prior on the same  
 220 data to choose proper values for the prior means and prior covariance matrix (alpha  
 221 mean and variance) to be specified in the parameter expanded run. We then used a  
 222 Cauchy prior as recommended for the parameter expanded run (Hadfield 2010), with  
 223 the alpha variance set to the square of the standard deviation in the posterior  
 224 distribution from the uninformative prior. The posterior sampling was run for 200 000  
 225 iterations with a burn-in of 40 000 and a thinning of 100. The MCMC-chain was  
 226 plotted and inspected for proper mixing, and autocorrelation remained low ( $< 0.1$ )  
 227 between successive samples in the chain. Three chains were run to ensure consistency  
 228 in parameter estimation. Model selection for these models was performed based on  
 229 DIC.

230

231 Finally, we addressed whether the variation among Italian sparrow populations is  
 232 aligned with the axis of parental divergence, or if the phenotypic values attained  
 233 deviate from this. We used PAST (Hammer et al. 2001) to estimate RWs and centroid  
 234 size for a dataset including both the Italian sparrow populations and one reference  
 235 population of each parent species. For size, we used an ANOVA with centroid size  
 236 from this analysis as response variable, and species as a grouping factor. For shape,  
 237 we performed a discriminant function analysis based on parental values only in PAST  
 238 (Hammer et al. 2001), and then transformed RW scores for the Italian sparrow  
 239 individuals into discriminant scores using the factor loadings of the discriminant axis  
 240 between parent species. We then tested whether the position along the score axis was  
 241 affected by hybrid index, thus reflecting a correlation between genomic and  
 242 phenotypic similarity to the parent species using a linear regression. This will shed

light on whether genomic composition constrains phenotypic adaptation within the Italian sparrow.

## Results

Sex did not significantly affect beak size or shape, and was not included in any of the best models for the dataset with both females and males in all populations (Supplementary Table 3), therefore we proceeded with our analyses using the full dataset.

### *Population divergence in hybrid index, beak size and beak shape*

Independent island populations from Crete, Corsica and Sicily differ in the proportion of the genome inherited from house sparrow (Runemark et al. n.d.) (Figure 1b). Beak size varies between populations (size:  $F_{8,127}=18.75$ ;  $P=2e-16$ ; shape:  $F_{32,508}=2.81$ ;  $P=1.05e-06$ ; (Fig. 1c-e). These differences persist if population is nested within island both for size (island:  $F_{2,127}=22.56$ ,  $P=4.12e-09$ ; population nested within island  $F_{6,127}=17.48$ ,  $P=1.13e-14$ ) and shape (island:  $F_{8,250}=6.94$ ,  $P=2.97e-08$ ; population nested within island:  $F_{24,508}=1.69$ ,  $P=0.022$ ). The presence of significant variation within islands shows that differences do not merely reflect genomic composition (Fig. 1c-e), but are influenced by other factors.

### *Beak size*

Temperature seasonality was the factor best explaining population divergence in beak size, and had  $\Delta AICc$  of more than 6 to the second best model (Tables 1-2; Figure 2a). As all individuals in a population experience the same climate, we also tested which factors affect beak size at the individual level, excluding climate variables. The best

268 model for individual variation includes  $\delta^{15}\text{N}$ , genomic hybrid index and the  
 269 interaction between these factors (Figure 2b), reflecting that  $\delta^{15}\text{N}$  changes do not  
 270 affect individual beak size in the same manner across islands. Two models were  
 271 within  $\Delta\text{AICc}$  of 2 of this best model (Tables 1-2). One included sex and the  
 272 interaction between sex and hybrid index in addition to the abovementioned factors,  
 273 whereas the other included  $\delta^{15}\text{N}$ , genomic hybrid index, sex and the interaction  
 274 between genomic hybrid index and sex. Hence, patterns of individual beak size  
 275 variation are complex and no clear best explanatory variables emerge.

276

#### 277 *Beak shape: the major axis of divergence*

278 The best model for population divergence along the main axis of shape variation,  
 279 reflecting a change from a wide to a narrow basal part of the beak (Supplementary  
 280 Figure 2), included only  $\delta^{13}\text{C}$ , and explained the data significantly better than the  
 281 second best model ( $\Delta\text{AICc} > 2$ ; Tables 1-2; Figure 2c). Individual level variation in  
 282 beak shape was also best explained by  $\delta^{13}\text{C}$  differences (Figure 2d), with  $\Delta\text{AICc}$  to  
 283 the second best model of  $>4$  (Tables 1-2).

284

#### 285 *Beak shape: all significant axes of divergence*

286 The first four RWs reflecting beak shape variation deviated from the noise floor  
 287 (Supplementary Table 1; Supplementary Figure 2). The model best explaining this  
 288 shape variation included annual precipitation, genomic hybrid index and the  
 289 interaction between these terms (Supplementary Figure 3a-d; Tables 1-2). We also  
 290 tested which factors affect beak shape at the individual level, excluding climate  
 291 variables. Individual shape differences were best explained by a model including only  
 292  $\delta^{15}\text{N}$  (Supplementary Figure 3e-h; Tables 1-2).

293

# 294 *Parental phenotypes and the extent of genomic contingencies*

295 We estimated the axis discriminating the parent species based on the four RWs  
 296 deviating from the noise floor (Supplementary Table 4), and scored the hybrids on  
 297 this axis. We found a significant correlation between hybrid index and score along the  
 298 parental axis of variation (estimate=9.08±2.95;  $F_{1,199}=9.50$ ;  $P=0.002$ ;  $R^2=0.05$ ),  
 299 implying that populations that are genomically similar to house sparrows also have a  
 300 more house sparrow like beak shape. Breaking up shape into the individual axes of  
 301 variation, we find intermediacy and hence potential constraints only in the third and  
 302 fourth shape component, while Italian sparrows attain values outside of the parental  
 303 range for the first and second (Supplementary Figure 3). Centroid size was nearly  
 304 significantly correlated with hybrid index (estimate=0.11±0.058;  $F_{1,199}=3.64$ ;  
 305  $P=0.058$ ;  $R^2=0.01$ ; Supplementary Figure 4).

306

## 307 Discussion

308 Both beak size and beak shape vary significantly between Italian sparrow populations,  
 309 as well as between islands. Interestingly size and shape are not best explained by the  
 310 same factors at the population level. While beak size is strongly affected by  
 311 temperature seasonality, the main axis of beak shape variation is best explained by  
 312 variation in carbon isotopic ratios. Although ecological factors best explain beak  
 313 shape along the major axis of variation, beak shape divergence for all significant axes  
 314 of variation is significantly affected by genomic hybrid index, reflecting island of  
 315 origin and potentially contingencies. The fact that there is a correlation between  
 316 position along the discriminant axis separating the parent species' shape and the  
 317 genomic similarity to the parent species is also consistent with a role for

318 contingencies. Patterns of individual axes of variation do, however, suggest that there  
319 may be contingencies in some, but not all, directions of variation.

320

321 There are various reasons temperature regime could affect beak size. Temperature  
322 variation could affect the size spectrum of the available diet. There is mounting  
323 evidence that beaks play an important role in thermoregulation, as blood flow through  
324 the network of supportive blood vessels beneath the keratinized surface is augmented  
325 at high temperatures and restricted in the cold (Symonds and Tattersall 2010;  
326 Campbell-Tennant et al. 2015). For instance, beak sizes vary as expected from Allen's  
327 rule (Allen 1877), which posits that the relative size of body extremities is smaller in  
328 colder environments, for ectotherms to reduce thermoregulatory costs (Symonds and  
329 Tattersall 2010). Even if the effect of smaller beaks cannot explain a high proportion  
330 of total heat loss, as in the toucan (Tattersall et al. 2009), using the beak for  
331 thermoregulation could potentially be important during summers on these arid  
332 Mediterranean islands. Furthermore, the fitness advantage of large bill size could  
333 differ depending on local temperature profiles and humidity, even in small passerine  
334 birds (Gardner et al. 2016). Individual level divergence is affected by a more complex  
335 combination of factors, and no clear best model emerged, although both nitrogen  
336 isotopic composition and genomic hybrid index were included in all models. This  
337 relationship could therefore be complex and involve many factors of small effect or  
338 variables that we have not measured.

339

340 Annual precipitation pattern is the ecological factor best explaining beak shape.  
341 Interestingly, both general beak shape as well as how precipitation patterns affect  
342 beak shape, are significantly affected by genomic hybrid index. Precipitation patterns

could affect seed size (Moles et al. 2005) and the hardness of seeds (Mohamed-Yasseen et al. 1994). Seed size is known to affect beak size evolution in passerines (Grant and Grant 1993), including in sparrows (Riyahi et al. 2013). In addition, beak shape affects bite force (Herrel et al. 2005), and the correlation between annual precipitation and beak shape could reflect adaptation to deal with harder seeds. As the significant genomic hybrid index term and interaction between genomic hybrid index and annual precipitation suggest, the significant genomic hybrid index term may reflect a genomic contingency in form of an island specific beak shape and beak shape variation structure implying responses to the same selective environment differ. The same increase in annual precipitation does hence not result in the same shape response across the islands. The correlation between genomic similarity to a parent species and shape similarity to that species suggests that this potentially could be due to genomic contingencies.

Nitrogen isotopic composition is the only factor in the model that best explained individual beak shape differences along all dimensions. Differentiation in isotopic composition between a consumer and dietary items is low, predictable and conserved across trophic levels (i.e. typically 1 ‰ difference) (Peterson and Fry 1987). Therefore it allows for accurate discrimination of dietary contributions from different nitrogen sources (Newsome et al. 2007). Thus stable isotope signatures may reflect dietary differences in birds, which in turn may also influence beak shape (Neto et al. 2016). Beak specialization for foraging in different selective regimes are well-established in birds (Grant and Grant 1996; Benkman 2002; 2016). The Italian sparrow is an opportunistic human commensal species, which feeds both on wild seeds, crop plants and insects. Specialization enabling foraging on prey from different

368 trophic levels, or differences in proportions in individual diet within populations  
369 could potentially explain the effect of nitrogen isotopic composition on beak shape.  
370 Although all sampled individuals were breeding adults, stable isotope composition  
371 reflects diet at molt the previous autumn, and the birds could have belonged to  
372 different age classes at this point in time.  
373  
374 Interestingly, one of the genes that was most divergent between Crete and Sicily in a  
375 study of the genomic composition of the island populations was FGF10 (Runemark et  
376 al. n.d.), a candidate gene for beak shape shown to be important in beak divergence in  
377 Darwin's Finches (Lamichhaney et al. 2015). Together with the ecological  
378 differences and correlated beak morphology divergence, this genomic signature of  
379 selection on the genes affecting the phenotype makes a strong case that the sorting of  
380 parental variants allows hybrid species to locally adapt.  
381  
382 The island populations of Italian sparrow from this study have contingencies in the  
383 proportion of inheritance from each parent species (Runemark et al. n.d.), resulting  
384 from mosaic patterns of parental inheritance or conditions during initial hybridization  
385 and genome stabilization c.f. (Eroukhmanoff et al. 2013b). We find that hybrid taxa  
386 are intermediate between parent species for both size and shape, although not for all  
387 shape components. This is consistent with the pattern predicted for traits where  
388 directional selection contributes to parent species differences in which hybrids are  
389 expected to differentiate along the parental axis of divergence (Bailey et al. 2013).  
390 Furthermore, the proportion of the parental genome inherited from each species, here  
391 measured as hybrid index, was significantly correlated with similarity to the parent  
392 species beak shape. Taken together, that genomic hybrid index is involved in the best



393 model explaining population divergence in beak shape and is significantly correlated  
 394 with position along the parental axis of variation suggests that constraints may affect  
 395 evolutionary trajectories and evolutionary potential following hybridization. There  
 396 are, however, two shape dimensions that are transgressive. This demonstrates a  
 397 release of parental constraint for some components of shape, and is consistent with  
 398 the predicted patterns of divergence for traits under stabilizing selection in the parents  
 399 (Bailey et al. 2013).

400

401 In conclusion, this study provides evidence of adaptive local divergence within a  
 402 hybrid species, but shows that genomic contingencies could affect the evolutionary  
 403 potential to respond to selection in a hybrid species. Size and shape divergence are  
 404 best explained by different selective factors, with temperature patterns affecting size  
 405 and precipitation patterns and proportion inherited from different parent species  
 406 predicting shape. Interestingly, we only find evidence for constraint in shape and not  
 407 in size, consistent with patterns in the fossil record suggesting that size is more  
 408 evolvable than shape (Hunt 2007).

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## 632 Tables

633 **Table 1.** Model selection table. Dependent variable, replicated unit (reflecting  
 634 whether the analysis was performed at the population or individual level), explanatory  
 635 factors included in the model, whether a random factor was included and the AICc  
 636 values and, when relevant, importance values the model selection was based on are  
 637 included. Each set of tests has its own headline, and the best model is presented in  
 638 bold text.

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Dependent variable(s)	Replicated unit	Factor	Random Factor	AICc	Importance AICc
<b>Size analyses at the population level</b>					
Size	Population	$\delta^{13}\text{C}$	None	3.63	0.085
Size	Population	$\delta^{15}\text{N}$	None	3.1	0.129
Size	Population	Annual_Temp	None	2.3	0.18
Size	Population	Annual_Prec	None	2.7	0.155
<b>Size</b>	<b>Population</b>	<b>Temp_Seas</b>	<b>None</b>	<b>-4</b>	<b>0.84</b>
Size	Population	Prec_Seas	None	3	0.135
Size	Population	Island	None	7.2	0.019
<b>Size analyses at the individual level</b>					
Size	Individuals	$\delta^{13}\text{C}$	Population	-140.6	<0.01
Size	Individuals	$\delta^{15}\text{N}$	Population	-142.5	0.0126
Size	<b>Individuals</b>	$\delta^{15}\text{N}+\text{HI}+ \delta^{15}\text{N}\times\text{HI}$	<b>Population</b>	<b>-166.58</b>	
Size	Individuals	$\delta^{15}\text{N}+\text{Sex}+\text{HI}+ \delta^{15}\text{N}\times\text{HI}+ \text{HI}\times\text{Sex}$	Population	-165.59	
Size	Individuals	$\delta^{15}\text{N}+\text{HI}+\text{Sex}+\text{HI}\times\text{Sex}$	Population	-164.61	
<b>Univariate shape analyses at the population level</b>					
<b>RW1</b>	<b>Population</b>	$\delta^{13}\text{C}$	<b>None</b>	<b>-42.4</b>	<b>0.737</b>
RW1	Population	$\delta^{15}\text{N}$	None	-36	0.103
RW1	Population	Annual_Temp	None	-41.8	0.678
RW1	Population	Annual_Prec	None	-36.1	0.106
RW1	Population	Temp_Seas	None	-35.7	0.088
RW1	Population	Prec_Seas	None	-40.2	0.476
RW1	Population	Island	None	-36	0.103
<b>Univariate shape analyses at the individual level</b>					
<b>RW1</b>	<b>Individuals</b>	$\delta^{13}\text{C}$	<b>Population</b>	<b>-337.04</b>	<b>0.176</b>
RW1	Individuals	$\delta^{15}\text{N}$	Population	-311.9	<0.01
RW1	Individuals	$\delta^{13}\text{C}+\text{HI}$	Population	-335.81	
RW1	Individuals	$\delta^{13}\text{C}+\text{Sex}$	Population	-334.94	
<b>Multivariate shape analyses at the population level</b>					
RW1-4	Population	$\delta^{13}\text{C}$	None	-240.09	
RW1-4	Population	$\delta^{15}\text{N}$	None	-231.09	
RW1-4	Population	Annual_Temp	None	-239.47	
RW1-4	Population	Annual_Prec	None	-243.55	
RW1-4	Population	Temp_Seas	None	-239.42	
RW1-4	Population	Prec_Seas	None	-237.15	
<b>RW1-4</b>	<b>Population</b>	<b>Annual_Prec<math>\times</math>HI</b>	<b>None</b>	<b>-312.43</b>	
RW1-4	Population	Annual_Prec+HI	None	-284.05	
<b>Multivariate shape analyses at the individual level</b>					
RW1-4	Individuals	$\delta^{13}\text{C}$	Population	5792.000	
<b>RW1-4</b>	<b>Individuals</b>	$\delta^{15}\text{N}$	<b>Population</b>	<b>5788.888</b>	
RW1-4	Individuals	$\delta^{15}\text{N}+ \text{HI}$	Population	5790.069	
RW1-4	Individuals	$\delta^{15}\text{N}+ \delta^{13}\text{C}$	Population	5788.191	
RW1-4	Individuals	$\delta^{15}\text{N}\times\text{HI}$	Population	5792.283	
RW1-4	Individuals	$\delta^{15}\text{N}\times\delta^{13}\text{C}$	Population	5789.635	
RW1-4	Individuals	$\delta^{15}\text{N}\times\delta^{13}\text{C}\times\text{HI}$	Population	5792.25	
RW1-4	Individuals	$\delta^{15}\text{N}+ \delta^{13}\text{C}+ \text{HI}$	Population	5790.186	
RW1-4	Individuals	$\delta^{15}\text{N}\times\delta^{13}\text{C}+ \text{HI}$	Population	5790.897	

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644 **Table 2.** Properties of the best models. *F*-values, degrees of freedom, *P*-values (for lm  
645 and lmer models) and pmcmc values (for the MCMCglmm model) and model  $R^2$  for  
646 the models where it is applicable.

Dependent variable	Factor	Estimate	<i>F</i>	DF, error DF	<i>P</i> /pmcmc	Model $R^2$
<b>Population level analyses</b>						
<b>Size</b>	Temperature seasonality	0.052	10.26	1, 7	0.015	0.5945
<b>Warp1</b>	$\delta^{13}\text{C}$		8.01	1, 7	0.025	0.467
<b>Warp1-4</b>	Annual precipitation	-0.002	2.78	5, 3	0.21	0.8225
	HI	0.025				
	Annual precipitation×HI	-4.56e-05				
<b>Individual level analyses</b>						
<b>Size</b>	$\delta^{15}\text{N}$	-0.092	2.94	1, 128.89	0.09	0.7209
	HI	-1.28	2.72	1, 43.75	0.11	
	$\delta^{15}\text{N} \times \text{HI}$	0.16	2.89	1, 128.95	0.09	
<b>Warp1</b>	$\delta^{13}\text{C}$		4477 4.00	1, 29	0.008	0.0712
<b>Warp1-4</b>	$\delta^{15}\text{N}$	8.41	NA	1, 129	0.0368	NA

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## 648 Figures

649 **Figure 1.** Description of the study system and beak morphology divergence. The  
 650 Italian sparrow is a hybrid between the house sparrow and the Spanish sparrow. **A)**  
 651 Independent, genetically divergent populations are found on the islands of Crete,  
 652 Corsica and Sicily (Runemark et al. n.d.). Three populations were sampled from each  
 653 island, see Supplementary Table 1 for coordinates. **B)** Hybrid index, e.g. the  
 654 probability of house sparrow origin based on whole genome data, differs between  
 655 populations with Crete being most house-like and Sicily most Spanish-like. **C)** Pair-  
 656 wise mean beak shape differences between populations (size differences are scaled).  
 657 **D)** Population divergence in size is not merely reflecting island of origin. **E)** The  
 658 major axis of shape variation is not predicted by island of origin either.

659  
 660 **Figure 2.** Factors best explaining size and shape variation. **A)** Temperature  
 661 seasonality is the best predictor of centroid size at the population level, and the  
 662 relationship is highly significant ( $F_{1,7}=10.26$  ;  $P=0.015$ ;  $R^2=0.59$ ). **B)**  $\delta^{13}\text{C}$  best  
 663 explained population divergence along the main axis of variation ( $F_{1,7}=8.01$  ;  
 664  $P=0.025$ ;  $R^2=0.47$ ). **C)** At the individual level, centroid size was best explained by a  
 665 model including both  $\delta^{15}\text{N}$  and genomic hybrid index and their interaction, as the  
 666 relationship between  $\delta^{15}\text{N}$  and centroid size varied between islands (model  $R^2=0.72$ ).  
 667 **D)** Individual level shape divergence along the axis of largest variation was, as for the  
 668 population level, best explained by  $\delta^{13}\text{C}$  ( $R^2=0.07$ ).



