

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

118 variables previously shown to influence beak size in the Italian sparrow
119 (Eroukhmanoff et al. 2013a), and whole genome estimates of relative parental
120 proportions from an earlier study on the island populations (Runemark et al. n.d.) to
121 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 C^3 and 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

168 precipitation, temperature seasonality and precipitation seasonality from the
169 Worldclim database (Hijmans et al. 2005) using the R-packages raster (Hijmans and
170 van Etten 2016), rgdal (Bivand et al. 2016) and foreach (Calaway et al. 2015).
171 Population hybrid index estimates were retrieved from (Runemark et al. n.d.). They
172 were based on a whole genome ADMIXTURE analysis (Alexander et al. 2009), and
173 the mean population probability of house sparrow ancestry was used as an index. The
174 genomic hybrid index differs between all islands, and if beak shape similarity to the
175 parent species corresponds to genomic resemblance, this would be an important factor
176 in the models. Thus, these variables were used as explanatory factors in our models.
177
178 Centroid size and shape were used as dependent variables. Two models were run for
179 shape: One with only the main axis of divergence, RW1, explaining > 60% of the
180 variation in shape, and another including all four relative warps that deviate from the
181 noise floor. As climate is identical for all individuals within a population whereas diet
182 may vary between individuals within a population, one population level dataset was
183 created to address the effects of both diet and climate, and one individual level dataset
184 solely with individual diet estimates. To test which models best explain size and shape
185 we used a model selection framework based on applicable information criteria.

186

187 *Population level analyses*

188 For the population level analyses, we first tested which ecological factors best explain
189 population divergence in beak size and shape. For the models with centroid size as
190 dependent variable, AICc and importance were estimated using the R-package
191 MuMIn (Barton 2016). AICc is a version of Akaike's Information Criterion, (Akaike
192 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

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

245

246 Results

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

251

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

253 Independent island populations from Crete, Corsica and Sicily differ in the proportion
254 of the genome inherited from house sparrow (Runemark et al. n.d.)(Figure 1b). Beak
255 size varies between populations (size: $F_{8,127}=18.75$; $P=2e-16$; shape: $F_{32,508}=2.81$;
256 $P=1.05e-06$; (Fig. 1c-e). These differences persist if population is nested within island
257 both for size (island: $F_{2,127}=22.56$, $P=4.12e-09$; population nested within island
258 $F_{6,127}=17.48$, $P=1.13e-14$) and shape (island: $F_{8,250}=6.94$, $P=2.97e-08$; population
259 nested within island: $F_{24,508}=1.69$, $P=0.022$). The presence of significant variation
260 within islands shows that differences do not merely reflect genomic composition (Fig.
261 1c-e), but are influenced by other factors.

262

263 *Beak size*

264 Temperature seasonality was the factor best explaining population divergence in beak
265 size, and had $\Delta AICc$ of more than 6 to the second best model (Tables 1-2; Figure 2a).
266 As all individuals in a population experience the same climate, we also tested which
267 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 ΔAICc 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 ΔAICc 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

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

356

357 Nitrogen isotopic composition is the only factor in the model that best explained
358 individual beak shape differences along all dimensions. Differentiation in isotopic
359 composition between a consumer and dietary items is low, predictable and conserved
360 across trophic levels (i.e. typically 1 ‰ difference) (Peterson and Fry 1987).
361 Therefore it allows for accurate discrimination of dietary contributions from different
362 nitrogen sources (Newsome et al. 2007). Thus stable isotope signatures may reflect
363 dietary differences in birds, which in turn may also influence beak shape (Neto et al.
364 2016). Beak specialization for foraging in different selective regimes are well-
365 established in birds (Grant and Grant 1996; Benkman 2002; 2016). The Italian
366 sparrow is an opportunistic human commensal species, which feeds both on wild
367 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).

409 Literature cited

410

411 Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J.
412 Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Dieckmann, F.
413 Eroukhmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C.
414 Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M.
415 Möst, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G.
416 Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M. Szymura, R. Väinölä, J. B. W.
417 Wolf, and D. Zinner. 2013. Hybridization and speciation. *J Evol Biol* 26:229–246.

418

419 Akaike, H. 1974. A New Look at the Statistical Model Identification. *IEEE Trans*
420 *Autom Control* 19:716–723.

421

422 Alexander, D. H., J. Novembre, and K. Lange. 2009. Fast model-based estimation of
423 ancestry in unrelated individuals. *Genome Res* 19:1655–1664.

424

425 Allen, J. A. 1877. The influence of Physical conditions in the genesis of species.
426 *Radical Review* 1: 108–140.

427

428 Bailey, R. I., F. Eroukhmanoff, and G.-P. Sætre. 2013. Hybridization and genome
429 evolution II: Mechanisms of species divergence and their effects on evolution in
430 hybrids. *Curr Zool* 59:675–685.

431

432 Barton, K. 2016. Package “MuMIn.” R-package 1–63.

433

434 Bates, D., M. Maechler, B. Bolker, S. Walker, R. Haubo Bojesen Christensen, H.
435 Singman, B. Dai, G. Grothendieck, and P. Green. 2016. Package “lme4.” R-package
436 1–113.

437

438 Benkman, C. W. 2016. Adaptation to Single Resources and the Evolution of Crossbill
439 (*Loxia*) Diversity. *Ecol Monograph* 63:305–325.

440

441 Benkman, C. W. 2002. Divergent selection drivest the adaptive radiation of crossbills.
442 *Evolution* 57:1176–1181.

443

444 Bivand, R., T. Keitt, B. Rowlingson, E. Pebesma, M. Sumner, R. J. Hijmans, and E.
445 Rouault. 2016. Package “rgdal.” R-package 1–53.

446

447 Calaway, R., R. Analytics, and S. Weston. 2015. Package “foreach.” R-package 1–10.

448

449 Campbell-Tennant, D. J. E., J. L. Gardner, M. R. Kearney, and M. R. E. Symonds.
450 2015. Climate-related spatial and temporal variation in bill morphology over the past
451 century in Australian parrots. *J Biogeogr.* 42:1163–1175.

452

453 Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (Δ
454 $15N$ and $\Delta 13C$): the effect of diet isotopic values and applications for diet
455 reconstruction. *J Appl Ecol* 46:443–453.

456

- 457 Derryberry, E. P., N. Seddon, S. Claramunt, J. A. Tobias, A. Baker, A. Aleixo, and R.
458 T. Brumfield. 2012. Correlated evolution of beak morphology and song in the
459 neotropical woodcreeper radiation. *Evolution* 66:2784–2797.
460
461 Dittrich-Reed, D. R., and B. M. Fitzpatrick. 2012. Transgressive Hybrids as Hopeful
462 Monsters. *Evol Biol* 40:310–315.
463
464 Dumont, E. R., L. M. Dávalos, A. Goldberg, S. E. Santana, K. Rex, and C. C. Voigt.
465 2012. Morphological innovation, diversification and invasion of a new adaptive zone.
466 *Proc Biol Sci* 279:1797–1805.
467
468 Elgvin, T. O., J. S. Hermansen, A. Fijarczyk, T. Bonnet, T. Borge, S. A. Saether, K.
469 L. Voje, and G.-P. Sætre. 2011. Hybrid speciation in sparrows II: a role for sex
470 chromosomes? *Mol Ecol* 20:3823–3837.
471
472 Eroukhmanoff, F., J. S. Hermansen, R. I. Bailey, S. A. Saether, and G.-P. S. A. tre.
473 2013a. Local adaptation within a hybrid species. *Heredity* 111:286–292.
474
475 Eroukhmanoff, F., R. I. Bailey, and G.-P. Sætre. 2013b. Hybridization and genome
476 evolution I: The role of contingency during hybrid speciation. *Curr Zool* 59:667–674.
477
478 Ferrio, J. P., and J. Voltas. 2005. Carbon and oxygen isotope ratios in wood
479 constituents of *Pinus halepensis* as indicators of precipitation, temperature and vapour
480 pressure deficit. *Tellus* 57B:164–173.
481
482 Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York.
483
484 Gardner, J. L., M. R. E. Symonds, L. Joseph, K. Ikin, J. Stein, and L. E. B. Kruuk.
485 2016. Spatial variation in avian bill size is associated with humidity in summer among
486 Australian passerines. *Clim Change Respons* 3:1–11.
487
488 Grant, B. R., and P. R. Grant. 1993. Evolution of Darwin's Finches Caused by a Rare
489 Climatic Event. *Proc R Soc Lond B Biol Sci* 251:111–117.
490
491 Grant, P. R., and B. R. Grant. 2006. Evolution of Character Displacement in Darwin's
492 Finches. *Science* 313:224–226.
493
494 Grant, P. R., and B. R. Grant. 1996. High Survival of Darwin's Finch Hybrids: Effects
495 of Beak Morphology and Diets. *Ecology* 77:500–509.
496
497 Grant, P. R., and B. R. Grant. 2008. *How and why species multiply*. Princeton
498 University Press, Princeton.
499
500 Hadfield, J. 2010. MCMC Methods for Multi-response Generalized Linear Mixed
501 Models: The MCMCglmm R Package. *J Stat Softw* 33:1–22.
502
503 Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological statistics
504 software package for education and data analysis. *Paleontol Electronica* 4:1–9.
505

- 506 Hermansen, J. S., S. A. Sæther, T. O. Elgvin, T. Borge, E. Hjelle, and G.-P. Sætre.
507 2011. Hybrid speciation in sparrows I: phenotypic intermediacy, genetic admixture
508 and barriers to gene flow. *Mol Ecol* 20:3812–3822.
509
- 510 Herrel, A., J. Podos, S. K. Huber, and A. P. Hendry. 2005. Bite performance and
511 morphology in a population of Darwin's finches: implications for the evolution of
512 beak shape. *Funct Ecology* 19:43–48.
513
- 514 Hijmans, R. J., and J. van Etten. 2016. Raster. R-package 1–244.
515
- 516 Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high
517 resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–
518 1978.
519
- 520 Huber, S. K., and J. Podos. 2006. Beak morphology and song features covary in a
521 population of Darwin's finches (*Geospiza fortis*). *Biol J Linn Soc* 88:489–498.
522
- 523 Hunt, G. 2007. The relative importance of directional change, random walks, and
524 stasis in the evolution of fossil lineages. *Proc Natl Acad Sci U S A* 104:18404–18408.
525
- 526 Hunter, J. P. 1998. Key innovations and the ecology of macroevolution. *Trends Ecol*
527 *Evol* 13:31–36.
528
- 529 Jarvis, E. D., S. Mirarab, A. J. Aberer, B. Li, and P. Houde et al. 2014. Whole-
530 genome analyses resolve early branches in the tree of life of modern birds. *Science*
531 346:1320-1331.
532
- 533 Lamichhaney, S., F. Han, J. Berglund, C. Wang, M. S. Almén, M. T. Webster, B. R.
534 Grant, P. R. Grant, and L. Andersson. 2016. A beak size locus in Darwin's finches
535 facilitated character displacement during a drought. *Science* 24:284–293.
536
- 537 Lamichhaney, S., J. Berglund, M. S. Almén, K. Maqbool, M. Grabherr, A. Martinez-
538 Barrio, M. Promerová, C.-J. Rubin, C. Wang, N. Zamani, B. R. Grant, P. R. Grant, M.
539 T. Webster, and L. Andersson. 2015. Evolution of Darwin's finches and their beaks
540 revealed by genome sequencing. *Nature* 518:371–375. Nature Publishing Group.
541
- 542 Mallarino, R., O. Campas, J. A. Fritz, K. J. Burns, O. G. Weeks, M. P. Brenner, and
543 A. Abzhanov. 2012. Closely related bird species demonstrate flexibility between beak
544 morphology and underlying developmental programs. *Proc Natl Acad Sci U S A*
545 109:16222–16227.
546
- 546 Mallet, J. 2007. Hybrid speciation. *Nature* 446:279–283.
547
- 548 Mallet, J. 2005. Hybridization as an invasion of the genome. *Trends Ecol Evol*
549 20:229–237.
550
- 551 Miraldo, A., and I. A. Hanski. 2014. Competitive Release Leads to Range Expansion
552 and Rampant Speciation in Malagasy Dung Beetles. *Syst Biol* 63:480–492.
553
- 553 Mohamed-Yasseen, Y., S. A. Barringer, W. E. Splittstoesser, and S. Costanza. 1994.
554 The role of seed coats in seed viability. *Bot. Rev* 60:426–439. Springer-Verlag.

- 555 Moles, A. T., D. D. Ackerly, C. O. Webb, J. C. Tweddle, J. B. Dickie, A. J. Pitman,
556 and M. Westoby. 2005. Factors that shape seed mass evolution. *Proc Natl Acad Sci U*
557 *S A* 102:10540–10544.
558
- 559 Neto, J. M., L. de Oliveira Gordinho, B. Vollot, M. Marín, J. S. Monrós, and J.
560 Newton. 2016. Stable isotopes reveal differences in diet among reed bunting
561 subspecies that vary in bill size. *J Avian Biol* 47:1–11.
562
- 563 Newsome, S. D., C. Martinez del Rio, S. Bearhop, and D. L. Phillips. 2007. A niche
564 for isotopic ecology. *Front Ecol Environ* 5:429–436.
565
- 566 Peterson, B. J., and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. *Ann Rev Ecol*
567 *Syst* 18:293–320.
568
- 569 Ravinet, M., P. A. Prodöhl, and C. Harrod. 2012. Parallel and nonparallel ecological,
570 morphological and genetic divergence in lake -stream stickleback from a single
571 catchment. *J Evol Biol* 26:186–204.
572
- 573 Rieseberg, L. H. 2003. Major Ecological Transitions in Wild Sunflowers Facilitated
574 by Hybridization. *Science* 301:1211–1216.
575
- 576 Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation,
577 adaptation and speciation. *Heredity* 83:363–372.
578
- 579 Rohlf, J. F. 1998. On Application of Geometric Morphometrics to Studies of
580 Ontogeny and Phylogeny. *Syst Biol* 47:147–158.
581
- 582 Runemark, A., C. N. Trier, F. Eroukhanoff, J. S. Hermansen, M. Matschiner, M.
583 Ravinet, and G.-P. Sætre. n.d. Variation and constraint in hybrid genome formation.
584 In review, *Nature Ecol Evol*
585
- 586 Runemark, A., K. Sagonas, and E. I. Svensson. 2015. Ecological explanations to
587 island gigantism: dietary niche divergence, predation, and size in an endemic lizard.
588 *Ecology* 96:2077–2092.
589
- 590 Runemark, A., M. Brydegaard, and E. I. Svensson. 2014. Does relaxed predation
591 drive phenotypic divergence among insular populations? *J Evol Biol* 27:1676–1690.
592
- 593 Schluter, D. 2009. Evidence for Ecological Speciation and Its Alternative. *Science*
594 323:737–741.
595
- 596 Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press,
597 Oxford.
598
- 599 Seehausen, O. 2013. Conditions when hybridization might predispose populations for
600 adaptive radiation. *J Evol Biol* 26:279–281.
601
- 602 Singman, H., J. Westfall, F. Aust, S. Højsgaard, J. Fox, M. A. Lawrence, and U.
603 Mertens. 2016. Package “afex.” 1–45.
604

- 605 Stewart, G. R., M. H. Turnbull, S. Schmidt, and P. D. Erskine. 1995. ^{13}C Natural
606 Abundance in Plant Communities Along a Rainfall Gradient: a Biological Integrator
607 of Water Availability. *Aust J Plant Physio.* 22:51–6.
608
- 609 Symonds, M. R. E., and G. J. Tattersall. 2010. Geographical Variation in Bill Size
610 across Bird Species Provides Evidence for Allen’s Rule. *Am Nat* 176:188–197.
611
- 612 Tattersall, G. J., D. V. Andrade, and A. S. Abe. 2009. Heat Exchange from the
613 Toucan Bill Reveals a Controllable Vascular Thermal Radiator. *Science* 325:468–
614 470.
615
- 616 R-core team 2013. R: A language and environment for statistical computing. R
617 Foundation for Statistical Computing, Vienna, Austria.
618
- 619 Trier, C. N., J. S. Hermansen, G.-P. Sætre, and R. I. Bailey. 2014. Evidence for Mito-
620 Nuclear and Sex-Linked Reproductive Barriers between the Hybrid Italian Sparrow
621 and Its Parent Species. *PLoS Genet* 10:e1004075–10.
622
- 623 Wagenmakers, E.-J., and S. Farell. 2004. AIC model selection using Akaike weights.
624 *Psychon Bull Rev* 11:192–196.
625
- 626 Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. *Geometric*
627 *Morphometrics for Biologists: A Primer*. Elsevier Academic Press, New York.
628
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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.
639

640

Dependent variable(s)	Replicated unit	Factor	Random Factor	AICc	Importance AICc
Size analyses at the population level					
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
Size	Population	Temp_Seas	None	-4	0.84
Size	Population	Prec_Seas	None	3	0.135
Size	Population	Island	None	7.2	0.019
Size analyses at the individual level					
Size	Individuals	$\delta^{13}\text{C}$	Population	-140.6	<0.01
Size	Individuals	$\delta^{15}\text{N}$	Population	-142.5	0.0126
Size	Individuals	$\delta^{15}\text{N}+\text{HI}+\delta^{15}\text{N}\times\text{HI}$	Population	-166.58	
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	
Univariate shape analyses at the population level					
RW1	Population	$\delta^{13}\text{C}$	None	-42.4	0.737
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
Univariate shape analyses at the individual level					
RW1	Individuals	$\delta^{13}\text{C}$	Population	-337.04	0.176
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	
Multivariate shape analyses at the population level					
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	
RW1-4	Population	Annual_Prec \times HI	None	-312.43	
RW1-4	Population	Annual_Prec+HI	None	-284.05	
Multivariate shape analyses at the individual level					
RW1-4	Individuals	$\delta^{13}\text{C}$	Population	5792.000	
RW1-4	Individuals	$\delta^{15}\text{N}$	Population	5788.888	
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
Population level analyses						
Size	Temperature seasonality	0.052	10.26	1, 7	0.015	0.5945
Warp1	$\delta^{13}\text{C}$		8.01	1, 7	0.025	0.467
Warp1-4	Annual precipitation	-0.002	2.78	5, 3	0.21	0.8225
	HI	0.025				
	Annual precipitation \times HI	-4.56e-05				
Individual level analyses						
Size	$\delta^{15}\text{N}$	-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	
Warp1	$\delta^{13}\text{C}$		4477 4.00	1, 29	0.008	0.0712
Warp1-4	$\delta^{15}\text{N}$	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 $\delta^{13}\text{C}$ ($R^2=0.07$).



