Evolutionary Ecology Research, 2012, **14**: 365–380

Individual specialization and the seeds of adaptive radiation in Darwin's finches

Luis Fernando De León[1,2,3], Gregor Rolshausen[1],
Eldredge Bermingham[3], Jeffrey Podos[4] and
Andrew P. Hendry[1]

[1]Redpath Museum and Department of Biology, McGill University, Montreal, Quebec, Canada, [2]Instituto de Investigaciones Científicas y Servicios de Alta Tecnología-AIP (INDICASAT-AIP), Panamá, República de Panama, [3]Smithsonian Tropical Research Institute, Balboa, República de Panama and [4]Department of Biology, University of Massachusetts, Amherst, Massachusetts, USA

ABSTRACT

Background: Empirical and theoretical studies suggest that individual specialization can be an important force in evolutionary diversification. However, few studies of natural populations have explicitly considered the impact of individual specialization on adaptive divergence.

Questions: To what extent do individuals within a bimodal Darwin's finch population specialize on different resources? Is this individual specialization likely to enhance adaptive divergence?

Field site: El Garrapatero, Santa Cruz Island, Galápagos, Ecuador.

Organism: A population of the medium ground finch, *Geospiza fortis*, showing large – and bimodal – morphological and genetic variation resulting from ecologically based adaptive divergence.

Methods: We described the diets of individual *G. fortis* through feeding observations in the wild. We calculated several indices of individual specialization. We then examined the relationship between individual specialization, adaptive morphological traits (beak and head dimensions), and neutral genetic variation (microsatellites). We also performed a cluster analysis on the basis of individual foraging observations and asked whether the clusters were morphologically and genetically divergent.

Results: We found significant levels of individual specialization and expected, but weak, associations between individual diet differences, morphological traits, and neutral genetic variation. The cluster analysis yielded two distinct diet-clusters of individuals that differed in morphological traits but not in neutral genetic markers. In the early stages of adaptive radiation, individual specialization appears to be associated with morphological divergence but not neutral genetic divergence.

Keywords: adaptive radiation, Darwin's finches, ecological speciation, Galápagos, individual specialization, intraspecific competition, niche variation.

Correspondence: L.F. De León, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología-AIP (INDICASAT-AIP), PO Box 0843-01103, Panamá 5, República de Panama. e-mail: luis.deleonreyna@gmail.com

INTRODUCTION

Adaptive radiations are composed of different species occupying different niches (Simpson, 1953; Schluter, 2000; Losos, 2009) but how did this diversification proceed? That is, how did a single species originally adapted to an ancestral niche subsequently diversify into multiple cohabiting species, each adapted to a different derived niche? In one widely accepted – and probably common – scenario, different subsets of the original species became physically isolated in locations with different resources or habitats. These different conditions imposed divergent selection that caused adaptive divergence between the populations. At some later time, the physical barrier that separated the populations disappeared or was circumvented by dispersal, leading to secondary contact. If the populations had diverged sufficiently in allopatry, they could then coexist as separate species in sympatry (Schluter, 2000; Coyne and Orr, 2004; Rundle and Nosil, 2005; Grant and Grant, 2008; Price, 2008). In addition, the period of secondary contact might be characterized by competition that caused disruptive selection and promoted further adaptive divergence and reproductive isolation (Lack, 1947; Schluter, 2000; Grant and Grant, 2008). A less widely accepted – and probably less common – scenario starts with the ancestral species being subject to disruptive selection in the absence of physical barriers (i.e. sympatry). This disruptive selection is believed to be caused by competition for shared resources, and it favours individuals that use different resources or habitats, thus promoting adaptive divergence and building reproductive isolation *in situ* (Rueffler *et al.*, 2006; Bolnick and Fitzpatrick, 2007).

Both of the above scenarios require individual variation in niche use (e.g. different resources or habitats) upon which divergent or disruptive selection can act. The raw material for, and also the beginnings of, adaptive radiation is therefore the specialization of different individuals within a population on different niches. This 'individual specialization' appears to be a widespread phenomenon across multiple taxa (Bolnick *et al.*, 2003), and it has attracted increasing interest in the study of evolutionary diversification (Smith and Skúlason, 1996; Bolnick *et al.*, 2003, 2007). Indeed, individual specialization forms the core of theoretical models of adaptive diversification (Wilson and Turelli, 1986; Rueffler *et al.*, 2006; Abrams *et al.*, 2009; Day and Young, 2009). In addition, empirical studies show that individual specialization can generate and maintain morphological and genetic variation (Bolnick and Paull, 2009; Agashe and Bolnick, 2010), promote the evolution of niche expansion (Svanbäck and Persson, 2004; Bolnick *et al.*, 2007; Svanbäck and Bolnick, 2007), and enhance adaptive divergence (Bolnick, 2001; Martin and Pfennig, 2009). Our goal is to consider these ideas in the context of the adaptive radiation of Darwin's finches.

Darwin's finches

Darwin's finches of Galápagos, Ecuador, are an adaptive radiation in which different species show beak morphologies well suited for exploiting different food resources, such as various seeds, fruits, insects, and nectar (Lack, 1947; Bowman, 1961; Abbott *et al.*, 1977; Schluter and Grant, 1984; Grant, 1999; Schluter, 2000; Grant and Grant, 2008). In the part of this radiation (the granivorous ground finches) on which our research focuses, beak size and shape are differentially suited for feeding on seeds of different size and hardness (Lack, 1947; Bowman, 1961; Abbott *et al.*, 1977; Schluter and Grant, 1984; Grant, 1999; Schluter, 2000; Grant and Grant, 2008). The small ground finch (*Geospiza fuliginosa)* has a small beak and feeds mostly on small and soft seeds. The medium ground finch (*Geospiza fortis*) has an intermediate beak and feeds mostly on intermediate seeds. The large ground finch (*Geospiza magnirostris*) has a large beak and feeds mostly on large and hard seeds. A closely related species, *Geospiza scandens*, feeds much more often on the nectar and pollen of *Opuntia* cactus.

Interspecific niche specialization is thus a defining feature of the adaptive radiation of Darwin's finches. We suggest that these differences likely originated from intraspecific (individual) specialization during the initial stages of population divergence. To date, individual specialization has been considered for Darwin's finches in some instances. First, Werner and Sheery (1987) found high individual specialization in the generalist Cocos finch (*Pinaroloxias inornata*). They suggested that this specialization is related to behaviour rather than morphology, given the low morphological variation found in this species (Werner and Sherry, 1987; Grant, 1999). Second, Grant *et al.* (1976) and Price (1987) found strong positive associations between individual diets and individual beak morphology in a population of *G. fortis* on the small island of Daphne Major. They suggested that individual specialization explains the high morphological variability in this population (Grant *et al.*, 1976; Price, 1987).

None of the above populations show any signs of divergence into separate groups and the relevance of their individual specialization to adaptive radiation is not particularly clear. This relevance can be made stronger by examining populations in the early stages of adaptive radiation, such as seems to be the case for some *G. fortis* on Santa Cruz Island (Fig. 1). Local populations of this species have highly variable, and sometimes bimodal, distributions of beak morphology (Hendry *et al.*, 2006; De León *et al.*, 2011), bite force (Herrel *et al.*, 2005a), and song characteristics (Podos *et al.*, 2004; Huber and Podos, 2006; Podos, 2007; Herrel *et al.*, 2009). In studies of one bimodal population (El Garrapatero, Santa Cruz), the large and small beak size morphs were seen to mate assortatively (Huber *et al.*, 2007), experience disruptive selection (Hendry *et al.*, 2009), have different diets (De León *et al.*, 2011), and show modest differentiation at neutral genetic markers (Huber *et al.*, 2007; De León *et al.*, 2010). These patterns are those expected during the

Fig. 1. Males of the small and large beak size morphs in *G. fortis* from Santa Cruz Island (upper panel). Lower panel shows some common seeds and fruits in the diet of *G. fortis*. From left to right they are: *Scutia spicata*, *Tournefortia pubescens*, and *Bastardia viscosa*. Photograph by L.F. De León.

early stages of speciation, and this population is therefore a suitable place to consider the potential role of individual niche (diet) specialization in adaptive radiation. Note, however, that we have no indication of whether this population is likely to continue its divergence – as opposed to being caught at some intermediate stage. We also do not know the initial origins, such as allopatry, parapatry or sympatry, of the divergence in this population. These topics are discussed at length in our previous publications.

Our study

We start by using a series of niche specialization indices to quantify the degree of individual specialization in *G. fortis* at El Garrapatero. We then test for patterns that would be expected if individual specialization is a contributor to adaptive radiation, which our previous work suggests is continuing in this population. First, we ask whether diet differences among individuals are associated with morphological and/or neutral genetic differences among those individuals. Our expectation is that individuals with more divergent diets will also be more morphologically and genetically divergent. We then use cluster analysis of foraging observations to test whether individual specialists form two emerging niche-use clusters. Our expectation is that individuals assigned to different diet-clusters will show differences in foraging-related morphological traits (e.g. beak dimensions). Finally, we compare the resulting diet-clusters to see if they are associated with differences in foraging morphology or neutral genetic markers. Failure to find strong individual specialization, and expected associations between diet differences and trait differences, would reject individual specialization as a contributor to the ongoing diversification of this group.

MATERIALS AND METHODS

Sampling, morphology, and genetics

We studied the diets of individual *G. fortis* at the El Garrapatero site on the eastern side of Santa Cruz Island, Galápagos, Ecuador. All data were collected between January and April over five consecutive years (2003 to 2007; Table 1). *Geospiza fortis* were captured using mist nets and immediately banded with unique combinations of three coloured leg bands plus a metal leg band with a unique code. These bands enabled us to avoid unknowingly re-measuring the same birds, and also to individually identify free-ranging birds as they foraged in the wild. For each bird, beak dimensions were measured following Grant *et al.* (1985) and head dimensions were measured following Herrel *et al.* (2005b). The specific measurements included beak length (anterior edge of nares to anterior tip of upper mandible), beak depth (at the nares), beak width (at the base of the lower mandible), head length (from the tip of the upper mandible to the back of the head), head depth (at the deepest part of the head posterior to the orbits), and head width (the widest part posterior to the orbits). As in our previous studies, all beak measurements were taken three times, and the median was used for analysis.

Blood samples were taken from each bird by pricking the ulnar vein with a needle, and blotting one or a few drops of blood onto filter paper treated with EDTA. DNA was amplified by PCR and screened for allelic variation at 11 di-nucleotide microsatellite loci: Gf03, Gf04, Gf05, Gf07, Gf08, Gf09, Gf10, Gf11, Gf12, Gf13, and Gf16 (Petren, 1998). More details on these genetic methods are provided in De León *et al.* (2010). For analyses in the present study, we calculated individual pairwise genetic distance matrices based on two commonly used microsatellite distance metrics: F_{ST} and Slatkin's R_{ST} (Slatkin, 1995).

Diet

Feeding observations were conducted during morning and afternoon walks through the study area. Each time a banded bird was seen, it was followed and observed through binoculars until a foraging event occurred. This technique works well because Darwin's finches are very tame and can be easily observed from short distances (2–5 m) while engaged in normal feeding behaviour (Lack, 1947; Grant, 1999). For each feeding observation, we recorded the food item (e.g. plant species) and, if applicable, the specific plant part (e.g. seeds, fruits, or leaves). Plant identification was made by reference to Wiggins and Porter (1971), and by comparison with seed collections at the Charles Darwin Research Station, Santa Cruz. When a specific food item could not be identified to the species level, we used more inclusive categories, including 'grass' (several Gramineae species with small and soft seeds), 'ground' (unidentified small seeds), and 'arthropods' (e.g. butterfly larvae, spiders, and grasshoppers). After making one foraging observation of a given bird, we ceased observation of that bird and searched for other banded birds. This 'point observation' procedure was chosen to increase independence among the observations for a given bird – as opposed to methods used in previous studies that followed individual birds continuously for longer periods of time (Abbott *et al.*, 1977; Smith *et al.*, 1978). Nevertheless, estimates from the two methods should be equivalent in the sense that each allows the calculation of the proportion of time spent feeding on a particular food type. More details on the foraging observations are provided in De Leon *et al.* (2011). For the present study, only adult birds with at least six feeding observations were included in the analyses. Also, all analyses incorporated diet data to the finest possible scale of resolution, i.e. including specific plant parts (e.g. seed, fruit, and leaves) when applicable.

Data analysis

Morphological variation was first summarized through principal components analysis (PCA), with separate analyses for beak dimensions and head dimensions. Following the usual conventions and consistent with trait loadings (not shown), we interpret the first principal component (PC1) as size and the second component (PC2) as shape. In addition, we classified individuals into small or large beak size classes ('morphs') by using PC1 for beak dimensions in a cluster analysis to determine the best cut-off between large and small beak size classes. Huber *et al.* (2007) provide more details on this method.

Indices of niche use were calculated using the software IndSpec 1.0 (Bolnick *et al.*, 2002), and are based on proportional similarities/differences in the frequencies of foraging on different food types. Total niche width (TNW) for the population was calculated (Roughgarden, 1979) and then divided into two components: within-individual niche variation (WIC; reflecting the within-individual variation in diet) and between-individual niche variation (BIC; reflecting the between-individual variation in diet). The ratio WIC/TNW was used to describe the among-individual variation in diet, and it reflects the narrowness of the individual's niche (i.e. individual specialization) relative to that of the entire population (Bolnick *et al.*, 2002; Araújo *et al.*, 2011). We next described variation in resource use among individuals by calculating the proportional similarity index across the whole population (*PSi*) and a pairwise diet similarity matrix between all individuals (*PSij*) (Schoener, 1968; Bolnick *et al.*, 2002). The *PSi* estimates specialization for each individual relative to the population as a whole, and Mann-Whitney *U*-tests can be used to test for differences in *PSi* between groups of individuals within the population (Araújo *et al.*, 2007); here the small and large beak morphs. Finally, we estimated the predominance of individual specialization by using a likelihood index (*Wi*) that estimates the probability that the diet of individuals is drawn from the population's diet as a whole (Petraitis, 1979; Bolnick *et al.*, 2002). All of the above indices range from 0 (indicating high individual specialization) to 1 (indicating complete generalization). Statistical significance for all indices was obtained through 1000 Monte Carlo permutations implemented in IndSpec 1.0 (Bolnick *et al.*, 2002). To further aid interpretation of the *PSi*, we also calculated an adjusted *PSi* value ($E = 1 - \text{mean}[PSij]$) following Araújo *et al.* (2009). This value was then scaled to the null model value. In this case, higher values of E indicate higher levels of individual specialization. Associations between indices of individual diet specialization, morphological variation, and genetic variation were scrutinized through correlations among distance matrices (*PSij*, PC1 for beak and head dimensions, F_{ST}, and R_{ST}) based on Mantel tests (Mantel, 1967).

We next considered influences surrounding the possibility of diet-clusters within the populations. To test for such clusters, we used the feeding observation matrix [standardized using the Hellinger transformation (Legendre and Gallagher, 2001)] in a non-hierarchical cluster analysis that separates individuals into different diet-clusters. The appropriate number of K diet-clusters was then objectively evaluated based on the Calinski-Harabasz criterion (Calinski and Harabasz, 1974), analysing $K = 1$ to $K = 10$ clusters. Two diet-clusters were identified (see Results) and we next compared these clusters for their level of individual specialization (based on the *PSi* matrix), their foraging-related morphological traits (beak PC1 and head PC1), and their average pairwise genetic distances (F_{ST} and R_{ST}). We assessed statistical significance by permuting the individual composition of each diet-cluster while keeping cluster sizes constant. Distances between diet-cluster centroids were interpreted as revealing the magnitude of divergence for the variable in question. Finally, we used a chi-squared test to evaluate correspondence between individuals assigned to the two beak morphs and individuals assigned to the two beak clusters. Statistical analyses were performed with the R software package using the 'vegan' library (R Development Core Team, 2010).

RESULTS

Individual specialization in *G. fortis*

We obtained independent feeding observations across 152 birds. From these birds, we analysed a subset of individuals ($n = 96$; Table 1) with at least six feeding observations each. In addition, for analyses with genetic data, we included only individuals with complete genotypic data at all ten loci ($n = 80$). Some of the morphological data have been reported previously (Herrel *et al.*, 2005a; Hendry *et al.*, 2006), the microsatellite data are a subset of those reported in De León *et al.* (2010), and some of the diet data were reported in De León *et al.* (2011).

Table 2 summarizes the different niche indices, both across all birds and within each designated beak size morph (small or large). All of the WIC/TNW ratios were lower than expected by chance, indicating that the total diet niche width of the population was significantly influenced by individual specialization. The observed proportional similarity index (mean observed $PSi = 0.47$) was also significantly – if only marginally – lower than

Table 1. Summary of feeding observations of individually banded *G. fortis* in each year at El Garrapatero

Year	Total observations	Individual birds with > 5 feeding observations	Individual birds with complete genotypes
2003	88	13	11
2004	156	21	18
2005	245	23	19
2006	304	37	30
2007	17	2	2
Total	810	96	80

Table 2. Individual niche properties and estimates of individual specialization in *G. fortis* at El Garrapatero

Estimator	All birds	Small morph	Large morph
WIC	1.005	0.997	1.014
BIC	0.893	0.876	0.895
TNW	1.899	1.873	1.910
WIC/TNW	**0.528** (0.59)	**0.532** (0.59)	**0.531** (0.59)
IS	**0.463** (0.51)	**0.455** (0.50)	**0.442** (0.48)
Wi	0.361	0.368	0.381

Note: These analyses were performed across both beak size morphs (small and large) and also for each morph separately. The estimates represent total niche width (TNW), the within-individual component of niche width (WIC), the between-individual component of niche width (BIC), and the degree of individual specialization (WIC/TNW). Also shown is average individual specialization (*IS*) based on the proportional similarity index (Bolnick, *et al.*, 2002) and the likelihood index (*Wi*) (Petraitis, 1979; Bolnick, *et al.*, 2002). These last three estimators range from 0 (indicating complete specialization) to 1 (indicating complete generalization) and statistical significance for WIC/TNW and *IS* was obtained through 1000 Monte Carlo permutations implemented in IndSpec v.1.0 (Bolnick *et al.*, 2002). Values shown in **bold** are significantly lower ($P < 0.05$) than the expected null value shown in parentheses.

expected by chance (mean expected $PSi = 0.52$), indicating that individuals commonly exploit some resources not typical of the population as a whole. Adjusted PSi further revealed individual specialization ($E = 0.53$). However, the level of specialization appeared relatively low when scaled to the null model (0.09). Small and large beak size morphs did not differ in PSi ($U = 1052$, $P = 0.92$), suggesting that individual specialization was not limited to one of the morphs but was instead characteristic of both, as well as when the two were considered together as a single population. Finally, the likelihood index (Wi) also revealed individual specialization (Table 2). Despite the fact that average individual specialization was not particularly high with respect to the null expectation, individuals varied in their degree of specialization and frequency distributions of PSi and Wi clearly reveal a substantial number of individuals that show a very high levels of individual specialization, with values below 0.40 (Fig. 2). Taken together, these results confirm the presence of individual specialization in *G. fortis* at El Garrapatero.

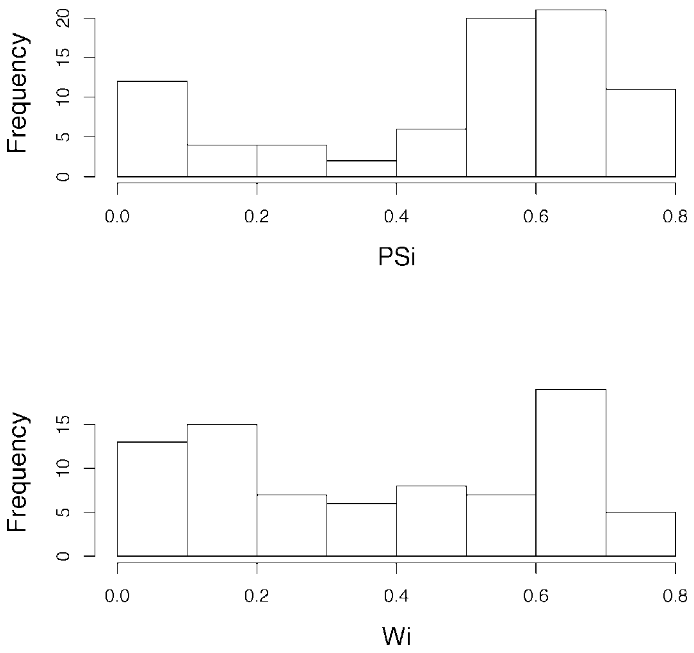

Fig. 2. The frequency distribution of overall individual diet specialization in *G. fortis* at El Garrapatero. The upper panel shows individual specialization based on the proportional similarity index (*PSi*) (Schoener, 1968; Bolnick *et al.*, 2002) and the lower panel shows individual specialization based on the likelihood index (*Wi*) (Petraitis, 1979; Bolnick *et al.*, 2002).

Across all birds, and momentarily ignoring statistical significance, individuals with more divergent diets were also more morphologically and genetically divergent, and individuals that were morphological divergent were more genetically divergent. Although all of these trends were as expected if individual specialization promotes adaptive divergence, few of them were statistically significant: beak size (PC1) against R_{ST} ($r = 0.142$, $P = 0.014$; Fig. 3) and F_{ST} ($r = 0.08$, $P = 0.100$); and *PSij* against head size ($r = -0.023$, $P = 0.67$), beak size ($r = -0.014$, $P = 140.39$), R_{ST} ($r = 0.013$, $P = 0.45$), and F_{ST} ($r = -0.049$, $P = 0.728$).

Cluster analyses on the foraging observation matrix revealed that $K = 2$ diet-clusters provided the best fit for our dataset (Fig. 4). Figure 5 shows the PCA biplot for the first two principal components and their five highest loadings with respect to feeding items. Individual diet specialization denoted via *PSi* was significantly lower within diet-cluster 1 ($PSi = 0.597$) than within diet-cluster 2 ($PSi = 0.105$; $t = -22.3$, $P < 0.001$). In addition, birds in the two diet-clusters differed in beak size ($P = 0.002$) and head size ($P = 0.010$), with larger birds in cluster 1 (Fig. 5). Furthermore, the chi-squared test indicated that the two beak size morphs were not randomly distributed between clusters (cluster 1: 29 large, 42 small; cluster 2: 3 large, 22 small; $\chi^2 = 5.686$, $P = 0.017$). We did not, however, find significant genetic differentiation between the diet-clusters (pairwise $F_{ST} = 0.006$, $P = 0.332$).

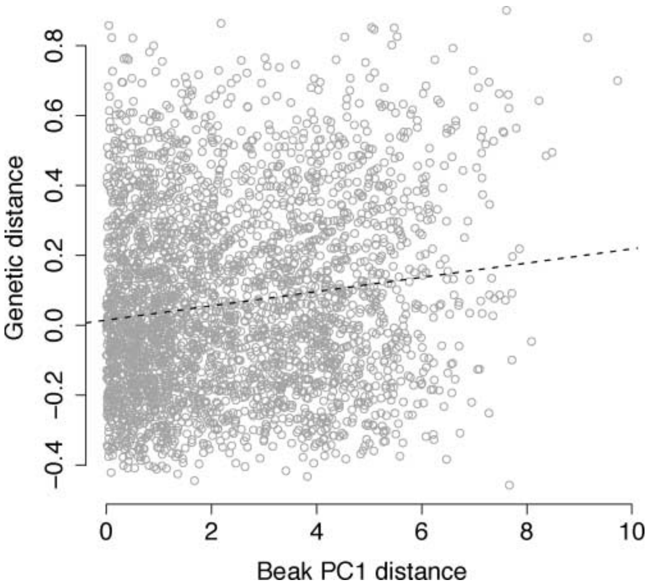

Fig. 3. Positive correlation between individual pairwise Euclidean distance in beak size (denoted as beak PC1) and individual pairwise genetic distance over 10 microsatellites (Slatkin's R_{ST}: $r = 0.142$, $P = 0.014$). Values for beak size versus F_{ST} (not shown in the plot) are $r = 0.08$, $P = 0.100$.

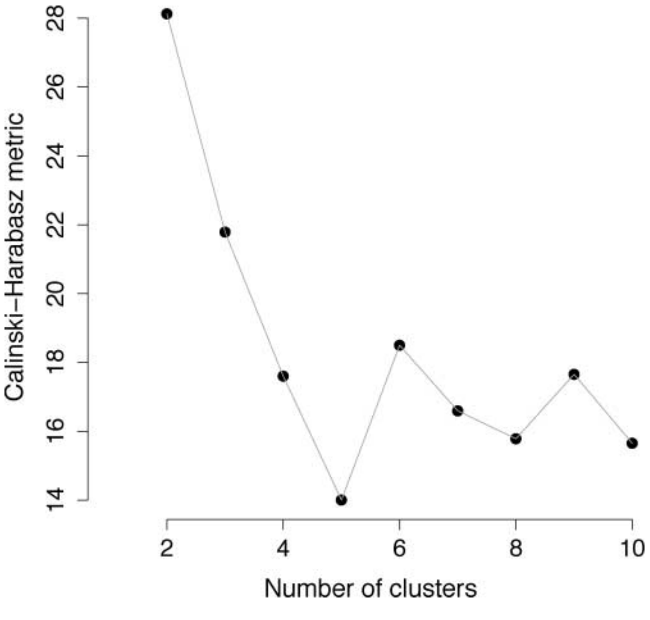

Fig. 4. Evaluation for the best fitting choice of K clusters separating individual finches on the basis of foraging observations. We chose $K = 2$ as the best fitting solution based on the Calinski-Harabasz metric (Calinski and Harabasz, 1974).

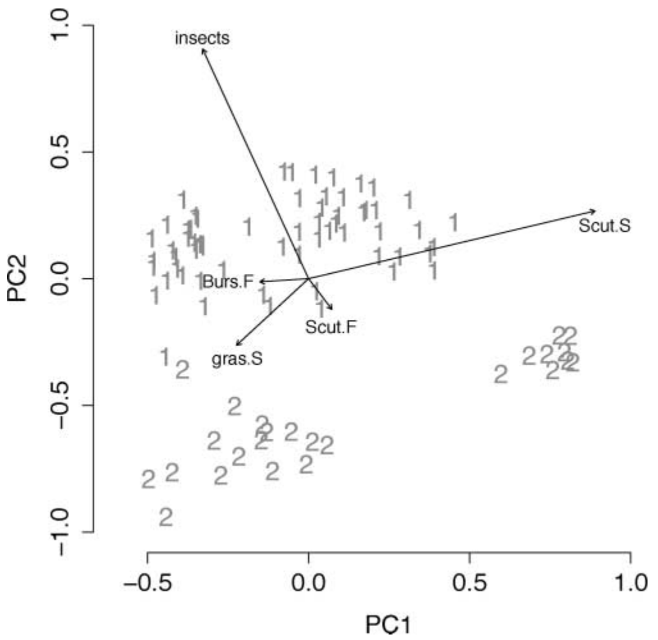

Fig. 5. Biplot of principal component analysis performed on the transformed foraging observation matrix. Individuals are plotted according to their respective assignment to one of the two diet-clusters. Arrows denote the five highest loadings for the first two principal components: insects, Scut.S = *Scutia spicata* seeds, Scut.F = *Scutia spicata* fruits, Burs.F = *Bursera graveolens* fruit, gras.S = grass seeds.

DISCUSSION

Individual specialization

Although the diets of Darwin's finches clearly vary among species (Lack, 1947; Bowman, 1961; Grant, 1999), they also can be quite variable within species (Grant *et al.*, 1976; Abbott *et al.*, 1977; Smith *et al.*, 1978; Price, 1987). Among the ground finches, *G. fortis* has the most variable diet, which also overlaps considerably with its smaller (*G. fuliginosa*) and larger (*G. magnirostris*) granivorous congeners. Each of these species, and the ground finch group as a whole, therefore might be considered generalists – at least within a broad class of seeds/fruits. And yet, a generalist group of this sort might be composed of a diversity of individual specialists, which in their differences might form the seeds of adaptive radiation. We considered this possibility in a population of Darwin's finches that is bimodal for the very traits that characterize differences between it and its granivorous congeners. We calculated formal indices of individual specialization, and compared these indices to the null expectation, as well as to foraging morphology and neutral genetic variation. Our premise is that information on individual specialization in this population can perhaps lend insight into the origins of diversification.

We found significant levels of individual specialization in *G. fortis* at El Garrapatero on Santa Cruz Island (Table 2). Average individual specialization according to all three indices was higher than expected by chance (Table 2). Although the difference between observed and expected random levels of individual specialization was small, it was also clear that many individuals showed very high levels of specialization (Fig. 2). These results conform to assertions that individual specialization is a common property of heterogeneous populations (Van Valen, 1965; Roughgarden, 1972; Bolnick *et al.*, 2003, 2007), including birds (Werner and Sherry, 1987; Araújo *et al.*, 2011). In general, individual specialization is thought to be the result of intraspecific competition that generates disruptive selection (Bolnick, 2001; Svanbäck and Bolnick, 2005, 2007; Martin and Pfennig, 2009; Agashe and Bolnick, 2010). Although we have not directly quantified competition within this population, we do know that disruptive selection among morphs is strong (Hendry *et al.*, 2009) and that competition influences beak size evolution in other *G. fortis* populations (Grant, 1999; Grant and Grant, 2002). It is therefore likely that competition is an important contributor to individual specialization in our study population.

There are several reasons why individual specialization is common in Darwin's finches. First, some Galápagos Islands – including Santa Cruz – show remarkable variation in plant species both within and between locations (Wiggins and Porter, 1971), thus allowing the potential for individuals to specialize on different foods. Second, the relative scarcity of competitor species on Galápagos should further allow the diversification of single species onto a diversity of resources [i.e. 'ecological release' (Lister, 1976)]. Third, intense periods of drought that prevent reproduction by most plants and insects lead to intense competition among finches for the rapidly diminishing seed bank (Grant *et al.*, 1976; Grant, 1999; Grant and Grant, 2002). Although these properties (a diversity of resources, a scarcity of other competitor species, and high competition within species) might lead to a population where everyone is a similar generalist, the outcome for Darwin's finches appears to be at least some individual specialization. And, as noted earlier, similar – although more qualitative – findings have been reported for the Cocos finch [*Pinaroloxias inornata* (Werner and Sherry, 1987)], another population of *G. fortis* (Price, 1987), and seemingly also *G. fuliginosa* (Kleindorfer *et al.*, 2006). The key question then becomes: what are the consequences of this individual specialization for adaptive radiation?

The seeds of adaptive radiation?

Our finding of significant individual specialization in a bimodal population supports theoretical predictions and empirical suggestions that individual specialization might be an important contributor to diversification (Wilson and Turelli, 1986; Skúlason and Smith, 1995; Bolnick *et al.*, 2003; Rueffler *et al.*, 2006; Day and Young, 2009). In particular, specialization by individuals on alternative resources can generate frequency-dependent competition that generates strong disruptive selection (Skúlason and Smith, 1995; Bolnick, 2001; Svanbäck and Bolnick, 2007; McCormack and Smith, 2008; Martin and Pfennig, 2009). This selection can then generate adaptive divergence and ecological speciation, perhaps even in sympatry (Schluter 1994; Dieckmann and Doebeli, 1999; Bolnick, 2004; Polechova and Barton, 2005; Rundle and Nosil, 2005). If this process is playing out in our study population, we would expect individual diets to correlate with the traits that influence foraging efficiency on different food types and that characterize the adaptive radiation. For ground finches of Galápagos, those traits are beak and head size (Schluter and Grant, 1984; Grant, 1999; Herrel *et al.*, 2005a, 2005b; Foster *et al.*, 2008).

In our study, all trends (ignoring statistical significance) were consistent with the expectation that individual specialization contributes to the diversification of adaptive traits within populations. That is, diet differences showed a positive trend with morphological and genetic differences among individuals. In addition, greater divergence in beak size was significantly associated with more marked differences (Fig. 3). However, all of these trends were very weak and not statistically significant – as is often the case in comparable analyses of other taxa (e.g. Araújo *et al.*, 2009; Bolnick and Paull, 2009; Agashe and Bolnick, 2010). The weakness of such trends might reflect noise in the estimation of individual diets, traits, and genetic variation. In our study, for example, more feeding observations per individual would likely have increased the precision of estimates of individual diets and diet specialization. In addition, individual specialization and within-population divergence might often be influenced by unmeasured traits. Although beak morphology is expected to be the most relevant trait in Darwin's finches (Lack, 1947; Bowman, 1961; Grant and Grant, 2002, 2008; Huber *et al.*, 2007; Hendry *et al.*, 2009), other morphological traits could also be important, as could cultural (Estes *et al.*, 2003), developmental, or stochastic effects. Finally, larger sample sizes might well have converted our non-significant trends into significant ones.

In adaptive radiation, diversification in traits is expected to eventually become associated with reproductive isolation and therefore genetic divergence. We tested whether this effect was evident in the context of individual specialization by asking whether individuals that differed more in diets were also more genetically divergent. Again, although just such a trend was suggested, it was very weak and not statistically significant. We suggest that individual specialization within a population is probably too early in the process of divergence to be strongly associated with genetic divergence in neutral markers. The reason is that neutral markers can be homogenized even in the presence of partial ecologically based reproductive barriers (Thibert-Plante and Hendry, 2010).

If individual specialization is to lead to diversification, then all of the patterns expected at the individual level should amplify as a population starts to become bimodal in adaptive traits. We therefore first asked whether our population, known to be bimodal for beak size (Hendry *et al.*, 2006), bite force (Herrel *et al.*, 2005a), and mating signals (Huber and Podos, 2006), was also bimodal for diet; and, indeed, we found that two diet-clusters was the best fit to the data (Fig. 4). We also found that birds making up the two diet-clusters differed in adaptive traits (beak PC1 and head PC1), although not in neutral genetic variation. As explained above, the differences in foraging morphology are expected in the case of adaptive radiation, and the lack of differences in neutral genetic markers is also expected for populations in the early stages of divergence (Thibert-Plante and Hendry, 2010). Overall, the stronger associations between diet and morphology when considering diet-clusters than when considering pairwise differences among individuals suggests that the diet-clusters might represent the beginnings of divergence.

Although our results are broadly consistent with the idea that individual specialization contributes to the early stages of adaptive radiation, additional work is necessary before definitive conclusions can be drawn. One reason is that individual specialization has been inferred also for finch populations that are not bimodal, such as *Pinaroloxias inornata* on Cocos Island (Werner and Sheery, 1987) and *G. fortis* on Daphne Major (Grant *et al.*, 1976; Price, 1987). In addition, we find substantial individual specialization even *within* the two beak size morphs at El Garrapatero. It is therefore clear that individual specialization is not sufficient on its own to cause diversification. Another reason is that associations between diets and traits that we observed were very weak and often non-significant, which means that they might not have been sufficiently important to play a critical role in diversification. More definitive evidence would come from a demonstration that individual specialization in El Garrapatero *G. fortis* is higher than that in bird populations that are not in the process of diversifying. We therefore encourage comparative analyses of individual speciation between populations that appear to represent different stages in progress towards ecological speciation and adaptive radiation.

General implications

Our results fit the paradigm of adaptive radiation in Darwin's finches (Lack, 1947; Bowman, 1961; Grant, 1999; Schluter, 2000; Grant and Grant, 2008). However, we here complement previous studies by providing evidence that individual specialization might promote and maintain the initial stages of divergence. In particular, individuals that differed more in key foraging traits (beak size and head size) differed more in neutral genetic markers. We also found a tantalizing trend towards birds with more divergent diets also being more morphologically and genetically distinct, although the association was weak and non-significant. A stronger result was seen in that individuals could be grouped by their diets into two clusters and the birds in these clusters differed in foraging traits (beak and head size), although not in neutral genetic markers. The observed morphological variation parallels the same axis of divergence observed among the ground finch species (Hendry *et al.*, 2006; Foster *et al.*, 2008), and because beak size differences are highly heritable both within and between species (Boag and Grant, 1978; Boag, 1983; Keller *et al.*, 2001; Abzhanov *et al.*, 2004), we expect similar parallelism in the ecological factors promoting the initial stages of divergence. Although divergence in resource use among individuals within populations appears to play an important role in promoting the initial stages of adaptive divergence in Darwin's finches, more data would help to refine these assertions.

ACKNOWLEDGEMENTS

The Galápagos National Park Service and the Charles Darwin Research Station provided logistical support and permits. E. Hilton, M. Rossi-Santos, D. Ruiz, A. Gabela, P. Kelley, M. Hendry, S. Huber, K. Huyghe, B. Vanhooydonck, and D. Delaney provided field assistance. J. Raeymaekers provided useful suggestions for data analysis. D. Sharpe provided useful comments on an earlier version of the manuscript. Funding was provided by the Secretaría Nacional de Ciencia, Tecnología e Innovación and the Instituto para la Formación y Aprovechamiento de los Recursos Humanos, Panamá (L.F. De León); the US National Science Foundation (J. Podos); the Natural Sciences and Engineering Research Council of Canada (A.P. Hendry); and the Smithsonian Tropical Research Institute (E. Bermingham). We thank the associated editor and anonymous reviewers for providing valuable comments on an earlier version of the manuscript.

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