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A geometric morphometric appraisal of beak shape in Darwin's finches

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Abstract

Beak size and shape in Darwin's finches have traditionally been quantified using a few univariate measurements (length, depth, width). Here we show the improved inferential resolution of geometric morphometric methods, as applied to three hierarchical levels: (i) among seven species on Santa Cruz Island, (ii) among different sites on Santa Cruz for a single species (*Geospiza fortis*), and (iii) between large and small beak size morphs of *G. fortis* at one site (El Garrapatero). Our results support previous studies in finding an axis of shape variation (long/shallow/pointy vs. short/deep/blunt) that separates many of the species. We also detect additional differences among species in the relative sizes and positions of the upper and lower mandibles and in curvature of the mandibles. Small-scale, but potentially relevant, shape variation was also detected among *G. fortis* from different sites and between sympatric beak size morphs. These results suggest that adaptation to different resources might contribute to diversification on a single island.

Introduction

Adaptive radiation occurs when a single ancestral species diversifies into multiple species that occupy different ecological niches (Simpson, 1944; Lack, 1947; Grant, 1986; Schluter, 2000). Some classic vertebrate examples include cichlid fishes of the African Great Lakes (Fryer & Iles, 1972), honeycreepers of Hawaii (Amadon, 1950; Freed *et al.*, 1987), threespine stickleback fishes of the northern hemisphere (Bell & Foster, 1994; McKinnon & Rundle, 2002), and Darwin's finches of the Galápagos (Lack, 1947; Bowman, 1961; Grant, 1986). Patterns of morphological diversity in these and other adaptive radiations have long been quantified on the basis of univariate measurements of key traits. These traditional methods usually involve the analyses of linear distance measurements, from which the first combined axis (PC1) is related to overall size, and the other orthogonal axes collectively stand for something that is somehow 'not size' (Klingenberg, 1996). These other axes, however, do not correspond to any particular interpretable 'shape', and the actual form cannot be recovered or visualized.

Some limitations of these traditional univariate measurements can be circumvented through the application of geometric morphometrics, which examines associations among an entire set of landmark points (Rohlf & Marcus, 1993; Marcus *et al.*, 1996; Zelditch *et al.*, 2004). Geometric morphometrics thus define 'shape' explicitly with respect to proportions and relative arrangements of parts that are invariant under scaling, and therefore yield shape variables directly related to geometric associations among parts (Rohlf & Marcus, 1993; Klingenberg, 1996). Two additional advantages of geometric morphometrics are improved statistical power and fewer *a priori* assumptions about what should be measured (Marcus *et al.*, 1996; Zelditch *et al.*, 2004). In recognition of these advantages, geometric morphometrics has now been applied to several aspects of the classic adaptive radiations, such as jaw structure in cichlid fishes (Kocher *et al.*, 2002), body shape in threespine stickleback (Walker, 1997; Taylor *et al.*, 2006), and head and jaw structure in plethodontid salamanders (Adams & Rohlf, 2000). These new analyses often support earlier conclusions based on univariate measurements, and sometimes also generate new insights. For example, geometric morphometrics has revealed formerly cryptic

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differences between beetle taxa (Adams & Funk, 1997) and has detected functionally important differences in anatomical features of salamanders (Adams & Rohlf, 2000).

Surprisingly, some classic adaptive radiations have still not been viewed through the lens of geometric morphometrics. In Darwin's finches, for example, all quantitative evaluations of morphological variation have thus far been based on univariate measurements of beak length, depth and width (Lack, 1947; Bowman, 1961; Grant *et al.*, 1985; Grant, 1986; Hendry *et al.*, 2006). Although Bowman (1961) conducted more comprehensive analyses of beak shape, these descriptions were entirely qualitative. Our aim in the present study was to initiate a geometric morphometric appraisal of beak shape variation in Darwin's finches. We analysed only a subset of the radiation (see below), and so the value of our study is in (1) demonstrating the utility of geometric morphometrics for informing beak shape variation in Darwin's finches, and (2) generating hypotheses for future studies.

Darwin's finches

Darwin's finches comprise 13 (or 14) recognized species on the Galápagos Islands and one on Cocos Island (Lack, 1947; Bowman, 1961; Grant, 1986; Grant & Grant, 2002). In Galápagos, these species can be divided into several major groups that differ markedly in beak form and function. The vegetarian finch (*Platyspiza crassirostris*) feeds mainly on plant matter and has a stout beak used for crushing vegetation. The warbler finches, *Certhidea olivacea* and *Certhidea fusca*, have small and pointed beaks used to probe and manipulate insect prey. The tree finches (*Camarhynchus parvulus*, *Camarhynchus pauper*, *Camarhynchus psittacula*, *Cactospiza heliobates* and *Cactospiza pallida*) feed on a variety of foods (insects, fruits and seeds) and have triangular beaks well suited for tip-biting. The ground finches (*Geospiza magnirostris*, *Geospiza fortis*, *Geospiza fuliginosa*, *Geospiza scandens*, *Geospiza difficilis* and *Geospiza conirostris*) are mostly granivorous, and often use the base of their robust beaks to crush seeds.

Variation in beak shape and function is also evident within these broad groupings, the ground finches of Santa Cruz being an exemplar (Lack, 1947; Bowman, 1961; Grant, 1986; Herrel *et al.*, 2005a). *G. magnirostris* have large/deep beaks used for feeding on large/hard seeds. *G. fuliginosa* have small/shallow beaks used for feeding on small/soft seeds. *G. fortis* have intermediate beaks used for feeding on intermediate seeds. *G. scandens* have long/shallow beaks often used for probing the flowers of *Opuntia* cacti. Although the diet of these species overlaps considerably during the wet season, substantial differences are evident in the dry season, when food tends to be scarce (Abbott *et al.*, 1977; Smith *et al.*, 1978). This dramatic variation in beak form and function provides an interesting substrate for the application of geometric morphometrics.

Our study

We begin by examining beak shape variation among seven species on Santa Cruz Island. As noted above, previous quantitative studies have focused on univariate measurements of beak length, depth and width. These measurements are usually combined into principal components, with beak size (PC1) separating some of the species. This variation has been the subject of many studies, and will therefore not be a focus of our work. The second principal component (PC2) in previous analyses generally captures variation from long/shallow beaks to short/deep beaks. This axis can discriminate some, but not all, of the species (see Fig. 6 in Grant *et al.*, 1985; and Figs 21 and 22 in Grant, 1986). It therefore seems profitable to determine whether geometric morphometrics can reveal additional differences and better discriminate among the species based on beak shape.

We next examine beak shape variation in a single species (*G. fortis*) found at different sites on Santa Cruz, which may inform the geographical context for speciation. The traditional view holds that Darwin's finch speciation begins in allopatry (i.e. on different islands) and may then be completed during secondary contact (Lack, 1947; Schluter *et al.*, 1985; Grant, 1986; Petren *et al.*, 2005; Grant & Grant, 2006). Without questioning this well-supported model, we suggest that diversification may also be possible on a single island. Some Galápagos islands are reasonably large and manifest dramatic variation in climate: for example, from arid zones near the coast to consistently wet areas in the highlands. This variation in climate leads to spatial variation in vegetation (Wiggins & Porter, 1971), which should then drive adaptive divergence of finch beaks (Bowman, 1961; Abbott *et al.*, 1977; Kleindorfer *et al.*, 2006). If this divergence in beak morphology contributes to reproductive isolation, as is thought to be the case for Darwin's finches (Grant, 1986; Podos, 2001), the stage is set for parapatric speciation along ecological gradients (Doebeli & Dieckmann, 2003).

Finally, we test for beak shape variation between different morphs of *G. fortis* at a single site. Beak size in *G. fortis* on Santa Cruz can be extremely variable and sometimes even bimodal, with large and small modes linked by fewer intermediates (Ford *et al.*, 1973; Hendry *et al.*, 2006). Studies at El Garrapatero suggest that these two morphs are maintained through disruptive selection and assortative mating in sympatry (Hendry *et al.*, 2006; Huber & Podos, 2006; Huber *et al.*, 2007; A.P. Hendry & J. Podos, unpublished data). We here ask whether the two beak size morphs at El Garrapatero also differ in beak shape, which may yield some additional insights into divergent selection caused by different foraging modes (Bowman, 1961; Grant, 1986). We do not perform similar analyses at the other collection sites – where bimodality is not strong (Hendry *et al.*, 2006).

Materials and methods

Study sites and field methods

Our analyses focus on Santa Cruz, the second largest (986 km^2) and fourth highest (870 m) island in Galápagos (Grant, 1986). Santa Cruz has several different vegetation zones and habitats (Wiggins & Porter, 1971) that support a total of nine recognized finch species (Grant, 1986). In 2003 and 2004, we used mist nets to capture finches at four different sites that span a range of elevations and locations: Academy Bay, Los Gemelos, El Garrapatero and Borrero Bay (Fig. 1). Species were identified by eye based on previously established differences in body size and shape, plumage characteristics, and beak size and shape (Bowman, 1961; Grant, 1986).

Following Grant (1986), each bird was measured for beak length (rostral edge of nares to tip of upper mandible, i.e. the culmen), beak depth (at the nares) and beak width (base of lower mandible, i.e. the gonys). The profile of the beak was digitally photographed with a Nikon Coolpix 995 camera (Nikon, Tokyo, Japan), most of the birds (70.65%) were banded, and all birds were released at the site of capture. We later examined the photographs and retained those where the beak was perpendicular to the camera, in focus and closed. These criteria left us with a total of 532 birds of seven species (Table 1). The remaining two species found on Santa Cruz (*C. pallida* and *C. psittacula*) were too rare in our collections to be analysed.

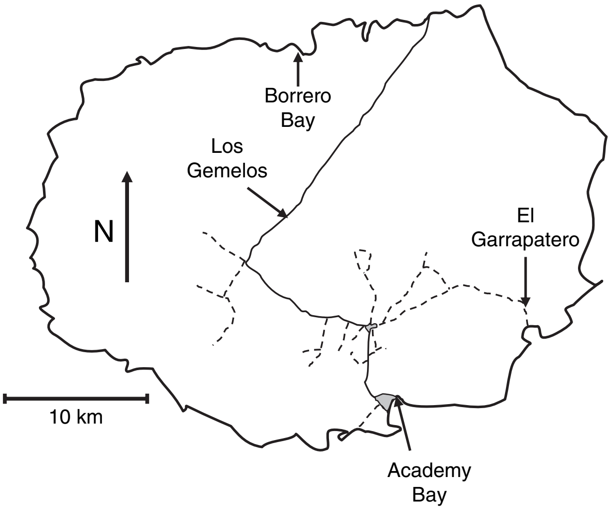

Fig. 1 The four study sites on Santa Cruz Island, Galápagos, Ecuador: Academy Bay (AB), Borrero Bay (BB), El Garrapatero (EG), and Los Gemelos (LG). Grey shading represents human settlements, solid lines represent paved roads, and dotted lines represent gravel roads and major trails.

Table 1 Sample sizes for each species from each site on Santa Cruz Island, Galápagos.

Species	Academy Bay	Borrero Bay	El Garrapatero	Los Gemelos	Species total
Platyspiza crassirostris	25	0	0	0	25
Geospiza fortis	186	62	79	0	327
Geospiza fuliginosa	39	22	23	4	88
Geospiza magnirostris	16	1	0	0	17
Certhidea olivacea	0	0	0	14	14
Camarhynchus parvulus	12	1	3	8	24
Geospiza scandens	18	1	18	0	37
Site total	296	87	123	26	532

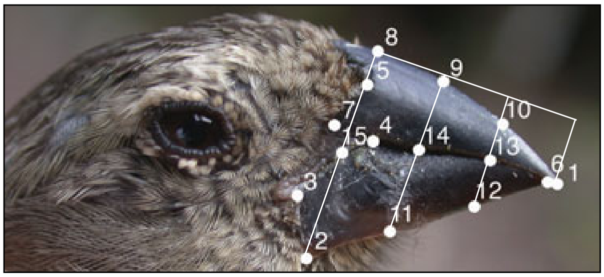

Fig. 2 Landmarks and semilandmarks used for geometric morphometrics. Landmarks are clear homologous points: 1, culmen (upper mandible) tip; 2, corner of mandibular rami; 3, junction of the pars mandibularis and the malar region of the head; 4, point of maximum height of lower mandible in the vertical plane; 5, rostral-most point of the nares; 6, gonys (lower mandible) tip; and 7, caudal-most point on the angle of the lower portion of the upper mandible. Semilandmarks (points 8–15) are positioned where the standardized grid (white lines) intersects the margins of the beak (see text for details).

Geometric morphometrics

TPSDIG (Rohlf, 2004) was used to place, on each beak image (Fig. 2), seven 'landmarks' (discrete homologous points) and eight 'semilandmarks' (points on an outline determined by extrinsic criteria; Zelditch *et al.*, 2004). The semilandmarks were important for quantifying shape in areas of the beak that lack clear homologous points (Fig. 2). The semilandmarks were placed by reference to a standardized grid superimposed onto each image. This grid was defined by first positioning a line so that it passed through landmarks 2 and 5, and by then positioning additional parallel lines that divided the beak into one-third portions along this length (Fig. 2). Semilandmarks were placed where the lines of this grid intersected the dorsal outline of the upper mandible, the ventral outline of the lower mandible, and the junction between the two mandibles. The upper mandible can overlap the lower mandible along this junction, and so the shape of lower mandible here represents that which is not obscured beneath the upper mandible.

TPSRELW (Rohlf, 2004) was used to rotate, translate and scale landmark coordinates (semilandmarks were 'slid' along the beak contours) through generalized least squares superimposition (Bookstein *et al.*, 1985; Bookstein, 1991; Marcus *et al.*, 1996). This procedure removes all isometric effects of body size, but allometric effects may still remain (Bookstein, 1996; Klingenberg, 1996). TPSRELW also calculates, for each individual, a series of shape variables often referred to as 'uniform components' (affine) and 'partial warps' (non-affine). The program also extracts scores for each individual along a series of relative warp (RW) functions, which are principal component axes constructed from the uniform components and partial warps.

Geometric morphometric analyses usually assess and control for allometry by reference to centroid size (Bookstein, 1996), which could not be obtained here because of logistical constraints that prevented inclusion of a standardized scale in each image. Allometry was instead evaluated by reference to PC1 based on univariate measurements of beak length, depth and width. Of the total variation in these measurements, PC1 explained 91.04% in analyses of the different species, 84.51% in analyses of *G. fortis* at the three collection sites, and 85.25% in analyses of the large and small *G. fortis* morphs at El Garrapatero.

Statistical analyses

Statistical analyses were conducted in SPSS (v. 14) and JMP (v. 4). Analyses proceeded through three hierarchical levels: (1) variation among the seven species pooled across sites, (2) variation among *G. fortis* from the different sites, and (3) variation between large and small *G. fortis* beak size morphs at El Garrapatero. For the last of these levels, we use variation along PC1 to determine a reasonable dividing point between the large and small morphs. We here use PC1 = 0.75, which represented a clear division in the frequency histogram, and was similar to previous analyses (Hendry *et al.*, 2006; Huber & Podos, 2006; Huber *et al.*, 2007).

At each hierarchical level, we compared 'groups' (species, *G. fortis* sites, or *G. fortis* beak size morphs) with two complementary approaches: (1) univariate analyses based on the principal component axes of shape variation (RWs) and (2) multivariate analyses based on all the shape variables. The univariate analyses consider major shape variables as defined and calculated without reference to group identity (RWs 1–3). These analyses are used for assessing how specific orthogonal aspects of shape variation differ among particular groups. In contrast, the multivariate analyses identify aspects of shape variation that maximize discrimination between the groups. These two approaches have different strengths, and using both allows more robust inferences than would be the case with either alone.

In the univariate analyses, we first test whether average scores along a RW axis differ significantly among groups (ANOVA followed by *post hoc* Tukey tests). We then test for allometric effects of beak size by adding PC1 as a covariate, as well as the interaction between group and PC1 (ANCOVA). Finally, we remove the interaction term (but not PC1) to test whether RW scores differ among groups after controlling for allometric effects of beak size (ANCOVA followed by comparison of adjusted mean values using the Bryant & Paulson (1976) generalization of the Tukey test). The interaction term was occasionally significant, in which case the comparison of adjusted means is made with appropriate cautionary notes.

In the multivariate analyses, we first use MANOVA to test for a significant effect of group, and to extract canonical variate (CV) axes that best discriminate among the groups. Shape variation along these CV axes is then visualized with TPSREGR (Rohlf, 2004). We next test and control for allometric effects of beak size by adding PC1 as a covariate, as well as the interaction between PC1 and group. CV axes are again extracted and visualized with TPSREGR. Note that shape deformations along CV axes should be viewed with caution because they distort shape space (D. Adams, pers. comm.).

MANOVA is also used to determine how well various size and shape variables discriminated among groups. We specifically examine discriminant function classification patterns based on (1) the three univariate traits (beak length, depth, and width), (2) geometric morphometric shape variables plus beak size (all partial warps and uniform components plus PC1), (3) geometric morphometric shape variables without isometric effects of beak size (all partial warps and uniform components), and (4) geometric morphometric shape variables without isometric and allometric effects of beak size. For this last analysis, discriminant functions cannot be analysed with a covariate, and so we instead used residual scores for partial warps and uniform components calculated in a MANCOVA with PC1 as the predictor variable. Only the third and fourth of these analyses were performed for the *G. fortis* beak size morphs, because they were explicitly defined based on beak size. (Note that identical results are obtained if we use all of the relative warps in discriminant functions instead of the partial warps and uniform components.)

Results

Species

Beak shape differed among the species in a variety of complex ways (Fig. 3). To provide a tractable presentation of the most interesting patterns, we here identify and discuss three major aspects of shape variation. The first represented variation from short/deep/blunt beaks to long/shallow/pointed beaks. This aspect of shape was nicely captured by RW1 (Fig. 4), which explained 63.6%

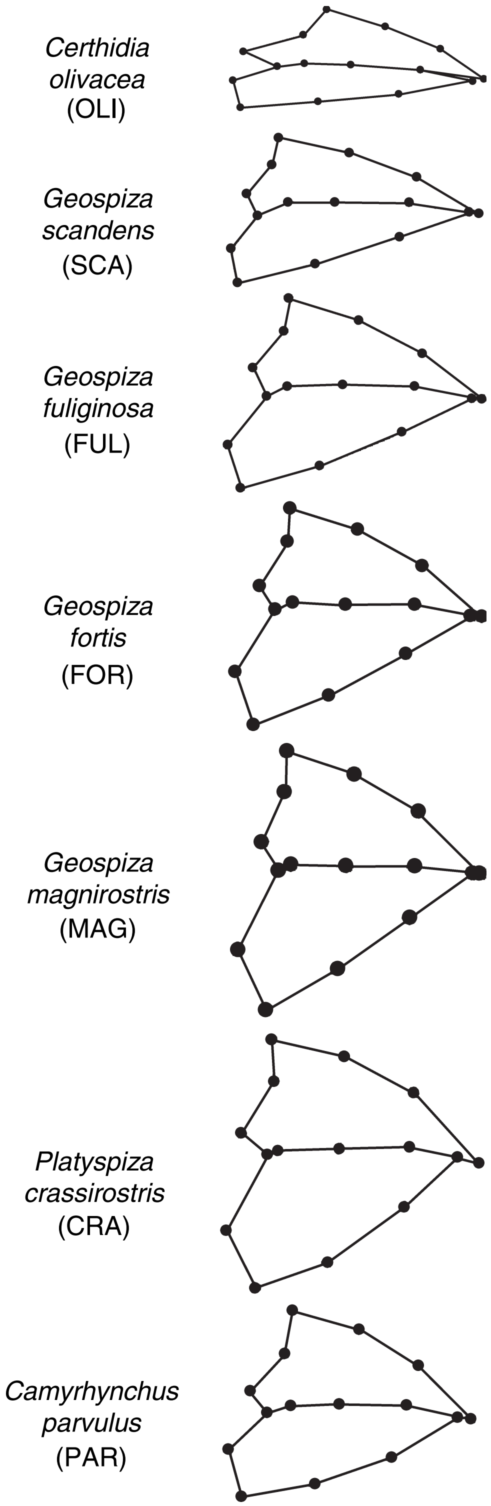

Fig. 3 The average beak shape of each species used in our analysis. Shown is the consensus configuration from TPSRELW when each species is analysed separately. Beaks are scaled to the same length to better illustrate shape differences – but note that overall size differences have no meaning (nor do different sizes of the points and lines).

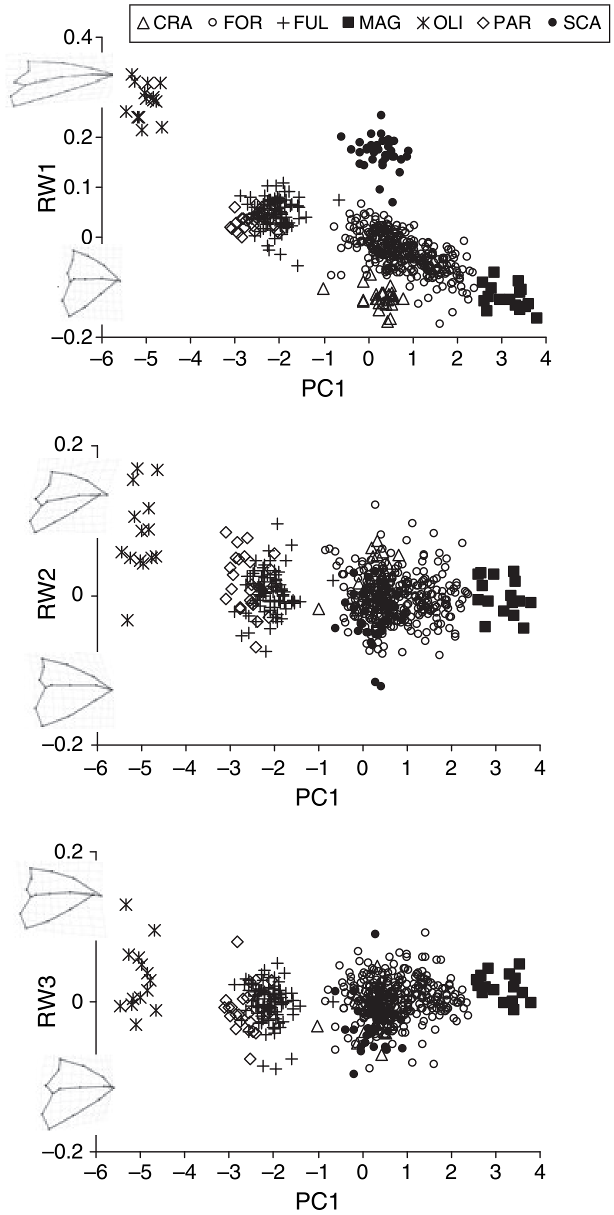

Fig. 4 Beak shape (RWs 1–3) and size (PC1) variation within and among the seven species. Also shown are visual representations of extreme scores along each RW axis (from TPSREGR). The grid deformation for each species representing its average in the shape space defined by RW1 and RW2 is shown in Fig. S4.

of the total shape variation, and by CV1 (Fig. 5). Thus, the major axis of shape variation in the entire dataset (RW1) was similar to the axis that best discriminated among the species (CV1). Based on statistical analyses of RW1 (Tables 2 and 3), coupled with visual examination of CV1 (Fig. 5), we infer that species have increasingly long/shallow/pointed beaks in the following order: *P. crassirostris/G. magnirostris*, *G. fortis*, *C. parvulus/*

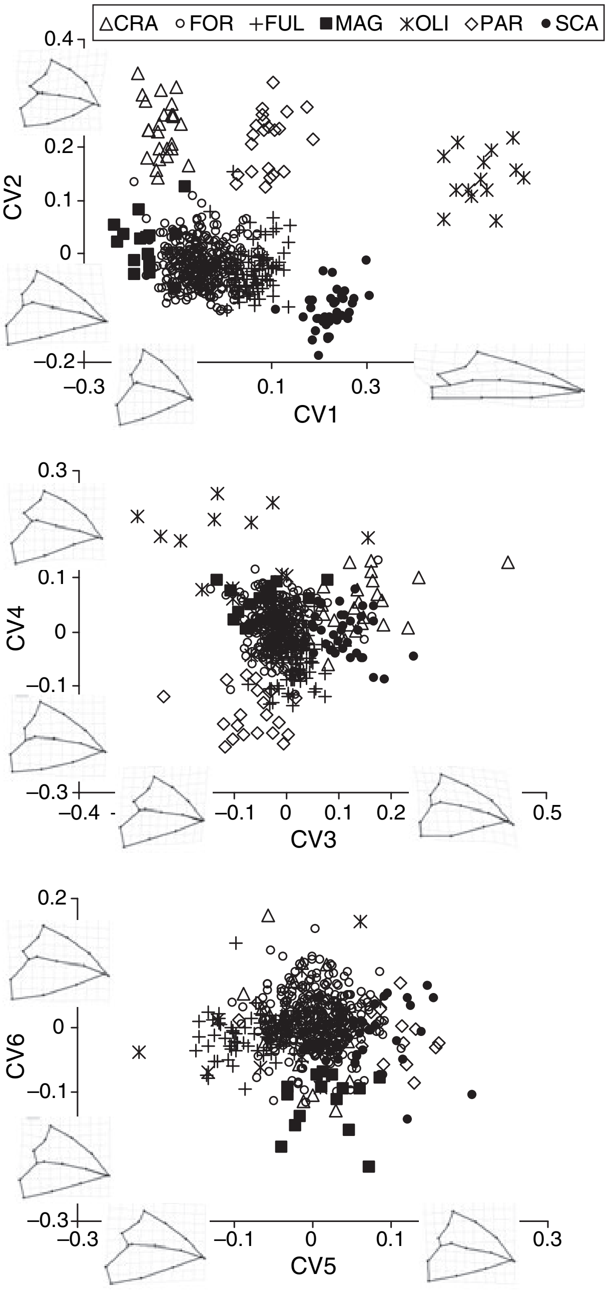

Fig. 5 Beak shape variation within and among species as represented by the six canonical variates (CVs) from a MANOVA with species as a predictor variable and all partial warps and uniform components as response variables. Also shown are visual representations of extreme scores along each CV axis (from TPSREGR).

G. fuliginosa, *G. scandens* and *C. olivacea* (species here separated by a slash were not significant different). This aspect of shape variation was influenced by allometry, as revealed through a significant effect of PC1 on RW1 (Table 2) and in MANCOVA (Table 4). Controlling for this variation by leaving PC1 in the analyses led to three basic differences from the above results, all of which were more obvious for RW1 than for MANCOVA. First, *P. crassirostris* was now seen to have a longer/shallower/pointier beak than *G. magnirostris* (Table 3; Fig. S1). Secondly, the three granivorous ground finches (*G. fuliginosa*, *G. fortis* and *G. magnirostris*) became much more difficult to separate (Table 3). Thirdly, the original difference between *C. olivacea* and *G. scandens* (see above) was reduced in MANCOVA (Fig. S1) and reversed for RW1 (Table 3).

A second major aspect of shape variation was the relative robustness and caudal/rostral positioning of the upper versus lower mandibles. This aspect of shape was captured mostly by RW2 and partly by RW3 (Fig. 4), which explained 10.9% and 7.3% of the total shape variation respectively. The only species clearly distinguishable along these axes was *C. olivacea*, which have the relatively most robust and rostrally-displaced upper mandible. This result is of particular interest in comparison to the other species (*G. scandens*) with a long/shallow/pointed beak (Table 3; Fig. 4). In MANOVA, relative size and position of the mandibles was captured by several CVs, along which *C. olivacea* was again quite distinct (Fig. 5). This aspect of shape variation was not strongly influenced by allometry – because PC1 did not influence variation along RW2 or RW3 (Table 2). Moreover, MANCOVAS that included PC1 continued to clearly separate *G. scandens* and *C. olivacea*, particularly along CV5, which nicely captured relative sizes of the two mandibles (Fig. S1).

A third major aspect of shape variation was curvature of the dorsal and ventral profiles of the beak. This variation was not captured by any of the major RWs, but was clearly seen in CV2 (Fig. 5). Species with curved beak profiles included *P. crassirostris*, *C. parvulus* and *C. olivacea*. The other species, particularly *G. scandens*, had relatively straight beak profiles. Here, then, is another way in which the two species with long/shallow/pointed beaks (*G. scandens* and *C. olivacea*) differ from each other. Note that CV2 was also excellent at discriminating between species that were difficult to separate on the other axes (see above). Specifically, *C. parvulus* has a more curved beak profile than does *G. fuliginosa*, and *P. crassirostris* has a more curved beak profile than does *G. magnirostris*. Allometry was not the primary driver of these differences because they remained, albeit weaker, when PC1 was included in MANCOVA (Fig. S1).

Discriminant analysis based on the three classic univariate measurements (beak length, depth and width) successfully classifies 83.0% of the birds back to the correct species. Most misclassifications occurred between *C. parvulus* and *G. fuliginosa* (Table 5) – because a major difference between them (curvature of the beak profile) is not captured by univariate measurements. In contrast, discriminant analysis based on geometric morphometrics plus PC1 correctly classifies 98.3% of the birds. If

Table 2 Univariate statistical analysis of variation along the first three principal component axes of shape variation (RWs) among the seven species, among *Geospiza fortis* from different sites, and between the two *G. fortis* beak size morphs at El Garrapatero.

	RW1			RW2			RW3		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
Species									
ANOVA									
Species	451.23	6, 516	<0.001	25.03	6, 516	<0.001	14.45	6, 516	<0.001
ANCOVA									
Species	164.90	6, 509	<0.001	3.51	6, 509	0.002	5.50	6, 509	<0.001
PC1	3.58	1, 509	0.059	0.62	1, 509	0.430	0.04	1, 509	0.850
Interaction	2.93	6, 509	0.008	1.77	6, 509	0.104	0.36	6, 509	0.902
ANCOVA									
Species	328.94	6, 515	<0.001	15.07	6, 515	<0.001	14.46	6, 515	<0.001
PC1	177.68	1, 515	<0.001	0.06	1, 515	0.80	0.343	1, 515	0.558
G. fortis sites									
ANOVA									
Site	13.14	2, 312	<0.001	0.46	2, 312	0.632	0.15	2, 312	0.859
ANCOVA									
Site	16.18	2, 309	<0.001	0.41	2, 309	0.664	0.18	2, 309	0.833
PC1	84.75	1, 309	<0.001	29.88	1, 309	<0.001	27.93	1, 309	<0.001
Interaction	2.08	2, 309	0.126	4.55	2, 309	0.011	0.42	2, 309	0.655
ANCOVA									
Site	16.25	2, 311	<0.001	0.40	2, 311	0.668	0.18	2, 311	0.839
PC1	96.75	1, 311	<0.001	31.52	1, 311	<0.001	34.88	1, 311	<0.001
G. fortis morphs									
ANOVA									
Morph	66.13	1, 76	<0.001	2.82	1, 76	0.097	0.14	1, 76	0.705
ANCOVA									
Morph	4.44	1, 74	0.038	0.05	1, 74	0.818	0.00	1, 74	0.972
PC1	0.67	1, 74	0.414	0.82	1, 74	0.368	0.00	1, 74	0.953
Interaction	0.51	1, 74	0.478	1.46	1, 74	0.230	0.17	1, 74	0.685
ANCOVA									
Morph	6.793	1, 75	0.011	1.59	1, 75	0.211	0.39	1, 75	0.54
PC1	3.591	1, 75	0.062	6.26	1, 75	0.014	0.25	1, 75	0.62

Shown are analyses of species differences before (ANOVA) and after (ANCOVA) controlling for allometric effects of beak size by including PC1. The latter analyses show results with and without the interaction between PC1 and each RW.

Tukey's *post hoc* tests are shown for species in Table 3 and for *G. fortis* sites in Table 6.

Table 3 Estimated marginal means for relative warp scores in beak shape comparisons among Darwin's finch species.

Species	RW1 (ANCOVA)	RW1 (ANOVA)	RW2 (ANOVA)	RW3 (ANOVA)
Platyspiza crassirostris	−0.11328^{a}	−0.12169^{a}	0.02006^{b}	−0.01658^{a,b}
Geospiza fortis	−0.00731^{c}	−0.03065^{b}	−0.00452^{a,b}	0.00361^{b,c}
Geospiza fuliginosa	−0.01599^{c}	0.04770^{c}	0.00401^{b}	−0.00205^{b,c}
Geospiza magnirostris	−0.02548^{b,c}	−0.12149^{a}	−0.00198^{a,b}	0.01895^{c,d}
Certhidea olivacea	0.11935^{d}	0.27236^{e}	0.08288^{c}	0.03109^{d}
Camarhynchus parvulus	−0.0472^{b}	0.03239^{c}	0.01776^{b}	−0.00928^{a,b}
Geospiza scandens	0.17716^{e}	0.16899^{d}	−0.02641^{a}	−0.02872^{a}

When the effect of beak size (PC1) is significant in ANCOVA, results are shown for analyses with (ANCOVA) and without (ANOVA) this covariate.

Homogenous subsets based on *post hoc* tests are indicated with letter superscripts.

isometric aspects of beak size are removed by excluding PC1, classification success is 92.9%, with additional mistakes between *G. fortis* and *G. fuliginosa* (Table 5). If allometric aspects of beak size are then removed by the use of residuals, classification success is 86.6%, with additional mistakes between *G. fortis* and *G. fuliginosa*. Overall, geometric morphometrics allows more accurate classification of species by beak shape (even when all size effects are removed) than do the classic univariate traits (even when size effects are included).

G. fortis spatial variation

Geospiza fortis from the different sites varied subtly in beak length/pointiness. This aspect of the shape was nicely captured by RW1 (35.7% of the variation; Fig. 6) and by CV1 (Fig. 7). Based on statistical analyses of variation in

Table 4 MANCOVA analyses examining how total shape variation is explained by group (species, *Geospiza fortis* sites, or El Garrapatero *G. fortis* beak size morphs), beak size (PC1), and their interaction.

	Wilk's partial λ	F	df (numerator, denominator)	P	Partial variance explained (%)
Species					
Species	0.074	10.20	156, 2847	<0.001	35.2
PC1	0.841	3.52	26, 484	<0.001	15.9
Interaction	0.525	2.12	156, 2847	<0.001	10.2
G. fortis sites					
Sites	0.616	2.99	52, 568	<0.001	21.5
PC1	0.432	14.35	26, 284	<0.001	56.8
Interaction	0.770	1.53	52, 568	0.012	12.3
Beak size morphs					
Morph	0.628	1.12	26, 49	0.361	37.2
PC1	0.517	1.76	26, 49	0.043	48.3
Interaction	0.650	1.02	26, 49	0.469	35.0

RW1 (Tables 2 and 6), coupled with visual examination of CV1 (Fig. 7), we infer that *G. fortis* at Academy Bay have the shortest and most blunt beaks. A further difference, revealed by MANOVA, is that El Garrapatero *G. fortis* have the most curved upper mandible (CV2 in Fig. 7). Although these aspects of shape were influenced by allometry (RWs: Table 2; MANOVA: Table 4), the differences described above persisted after controlling for this effect (Table 6; Fig. S2). It is important to recognize, however, that although the shape differences were statistically significant, they were subtle – as can be seen in the overlap among birds from the different sites (Figs 6 and 7).

Discriminant analysis based on the three classic univariate measurements was essentially random (31.7% success; Table 7). In contrast, discriminant analysis based on geometric morphometric variables plus PC1 correctly classified two-thirds of the birds, whether including beak size (63.5%), excluding isometric effects of beak size (63.1%), or also excluding allometric effects of beak size (63.5%). Similarity among these last three analyses confirms that beak size does not drive the subtle shape differences among sites.

Beak size morphs

The two beak size morphs were similar in beak shape, except that the small morph had a slightly longer/pointier beak. This aspect of shape variation was nicely captured by RW1 (32.8% of the variation; Fig. 8) and by MANOVA (Wilk's partial $\lambda = 0.259$, $F_{26,51} = 5.61$, $P < 0.001$, partial variance explained = 74.1%; classification success = 93.6%; Fig. S3). This variation was strongly influenced by allometry (Table 2; Fig. 8), which was difficult to fully evaluate given the non-overlapping size ranges (by definition). Inclusion of PC1 weakened but did not eliminate the differences in RW analyses (Table 2) and in MANCOVA (Wilk's partial $\lambda = 0.907$, $F_{26,51} = 0.200$, $P = 1.00$, partial variance explained = 9.3%; classification success = 70.5%; Fig. S3).

Discussion

Species

Our first major result is that geometric morphometrics substantially improve discrimination among the species based on beak dimensions. On the one hand, analysis based on univariate measurements of beak length, depth and width classified 83.0% of the individuals back to the correct species. On the other hand, comparable analyses of geometric morphometric data correctly classified 98.3% of the individuals. Indeed, geometric morphometric analyses were as good at discriminating species even without beak size effects as were the univariate measurements with beak size effects (Table 5). This comparison is the first of several pointing to the potential

Table 5 Results of discriminant analyses comparing species using the different morphological data sets explained in *Materials and methods*.

	Univariates	Geometric morphometrics with size	Geometric morphometrics without isometric size	Geometric morphometrics without allometric size
Response variables	Beak length, depth, and width	Partial warps, uniform components, and PC1	Partial warps and uniform components	Residual partial warps and uniform components
Wilk's partial λ	0.036	0.001	0.003	0.008
F	179.86	43.29	33.22	43.29
df (numerator, denominator)	18, 1454	162, 2887	156, 2941	156, 2888
P	<0.001	<0.001	<0.001	<0.001
Partial variance explained (%)	66.9	70.1	63.0	55.6
Classification success (%)	83.0	98.3	92.9	86.6
Most misclassified	PAR (19.5% to FUL)	PAR (8.3% to FUL)	FOR (5.8% to FUL)	FUL (14.9% to FOR)
Next most misclassified	FUL (25.0% to PAR)	FOR (1.6% to MAG)	PAR (8.3% to FUL)	FOR (10.6% to FUL)

'Most misclassified' indicates the species that was most often classified to another species, and the percentage of that species misclassified most often to a particular species. 'Next most misclassified' provides the same information for the species next most often misclassified to another species.

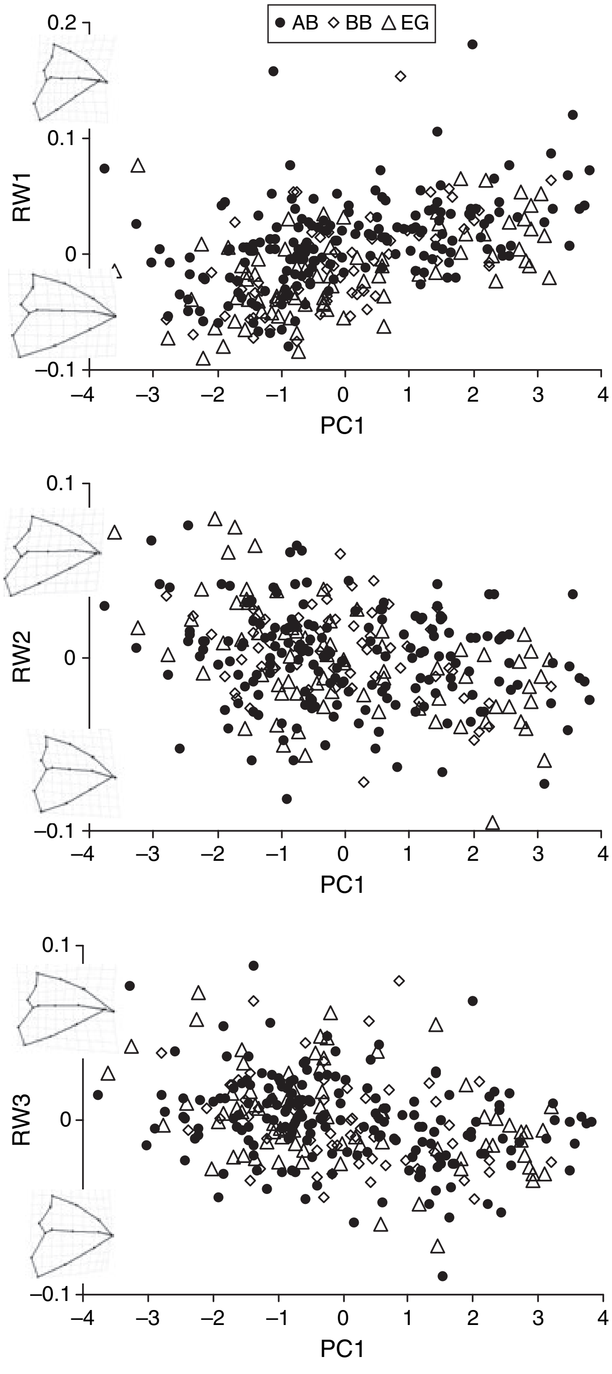

Fig. 6 Beak shape (RW1–3) and size (PC1) variation within and among the three *G. fortis* sites. Also shown are visual representations of extreme scores along each RW axis (from TPSREGR).

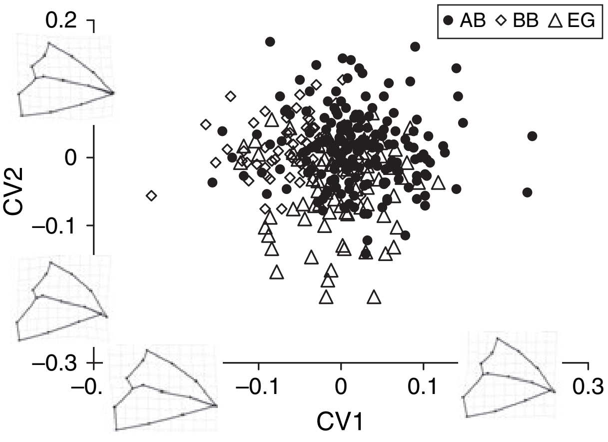

Fig. 7 Beak shape variation within and among the three *G. fortis* sites as represented by the two canonical variates (CVs) from a MANOVA with site as a predictor variable and all partial warps and uniform components as response variables. Also shown are visual representations of extreme scores along each CV axis (from TPSREGR).

value of geometric morphometrics for appraising the Darwin's finch radiation. Our second major result is that the previously described differences among species are upheld when viewed through the lens of geometric morphometrics. Our third major result is that geometric morphometrics provide a quantitative demonstration of additional shape differences, some of which were suggested by the qualitative analysis of Bowman (1961). We now discuss three major aspects of shape variation that serve to delineate the different species.

First, virtually all of the previously quantified variation in Darwin's finch beak shape is here captured by a single morphological axis, which ranges from short/deep/blunt beaks to long/shallow/pointy beaks (RW1 in Fig. 4 and CV1 in Fig. 5). Species differences along this axis generally parallel those described in earlier work (Grant *et al.*, 1985; Grant, 1986), and so are not discussed in detail. Importantly, however, our new results inform the nature of these differences by revealing the role of allometry. Specifically, some species (*G. magnirostris* vs. *P. crassirostris*) were best distinguished after controlling for allometry, whereas others (*G. magnirostris* vs. *G. fortis* vs. *G. fuliginosa*) were best distinguished before controlling for allometry. With regard to this latter grouping, our quantitative analysis supports the qualitative observation of Bowman (1961, p. 140) that beak depth increases (relative to beak length) from *G. fuliginosa* to *G. fortis* to *G. magnirostris*. Our analysis extends this observation by showing that the differences can be attributed to allometry – because they largely disappear when beak size is added as a covariate (Table 3). In short, granivorous *Geospiza* species with larger beaks do indeed have relatively deeper beaks (presumably to resist the greater forces generated when crushing larger/harder seeds: Bowman, 1961; Herrel *et al.*, 2005a) – and these differences are an extension of within-species allometry.

A second notable aspect of shape variation among species, one not emphasized in previous quantitative work, is the relative size and caudal/rostral positioning of

Table 6 Estimated marginal means for relative warp scores in beak shape comparisons among *Geospiza fortis* sites.

Location	RW1 (ANCOVA)	RW1 (ANOVA)	RW2 (ANCOVA)	RW2 (ANOVA)	RW3 (ANCOVA)	RW3 (ANOVA)
Academy Bay	0.00925[a]	0.00933[a]	−0.00036	−0.00042	−0.00038	−0.00026
Borrero Bay	−0.00765[b]	−0.00853[b]	0.00240	0.00281	−0.00094	−0.00055
El Garrapatero	−0.01556[b]	−0.01582[b]	−0.00172	−0.00164	0.00132	0.00084

When the effect of beak size (PC1) is significant in ANCOVA, results are shown for analyses with (ANCOVA) and without (ANOVA) this covariate. Homogeneous subsets based on *post hoc* tests are indicated with letter superscripts. Superscripts are not shown when the group effect is not significant in the original AN(C)OVA.

Table 7 Results of discriminant analyses comparing *G. fortis* sites using the different morphological data sets explained in *Materials and methods*.

	Univariates	Geometric morphometrics with size	Geometric morphometrics without isometric size	Geometric morphometrics without allometric size
Response variables	Beak length, depth, and width	Partial warps, uniform components, and PC1	Partial warps and uniform components	Residual partial warps and uniform components
Wilk's partial λ	0.961	0.618	0.651	0.618
F	2.075	2.88	2.69	3.00
df (numerator, denominator)	6, 620	54, 572	52, 584	52, 574
P	0.054	<0.001	<0.001	<0.001
Partial variance explained (%)	2.0	21.4	19.3	21.4
Classification success (%)	31.7	63.5	63.1	63.5
Most misclassified	EG (39.5% to BB)	AB (22.6% o EG)	EG (53.5% to AB)	AB (22.6% to EG)
Next most misclassified	BB (22.6% to AB)	EG (26.3% to AB)	AB (20% to EG)	EG (26.3% to AB)

'Most misclassified' indicates the site from which birds were most often classified to another site, and the percentage of birds at that site misclassified most often to a particular site. 'Next most misclassified' provides the same information for the site from which birds were next most often misclassified to another site.

the upper and lower mandibles. The most interesting observation here is the dramatic difference between two species that were not separated on the primary axis of variation. Specifically, *G. scandens* has a relatively small and caudally displaced upper mandible, whereas *C. olivacea* has a relatively small and caudally displaced lower mandible. This difference was qualitatively noted by Bowman (1961, p. 139) and probably reflects selection because of their very different diets, with *C. olivacea* specializing on insects and *G. scandens* specializing on the nectar, pollen and seeds of *Opuntia* cactus (Bowman, 1961; Grant, 1986). It would be interesting to now quantify how these aspects of beak shape influence feeding mechanics and natural selection on different foods.

A third notable aspect of shape variation is curvature of the beak profile (CV2 in Fig. 5). Specifically, the four *Geospiza* species have relatively straight beak profiles in comparison with the other species. Bowman's (1961) qualitative analysis emphasized this particular feature of *Geospiza*, but subsequent quantitative analyses have not addressed it. Bowman (1961, pp. 141–156) argued that curved profiles are better suited for resisting fracture forces incurred during tip-biting, whereas *Geospiza* more commonly crack seeds near the base of the beak (Bowman, 1961; Herrel *et al.*, 2005b). Geometric morphometrics may therefore provide a way to quantify this aspect of shape variation for studies of feeding behaviour and natural selection. Predictions might be that *Geospiza* individuals with greater beak curvature will employ more tip biting, and that these individuals will be disfavoured when foraging on large/hard seeds.

G. fortis spatial variation

Geospiza fortis on Santa Cruz are highly variable in beak size and shape (Lack, 1947; Ford *et al.*, 1973; Grant & Grant, 1989; Hendry *et al.*, 2006), perhaps due to some combination of hybridization between species, gene flow between *G. fortis* populations, or weak stabilizing selection. Although most of the variation is found within sites, some differences are evident among sites. With respect to beak size, Borrero Bay has the fewest large-beaked *G. fortis*, perhaps because of a relative scarcity of large/hard seeds (Hendry *et al.*, 2006). With respect to beak shape, our present analysis shows that Academy Bay

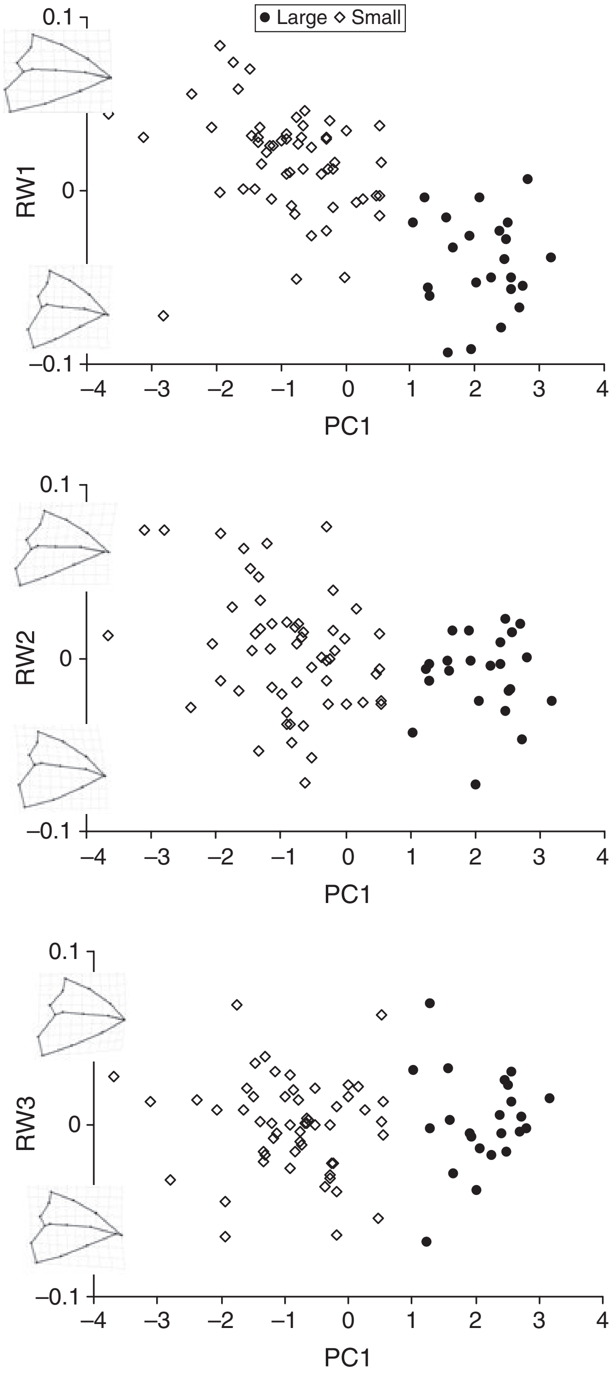

Fig. 8 Beak shape (RW1–3) and size (PC1) variation within and between the two *G. fortis* morphs. Also shown are visual representations of extreme scores along each RW axis (from TPSREGR).

G. fortis have the shortest/bluntest beaks, whereas El Garrapatero *G. fortis* have the most curved beak profile (Figs 6 and 7). These differences were subtle and detectable only using geometric morphometrics (Table 7). It seems likely that these spatial differences, although subtle, have a genetic basis – because beak size and shape show little environmental influence (Keller *et al.*, 2001). If so, *G. fortis* may vary adaptively in beak morphology not only among islands (Bowman, 1961; Grant *et al.*, 1985; Schluter *et al.*, 1985; Grant, 1986), but also among sites on a single island.

The most likely explanation for among-site differences in beak shape is adaptation to spatial differences in food resources. For example, Kleindorfer *et al.* (2006) showed that spatial variation in *G. fuliginosa* beak dimensions on Santa Cruz was associated with spatial variation in their diets. We expect a similar effect for *G. fortis* given that vegetation and finch diets differ dramatically among the sites (Abbott *et al.*, 1977; Smith *et al.*, 1978; L. De Léon & A. Hendry, unpublished data). Although it is not possible at present to identify the specific feeding differences that cause the specific beak shape differences, some sort of adaptive divergence does seem possible (see also Grant *et al.*, 1976; Hendry *et al.*, 2006), particularly in the light of new evidence that *G. fortis* dispersal on Santa Cruz is spatially restricted (A. Gabela and J. Podos, unpublished data). If divergence in beak morphology then contributes to mating isolation, as is thought to be the case (Grant & Grant, 1997; Podos, 2001), parapatric speciation may commence (Doebeli & Dieckmann, 2003). Hinting at this possibility, El Garrapatero *G. fortis* respond more strongly to the songs of local birds than to the songs of Academy Bay *G. fortis* (Podos, 2007). In short, the modest spatial variation in beak shape may reflect adaptive divergence and may contribute to spatial restrictions in gene flow.

Beak size morphs

Bimodal populations provide exceptional opportunities to study the early stages of adaptive divergence and speciation. This point was argued by Ford *et al.* (1973) for the large and small *G. fortis* beak size morphs historically present at Academy Bay. Although the bimodality at Academy Bay has since weakened, it remains strong at El Garrapatero (Hendry *et al.*, 2006), where the two morphs mate assortatively and show limited gene flow (Huber *et al.*, 2007). These phenomena are presumably driven by adaptation to different food types, a hypothesis that we are currently testing. The present study may aid this parallel endeavour – because beak shape is clearly linked to foraging and natural selection (Bowman, 1961; Grant, 1986; Grant & Grant, 2006).

We found that the small morph at El Garrapatero has a relatively longer/pointier beak than does the large morph (Fig. 8). Remarkably, individual birds could be correctly classified to their morph 93.6% of the time based on beak shape, with isometric effects of beak size removed. Much of this difference was the result of allometry – because controlling for this effect reduced classification success to 70.5%. Moreover, we are not entirely confident that all of the allometric effects of beak size were removed (see *Results*). Interestingly, the apparent influence of allometry on differences between the *G. fortis* morphs closely matches our results in comparisons among the granivorous ground finches (*G. fuliginosa*, *G. fortis* and *G. magnirostris*). This parallelism suggests that the large and small beak size morphs are diverging along the same morphological axis that separates the three granivorous ground finches. This result supports previous arguments that studying morphological divergence and reproductive isolation between the sympatric morphs might inform factors that drive the radiation of ground finches (Hendry *et al.*, 2006; Huber *et al.*, 2007).

Conclusions

Our geometric morphometric appraisal of beak shape in one part of the Darwin's finch radiation generated several insights. First, we were able to achieve better discrimination among species than was possible with the traditional measurements of beak length, depth and width. Secondly, traditional beak shape distinctions among the species were here captured by a single beak shape axis ranging from birds with long/shallow/pointy beaks to short/deep/blunt beaks. Thirdly, additional differences among the species relate to the relative size, position and curvature of the upper and lower mandibles. These newly quantified differences support and extend the qualitative assessments of Bowman (1961). They also generate new hypotheses, and suggest ways to test them in natural populations of Darwin's finches. Fourthly, we find subtle, but significant, beak shape differences between *G. fortis* from different sites on the same island, and between large and small beak size morphs of *G. fortis* at a single site (El Garrapatero). These within-species differences suggest that within-island diversification, whether sympatric or parapatric, may be possible in Darwin's finches.

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Supplementary material

The following supplementary material is available for this article:

Figure S1 Beak shape variation within and among species as represented by the six canonical variates (CVs) from a MANOVA with species, PC1, and their interaction as predictor variables.

Figure S2 Beak shape variation within and among the three *G. fortis* sites as represented by the two canonical variates (CVs) from a MANOVA with site, PC1, and their interaction as predictor variables.

Figure S3 Visual representations of extreme scores along the CV axis in analyses of the E1 Garrapatero beak size morphs.

Figure S4 Deformations from the consensus form showing the average shape for each species in the shape space defined by RW1 and RW2.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1420-9101.2007.01449.x

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